HIGH DOSE INTRA-VENOUS VITAMIN C ADMINISTRATION TO PREVENT MORTALITY IN PATIENTS WITH SEPSIS: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL

DISSERTATION SUBMITTED TOWARDS PARTIAL FULFILLMENT OF THE RULES AND REGULATIONS FOR THE M.D. GENERAL MEDICINE EXAMINATION OF THE TAMIL NADU DR. M.G.R. UNIVERSITY, CHENNAI TO BE HELD IN MAY 2020

REGISTRATION NUMBER: 201711452

CERTIFICATE

This is to certify that this dissertation titled **"High dose intra-venous Vitamin C administration to prevent mortality in patients with sepsis: a randomized, double-blind, placebo-controlled trial"** is a bonafide work of Dr. Amith Balachandran (Registration Number : 201711452) carried out under my guidance towards partial fulfillment of rules and regulations for M.D. General Medicine Examination of the Tamil Nadu Dr. M.G.R. University, Chennai to be held in May 2020.

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DECLARATION

This is to certify that this dissertation titled **"High dose intra-venous Vitamin C administration to prevent mortality in patients with sepsis: a randomized, double-blind, placebo-controlled trial"** which is submitted by me towards partial fulfillment of rules and regulations for M.D. General Medicine Examination of the Tamil Nadu Dr. M.G.R. University, Chennai to be held in May 2020 comprises of original work done by me. The information taken from other sources has been duly acknowledged and cited.

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LIST OF ABBREVATIONS USED

AGE	Acute gastroenteritis
AKI	Acute kidney injury
APACHE	Acute physiology and chronic health evaluation
ARDS	Acute respiratory distress syndrome
BC	Before Christ
CAUTI	Catheter associated urinary tract infection
CI	Confidence interval
CKD	Chronic kidney disease
CLD	Chronic liver disease
CLP	Caecal ligation and perforation
CONSORT	Consolidated Standards of Reporting Trials
COPD	Chronic obstructive pulmonary disease
CRP	C reactive protein
CTRI	Clinical trial registry of India
CVA	Cerebrovascular accident
DBP	Diastolic blood pressure
DHAA	Dihydro ascorbic acid
DVT	Deep vein thrombosis
e.g.	example
ECF	Extracellular fluid
FIP	Faecal-induced peritonitis
G6PD	Gluclose-6-phosphate dehydrogenase
GCS	Glasgow comma scale
GLUTs	Glucose transporters
HDU	High dependency unit
HIF	Hypoxia-inducible factor
i.e.	*id est*(that is)
ICAM	Intercellular Adhesion Molecule
ICTRP	International Clinical Trials Registry Platform
ICU	Intensive care unit
IL	Interleukin
IRB	Institutional review board
ITT	Intention-to-treat
IV	Intravenous
IVIG	Intravenous Immunoglobulin
KDIGO	Kidney Disease: Improving Global Outcomes
LFT	Liver function test
LOS	Length of stay
LPS	Lipopolysaccharide
MAP	Mean arterial pressure
mcg	microgram
MDR	Multi-drug resistant

MODS	Multiorgan dysfunction syndrome
NF-κβ	Nuclear Factor kappa-light-chain-enhancer of activated B cells
NIV	Non-invasive ventilation
OP	Organophosphate
PAM	Peptidyl glycine α-amidating monooxygenase
PCR	Polymerase chain reaction
PLEX	Plasma exchange
PMN	Polymorphonuclear
POC	Point of care
RBCs	Red blood cell
RCT	Randomised control trial
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RR	Respiratory rate
RRT	Renal replacement therapy
SBP	Systolic blood pressure
SD	Standard deviation
SIRS	Systemic inflammatory response syndrome
SOFA	Sequential organ failure assessment
TLR	Toll like receptor
TNF-α	Tumour necrosis factor alpha
UTI	Urinary tract infection
VAP	Ventilator associated pneumonia
VCAM	Vascular cell adhesion molecule
WBC	Whole blood cell
μmol	micromole

1. INTRODUCTION

Sepsis is a constellation of physiological, pathological and biochemical abnormalities, induced by an infection. Sepsis is currently defined as “a life threatening Organ Dysfunction secondary to a dysregulated host response to infection (1)”. It is a major healthcare concern, leading to substantial healthcare costs. Although it is difficult to find the exact incidence, good statistical estimates deduce that sepsis is a leading cause for mortality and critical illness around the world (1). Sepsis survivors also have chronic psychological, physical and cognitive disabilities (2).

According to current understanding, it’s not just the infection, but also the body’s uncontrolled response to it , that manifests as a sepsis syndrome (1). It causes an overwhelming inflammatory response, endothelial dysfunction and microangiopathic response, and can affect almost all organ systems. Although a number of interventions have studies in Sepsis, the backbone of current therapy is still comprised of only antibiotics and supportive care. The mortality from Sepsis remains to be high (3).

Ascorbic acid or Vitamin C is an essential micronutrient that has significant reducing property. It has a number of biological functions including role in norepinephrine synthesis and synergism with glucocorticoids (4). A number of pre-clinical and clinical studies have thrown light to the possible benefits of Vitamin C in Sepsis. Vitamin C is cheap and largely safe. Despite a strong biological plausibility and promising data from observational studies, we lack robust evidence for routine use of Vitamin C in sepsis(5). If the benefit of Vitamin C in sepsis is proven, this could potentially be a cheap by effective intervention that can prevent a substantial number of deaths.

This single centre randomised control trial was conducted to evaluate the effect of Vitamin C administration in preventing Sepsis related mortality. To the best of our knowledge, there are few published randomised control trials till addressing this research question. This study is hence expected to bridge a large knowledge gap in this regard.

2. AIMS & OBJECTIVES

2.1 AIM

To study the effect of high dose intravenous Vitamin C administration in adult patients with sepsis

2.2 OBJECTIVES

a) To evaluate the effect of high dose intravenous Vitamin C administration on all cause in-hospital mortality in adult patients with sepsis

b) To evaluate the effect of Vitamin C administration on time to ventilator independence, time to vasopressor independence, length of ICU stay, length of hospital stay and new onset organ dysfunction in adult patients with sepsis

c) To identify the adverse effects of Vitamin C administration in sepsis

3. REVIEW OF LITERATURE

The review of literature was done through a comprehensive search of available literature indexed in PubMed till 1/10/2019 and is discussed under 18 subheadings

3.1 HISTORICAL PERSPECTIVES OF SEPSIS

A syndrome of fever and suppuration produced by a disease-producing entity that originated in the intestine and later spread to the rest of the body was first outlined in the Ebers Papyrus (1550 BC) (6). The word sepsis is derived from Greek and is first noted in the Epics of Homer and denotes rotting of flesh(7).

Eventually, Celsus identified the cardinal features of inflammation, the pathogenetic mechanisms of which are now known to play a key role in sepsis as well. The first definition of Sepsis is usually attributed to Hippocrates (460-370 BC). He defined Sepsis as “the process of death and decay associated with illness, putrefaction and a foul smell.” (8).

Schottmueller, in 1914 postulated that systemic symptoms and signs are caused by the release of pathogenic organisms into the blood (9). Pfeiffer demonstrated that the syndrome caused by experimental infection can be seen in the absence of a viable organism as well, proposing that some toxic factor of the organism or released by the organism could be responsible for the same. This lead to the concept of endotoxin and exotoxin (10).

Subsequent preclinical and clinical experiments helped to elucidate the role of Microbial endotoxins and exotoxins and host cytokines and chemokines in Sepsis. Histopathological changes in various organs and their molecular mechanisms were studied. The insights into the pathogenesis of Sepsis also helped pin-point possible targets of therapy. The story of sepsis in last 100 years, thus, has been a fascinating tale of discovery and man's never-ending attempt to conquer it(9) . Although there has been remarkable progress in our understanding and management, the adverse outcomes remain unacceptably high.

3.2 THE EPIDEMIOLOGY OF SEPSIS

The incidence of sepsis as reported by publications from around the world is increasing, probably due to increased lifespan leading to increase in aged population with multi-morbidities, and increased recognition of the syndrome(1) .From the data available from the Global Burden of Disease studies(11) , Adhikari et al estimated the global burden of sepsis to be between 15 and 19 million cases per year, of which approximately 5 million are in East Asia, 4 million in South Asia, and 2 million in sub-Saharan Africa(12) . According to this estimation 23% of Deaths in the world can be attributed to Infections. Numerous studies have tried to estimate the incidence of Sepsis around the world and have reported the incidence of severe Sepsis to be 51 cases per 100,000 population in England, Wales, and Northern Ireland, and 135 cases per 100,000 population in Taiwan. Of every 100,000 men in Spain, 114 develop sepsis during their lifetime. 9% of all ICU admissions in China are due to Sepsis (13). In the United states, Martin et al concluded that the population-adjusted incidence of Sepsis had increased

by 8.7% per year between 1979 and 2000, in an analysis of hospital discharge data for 750 million hospitalizations in the country. The analysis estimated a rate of about 1,665,000 case per annum events of sepsis between these years(13) .

An analysis of 1794 patients from 62 countries, of which 70 were from India, showed that 39% of patients who presented to the hospital with sepsis, progressed to septic shock. 86% of these patients required ICU admission(14) . In a study from 150 ICUs of 16 Asian countries, 10.9% of all ICU admissions were for Sepsis(15) . In another study that included 10,069 patients requiring ICU care, 29.5%(2973 patients) had sepsis on admission or during ICU stay(16) . Another retrospective analysis of an international database including the countries of the United States, Australia, Germany, Norway, Taiwan, Sweden and Spain found a global incidence of 437 per 100,000 person-years for sepsis between 1995 and 2015. However, this rate was not representative of the scenario in middle and low-income countries (17).

According to the 'Intensive Care in India: The Indian Intensive Care Case Mix and Practice Patterns Study'(INDICAPS(18)), that looked at the case mix among 4209 patients from 120 Indian ICUs, 28.3% of patients admitted to the ICU developed severe sepsis or septic shock during ICU stay. Of the 3115 patients admitted to Medical ICUs, 404 were admitted primarily for the management of sepsis. About 12.2% of patients of the INDICAPS cohort developed an infection in the ICU. In a multivariate regression analysis, severe sepsis or septic shock during ICU stay was found to be an independent risk factor for mortality. In another single-center Indian observational study, of 4711

admissions to ICU over a 5-year period, 282 were with severe sepsis. The predominant infection site was the respiratory tract and most common pathogens were Gram-negative microbes, among which *Acinetobacter baumanni* was the most common. ICU mortality, hospital mortality, and 28-day mortality in this cohort of 282 patients with severe sepsis were 56%, 63.6%, and 62.8% respectively (19).

The severity of sepsis has also increased over the years. In the US in 1993, an estimated 72.4% with Sepsis had only a single organ dysfunction, as compared to 58.2% in 2003. There was a 1.3, 1.9 and 2.7-fold increase in the proportion of patients with 2,3 or 4 organ dysfunction, respectively. The age-adjusted mortality rate related to severe sepsis increased at an annual rate of 5.6% from 1993 to 2003. It is, however, reassuring to note that the fatality has dropped and there is increased survival(20) . Another notable US based retrospective population-based analysis reported increased rates of sepsis and septic shock from 13 to 78 cases per 100,000 between 1998 and 2009 .The age-adjusted hospital mortality during this time associated with septic shock dropped from 40.4% to 31.4% (21). Possible reasons for increased rate of sepsis include advancing age, immunosuppression, and multidrug-resistant infection.

The incidence of sepsis is reported to be greatest in the winter, and is postulated to be due to increased prevalence of respiratory tract infections. The case fatality rate of sepsis was also seen to be 13% greater in winter as compared to summer, despite similar severity of the illness(22). The geriatric age group (≥65 years of age) seems to account for 60 – 85 % of all sepsis episodes across various publications. With an increase in

aging population worldwide, it is expected that sepsis incidence will continue to rise. Females had lower age-specific incidence and mortality, but this difference could be explained by the differences in site of infection and underlying disease (23–25).

The organisms causing sepsis vary. While gram-positive infections like *Staphylococcus* spp. , seem to contribute to a large proportion of Sepsis in the west, gram-negative infections probably predominate in the Indian scenario(19,25) . Sepsis can also be caused by polymicrobial infections. This trend is increasingly seen in elderly individuals.

The emergence of multi-drug resistant (MDR) organisms has posed another significant challenge in the management of sepsis. A study reported a rise from 1% to 16% of MDR gram-negative bacteria over an 8.5 year period(26). Elderly patients are at particularly high risk of harboring multidrug-resistant, gram-negative bacteria(27). The incidence of fungal sepsis, especially *Candida* spp., has also been on the rise(28).

3.3 OUTCOMES OF SEPSIS

Although estimation of the outcomes of Sepsis in patients is difficult due to paucity of worldwide data of representative population, there is a general consensus among the medical fraternity that, this condition is associated with significant adverse outcomes – which may be death, organ dysfunction or disability.

Studies from the United States project sepsis to be one of the top 10 leading contributors to mortality. The number of deaths from Sepsis have nearly tripled from 1979 to 2000, notably in parallel to the increase in incidence of sepsis (24). Studies have also looked at pitfalls in management being cause of sepsis mortality and concluded that mortality is due to the disease severity itself, and was possibly not preventable by any known intervention. For example, in a study from the US, 264 of the 300 deaths due to sepsis 88.0% were concluded to be unavoidable(29).

In India, with the available evidence, it is reasonable to conclude that more than a fourth of patients with Sepsis die, despite care in critical care units. In the ICON audit, the ICU and Hospital mortality rates of patients with Sepsis were 25.8% and 35.3% , respectively in patients with sepsis as compared to 16.2% and 22.4% in the whole ICU population (16). This indicates that our battle against Sepsis is not as effective as it is in other critical illnesses. In a cohort of 1285 patients from 150 ICUs of 60 Asian countries with severe sepsis, there was 36.7% and 44.5% ICU mortality and in-hospital mortality, respectively (15).

In the INDICAPS Study, the mean APACHE score at admission was the notably the highest (21.9+/-9.5) among patients who were admitted to ICU for primary management of sepsis. Of these 29.7% did not survive through the ICU course and the in-hospital mortality was 32.2% (18). The mortality from severe sepsis(34%) was similar to the mortality described in a study from 150 Asian ICUs(15)(15), but higher than the mortality rates from the IMPRESS study (Global – 28.4% , Asia – 30.8%)(14),

and ICON Study(25.8%)(16). In a multi-variate regression analysis of the INDICAPS Cohort, severe sepsis or septic shock during ICU stay was found to be an independent risk factor for mortality.

In another single-center Indian observational study, of 4711 admissions to ICU over a 5-year period, 282 were with severe sepsis. ICU mortality, hospital mortality, and 28-day mortality among these patients were 56%, 63.6%, and 62.8%, respectively(19).One also needs to assume that the actual mortality from Sepsis in developing countries including India is underreported, as a lot of patients with Sepsis are managed in hospital wards, and not in critical care units. The mortality in Nosocomial Sepsis has been reported to be 14.6% to 33% from around the world(30–33). In a single-centre prospective study from a south Indian tertiary hospital, the mortality of nosocomial sepsis was 22%(33)

The long term physical and psychological disabilities and cognitive problems in survivors of sepsis have also come to light in recent research. These have significant social and health care implications. In a large American cohort of sepsis survivors, that aimed at addressing this issue, incident severe sepsis caused statistically and clinically significant rise in moderate to severe disability. For instance, the prevalence of moderate to severe disability increased from 6.1% among eventual Sepsis survivors just before severe sepsis, to 16.7% (95% CI: 13.8%, 19.7%) after severe sepsis(2) . Patients who survived sepsis are also known to have a lesser lifespan, worse physical

function, worse health related quality of life and poorly perceived general health after sepsis(34,35).

Follow up studies done in cohorts of sepsis survivors have also shown up to tripling in odds of moderate or severe cognitive impairment after surviving an event of severe sepsis. In one such American studies done in an older cohort of sepsis survivors, severe sepsis was found to be associated with accrual of 1.5 new functional limitations in individuals with no, mild or moderate pre-existing functional limitations. These new disabilities were also significantly larger than those seen after hospital admissions for other conditions. It was also demonstrated that these disabilities translate to significant burden in terms of care-giver time, nursing home admissions, depression, and long term mortality (2). In addition, sepsis has been identified as a major risk factor leading to discharge to hospice facilities and 30—day readmissions in the west(36).

3.4 THE ECONOMICS OF SEPSIS

Sepsis is not only a challenge to the patients and the doctors, but also the hospital and health care system(37). The direct cost of caring for patients with sepsis has been shown to be 6-fold higher than caring for ICU patients without sepsis(38). According to data from the US, each septic patient consumes, during hospitalization, about US$ 25,000, corresponding to approximately $ 17 billion annually(23). A systematic analysis of 37 studies on cost and cost effectiveness analysis in Sepsis, estimated the median of mean ICU cost of Sepsis to be $27,461 per patient and the median of the mean hospital-wide

cost of sepsis per patient to be $32,421 (2014 dollars)(39). A study from the Christian Medical College Hospital, Vellore has also noted that Hospital acquired infections, a proportion of which leads on to sepsis, causes significantly increased length of stay in hospital and significantly higher hospital costs, although mortality was not changed(40). As Silvia and Araujo rightly conclude, management of sepsis is not only challenging, but also costly(41). The presence of co-morbid illnesses, and acuity and severity of illness play a large role in deciding the healthcare cost in sepsis(37).

Many newer modalities that have stood the test of scientific analysis in Sepsis care have failed the test of cost effectiveness analysis(13). Usage of newer modalities, with either good or limited data on benefit in Sepsis on a routine basis in Indian ICUs, is limited by the economic constraints. It is in this setting that the need for inexpensive and novel treatment modalities in Sepsis arises. Vitamin C is such an inexpensive drug that has the potential to bridge the gap for a safe, effective, and cheap intervention in Sepsis.

The impact of Sepsis is so profound that September 13th of every year is commemorated as 'The world Sepsis Day' since 2012, to raise awareness about this deadly illness(42).

3.5 PATHOPHYSIOLOGY OF SEPSIS

The understanding of the pathophysiology of Sepsis, its molecular mechanisms and microbe specific mechanisms are ever evolving. The normal response of human body to infection attempts localizing and controlling the microbial invasion, while initiating the tissue repair, through a complex cascade of mechanisms. This mechanism involves

recruitment of phagocytes – both fixed and circulating, as well as release of chemical mediators - both anti-inflammatory and pro-inflammatory. These normal responses may be able to successfully fight off infections and localize them – hence not all infections progress to sepsis. According to our current understanding, sepsis sets in when the host response to fight an infection becomes so overwhelming that these response mechanisms damage normal host tissue(1). In other words, when the response to infection becomes generalized and involves the normal organs and tissue remote to the site of infection, sepsis ensues.

3.5.1 Normal Host Response To Infection

When a microbe enters the body, the components of the microbe are recognised by the body's innate immune cells, especially the macrophages. This is made possible by several mechanisms including recognition of Pathogen specific molecular patterns (PAMPs) and endogenous danger signals (Alarmins or Danger associated Molecular patterns-DAMPs) by Pathogen recognition receptors (PRRs) present on host immune cell surface; and binding of various myeloid receptors (TREM-1, MDL-1) to microbial components. The most widely known PRRs are toll like receptors (TLRs) (43,44). Microparticles from circulating and endothelial cells also participate in an inflammatory response.

This binding of immune cell receptors to microbes leads to a cascade of effects like:

- NF-κβ (cytosolic nuclear factor-kb) activation by TLRs results in induction of large sets of genes that code for cytokines, chemokines (ICAM-1, VCAM-1) and nitric oxide.

- Activation of Polymorphonuclear leucocytes (PMNs), which express adhesion molecules causing their aggregation and margination to vascular endothelium. The PMNs then go through rolling, adhesion, diapedesis, and chemotaxis , and move to the injury site(45).

- PMNs release chemical mediators at the injury site causing the cardinal signs of local inflammation, in an attempt to fight the infection.

- Pro-inflammatory mediators like TNF-α and IL-1; and anti-inflammatory mediators like IL-10 and IL-6 secreted by macrophages regulate the process of local inflammation(46).

If this process is successful and well regulated, the infectious insult is overcome and homeostasis is restored. Tissue repair and healing begin.

3.5.2 Progression Of Infection To Sepsis

Sepsis ensues when the release of pro-inflammatory mediators, as a response to infection, exceeds the boundaries of local injury. It is often conceptualized as malignant intravascular inflammation(47). The cause for this overspill of inflammation is likely multifactorial, including the effects of the microorganism, excess secretion of pro-inflammatory mediators and dysregulated complement activation.

- **Microbial Factors:** Bacterial endotoxins(cell wall components) and exotoxins(secretory products) may lead to progression of a local infection to sepsis as exemplified by the observations that endotoxin is present in the blood of septic patients,

the levels of plasma endotoxin correlates with severity of sepsis and organ dysfunction, and infusion of endotoxin to humans reproduces many features of sepsis (48).

- **Pro-inflammatory mediators:** Cytokines like TNF-α and IL-1, if secreted in large quantities, may spill in the bloodstream contributing to progression to sepsis. This can cause pyrexia, shock, leucocytosis, induction of other cytokine secretion, and activation of coagulation and fibrinolysis. It is known that patients with Sepsis have higher levels of circulating TNF-α, infusion of the same reproduces clinical features of septic shock, and antibodies against the same protects animals from endotoxin mediated damage(49) .

- **Activation of the complement system:** The complement system is activated by the cytokines released in sepsis. This may lead to enhanced inflammatory response, vascular leak, and add to sepsis related mortality. Inhibition of Complement system in experimental models of sepsis has led to decreased inflammation and mortality(50) **.**

- **Genetic susceptibility:** Various single nucleotide Polymorphisms (SNP), especially the ones involving genes coding for cytokines, cell surface receptors, lipopolysaccharide ligands etc. have been associated with increased susceptibility to infection and poor outcomes. Thus genetic factors may play a role in determining progression to sepsis(51).

3.5.3 *Cellular Injury And Organ Dysfunction*

In sepsis, cellular injury precedes organ dysfunction. The cellular injury is postulated to be caused due to factors like(52)

- **Tissue Ischemia**: Microcirculatory and endothelial dysfunction may contribute to this.
- **Alteration of mitochondrial function and cytopathic effect:** There is direct cytokine mediated inactivation of respiratory enzyme complexes, oxidative stress damage, and mitochondrial DNA breakdown that has been demonstrated ins sepsis
- **Accelerated apoptosis:** The cell and tissue damage in turn translates into clinically relevant organ dysfunctions.

The organ specific manifestations of Sepsis briefly include the following:

- **Circulation:** There is systemic vasodilation, that may result in shock. Prostacyclin and nitric oxide (NO) are the major mediators causing vasodilation. Impaired compensatory secretion of antidiuretic hormone (vasopressin) may contribute to this effect. Hypotension may also be due to redistribution of intravascular fluid. Sepsis causes a decrease in the number of functional capillaries, thereby causing inability to extract oxygen maximally at tissue level (53).
- **Heart :** Sepsis can cause decreased myocardial contractility, and that can add an element of cardiogenic shock(54).
- **Respiratory system:** There is endothelial injury, disturbed capillary blood flow, and enhanced microvascular permeability in the pulmonary vasculature causing interstitial and alveolar pulmonary edema. This causes ventilation-perfusion mismatch and hypoxemia manifesting as an Acute Respiratory Distress syndrome(53).

- **Gastro-Intestinal Tract:** The circulatory compromise may depress the normal barrier function of the gut, allowing translocation of bacteria and endotoxin into the systemic circulation and possibly extending the septic response(55).
- **Renal System:** An acute Kidney Injury is the end result of all the Sepsis related insult to the Kidney, and is caused by numerous mechanisms. Acute tubular necrosis due to hypoperfusion and hypoxemia is probably the major mechanism. Other mechanisms include direct renal vasoconstriction, cytokine mediated damage, systemic hypotension, neutrophil mediated injury etc.(56).
- **Nervous system:** Sepsis induced neurological disturbances are attributed to the changes in alterations in cell signaling and metabolism. Blood brain barrier disruption and mitochondrial disruption may also contribute. Clinically, this is manifested as Septic encephalopathy(57).
- **Immune System :** early pro-inflammatory state in severe sepsis often develops into a later and prolonged state of immune system dysfunction(46).
- **Hematopoietic system:** Sepsis is known to cause bone marrow suppression, and marked cytopenia. The mechanisms are yet to be fully elucidated(58).

3.6 CLINICAL PRESENTATION AND DIAGNOSIS OF SEPSIS

Patients with sepsis present with both infection site -specific features (e.g.: Cough, Expectoration, Crepitations and bronchial breath sounds in case of pneumonia), and features of multiple organ dysfunction (hypoxemia, oligo-anuria, encephalopathy etc.). They may have hyperthermia or hypothermia, tachycardia, tachypnoea, and often shock.

There are no laboratory parameters specific to sepsis. Leucocytosis, left shift, hyperglycaemia, elevated CRP, arterial hypoxemia, elevated creatinine, coagulation abnormalities, thrombocytopenia, hyperbilirubinemia, features of adrenal insufficiency, and sick euthyroid syndrome can be present, depending on the severity and organ systemic involved(59).

Hyperlactatemia is a marker of organ hypoperfusion and has been included as an important variable to define severity of sepsis(3). Procalcitonin is the latest addition to the diagnostic armamentarium in sepsis. Elevated procalcitonin has been shown to be associated with bacterial infection and sepsis. However a large metanalysis has shown that procalcitonin may not readily distinguish systemic inflammation due to infection from other causes (60). The gold standard to diagnose the etiological agent would be culture of the blood or affected body fluid/tissue.

A constellation of clinical, physiological, microbiological, laboratory, and radiological data is thus required for the diagnosis of sepsis. The diagnosis is made empirically at the bedside upon presentation more often than not. This is later confirmed retrospectively when follow-up data returns (e.g. positive blood cultures), or there is evident response to antibiotics(59).

3.7 NEW DEFINITION OF SEPSIS & IMPLICATIONS IN THIS STUDY:

Sepsis is immediately recognizable however unusually challenging to define(8). The first definition of Sepsis is usually attributed to Hippocrates (460-370 BC). He defined Sepsis as "the process of death and decay associated with illness, putrefaction and a foul smell"(8).

It was in the 1980s, when a 'Sepsis syndrome" based criteria was introduced and all the trials started using these criteria for patient recruitment(61). Dissatisfaction with the sepsis syndrome criteria and an emerging need articulated by several pharmaceutical companies planning trials of novel mediator-targeted therapy prompted the American College of Chest Physicians and the Society of Critical Care Medicine to host a consensus conference outside Chicago in August 1991(62).The aim of the conference was to develop new definitions for sepsis and organ failure, and criteria for the use of novel therapies. The concept of SIRS emerged after this and sepsis was considered to be SIRS caused due to an infection.

The 2001 definitions conference reaffirmed the concepts and terms from the 1991 conference, but proposed an expanded set of criteria to define SIRS, and presented a template for a novel stratification system for sepsis—the predisposition, insult, response, organ dysfunction (PIRO) model (63). In view of the ease of use, the 1991 SIRS based criteria remained the standard inclusion criteria for most trials done in sepsis(8,64–66).

Table 1: A Comparison of Sepsis 1,2 and 3 Definitions of Sepsis (40)

First Sepsis Definitions 1992	*Second Sepsis Definitions 2003*	*Third Sepsis Definitions 2016*
Documented/suspected infection and SIRS criteria (2 or more of the following): -Temperature > 38˚C or < 36˚C -Heart rate > 90/min -Respiratory rate > 20/min or Pa_{CO_2} < 32 mmHg (4.3 kPa) -Leukocytosis > 12,000/μl or leukopenia < 4,000/ μl	**Documented/suspected infection and Some of the following:** **General variables** -Fever (core temperature > 38.3˚C) -Hypothermia (core temperature < 36˚C) -Heart rate > 90/min -Tachypnea -Altered mental status -Significant edema or positive fluid balance -Hyperglycemia in the absence of diabetes **Inflammatory variables** -Leukocytosis (WBC count > 12,000/μL) -Leukopenia (WBC count < 4000/μL) -Normal WBC count with >10% immature forms -Plasma C-reactive protein increase -Plasma procalcitonin increase **Hemodynamic variables** -Arterial hypotension -Sv_{O_2} > 70% -Cardiac index > 3.5 L/min/m^2 **Organ dysfunction variables** -Arterial hypoxemia -Acute oliguria -Creatinine increase > 0.5 mg/dL -Coagulation abnormalities -Ileus -Thrombocytopenia -Hyperbilirubinemia **Tissue perfusion variables** -Hyperlactatemia (>1 mmol/L) -Decreased capillary refill or mottling	**Documented/suspected infection and A change of 2 or more in the total SOFA score:** - Respiratory ($Pa_{O_2}/F_{I_{O_2}}$) - Nervous System (Glasgow coma scale) - Cardiovascular (MAP or vasopressor requirement) - Liver (Bilirubin) - Coagulation (Platelets) - Renal (Creatinine or urine output) **Screening for patients likely to have sepsis:** 2 out of 3 criteria of quick SOFA (qSOFA): - Alteration in mental status - Systolic blood pressure < 100 mmHg - Respiratory rate < 22/min

The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) initiative introduced a new definition for sepsis, defining it as “life-threatening organ dysfunction caused by a dysregulated host response to infection”(1) . This definition identifies the organ dysfunction as the clinical phenotype of Sepsis, and eliminated the need for the terminology of Severe Sepsis. In addition, by describing the host response as dysregulated, the definition acknowledges the apparent paradox that manifestations of over-activation and suppression of the immune response could coexist and that the resulting syndrome was neither hyperinflammation nor immunosuppression, but rather something more complex(8). Whereas the SIRS based criteria included presence of

organ dysfunction as a mandate for severe sepsis, according to Sepsis 3 definition, organ dysfunction as defined by an increase in SOFA score as a prerequisite for Sepsis itself. Septic shock was recognized as a subset of Sepsis, where underlying cellular and metabolic changes, and circulatory abnormalities are profound enough to increase mortality substantially. This is identified when Sepsis is associated with persisting hypotension requiring Vasopressors to maintain MAP ≥ 65 mm Hg and having a serum lactate of > 2mmol/L despite adequate volume resuscitation.

Multiple validation studies have suggested that the SOFA based definition of Sepsis is superior to the SIRS based definition as it better correlates with the outcome(67–70). In contrast with the original consensus definition, which was created purely from expert opinion, the Sepsis-3 Task Force sought to use a data-driven approach to support their criteria(71). However, there are concerns regarding the use of the same in clinical research(72,73).We used the Sepsis 3 definition for defining the inclusion criteria in our trial.

This has a few implications. Firstly a direct comparison with other studies that used other interventions in Sepsis may not be possible(72). Secondly, the retrospective study based on which the sample size for this study is calculated uses the 1991 consensus criteria of sepsis, severe sepsis and septic shock. This study has only included patients with severe sepsis and septic shock(74). However , the presence of organ dysfunction, which was the defining entity for severe sepsis in the earlier definition(62), is currently a prerequisite for the definition of sepsis itself(1). Hence it seems reasonable and logical

to assume that our future cohort of patients with sepsis (as defined by Sepsis 3) are more likely to match the definition of ‘severe sepsis’ and ‘septic shock’ as per the 1991 consensus criteria(62); a sample size calculation based on this could be valid.

3.8 CURRENT MANAGEMENT OF SEPSIS AND STUDIED INTERVENTIONS:

The early management of patients with sepsis centres on the administration of antibiotics, IV fluids, and vasoactive agents, followed by source control. Unfortunately, there is no high-quality evidence (from one or more randomized controlled trials) demonstrating that any of these interventions alters outcome(75).It is, however, likely that the early detection of sepsis with the timely administration of appropriate antibiotics is the single most important factor in reducing morbidity and mortality in sepsis(76). It has become increasingly apparent that in many patients there is a long delay in both the recognition of sepsis, and the initiation of appropriate therapy. This has been demonstrated to translate into an increased incidence of progressive organ failure and a higher mortality(77,78).

The consensus guidelines based on current evidence recommend IV crystalloid resuscitation; early administration of IV antibiotics; source control measures; appropriate Vasopressor use; Corticosteroid use (in case of septic shock refractory to vasopressors); Judicious use of blood and blood products; and other supportive measures as indicated like Renal replacement therapy, ulcer and DVT prophylaxis. Therapies like IVIG, Glutamine and Arginine supplementation, and Omega 3 fatty acid supplementation are not recommended in the guidelines. Anti-oxidants are also not a part of consensus guidelines in Sepsis(3).

A number of Immune modulators have undergone Phase 2 or 3 clinical trials during the past 30 years and have either failed to show any clinical benefit, or have shown an initial benefit but failed to show consistent benefit in later larger trials(79). A list of interventions in Sepsis that have undergone the test of research and failed is compiled in table 2 (79) .

Table 2: Interventions Studied in Sepsis with Negative Result (79)

High-dose steroids	PMX-b-conjugants
Escherichia coli J5 antisera	PMX-b-columns
TNF mAb	Eritoran tetrasodium
Chimeric TNF mAb	Anti-CD14 mAb
Humanized TNF mAb	Interleukin-1 receptor antagonist
TNF-antigen binding fragment of immunoglobulin	PAF receptor antagonists
sTNFR1:Fc	IV immunoglobulin
sTNFR2:Fc	PAF-acetyl hydrolase
Anti-lipid A E5 mAb	Ibuprofen
Anti-lipid A HA1A mAb	Anti-β2 integrin
Bactericidal permeability increasing protein	Albumin hemoperfusion
Heparin	Nitric oxide synthase inhibitors
Antithrombin	Growth hormone
Complement component 1 esterase inhibitor	Tight glycaemic control
Tissue factor pathway inhibitor	Stress dose steroids
Recombinant human activated protein C	Bradykinin inhibitors
Nematode anticoagulant protein c2	TAK (Takeda) 242
Recombinant high-density lipoprotein and phospholipid	Lactoferrin
complexes	Etanercept

It is evident that the evidence-based options available for Sepsis are limited. Rapid fluid resuscitation, Vasopressor and Ventilatory support, and antibiotic therapy form the integral part of sepsis care; they are administered using a “do no harm” strategy(80)(80). Newer interventions are the need of the hour, considering the high incidence and mortality of the condition .

3.9 VITAMIN C IN HEALTH AND DISEASE

Vitamin C is an essential micronutrient and has a number of biological functions in human body (81). Most mammalian species synthesize ascorbic acid de novo from glucose in the liver, through a biosynthetic pathway involving gulono-gamma-lactone oxidase for the terminal step. But primates and guinea pigs are absolutely dependent on exogenously supplied dietary vitamin C due to inactivation of the gulono-gammalactone oxidase gene by mutation(82). Consequently, when humans do not ingest vitamin C in their diets, a deficiency state occurs that manifests as scurvy.

The Recommended daily intake of Vitamin C is in the range of 75–110 mg/day(83). Critically ill patients probably require significantly higher intakes of ascorbate due to enhanced metabolic turnover of vitamin C during the severe inflammatory response. In healthy fasting humans, circulating levels of ascorbate are typically in the range of 50–70 μmol/l, whereas levels <23 μmol/l are considered marginally deficient (or hypovitaminosis C), and levels <11 μmol/l are considered severely deficient and potentially scorbutic (84).

Severe vitamin C deficiency has been known for many centuries as the potentially fatal disease — scurvy. By the late 1700s the British navy was aware that scurvy could be cured by eating oranges or lemons, even though ascorbic acid would not be isolated until the early 1930s. Symptoms of scurvy include subcutaneous bleeding, poor wound closure, bruising easily, hair and tooth loss, and joint pain and swelling (85).The response of scurvy to Vitamin C supplementation Is dramatic. It is, in fact, interesting

that the probable first known clinical trial in history was performed by James Lind in Scurvy(86).

Apart from Scurvy, Vitamin C has been postulated to be of use in prevention and treatment of several other diseases. Prospective cohort studies and randomized control trials have shown preventive benefit of Vitamin C supplementation in heart failure, strokes, Hypertension, multiple cancers, Alzheimer's disease, Gout and cataract(Reviewed in 85).There have been propositions that genetic factors may contribute to Vitamin C deficiency and Scurvy, in addition to dietary deficiency(87).

Effect of Vitamin C supplementation on mortality has also been studied. In the Vitamins and Lifestyle Study; 55,543 participants were followed up for 5 years; it was found that Vitamin C supplementation was associated with a small decrease in mortality — although there was no association with cardiovascular or cancer specific mortality(88). In the EPIC-Norfolk multicenter prospective cohort study, there was a strong inverse association between plasma ascorbic acid level and mortality from all-causes, CVD, and ischemic heart disease (89). A dose-response decrease in cancer and overall mortality risks with higher vitamin C levels was observed in NHANES III that studied 16,008 adults (90).

With respect to disease treatment, there is evidence of benefit from Vitamin C to various extents through various mechanisms in a number of diseases – Heart failure, hypertension, Diabetes Mellitus, multiple cancers, common cold, asthma and lead

toxicity to name a few (Reviewed in 85). For example, a meta-analysis of 29 short-term trials indicated that vitamin C supplementation at a median dose of 500 mg/day for a median duration of eight weeks reduced blood pressure in both healthy, normotensive and hypertensive adults(91).

Thus, Vitamin C, through its various biological functions and actions on multiple biological pathways, has preventive and curative role in multiple conditions. The possible role of Vitamin C in sepsis and the evidence so far is discussed below.

3.10 VITAMIN C STRUCTURE AND METABOLISM

3.10.1 Vitamin C Structure

Vitamin C is the L -enantiomer of Ascorbate, with a chemical formula C6H8O6 and a molecular mass of 176.14 grams per mol. Its IUPAC name is (2*R*)-2-[(1*S*)-1,2-dihydroxyethyl]-3,4-dihydroxy-2*H*-furan-5-one(92) (92). Vitamin C can assume 3 redox forms in the body as shown in Figure 1 .More than 99% of Vitamin C in the body occurs in the form of Ascorbate anion in normal physiological conditions(4).

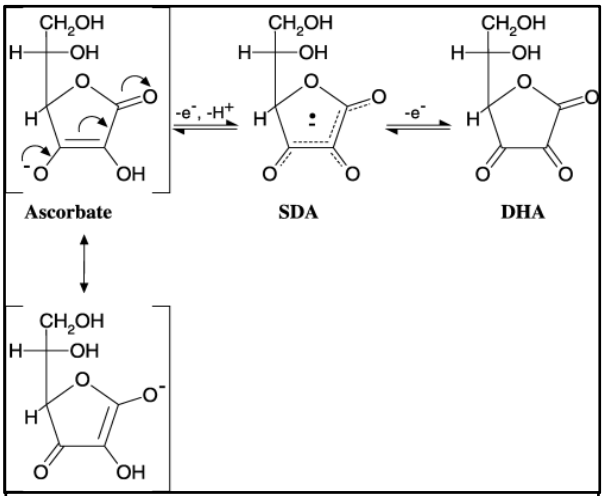

Figure 1:Structure of Ascorbic acid and its three redox States

(ascorbate, fully reduced form; SDA, monooxidized form; DHA, fully oxidized form) (92,93)

From a structural point of view, it is also one of the rare compounds containing a hydroxyl group that is so acidic as to be completely dissociated at neutral pH (carbon-3 hydroxyl pK_a=4.2)(93).

The electrons present in the ascorbate molecule possibly account for all its physiological effects. Vitamin C is a donor of electrons, making it a reducing agent. Vitamin C is generally termed as an anti-oxidant as the electrons from the molecule can reduce oxidized species. However this terminology is not entirely correct. Electrons from Vitamin C can reduce metals such as Iron and Copper leading to superoxide and hydrogen peroxide formation, and subsequent generation of ROS. Thus ascorbate, in special circumstances, generates oxidants(4).

3.10.2 Vitamin C Synthesis – Evolutionary Perspectives

Vitamin C is synthesised in many vertebrates. All plant species studies so far also synthesise Vitamin C. Yeasts form a C_5 analogue of ascorbate, D-dehydroascorbate. However, animals, plants, and fungi produce Vitamin C through different pathways(94). Vitamin C synthesis in animals has been well elucidated in Sea Lamprey, suggesting its evolution in early vertebrates. However the ability to biosynthesise Vitamin C was subsequently lost in a number of species — including certain species of fishes and birds, bats, Guinea pigs, and primates including humans(93). Fish, amphibians, and reptiles produce Vitamin C in the kidney; mammals synthesise it in the liver. The biosynthetic pathway and enzymes involved in Vitamin C biosynthesis in animals are illustrated in Figure 2.

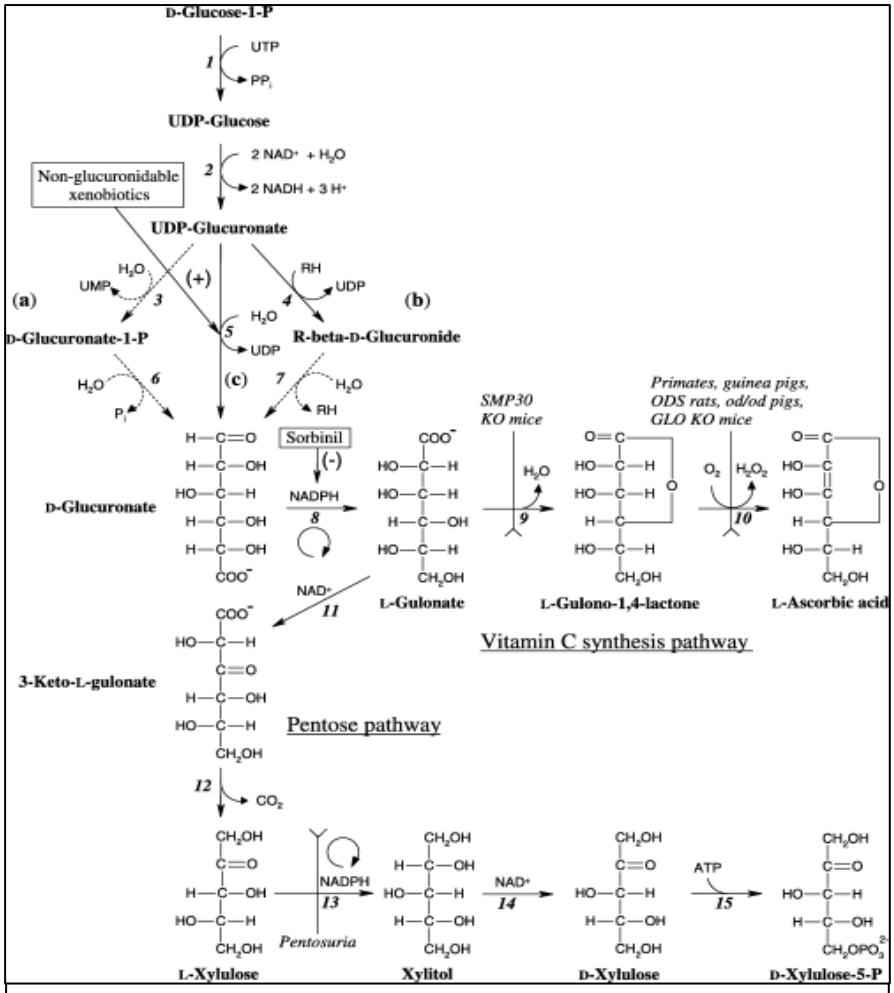

Figure 3: Vitamin C bio-synthesis in animals (93)

The reactions are catalyzed by the following enzymes: 1, UDP-glucose pyrophosphorylase; 2, UDP-glucose dehydrogenase; 3, nucleotide pyrophosphatase; 4, UDP-glucuronosyltransferase; 5, UDP-glucuronidase; 6, phosphatase; 7, β-glucuronidase; 8, glucuronate 9, gulonolactonase; 10, L-gulonolactone oxidase; 11, L-gulonate 3-dehydrogenase; 12, decarboxylase; 13, L-xylulose reductase; 14, xylitol dehydrogenase; 15, D-xylulokinase

Enzymological studies have identified GLO (L-gulonolactone oxidase, Enzyme No 10 in the pathway shown in Figure) deficiency — due to a mutation in the coding gene — as the reason for the inability of certain animals including man to synthesize their own vitamin C(95).

3.10.3Absorption And Transport Of Vitamin C

As discussed earlier, Ascorbic cannot be biosynthesized in human beings, making it an essential micronutrient or a Vitamin. Ascorbic acid and dehydroascorbic acid (DHAA or oxidized vitamin C) are dietary sources of vitamin C in humans(96).

Vitamin C uses 2 groups of transporters(96)

- Sodium dependent Vitamin C transporters (SVCT) 1 and 2 which are specific transporters for Ascorbic acid for intracellular influx. SVCT 1 is seen in small intestinal epithelium and PCT of Kidneys, and SVCT 2 is seen in other tissues.
- Glucose transporters (GLUTs) – that transport the ascorbate oxidation product DHA into the cells. Intracellularly, DHA is immediately reduced to ascorbate. This process of intracellular transport of Vitamin C is called Ascorbate recycling.

Gut absorption of Vitamin C occurs through SVCT1 from the small intestine. Some Ascorbic acid may be oxidized to DHA in the gut and transported by GLUT. After absorption, the highly soluble Vitamin C is distributed from blood throughout the extracellular fluid (ECF) (4,96) .

Tissue uptake of Vitamin C occurs mostly via SVCT 2. The tissue concentrations of Vitamin C depend on the concentration gradient, and in turn the Vitamin C intake. Tissue concentrations of vitamin C, are usually in milli molars and far higher than the concentration required for its action as a coenzyme. The major portion is in liver, brain and Kidneys(4,96).

Other than Saliva, red blood cells is the only body compartment that has lower Vitamin C levels as compared to plasma. RBCs obtain vitamin C via a DHA pathway(ascorbate recycling) since mature RBCs do not contain SVCT2(4). There is also constant efflux of Vitamin C from cells to plasma to maintain an equilibrium. The mechanism of Vitamin C efflux is unknown. SVCT 1 is also responsible for re-absorption of Vitamin C from the renal Proximal convoluted tubule(4).

3.10.4 Vitamin C Metabolism

Under normal physiological conditions, more than 99% of Vitamin C in the body occurs in the form of Ascorbate anion. From the double bond between Carbons 2 and 3 , it can sequentially donate two electrons, giving rise to ascorbate radical and Dehydroascorbic acid(DHA) respectively(4). DHA undergoes hydrolysis and irreversible ring rupture to form 2, 3-diketogulonic acid. The metabolic products of 2, 3-diketogulonic acid include oxalate, threonate, and possibly xylose, xylonic acid, and lynxonic acid — of which oxalate is clinically significant. DHA may be reduced back directly to ascorbic acid by enzyme-dependent mechanisms or sequentially to ascorbate radical and ascorbic acid by glutathione. The electron donor property of Vitamin C leads to its various physiological effects.

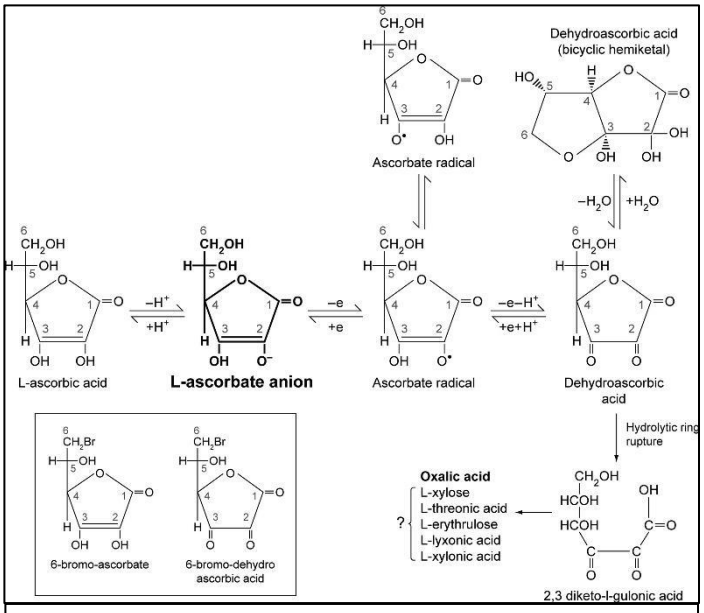

Figure 5: Vitamin C Structure and Metabolism(4)

3.11 FUNCTIONS OF VITAMIN C

Most function of Vitamin C in vivo can be attributed to its electron donating capacity. Ascorbate acts as an electron donor for 15 mammalian and three fungal enzymes(4).

Table 3:Well described Biological Actions of Vitamin C in mammals(4)

1. Co-Factor For Enzymes	
Enzyme	*Function*
Dopamine *β*-monooxygenase	Norepinephrine biosynthesis
Peptidylglycine *α*-amidating monooxygenase	Amidation of peptide hormones
Prolyl 4-hydroxylase(3 iso-enzymes)	Collagen hydroxylation
Prolyl 3-hydroxylase	Collagen hydroxylation
Lysyl hydroxylase	Collagen hydroxylation
Prolyl 4-hydroxylase	Hypoxia-inducible factor (HIF) hydroxylation
Asparaginyl hydroxylase or FIH-1 (factor inhibiting HIF)	Regulation of HIF
Trimethyllysine hydroxylase	Carnitine biosynthesis
γ-Butyrobetaine hydroxylase	Carnitine biosynthesis
4-Hydroxyphenylpyruvate dioxygenase	Tyrosine metabolism
Flavin adenine dinucleotide-dependent amine oxidase (lysine-specific demethylase 1)	Histone demethylation
2. Reducing Agent	
Site	*Action*
Small Intestine	Promotes Absorption of Iron
3. Anti-Oxidant Agent	
Site	*Action*
Cells	Regulate gene expression and mRNA translation, prevent oxidant damage to DNA and intracellular proteins
Plasma	Increase endothelium-dependent vasodilatation, reduce extracellular oxidants from neutrophils, reduce low density lipoprotein oxidation, quench aqueous peroxyl radicals and lipid peroxidation products
Stomach	Prevent formation of N-nitroso compounds
4. Pro-Oxidant	
Target	*Effect*
DNA	DNA Damage
Lipid hydroperoxidase	Decomposition of lipid peroxidase leading to DNA damage
Downstream targets of hydrogen peroxide	Damage to cancer cells

These enzymes play an important role in Norepinephrine biosynthesis, amidation of peptide hormones – predominantly hypothalamic and gastrointestinal, Collagen and HIF hydroxylation, Tyrosine metabolism, Histone demethylation, and Carnitine

biosynthesis. The reducing property of Vitamin C helps in Iron absorption. The electron donating property of Vitamin C can help the molecule function as an anti-oxidant or a pro-oxidant as discussed above – both these properties are demonstrated to have biological functions in-vitro, of which some are proven in-vivo. The well elucidated functions of Vitamin C in human body are summarized in Table 3 (4)

3.12 VITAMIN C IN SEPSIS – EVIDENCE FROM ANIMAL MODELS

The preliminary data on the benefits and potential mechanism of action of Vitamin C in Sepsis comes from experimental animal models of sepsis.

3.12.1 Stress Induces Vitamin C Production In Lower Animals

In animals that are able to synthesize Vitamin C, there is increased ascorbic acid production in liver when the animals are exposed to stress(97,98). So it is reasonable to assume that there is an increased need for Vitamin C in stressful conditions (this is probably a reflex protective mechanism) and that Vitamin C has a beneficial role in coping with stress. Enhanced mRNA expression of the ascorbate synthesizing enzyme gulonolactone oxidase in lipopolysaccharide-treated mice has also been observed(81). Other studies have shown up to an eight-fold enhancement in the synthesis of ascorbate in animals exposed to drugs, including hypnotics (sedatives), analgesics and muscle relaxants probably as a compensatory mechanism for the enhanced metabolism of ascorbate following drug administration (99). In a caecal-ligation and perforation model of rats compared against time matched controls, there was a 50% decrease in serum ascorbate levels and a 1000% increase in urinary ascorbate

levels associated with hypotension(100). Therefore, it is conceivable that patients with severe infection in intensive care may have enhanced ascorbate requirements, not only due to the infectious disease process, but also because of the concurrent administration of sedatives and other drugs(81).

3.12.2 Vitamin C Reverses Sepsis Induced Damage In Experimental Models Of Sepsis

In a caecal-ligation and perforation model of rats compared against time matched controls, there was a 50% decrease in serum ascorbate levels and a 1000% increase in urinary ascorbate levels associated with hypotension. This manifested as decreased perfused capillary density in skeletal muscles, indicating microvascular dysfunction(100). A bolus of IV Vitamin C was also shown to reverse these changes. It could thus be hypothesized that Sepsis induces urinary loss of Ascorbic acid and the clinical manifestations of Sepsis can, at least partially, be reversed by prompt supplementation of the same. Another CLP model has shown improved microvascular circulation even with delayed administration of Vitamin C(101). From CLP and feces injection into peritoneum (FIP) polymicrobial sepsis models in rats, there is also good evidence to show that Vitamin C improves arteriolar responsiveness, capillary blood flow, blood pressure, LFT, and overall survival in Sepsis (102–104). The findings were further hypothesized to be mediated by iNOS and eNOS dependent mechanisms.

In sepsis experimental models using sheep, IV Vitamin C was shown to prevent *E Coli* endotoxin induced lung injury(105). In another experimental model that evaluated benefit of various anti-oxidants in Lipo-polysaccharide induced sepsis in rats, antioxidants including Vitamin C were found to reverse hypotension induced after LPS

injection, reduced the quantity of exhaled NO & Plasma nitrate concentration, and prevented lung injury. There was an in-vivo attenuation of LPS induced oxygen radical release by WBCs of rat and mRNA expressions of inducible nitric oxide synthase (iNOS) and manganese superoxide dismutase (MnSOD) genes. A finding peculiar to Vitamin C was inhibition of expression of IL1-beta — which was also found with administration of superoxide dismutase (SOD) and Catalase(106). Another model using rat and mice also demonstrated the significant anti-inflammatory, hypotension reversing and radical scavenging role of Vitamin C in Sepsis(107).

3.12.3 Vitamin C Sufficient Animals Perform Better In Experimental Models Of Sepsis

In a study which looked at outcomes in ascorbate depleted and replete mice deficient in L-gulono-gamma-lactone oxidase (Gulo(-/-)) — the rate-limiting enzyme in ascorbate synthesis — infected with K. pneumoniae, the ascorbate depleted mice were found to be 3 times more likely to die from infection as compared to ascorbate replete mice(108).In FIP mice models, the 24 hour survival was 50% in mice which received 10mg/kg IV Vitamin C as compared to 19% in mice which received saline vehicle injection(104).

From the data from animal models of Sepsis that clearly demonstrated — decreased amount of serum Ascorbate in Sepsis (which could directly be attributed to the sepsis itself), decreased survival in Vitamin C Deficient Septic models, and improved outcomes with Vitamin C supplementation —it is rational to hypothesize that Vitamin C deficiency does correlate with Sepsis outcomes, and supplementation of the same may have a beneficial role in Sepsis.

3.13 POSTULATED MECHANISMS OF ACTION OF VITAMIN C IN SEPSIS

Multiple mechanisms have been postulated to explain the observed effects of Vitamin C in sepsis. The first and foremost theory is that Vitamin C is a powerful anti-oxidant and exerts effects to reverse the chemical medicated changes in Sepsis. Vitamin C, through its anti-oxidant effect is thought to induce an immediate as well as a delayed response in Sepsis(109). Data from animal models and pharmacological experiments have been used to elucidate the mechanisms of these effects. Other possible mechanisms are by exerting a synergistic effect with glucocorticoids, helping replenish catecholamines, and by exerting a bacteriostatic effect. These have been discussed below.

3.13.1 Anti-Oxidant Effects Of Vitamin C & Microvascular Changes:

As an immediate response to parenteral Vitamin C supplementation, reversal of hypotension, improvement in arteriolar responsiveness and prevention of microvascular redistribution of blood flow away from muscles (Non-vital organs) have been observed. The proposed mechanisms are

a) Prevention and reversal of tetrahydrobiopterin oxidation causes an increase in THB levels. This, in turn, causes increased NO levels in endothelial cells via THB dependent synthesis of NO by eNOS. This causes reversal of the maldistribution of capillary blood flow effected by decreased NO levels in Sepsis(104,109)

b) Scavenging of Superoxide and other ROS that would otherwise react with NO, decreasing its vasodilatory effect(109,110).

c) Prevention of Platelet aggregation, microthrombi formation and impaired blood flow by Vitamin C is observed in other prothrombotic patients. Ascorbic acid possibly inhibits expression and activation of NADPH oxidase, preventing local deficiency of NO; possibly has a similar action against Sepsis induced prothrombotic state and resultant microcirculatory changes(109,111).

3.13.2 Microvascular Changes Causing Persistent And Delayed Response

capillary perfusion defects in experimental sepsis have been prevented for up to days by a single dose of Vitamin C, even after the serum Ascorbate levels return to baseline(104). The postulated mechanisms are as follows

a) Higher retention of Intracellular ascorbate as compared to extracellular ascorbate(112).

b) Ascorbate inhibits induction of the p47phox subunit of NADPH oxidase enzyme in endothelial cells, causing decrease superoxide and ROS synthesis in long term(109,112).

c) Inhibition of hypoxia mediated induction and stabilization of HIF-1 alpha, thereby inhibiting the expression of HIF-1 sensitive genes, such as GLUT1 and iNOS (103,109,110).

d) Inhibition of Superoxide production and of Superoxide mediated expression of cell surface intercellular adhesion molecule 1 (ICAM-1). This in turn prevents ICAM-1 induced leukocyte adhesion and microcirculation alterations(109,112) .

3.13.3 Synergistic Role With Glucocorticoids

It is known that there can be a relative glucocorticoid deficiency in Sepsis and other critical illnesses; there is some evidence to suggest that Glucocorticoid supplementation may have a beneficial role in sepsis(113) Vitamin C may act synergistically with endogenous or exogenous glucocorticoids to prevent excessive activation of nuclear factor-κB (NF-κB). NF-κB has been implicated in the generation of "cytokine storm" in the early stages of sepsis. One theory even state that the observed benefit of glucocorticoids in sepsis is because it drives Vitamin C inside the cells by activating sodium-dependent vitamin C transporter, consequently increasing intra-cellular Vitamin C levels. Vitamin C is also shown to increase Glucocorticoid receptor function, and has been postulated to increase glucocorticoid binding — causing more glucocorticoid responsiveness in Sepsis. This may be because of the fact that oxidizing molecules decreases the binding of glucocorticoids to its receptors; Vitamin C, being a reducing molecule, reverses this(114,115) . Another interesting observation in this regard is a modest glucocorticoid sparing effect with Vitamin C in asthmatics(116). A study has shown that Vitamin C attenuates the serum cortisol reduction after induction of Anesthesia with etomidate(117).Vitamin C could also exert a similar mechanism in preventing sepsis related relative glucocorticoid deficiency.

3.13.4 Vitamin C And Vasopressors

Vitamin C has been linked with a decrease in the need for resuscitation fluids and exogenous vasopressors in various cohorts of critically ill patients(118,119).

Catecholamines are synthesized in the sympathetic nervous system and adrenal medulla. Interestingly, tissues that synthesize catecholamines have the highest concentration of Vitamin C(83). Catecholamines increase arterial pressure by binding to and activating α-adrenergic receptors on the smooth muscle cells of the vasculature, and can promote increased cardiac contractility and heart rate by binding to beta-adrenergic receptors of the cardiac muscle. Downregulation of beta-adrenergic receptors is an important mechanism for sepsis induced myocardial dysfunction.

Ascorbic acid is essential for 2 steps in synthesis of Catecholamines. It is a cofactor for the copper containing enzyme dopamine β-hydroxylase(120). Ascorbic acid also helps in the rate-limiting step of the synthesis of dopamine via recycling the enzyme cofactor tetrahydrobiopterin(121). Tetrahydrobiopterin is required by Tyrosine hydroxylase to hydroxylate amino acid L-tyrosine to form L-dopa (dopamine precursor). Ascorbic acid may also increase the synthesis of Tyrosine hydroxylase enzyme itself.

Vitamin C also binds to alpha and beta adrenergic receptors, and enhances their activity(122,123). Vitamin C increases potency and duration of beta-adrenergic agonistic effects in asthma and COPD, prevents tachyphylaxis, and reverses fade. These effects are probably caused by a novel mechanism involving phosphorylation of aminergic receptors, and have clinical and drug-development applications(123). Thus, appropriate supplementation of ascorbate in sepsis may support endogenous synthesis of vasoactive catecholamines, and possibly also facilitate adrenergic receptor binding(81). This may translate to clinically significant decrease in exogenous

vasopressor requirement in sepsis, prevention and early reversal of shock, and improved survival.

Vasopressin is a peptide hormone synthesized in the hypothalamus; stored and secreted from the posterior pituitary in response to decreased blood volume, decreased arterial pressure, or increased plasma osmolality. It acts via receptor mediated mechanisms to cause vasoconstriction and water retention (124). Circulating vasopressin levels increase dramatically during the initial phase of septic shock, but this is followed by a significant decline in the latter phase. Patients in late-phase septic shock have significantly lower levels of circulating vasopressin compared with patients in cardiogenic shock, despite similar severity of hypotension. This decline in circulating vasopressin levels after the onset of septic shock is due to depletion of pituitary stores and possibly impaired vasopressin synthesis as well (125,126) . Ascorbate is a cofactor for the copper-containing enzyme peptidyl glycine α-amidating monooxygenase (PAM that is required for the endogenous synthesis of vasopressin(127). Also, ascorbic acid is present in highest concentration in the pituitary gland where PAM is abundantly expressed(83). Thus, it seems possible that the Vasopressin depletion in sepsis is linked to lower ascorbic acid levels. An animal study supported the presumed link between ascorbate and Vasopressin biosynthesis by showing that centrally administered ascorbic acid enhances circulating levels Vasopressin and vasopressin mediated anti-diuresis(128).

Ascorbate enhances the synthesis of the vasopressors norepinephrine and vasopressin by acting as a cofactor for their respective biosynthetic enzymes. It may be hypothesized that administration of high-dose ascorbate in conditions of ascorbic acid deficiency (in sepsis) may support the endogenous synthesis of these vasoactive compounds and thus ameliorate the need for exogenously administered vasopressors, prevent or reverse shock, and improve outcomes. Ascorbate-dependent vasopressor synthesis represents a plausible physiological mechanism whereby ascorbate could act as an adjuvant therapy for severe sepsis and septic shock(81).

3.13.5 Vitamin C Mediated Inhibition Of Bacterial Replication

Vitamin C has been shown to prevent the growth of *Staphylococcus* spp. in vitro(129) .Vitamin C is also shown to inhibit growth of both — drug susceptible and drug resistant Mycobacterium tuberculosis , possibly by driving the Fenton reaction and generation of highly reactive hydroxyl radicals(130).

It is possible that the bacteriostatic mechanism may involve production of hydrogen peroxide during oxidation of ascorbate in culture medium(131). Although vitamin C itself is a powerful antioxidant, its aerobic metabolism increases oxidative stress on bacterial cells. Ascorbate reduces the valence of free transition metals, and then the reduced metals catalyze the production of hydrogen peroxide, which is a potent antibacterial agent. For instance, *E. coli* replication is inhibited by 25–50 μ*M* hydrogen peroxide (bacteriostatic action) and significant killing of the bacteria occurs at 500 μ*M* hydrogen peroxide (bactericidal action). It remains to be determined if the amount of

hydrogen peroxide generated during ascorbate oxidation is sufficient to explain the vitamin's bacteriostatic effect(131). Whether the bacteriostatic activity is present in-vivo is not clear.

3.13.6 Other Mechanisms

a) Prevention of increased endothelial permeability: Endothelial dysfunction in Sepsis causes increased capillary permeability. This may be caused by increased endothelial apoptosis. The preventive role of Vitamin C in endothelial cell apoptosis(132) could have a beneficial role in preventing endothelial dysfunction and tissue edema.

b) Reversal of arteriolar hypo -responsiveness to vasoconstrictors (norepinephrine, angiotensin, vasopressin), a major pathogenic mechanism (other than myocardial dysfunction) in septic shock. Animal and human studies have shown that infusion of ascorbate reverses this hypo responsiveness to vasopressors in inflammatory conditions/LPS induced sepsis models(102,104,133,134).

c) Promotion of endothelial cell function, proliferation, and survival; thus maintaining the barrier function of endothelium, and preventing endothelial dysfunction mediated syndromes like capillary leak and ARDS(135). A recent study, that used the electric cell-substrate impedance sensing method to assess the barrier function of endothelial cell monolayers after introducing Vitamin C and Hydrocortisone, found that the lung endothelial barrier function is preserved when both Vitamin C and Hydrocortisone are introduced(136).

d) Enhanced Neutrophil Function

Neutrophils activated by pathogenic organisms accumulate Vitamin C intracellularly. The Vitamin C concentrations in activated neutrophils increase upto 10-30 fold(137). This increased Vitamin C uptake, likely via ascorbate recycling, is possibly a defense mechanism to protect the neutrophil from Oxidative damage caused by products of Oxidative burst. There could also be a pro-oxidant role of Vitamin C in place here; helping increase oxidant generation, and ensuring oxidative killing of the microbe(4). The various postulated molecular mechanisms of beneficial action of Vitamin C in Sepsis are schematically represented in figure 4

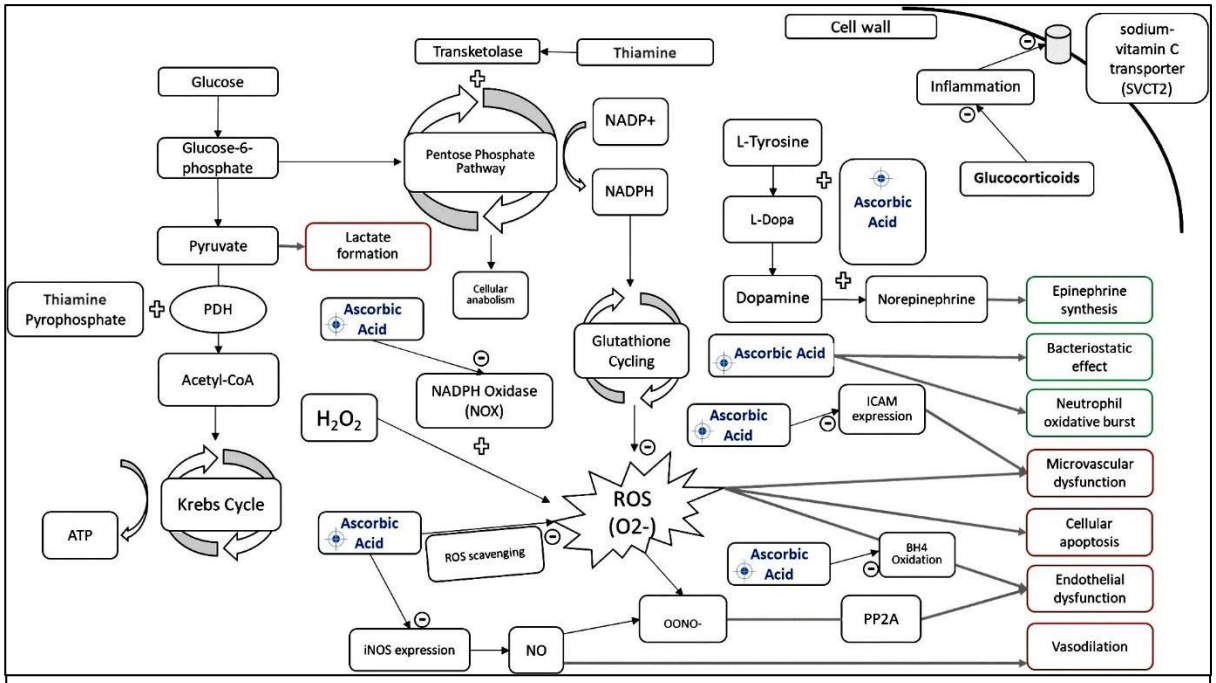

Figure 7:Suggested mechanisms for the efficacy of Ascorbic acid in sepsis(115)

PDH pyruvate dehydrogenase, ATP adenosine triphosphate, NADPH nicotinamide adenine dinucleotide phosphate, PP2A protein phosphatase 2, ROS reactive oxygen species, BH4 tetrahydrobiopterin, ICAM intercellular adhesion molecule. A circled minus sign indicates an inhibitory action; arrows indicate an activating action; green-outlined boxes indicate a beneficial effect of the medication combination; red-outlined boxes indicate a potentially harmful effect attenuated by the medication combination; The sites of action of Ascorbic acid is highlighted with an 'aim' sign

3.13.7 Role Of Thiamine Co-Administration

Thiamine pyrophosphate is a key coenzyme necessary for the function of glyoxylate aminotransferase, which catalyzes the breakdown of glyoxylate to carbon dioxide instead of oxalate. Thiamine deficiency states, therefore, may predispose to increased oxalate excretion(115). This was the biological rationale of adding Thiamine to Vitamin C in many studies using high dose Vitamin C(74).

3.14 VITAMIN C IN SEPSIS – EVIDENCE FROM HUMAN STUDIES

3.14.1 Vitamin Levels Are Lower In Septic And Critically Ill Patients

Vitamin levels are significantly lower in patients who are critically ill, more so in patients with sepsis as compared to healthy individuals(138–140). In an observational study, the median concentrations of total Vitamin C (Ascorbic acid + DHA) and Ascorbic acid were significantly lower(up to <25%) in critically ill patients than both healthy controls and participants with diseases like Diabetes and Gastritis (140).

In a prospective study of 56 patients with sepsis or severe sepsis and 6 healthy volunteers, it was observed that there was a sharp and persistent decrease in Vitamin C levels once sepsis sets in(141). A phase 1 trial assessing safety of Vitamin C in sepsis also noted that low plasma ascorbic acid levels are “a predictable feature in patients with severe sepsis”(142).The Vitamin C levels are often so low that Vitamin C levels are undetectable in septic patients(109)

Wilson et al, in a review article, postulate 2 causes which could contribute to low Vitamin C levels in Sepsis(109):

a) Degradation of Ascorbate or DHA while preparation and storage of TPN bags used to feed the critically ill.

b) Intracellular Vitamin C depletion – This may be due to:

- Inflammatory cytokine mediated inhibition of Uptake of Vitamin C by SVCT 2 expressing cells
- Acute hyperglycemic episodes (which are common in septic patients), and resultant competitive inhibition of DHA uptake by glucose or
- Oxidization of Ascorbate to DHA and irreversible oxidization of the latter, mediated by excessive reactive Oxygen species.

There are even case reports of scurvy, the disease caused by florid Ascorbate deficiency, in patients admitted to intensive care (143–145), indicating that the magnitude of Vitamin C deficiency can be as severe.

3.14.2 The Low Levels Of Vitamin C In Sepsis Co-Relate With Outcome

In a prospective analysis of 16 critically ill Surgical ICU patients at risk of developing multi organ failure, circulating Vitamin C levels were low in all patients who developed multi organ dysfunction syndrome (MODS)(146). Another observational study also concluded that the levels of total ascorbic acid(Ascorbic acid + DHA) and Ascorbic acid correlated with the severity of illness(140).

3.14.3 Critically Ill Patients Who Are Administered Vitamin C Have A Better Chemical Milieu

In a prospective, randomized, double-blinded placebo controlled clinical trial that compared 20 septic abdominal surgery patients, half of whom who received 3 IV doses of 450mg/day of Vitamin C and the other half placebo, there was a reduction in caspase-3 and poly(ADP-ribose) polymerase levels and the increment of Bcl-2 levels, which translates to an antiapoptotic effect on peripheral blood neutrophils. In light of evidence showing that Reactive oxygen species play a role in neutrophil apoptosis and MODS development, this finding takes relevance (147).

3.14.4 Vitamin C Supplementation Improves Outcomes In Sepsis And Related Conditions In Clinical Trials And Observational Studies

A randomized, double-blind, placebo-controlled trial studied the supplementation of 500 mg/d of vitamin C and 400 IU/d of vitamin E through enteral feeding in critically ill patients, and showed significant reduction in mortality in the Intervention arm(148)

Accordingly, in another prospective, randomized trial, supplementation with vitamin E (1 000 IU, 3 times a day per naso or orogastric tube) and vitamin C (1000 mg, 3 times a day, intravenously) was associated with a reduction in the occurrence of pulmonary morbidity and multiple organ failure, and with the duration of mechanical ventilation and length of ICU stay (149)

A single report (published as abstract only) of a clinical study of large intravenous doses of ascorbic acid and other antioxidants (tocopherol, N-acetyl-cysteine, selenium), in patients with established ARDS showed a 50% reduction in mortality(150)

A phase 1 clinical trial that primarily looked at the safety of Vitamin C in sepsis also showed prompt reduction in SOFA score, reduction in CRP and Procalcitonin levels, and reduction in thrombomodulin levels as compared to patients who received placebo(142) . All these have been found to be associated with better outcomes in sepsis in various studies(151–153). Moreover, administration of Vitamin C IV was found to be safe in doses up to 200 mg/kg/24 h.

In a study that randomized 28 patients in septic shock — who required a vasopressor drug to maintain mean arterial pressure >65 mmHg — to receive either 25 mg/kg intravenous ascorbic acid every 6 h or matching placebo for 72 h, mean dose and duration of vasopressors (primary outcome) and 28-day mortality (secondary outcome) was significantly lower in the ascorbic acid group (14.28% vs. 64.28%, respectively; $P = 0.009$). The duration was ICU stay was not significantly different in this study(154).

A Cochrane analysis that looked at preventive and therapeutic role of Vitamin C in pneumonia, found some benefit of Vitamin C in the treatment of Pneumonia, especially in those with low Vitamin C levels, based on 2 clinical trials that included a total of 197

patients. The authors concluded that supplementing pneumonia patients who have low plasma vitamin C levels may be reasonable because of its safety and low cost. Further superior quality evidence is required to recommend its routine use in the treatment of pneumonia and prevention. None of the five trials reported any noteworthy adverse effects of vitamin C(155).

The most recent publication that drew the attention of medical fraternity towards the beneficial role of Vitamin C in sepsis was published by Marik et al in Chest in 2017(74). This retrospective before-after clinical study 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) with a control group treated in a single ICU during the preceding 7 months. There were 47 patients in both treatment and control groups, with no significant differences in baseline characteristics between the two groups. They showed a whopping 31.9% decrease in Sepsis related mortality with this cocktail (8.5% (4 of 47) in the treatment group and 40.4% (19 of 47) than in the control group ($P < .001$)). 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. There was also a significantly decreased mean duration of weaning from Vasopressors in the treatment group (18.3 ±9.8 h after starting treatment with the vitamin C protocol vs 54.9 ± 28.4 h in the control group ($P < .001$)). The incidence of Acute Kidney Injury was also significantly decreased.

However, another similar retrospective analysis done in 94 patients, the “Triple Therapy” failed to show any significant mortality benefit. There was also no beneficial trend in ICU Length of Stay, need for vasopressors or need for RRT(156) . Thus most of the randomized control trial and observational data shows a trend towards benefit with Vitamin C, although the evidence is largely equipoise.

3.14.5 Metanalyses Have Supported Use Of Vitamin C In Critically Ill Individuals And Patients With Sepsis

Over the past 1 year, after Marik et al’s before - after study(74), at least 6 published meta analyses have assessed the usefulness of Vitamin C administration in Sepsis and critically ill individuals(157).

Putzu et al analysed 44 RCTS; 16 done in General ICU setting, and 28 in Cardiac surgery ICUs, including a total of 6455 patients. This analysis failed to show any mortality benefits with different regimes of Vitamin C supplementation , as compared to no supplementation(158).

However, another review of 8 RCTs and 4 observational studies that looked at 1210 participants with a mixture of conditions including burns, sepsis, other critical illnesses, critical injury, trauma and critical injury showed that IV Vitamin C administration at doses of 3-10 g per day decreased mortality, whereas doses beyond

this had no effect. The subgroup analyses also showed a trend towards decreased vasopressor and ventilator requirement(159).

Langois et al noticed a trend towards improved mortality in critically ill patients, including those with sepsis in his review, with high dose Vitamin C supplementation (160). Hemila and Chalker observed decreased ICU LOS and need for mechanical Ventilation with Vitamin C supplementation in the Critically ill(161) . Zang and Sativa, in their review, observed that in critically ill patients , Vitamin C supplementation decreased the need for Vasopressor support and mechanical ventilation . He also observed a trend of decreased fluid requirement and increased urine output(162).

Table 5:Summary of metanalyses of Vitamin C use in critically ill and septic patients(129)

Publication details	Title	Selection criteria (PICO)	Included studies	Subgroup analysis	Findings
Putzu et al.	The effect of vitamin C on clinical outcome in critically ill patients: A systematic review with meta-analysis of randomised controlled trials	P—adult critically ill patientsI—vitC (any regimen)C—placebo or no therapyO—mortality, acute kidney injury, supraventricular arrhythmia, ventricular arrhythmia, stroke, ICU LOS, hospital LOS	44 RCTs:16 in ICU setting (n = 2857)28 in cardiac surgery (n = 3598)	Mixed ICU vs burns vs sepsis/septic shock vs acute pancreatitisVitC alone vs enteral vitC vs IV vitC vs IV vitC > 5 g	ICU patients:X mortalityX acute kidney injuryX ICU or hospital LOSCardiac surgery:↓ postoperative atrial fibrillation↓ ICU and hospital LOS
Wang et al.	Effects of different ascorbic acid doses on the mortality of critically ill patients: a meta-analysis	P—critically ill patientsI—IV vitC (including co-administration of antioxidants)C—placebo or no interventionO—mortality, resuscitation fluid requirement, urine output, acute kidney injury, vasopressor requirement, duration of mechanical ventilation, ICU and/or hospital LOS	12 RCT, quasi-RCT, observational (n = 1210)	Low dose vs medium dose vs high doseBurn vs sepsis vs others	↓ mortality (doses of 3–10 g/day)X morality (< 3 g/day or ≥ 10 g/day)↓ duration of vasopressor support↓ duration of mechanical ventilationX acute kidney injuryX ICU or hospital LOSX fluid requirementX urine output
Hemila and Chalker	Vitamin C can shorten the length of stay in the ICU: A meta-analysis	P—ICU patientsI—vitCC—placebo or noneO—ICU LOS, duration of mechanical ventilation	18 controlled trials (n = 2004)including 13 cardiac surgery	IV vs oral1–2 days ICU vs 3–5 days ICU> 24 h ventilation vs < 24 h ventilation	↓ ICU LOS↓ duration of mechanical ventilation
Langlois et al.	Vitamin C supplementation in the critically ill: A systematic review and meta-analysis	P—ICU patientsI—vitC (enteral or parenteral)C—placebo or noneO—mortality, incident infections, ICU LOS, hospital LOS, duration of mechanical ventilation	11 RCTs9 RCTs with mortality (n = 1322)	Low dose vs high doseCombined therapy vs monotherapyOral/enteral vs parenteralNon-septic vs septicHigher-quality trials vs low-quality trials	X mortality↓ (trend) mortality (IV high dose vitC monotherapy)X infectionsX ICU or hospital LOSX duration of mechanical ventilation
Zhang and Jativa	Vitamin C supplementation in the critically ill: A systematic review and meta-analysis	P—critically ill adult patientsI—IV vitCC—placebo or no interventionO—mortality, duration of mechanical ventilation, duration of vasopressor support, fluid requirements, urine output	4 RCTs and 1 retrospective (n = 142)		X mortality↓ need for vasopressor support↓ duration of mechanical ventilation↓ (trend) fluid requirements↑ (trend) urine output
LiCrit Care	Evidence is stronger than you think: a meta-analysis of vitamin C use in patients with sepsis	P—patients with sepsisI—IV vitCC—placebo or noneO—mortality, ICU LOS, vasopressor duration	2 RCTs and 1 before-after		↓ mortalityX ICU LOS↓ vasopressor duration
Lin et al.	Adjuvant administration of vitamin C improves mortality of patients with sepsis and septic shock: A systems review and meta-analysis	P—patients with septic shock and severe sepsisI—vitCC—placeboO—mortality	4 RCTs and 2 retrospective studies (n = 109)	RCT vs retrospectiveHigh dose vs low dose	X mortality↓ mortality (doses of > 50 mg/kg/day)X ICU LOS

ICU intensive care unit, IV intravenous, LOS length of stay, PICO patients, intervention, comparator, outcome, RCT randomized controlled trial, vitC vitamin C

Li et al performed a meta-analysis of 3 studies that looked at the use of Vitamin C, specifically in patients with sepsis and concluded that there was a positive correlation between Vitamin C administration and good clinical outcomes — including mortality and decreased Vasopressor use. This study observed good consistency of evidence, but concluded that the inadequacies in study design and small sample size made it difficult to prove causal relationship(163). Lin et al also observed a significant mortality benefit with the use of high dose Vitamin C in patients with Sepsis(164). The results of these reviews have been summarised in Table 1.

3.15 VITAMIN C IN SEPSIS -DOSAGE & ADMINISTRATION

Having considered the possible role of Vitamin C in patients with sepsis, the ideal route of administration and dose needs to be decided as well. This decision requires an understanding of the pharmacokinetics and metabolism of Vitamin C , in the setting of critical illness(165).

3.15.1 Parenteral Vs Enteral Administration

Administering ascorbate parenterally rather than orally increases its plasma ascorbate concentration and its effects on microvascular function(109). For instance, when oral and intravenous routes of ascorbate administration (500 mg/day for 30 days) are compared in sedentary men, only intravenous ascorbate improves endothelium-dependent arteriolar function — as indicated by flow-mediated vasodilation(166).

Vitamin C plasma concentrations are tightly controlled when the vitamin is taken orally, even at the highest tolerated amounts. This is likely due to saturation of the active transport involved in Vitamin C absorption. It is to be noted that even a normal volunteer, taking 3 g of Vitamin C fourth hourly may reach a plasma level of only ~200 μM (4,167) . By contrast, intravenous administration bypasses tight control and results in concentrations as much as 70-fold higher than those achieved by maximum oral consumption. Both findings have clinical relevance(167). High concentrations of vitamin C are produced in extracellular fluid only with intravenous or parenteral administration of ascorbate(167).

Dosing and bio-distribution data in humans show that pharmacological concentrations of ascorbic acid can only be attained following intravenous administration. Parenteral rather than enteral nutrition may be required for optimal ascorbate status in critically ill patients because intravenously administered ascorbate bypasses the rate-limiting intestinal uptake of orally administered ascorbate(167).

3.15.2 Dosing Of Vitamin C In Sepsis

The Recommended daily intake of Vitamin C is in the range of 75–110 mg/day(83). However, critically ill patients probably require significantly higher intakes of ascorbate due to enhanced metabolic turnover of vitamin C during the severe inflammatory response(81). Vitamin C levels remain low in critically ill patients despite supplementing the recommended daily allowance(168). However, high dose intravenous administration was shown to replete Vitamin C levels within minutes(167).

The amount of vitamin C provided in standard parenteral nutrition multivitamin preparations (nominally 200 mg/day)is not adequate to normalize plasma vitamin C levels in critically ill patients, even when administered for 7 days(169). In a study that studied the therapeutic response to ascorbic acid supplementation in TPN in increasing doses to critically ill patients and those with infections, the initial mean ± SEM baseline plasma ascorbic acid concentration was depressed (0.11 ± 0.03 mg/dl) and unresponsive following 2 days on 300 mg/day supplementation (0.14 ± 0.03; P = 1.0) and only approached low normal plasma levels following 2 days on 1000 mg/day (0.32 ± 0.08; P = 0.36). A significant increase was noted following 2 days on 3000 mg/day (1.2 ± 0.03; P = 0.005). Hence maximal early repletion of this vitamin requires early rapid pool filling using supraphysiologic doses for 3 or more days(139). Long et al. demonstrated that up to 3000 mg/day ascorbate was required to return plasma levels of critically ill patients to normal (i.e. 68 μmol/l(139)).

It may hence be concluded that, for any meaningful pharmacological effect in Sepsis to occur, Vitamin C needs to be administered parenterally. The dose required is supra-physiological, and is likely to be > 15-20 times the EDA.

3.16 VITAMIN C - SAFETY

A study of intravenous ascorbate in patients with advanced malignancies reported that injection of 1.5 g ascorbate/kg body weight three times weekly is well tolerated(170). A Phase 1 safety trial conducted in Septic Patients showed that doses up to 200 mg/kg/24 h were well tolerated by septic patients with no serious adverse events (142). Nathens et al. administered 1 gram of ascorbic acid every 8 hours for 28 days to

surgically critically ill patients with no ill effects(149). Tanaka et al. administered 66 mg/kg/hour for 24 hours to patients with 50% surface area burns with no adverse events(118). Hoffer et al. intravenously administered up to 90 grams of ascorbic acid 3 times weekly to patients with advanced malignancy with no adverse events (170).

However, there are a few concerns with the use of high dose Vitamin C. Ascorbate is metabolized to oxalate, which accumulates as nephrotoxic calcium oxalate crystals (nephrolithiasis) in the kidneys of susceptible individuals, as reported in a recent case study(171) . Two large prospective cohort studies, one following 45,251 men for 6 years and the other following 85,557 women for 14 years, reported that consumption of ≥1,500 mg of vitamin C daily did not increase the risk of kidney stone formation compared to those consuming <250 mg daily(172,173) . On the other hand, two other large prospective studies reported that a high intake of ascorbic acid was associated with an increased risk of kidney stone formation in men(174,175) .We know that only <0.5% of the intravenously administered ascorbic acid is recovered as oxalate in the urine of humans with normal renal function (176). Septic patients may require high-dose vitamin C therapy for only a few days, until source control and antibiotic therapy have eliminated virulent bacteria and the adverse effects of bactericidal antibiotics have ceased. Such a brief course of ascorbate therapy may not elevate the risk of oxalate stone formation. This is validated by the experiences from the previous trials (118,142,149,177). From Marik et al's experience, the incidence of Sepsis related Acute Kidney Injury , in fact, decreased with Vitamin C supplementation(74). Although Marik used Thiamine along with the Vitamin C, due to its biological plausibility in decreasing

the Oxalate load, data from other studies show that administration of Vitamin C alone is also safe.

Another concern is that ascorbate exerts prooxidant effects as it donates electrons to transition metals (e.g., iron)(131), which then catalyze the synthesis of hydrogen peroxide. Hydrogen peroxide is generated in the interstitial fluid by oxidation of large amounts of exogenous ascorbate (178)and this may alter the function of some cells. Indeed, ascorbate kills cancer cells in culture and the underlying mechanism involves promotion of hydrogen peroxide formation in the culture medium, as evidenced by suppression of cell killing by extracellular catalase(179). However, these concerns have not been supported by observations in human studies. Repeated intravenous injections of 750–7,500 mg/day of ascorbate for 6 days did not induce pro-oxidant changes in the plasma in healthy volunteers(180).

Another concern with the use of Vitamin C in critical care setting is the possibility of Fictitious hyperglycemia. It has been noted that in-vitro, Ascorbic acid interferes with the blood glucose measurements of Hand-held glucose analyzers – often showing a higher than the correct value, and at times a lower value(181).

A direct comparison of Point of Care and laboratory reference glucose values in the patients receiving vitamin C infusion revealed that all the patients receiving Vitamin C infusion demonstrated falsely elevated POC glucose values during and/or immediately after the infusion period, with discrepancies ranging from 10 to 200 mg/dl. These

findings were irregular, unpredictable and unrelated to hemoglobin levels(182). In the trial that explored the usefulness of high dose Vitamin C infusion in burns victims also, a similar phenomenon of fictitious hyperglycemia was noted(183). This may result in unnecessary correction using Insulin, leading on the Iatrogenic hypoglycemia and associated adverse effects.

However, this phenomenon of false high glucose measurements with POC devices has been observed with higher doses of Vitamin C [66 mg/kg/h for 18-24 hours] and with Infusions. Once the infusion was over the POC value and the laboratory value of Plasma glucose was not statistically different (183). Hence caution will be advised regarding complete reliability on POC devices for glucose measurements, while the patient is being administered Vitamin C and immediately following this (184). We also believe that this phenomenon is less significant with bolus doses of Vitamin C, as in our study, and is unlikely to cause clinically significant harm from iatrogenic hypoglycemia since tight glycemic control is not a standard of care in our ICU, and some level of hyperglycemia is accepted in critically ill patients.

Even though the risk of adverse effects does exist, as with any other drug, the safety profile of Vitamin C to the best of our current knowledge can be labelled excellent. Compared to the potential benefit of Vitamin C, the potential harm is negligible.

3.17 VITAMIN C IN SEPSIS – KNOWLEDGE GAPS

Our knowledge regarding the usefulness of Vitamin C in sepsis is essentially derived from a group of animal studies that explains the biological plausibility, small observational studies that prove the validity of the idea, and a few clinical trials that shows some temporal relation and coherence. There is a significant deficiency of evidence to conclusively prove its efficacy in sepsis and support its routine use.

One postulated mechanism of possible beneficial role of Vitamin C in Sepsis is via a synergistic action along with glucocorticoids. A recent study by Marik et al. showed that Vitamin C and Hydrocortisone synergistically prevented LPS induced pulmonary endothelial barrier dysfunction (136) .However, it is of note that the preventive effect was not evident when Vitamin C was administered alone. Whether the beneficial role of Vitamin C is seen only in the presence of Hydrocortisone or if the endogenous steroids have the same synergistic effect along with Vitamin C is something that is not evident from the current knowledge(113) .

In Marik et al's before-after study(74), patients were also administered Hydrocortisone along with Vitamin C. Whether the benefits seen were due to individual effects of these two drugs or a synergistic action is not clear. This study is aimed to address the independent role of Vitamin C in Sepsis. A limitation of the study will be the fact that it won't address the synergistic role of Steroids and Vitamin C in Sepsis. This will require a factorial design, and may be planned after the completion of this trial and when more evidence is available from other studies.

After Marik et al's publication, a number of scientific forums, newspaper articles and letters to editors have discussed the concept widely(185–192). The study was small and the design poor, but it cannot be ignored that the treatment was cheap, the concept very much rational, the current evidence corroboratory, and the results magnanimous. In fact, even before the study was published, some experts had recommended Vitamin C supplementation in septic patients who are Vitamin C depleted(193).

There are no studies that compare ascorbate and DHA for efficacy in treating sepsis. Maximal uptake rates are higher for DHA than ascorbate in most mammalian cell types, when studied under glucose-free conditions(109) The most effective dose and ideal duration remains unknown. A number of Phase 2 and 3 randomized clinical studies with the aim of providing more evidence with respect to use of Vitamin C, with or without Hydrocortisone have been planned or are already enrolling participants(194–201)

3.18 RELEVANCE OF THIS STUDY

As of today, sepsis remains a major cause of mortality and morbidity despite use of an array of strategies that are proven to be helpful. Ascorbate depletion, poor arteriolar reactivity and capillary leak as well as plugging are associated with poor outcome in sepsis. The repletion of ascorbate levels in Sepsis occurs only when the disease resolves or with parenteral administration of the same at higher doses (131). From the available evidence, Vitamin C seems to be largely safe with no significant risk of major adverse events.

Based on the current available evidence, which is not generally considered “strong” by the medical fraternity, many ICUs are currently administering Vitamin C or a combination of Vitamin C and Steroids to patients with sepsis. Even in our ICU there is difference in practice among physicians and Units and some patients are being administered Vitamin C, usually along with Hydrocortisone and Thiamine. A conclusive answer regarding the usefulness of Vitamin C in sepsis can only come from Randomized Controlled Trials. The effect of interactions between Vitamin C and Glucocorticoids should be studied using a factorial design.

As Oudemans-van Straaten et al rightly summarize, “pragmatic multicentre trials are needed to confirm the benefit and to exclude unforeseen harm as was seen in previous sepsis trials using promising drugs. Studies should also determine optimal dose and treatment duration, whether normal or temporarily supernormal plasma concentrations should be obtained, whether intermittent (high peak concentrations) or continuous dosing (less renal excretion of vitamin C and oxalate) performs better, whether co-administration of Thiamine reduces oxalate excretion, and finally whether the combination with Hydrocortisone acts synergistically”(188).

Worldwide, this topic has become a matter of discussion and there is an urge for better study designs to address the issue from both proponents and opponents of the concept. In the current situation — where there is biological plausibility for benefit of Vitamin C in sepsis and some preliminary evidence and coherence of the concept from animal and observational studies regarding benefit of Vitamin C in sepsis, but at the same time

lack of compelling evidence to support its use a Standard of Care — a Randomized Control Trial to clarify the same is the need of the hour, especially taking into consideration the low cost and safety of the intervention and the large potential benefit as suggested by observational studies. This study is an attempt to bridge such knowledge gaps. To the best of our knowledge, this is the first trial planned to assess the use of Vitamin C in Sepsis in India. Along with data from other clinical trials from around the world (194–201), this trial is expected to bring significant clarity to the conundrum, and unveil the efficacy of a potential 'cure to sepsis'.

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4. METHODOLOGY

4.1 STUDY DESIGN

The study design chosen to address the question of efficacy of Vitamin C administration in Sepsis was a double-blind, placebo-controlled randomized control trial. It was a Phase 3 trial conducted in a single centre. This was an investigator initiated pragmatic trial.

4.2 ETHICAL APPROVAL & FUNDING

The study was approved by the Institutional Review Board – Silver of the Christian Medical College Vellore: IRB - 11125 dated 24/01/2018. The funding was from the Institutional Fluid /Major Research Grant (Fund number: 22Z469). There was no external funding. The study was registered with the Clinical Trial Registry of India (CTRI/2018/05/013994) and WHO International Clinical Trials Registry Platform's (ICTRP)(UTN U1111-1207-5230) . An amendment of the protocol was submitted to the IRB and approved on 28/8/2019 (A 28 – 28/08/2019). (IRB Approval, Amendment and Fund letter attached in Appendix).

4.3 SETTING

The study was undertaken in a 2,500-bed, private teaching hospital in India. The original protocol aimed at conducting the study in the Intensive care unit. The Medical Intensive Care unit has a total of 24 beds with 12 in the high dependency section and

12 in the intensive care section. The I.C.U follows a semi-open model. However, due to slow accrual of participants, a protocol amendment was requested and approved by the IRB on 28/8/2019, to include patients admitted to the medical wards as well.

4.4 STUDY PARTICIPANTS AND ELIGIBILITY CRITERIA

4.4.1 Inclusion Criteria:

Patients who are admitted under the Department of General Medicine of Christian Medical College, Vellore and met all the following criteria and are willing to consent for the study were included in the trial. Consent was taken from the patient or the closest relative if the patient cannot provide consent. Randomization and initiation of agent administration was done within 24 hours of admission to the ICU. Patients who develop sepsis while in hospital was also included and randomized within 24 hours of identification of sepsis. The inclusion criteria were

1) Completed the age of 18 years

2) Has the diagnosis of Sepsis. Sepsis was defined in accordance with the "Sepsis 3" Criteria as "as life threatening organ dysfunction due to a dysregulated host response to infection(1)". Sepsis was hence identified if there is organ dysfunction and a suspected or proven infection.

2a. **Organ dysfunction** is defined as an increase in SOFA Score of 2 or more(1). If there is no known pre-existing organ dysfunction, the baseline SOFA Score was assumed to be zero. This is also in accordance with the "Sepsis 3" definition(1).

2b. **Suspected infection** was defined when all of the below 3 criteria were met(59,68)(59,68)

- Clinical syndrome consistent with infection
- The diagnosis of sepsis was made by the treating physician
- Blood culture(s) is drawn and antibiotics initiated.

2c. **Proven infection** was identified using standard diagnostic criteria described for various infectious syndromes. The various infectious syndromes are defined as follows

- Primary blood stream infection was diagnosed when the patient has a growth of recognized pathogen in one or more blood cultures, or growth of a common skin contaminant in 2 or more blood cultures, in the absence of a recognized source or in-situ intravenous catheters(202)
- Catheter related blood stream infection was diagnosed, according to the IDSA guidelines(203), if any of the following three criteria is satisfied
  - The growth of an organism from a percutaneous blood culture as well as from a quantitative (>15,000 CFU) culture of the culture tip.
  - The growth of an organism from a simultaneous percutaneous and a catheter lumen blood culture, with the growth in the latter, at least 2 hours sooner.
  - The growth of an organism in quantitative percutaneous and a catheter lumen blood culture, with 3-fold greater CFU in the latter

- Community Acquired Pneumonia was diagnosed if the patient has the characteristic clinical syndrome (any 2 of the following : fever or hypothermia,

rigors, sweats, new onset cough, sputum production, chest discomfort/pleuritic chest pain, new onset breathlessness, focal crepitation, bronchial breath sounds) along with a demonstrable infiltrate on lung imaging(Chest Radiograph, CT Thorax or lung Ultrasound done by a trained radiologist or Critical Care Physician) (204). We decided to use Lung Ultrasound as a reliable imaging modality to identify lung infiltrates as it is commonly performed in our hospital, often available before Chest X-Ray and CT (more so in case of VAP/HAP as described below) and there is good evidence to suggest high accuracy of Lung Ultrasound in diagnosis of pneumonia as compared to Chest X Ray and CT Thorax (205,206)

- Hospital Acquired pneumonia was diagnosed if the patient develops, a new lung infiltrate on imaging (Chest X-Ray, CT Thorax or Lung Ultrasound performed by a trained Radiologist or Critical Care Physician) along with clinical evidence for infective origin of the infiltrate (new onset fever, purulent sputum, leukocytosis and decline in the oxygenation) at least 48 hours following hospital admission(207,208).
- Ventilator associated Pneumonia was diagnosed if a patient develops the same syndrome as HAP, at least 48 hours after Endotracheal Intubation(207,208)
- Urinary Tract Infection was diagnosed according to the 2008 CDC guidelines if any 1 of the following criteria is met(209)(209)
    - Patient has at least 1 sign/symptom of UTI with no other recognized cause (fever, urgency, frequency, dysuria, or suprapubic tenderness) and patient has

a positive urine culture, that is, 10^5 microorganisms per cc of urine with no more than 2 species of microorganisms.

- Patient has at least 2 signs/symptoms of UTI with no other recognized cause (fever, urgency, frequency, dysuria, or suprapubic tenderness) and at least 1 of the following:

  a. positive dipstick for leukocyte esterase and/ or nitrate

  b. pyuria (urine specimen with >10 white blood cell [WBC]/mm3 or >3 WBC/high-power field of unspun urine)

  c. organisms seen on Gram's stain of unspun urine

  d. at least 2 urine cultures with repeated isolation of the same uro-pathogen (gram-negative bacteria or Staphylococcus saprophyticus) with $102 colonies/mL in non-voided specimens

  e. $>10^5$ colonies/mL of a single uro-pathogen (gram-negative bacteria or S saprophyticus) in a patient being treated with an effective antimicrobial agent for a urinary tract infection

  f. physician diagnosis of a urinary tract infection

  g. physician institutes appropriate therapy for a urinary tract infection

- Catheter Associated Urinary Tract Infection was diagnosed when there is "Growth of $\geq 10^3$ colony forming units (cfu)/mL of uro-pathogenic bacteria in the presence of symptoms or signs compatible with UTI without other identifiable source in a patient with indwelling urethral, indwelling suprapubic, or intermittent catheterization." The compatible symptoms include fever, suprapubic or costovertebral angle tenderness, and otherwise unexplained

systemic symptoms such as altered mental status, hypotension, or evidence of a systemic inflammatory response syndrome(210).

- Meningitis was diagnosed if the patient meets either of the following 2 criteria(211).
    - Patient has organisms cultured from cerebrospinal fluid (CSF).
    - Patient has at least 1 of the following signs or symptoms with no other recognized cause: fever (38.8C), headache, stiff neck, meningeal signs, cranial nerve signs, or irritability and at least 1 of the following:
        a. increased white cells, elevated protein, and/ or decreased glucose in CSF
        b. organisms seen on Gram's stain of CSF
        c. organisms cultured from blood
        d. positive antigen test of CSF, blood, or urine
        e. diagnostic single antibody titer(IgM) or 4-fold increase in paired sera (IgG) for pathogen and if diagnosis is made antemortem, physician institutes appropriate antimicrobial therapy
- An Intracranial infection (brain abscess, subdural or epidural infection or encephalitis) was diagnosed if
    - there is positive culture form brain tissue or dura,
    - if there is direct visual (during surgery) or histopathologic evidence or
    - if there is least 2 of the following signs or symptoms with no other recognized cause: headache, dizziness, fever, localizing neurologic signs, changing level of consciousness, or confusion along with either evidence of

microorganism on microscopic examination of the tissue, positive blood or urine antigen of a compatible organism, radiographic evidence of an organism or there is serological evidence of a compatible organism(211)

- Skin and soft tissue infections are diagnosed, based on clinical observation using the following criteria(212).
    - Cellulitis: any spreading infection involving the dermis and subcutaneous tissues.
    - Abscess: any collection of pus within the dermis or subcutaneous tissues
    - Necrotizing soft tissue infections: a necrotizing infection involving any of the soft tissue layers, including the dermis, subcutaneous tissue, superficial or deep fascia, and muscle

- Infective Endocarditis is diagnosed as per modified Duke's criteria (213).
- Spontaneous bacterial peritonitis (SBP) is diagnosed in the presence of an elevated ascitic fluid absolute polymorphonuclear leukocyte (PMN) count (i.e., ≥250 cells/mm3 [0.25 x 109 /L]) without an evident intra-abdominal, surgically treatable source of infection(214).

4.4.2 ***Exclusion Criteria:***

1) Pregnant and lactating women
2) Patients who are enrolled to other interventional trials

3) Past or present of renal stones according to history, medical/laboratory records from elsewhere or hospital electronic medical records. Patients will not be screened for renal stones or oxaluria

4) Patients who were receiving Vitamin C for another indication (e.g.: Wound healing, Burn Injuries, along with Inj. Doxycycline). *Patients who are enrolled to the trial will be allowed to be initiated on Doxycycline if indicated and will be included in the ITT analysis. Such patients will be specially mentioned while reporting the results.*

5) Patients with known allergy to Vitamin C

6) Patients with known G-6-PD deficiency and Hemochromatosis (193,215)

7) Patients with KDIGO 3 Acute Kidney Injury or Class 4 or 5 CKD(193,216,217)

8) Patients who are receiving warfarin or related drugs, bortezomib or cyclosporine (215,218)

9) Patients who were once enrolled in the trial and has received at least one dose of the drug were excluded if they are readmitted to ICU with a new onset sepsis

4.5 INTERVENTION AND COMPARATOR AGENTS

4.5.1 Intervention Agent:

Standard Care and Intravenous Vitamin C 1.5 g every 6 hours for 4 days (16 doses)

The drug was purchased from Liv-Biopharma (Inj. Livocee(219)). The drug was packed in 20 ml single-dose vials, which contained 20 ml of Vitamin C solution for IV Administration at a concentration of 250 mg per ml. 1 dose of 1.5 g will hence require injection of 6 ml of the solution. 6 ml of the solution was reconstituted in 100

ml of Normal Saline or 5% Dextrose (decided by the ICU physician as per the electrolyte status of the patient) and administered immediately over 30 to 60 minutes as a slow infusion. In case of patients on fluid restriction, reconstitution in 50 ml of the solvent was allowed.

The dose of Vitamin C was adapted from the study of Marik et al, which is the most accepted and cited original research publication regarding the use of Vitamin C in Sepsis(74). Marik et al has used current evidence to estimated that IV Vitamin C at a dose of more than 3g/day is required to correct Vitamin C levels in septic patients, in whom the Vitamin C levels are predictably low. The group then decided to administer Vitamin C at 6g/day in 4 divided doses based on pharmacokinetic models, available clinical data etc.

4.5.2 Comparator Agent:

Standard Care and Intravenous Distilled Water based placebo every 6 hours for 4 days (24 doses)

An inactive compound, with similar appearance and consistency as the Intervention agent; which was packed in similar vials, each vial containing 20 ml of the solution and administered in a similar way as the Intervention agent. The placebo was prepared in the Pharmacy of Christian Medical College, Vellore and packaged in a sterile manner like that of the drug in 20 ml vials procured from the same company.

4.5.3 Standard Care :

Both the arms received standard care including IV fluids, antibiotics, vasopressors and other medications as per the treating team's decision. The treating physicians were

encouraged to follow the Surviving Sepsis Campaign (3)guidelines. The treating team was free to use Thiamine and Hydrocortisone according to individual preferences and unit policies. The trial was pragmatic in nature and the investigators did not interfere in the treatment decision other than administering the trial drug according to randomization.

4.6 RANDOMISATION AND ALLOCATION CONCEALMENT

4.6.1 Method Of Randomisation:

Computer generated block randomization in blocks of 2 & 4

Permutated block randomization of sizes 2 and 4 was done for treatment allocation using SAS 9.4. Randomization was done according to computer generated block randomization generated by an independent investigator who is not involved in enrollment of the participants or collecting data. The drug and placebo were coded in identical containers as A or B and the coding was known only to the independent investigator and the pharmacist preparing the drug and placebo. The random allocation sequence and coding was stored in a secure place and decoded only after data analysis.

4.6.2 Method Of Allocation Concealment:

Sequentially Numbered Sealed Opaque Envelopes

The investigator who generated the random allocation sequence prepared allocation card with the sequence of participant and the code A/B. The allocation cards were sealed in opaque envelopes. The envelopes had only the sequence number written on top. The envelope was opened after recruitment, and the preparation A/B was be administered to

the patient as per the allocation. The analysis was be done using the codes for each arm and A/B was be replaced with the drug/placebo only after analysis of primary outcome.

4.6.3 Blinding And Masking:

The patient, investigator, nurse administering the drug, and investigator conducting the statistical analysis were blinded to the intervention. The decoding of codes A/B for the intervention and placebo preparations was be done only after the statistical analysis of primary outcome. Masking was ensured by preparing the Placebo in similar vials as the drug (purchased from the same company) and ensuring similar packaging

4.7 OUTCOMES

4.7.1 Primary Outcome:

The primary outcome assessed was in hospital all-cause mortality, which is considered to be one of the most important sepsis outcomes(220)

4.7.2 Secondary Outcomes:

The following secondary outcomes were analyzed (220)

1) ICU Length of Stay (LOS)
2) Hospital Length of Stay (LOS)
3) Time to Ventilator independence in survivors who required mechanical ventilation/NIV at the time of randomization
4) Time to vasopressor independence in participants who survived and required vasopressors at the time of randomization

5) New onset organ dysfunction(221)

a) Cardiovascular system failure: Systolic blood pressure ≤90 mmHg or mean arterial pressure ≤70 mmHg for at least 1 h despite adequate fluid resuscitation, or the use of vasopressors to maintain arterial pressure above these levels or unexplained metabolic acidosis (pH ≤ 7.30 or base deficit ≥5.0 mmol/L) with plasma lactate greater than 1.5 times the upper limit of normal.

b) Renal failure: Urine output <0.5 mL/kg/h for 1 h, or serum creatinine levels greater than 2.5 times the upper limit;

c) Respiratory failure: PaO_2/FiO_2 ≤250 in the presence of other dysfunctional organs or ≤200 if only lung

d) Hematologic dysfunction: Platelet count <80,000/mm^3 or 50 % drop in preceding 3 days

e) Liver dysfunction: Serum bilirubin ≥2.0 mg/dL when bilirubin was <2.0 mg/dL at baseline

f) Central nervous system failure: Glasgow coma scale (GCS) ≤10 after randomization in patients who had baseline GCS >10.

Although the initial trial protocol aimed at looking at days alive and independent of dialysis, ventilator and vasopressor, this was not analyzed in view of non-availability of data.

4.8 SAMPLE SIZE CALCULATION

Based on the study by Marik et al(72) , the mortality in patients with sepsis in the intervention group and control group were 8.5% and 40.4% respectively. By assuming

power of 80% and 2- sided alpha error of 5%, applying the formula given below, 27 patients should be studied in each arm to prove the hypothesis.

Equation 1: Sample Size Calculation Formula

$H_o : P_1 = P_2$; $H_a : P_1 \neq P_2$

$$n = \frac{\left\{ Z_{1-\frac{\alpha}{2}} \sqrt{2\,\bar{P}\,(1-\bar{P})} + Z_{1-\beta} \sqrt{P_1\,(1-P_1) + P_2\,(1-P_2)} \right\}^2}{(P_1 - P_2)^2}$$

Where,

$$\bar{P} = \frac{P_1 + P_2}{2}$$

P_1 : Proportion in the first group

P_2 : Proportion in the second group

α : Significance level

$1-\beta$: Power

It must be noted that in the study used to calculate the sample size, the intervention group received Vitamin C, Thiamine and Hydrocortisone. For sample size calculation, we assumed that Vitamin C contributed to the entire mortality benefit shown in the study. We aimed to recruit 30 patients in each arm – i.e. a total of 60 patients in our study. The study was able to recruit 51 patients.

4.9 MONITORING AND SAFETY

4.9.1 Interim Analysis

No interim analysis was performed

4.9.2 Withdrawal Of Participants

Participants were withdrawn of the trial in the following situations

a) Voluntary withdrawal of consent by the patient or the closest relative

b) Anaphylaxis to the agent.

c) Renal calculi detected while the participant is receiving the agent. Participants were not routinely screened for renal calculi. However, it is a standard of care in our ICU that patients are screened for obstructed urinary system in case of an acute kidney

Injury that cannot be attributed to any other cause, or out of proportion to the obvious cause. If renal calculi are detected in such screening or abdominal imaging performed for any other indication, the participant was planned to be withdrawn from the study. No participants were withdrawn due to this indication.

d) If the patient was started on bortezomib, cyclosporine or warfarin or related drugs for any indication during the drug administration period

e) Discharged against medical advice or limitation of care decided after enrollment into the study. However, such participants were included in the intention-to-treat analysis

In case consent is not obtained for inclusion of the withdrawn patients for analysis, the participants were excluded from the analysis and their medical records were not accessed further for the purpose of the trial.

4.9.3 *Data Safety Monitoring Board*

The Institutional Data Safety Monitoring Board was requested to monitor the trial and was informed of the progress of trial and Serious Adverse events at regular intervals as instructed by the IRB

4.10 DATA COLLECTION AND STATISTICAL ANALYSIS

Data was collected by the primary investigator using printed Clinical Research Forms, by daily review of the participants, In patient chart and Electronic Medical records and laboratory database. The data was entered using Microsoft Excel software and analysed using SPSS 25.0. The data was screened for outliers and extreme values using Box-Cox plot and histogram (for shape of the distribution). Summary statistics was used for reporting demographic and clinical characteristics. t-test was used for analysis of

continuous data with Normal distribution and Mann-Whitney U test for data with non-Normal distribution with groups (intervention and placebo). Chi-square test was performed for categorical variables with groups (intervention and placebo). Differences were considered significant if p Value is <0.05. Multivariate analysis was performed based on the variables which are significant at Bivariate levels.

Subgroup analysis was done as decided a priori for patients for the following group of patients

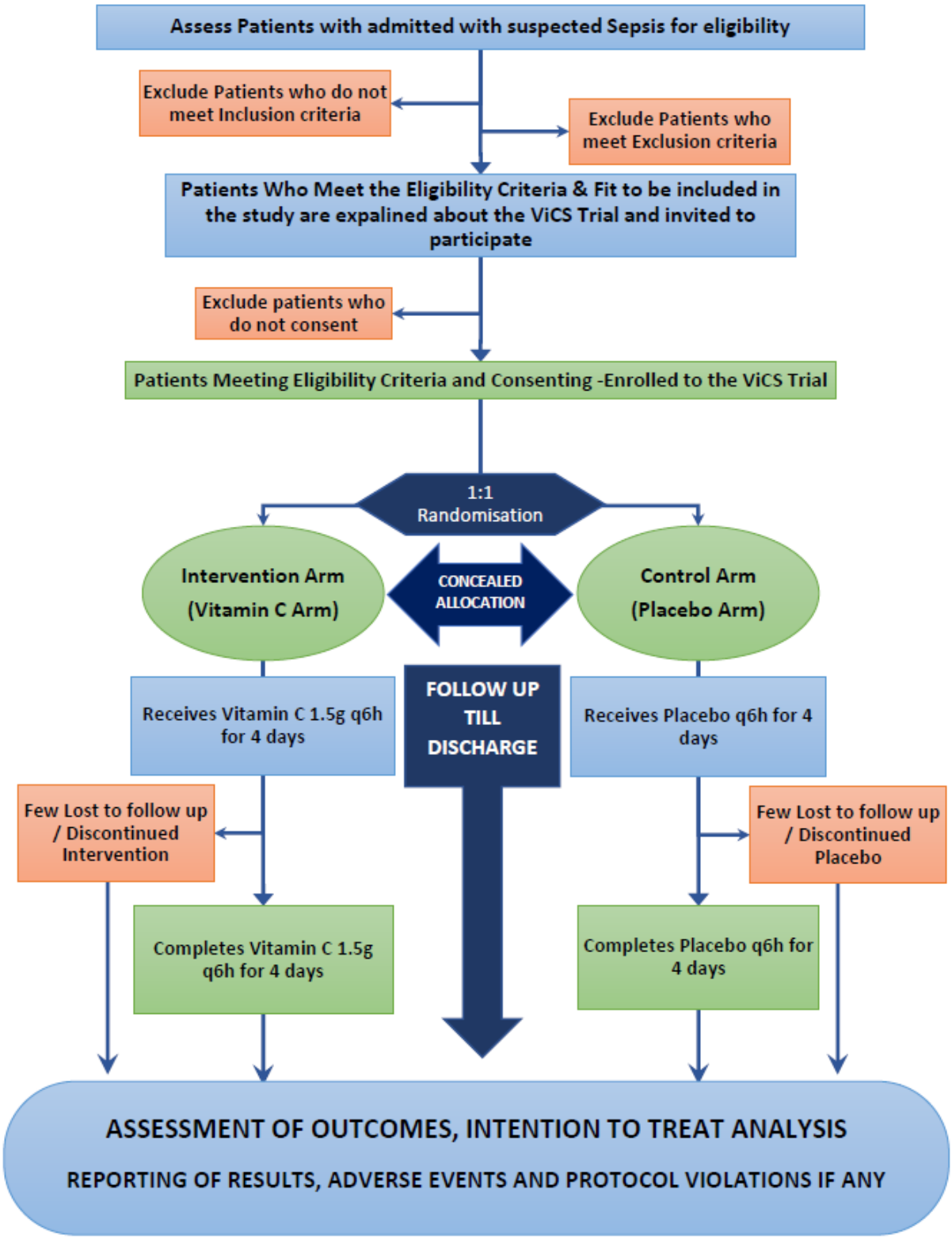
- Patients with and without septic shock at the time of enrolment. Septic shock was diagnosed as per the Sepsis 3 criteria (6)
- Patients with and without ARDS at the time of enrolment. ARDS was defined according to the Berlin definition (179)
- Patients with age groups of 18-30 years, 31-50 years and more than or equal to 51 years
- Males and females
- Patients with duration of symptoms of less than 48 hours before admission and more than 48 hours before admission
- Patients with blood or other usually sterile fluid culture (urine, sputum, CSF, ascitic or pleural fluid) positivity and patients who do not have a positive culture.
- Patients who received concomitant Hydrocortisone, Thiamine, both or none

Ad hoc subgroup analyses were also performed for the following groups

- Patients with or without AKI on presentation
- Patients with Pneumonia. This was because a Cochrane metanalysis had shown a possible beneficial effect of Vitamin C in treatment of Pneumonia(155)
- Patients with predicted mortality according to APACHE 2 score less than 50% and more than or equal to 50%
- Patients who received 1st dose of Vitamin C before or at 48 hours of symptom onset and more than 48 hours of symptom onset

Analysis of the secondary outcomes was done only in the participants who were alive and well at discharge (with the exception of days till death) and had the outcome data available.

4.11 DIAGRAMMATIC ALGORITHM OF THE STUDY



5. RESULTS

5.1 TRIAL OVERVIEW AND PATIENT RECRUITMENT

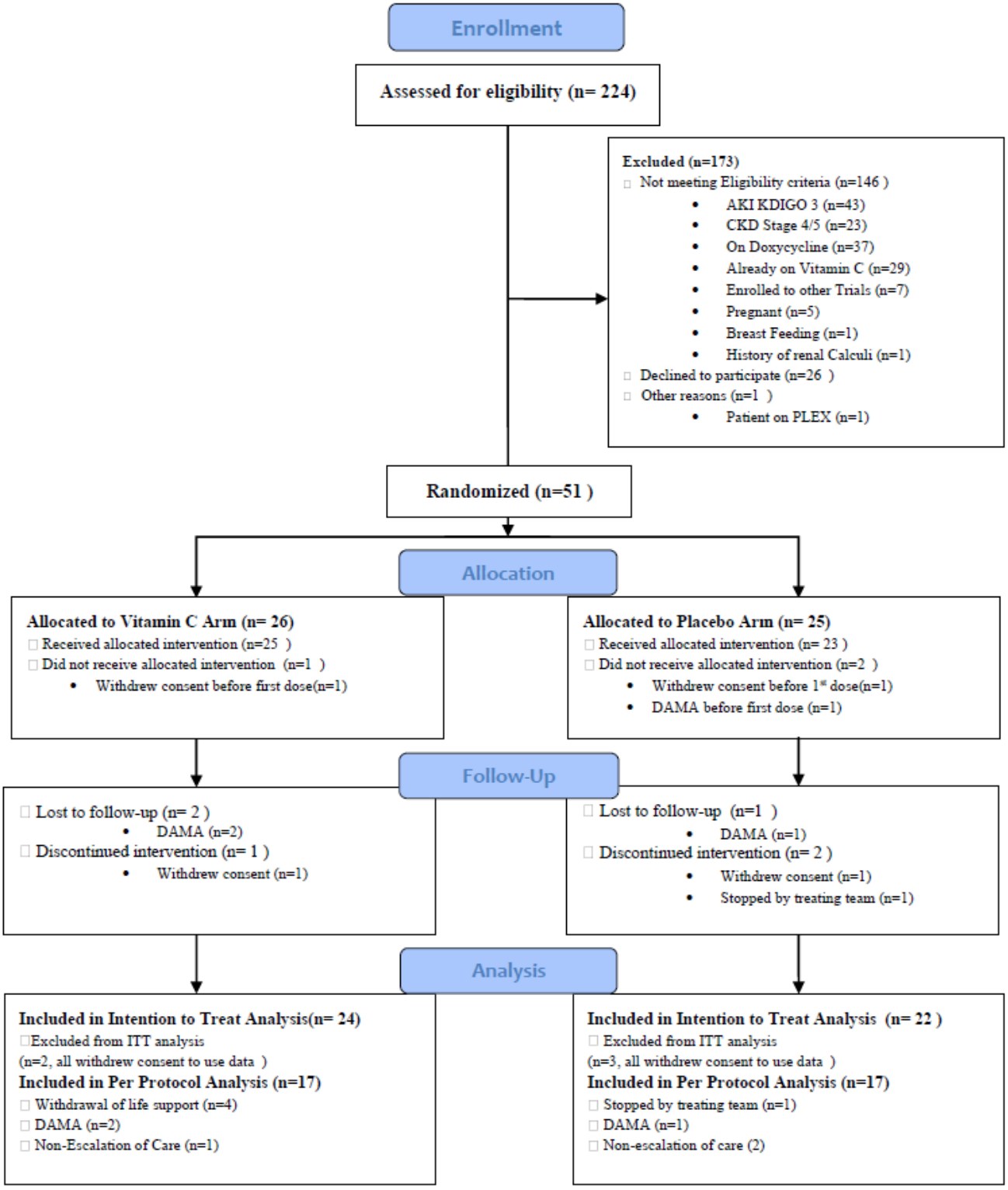
The trial was conducted from 21.09.2018 to 13.10.2019 and was recruited patients from the medical ICU for one year. From 08.09.2019, with an approved amendment of the trial protocol from the IRB, the trial started recruiting patients from the medical wards as well. This was due to slow accrual of participants.

A total of 224 patients were screened for eligibility to be included in the trial, of which 26 declined consent to participate and 173 did not meet the eligibility criteria. Further, 1 patient was excluded as the patient was initiated on PLEX, and Vitamin C kinetics is not well studied in patients on PLEX. The trial recruited a total of 51 patients – 26 were randomized to the Vitamin C arm and 25 to the placebo arm. Of these, 10(41.7%) and 13(59.1%) patients from intervention and control group respectively were recruited from ICU. Consent for participation was withdrawn by 2 patients from the intervention arm and 3 from the control arm at various stages. All these 5 participants declined consent for inclusion of their data in analysis as well. 24 patients in the intervention group and 22 in the control group were included in the intention-to-treat analysis.

A per-protocol analysis was also conducted by excluding the patients who were discharged against medical advice, whose life supports were withdrawn, who were planned for non-escalation of care, and who discontinued the study (but consented to use the data for analysis). The per protocol analysis included 17 patients in each arm.

The results are published in accordance with the CONSORT (Consolidated Standards of Reporting Trials) 2010 guidelines(222).

5.2 CONSORT FIGURE



5.3 PROTOCOL VIOLATIONS & STUDY DISCONTINUATION

There were 2 protocol violations in the included participants

- One patient who was randomized to the placebo group received therapeutic ascorbic acid for 2 days after the study period was over. This was due to a miscommunication between the investigators and the treating team. The patient was, however, included in intention-to-treat analysis. The patient was also planned for non-escalation of care, and was hence excluded from the per-protocol analysis.
- One patient assigned to the placebo group was on doxycycline. Patients on doxycycline were planned to be excluded from the study, as doxycycline injection is usually co-administered with ascorbic acid. However, the brand of doxycycline that this patient received did not contain ascorbic acid. The patient was included in the intention-to-treat and per protocol analysis.

A total of 2 patients, who were randomized and assigned to a group, withdrew consent to participate and include data in analysis before the first dose was administered. Two patients, with one patient assigned to Vitamin C and control arm each, withdrew consent for participation and inclusion in analysis during the study period. One patient in placebo arm was withdrawn from the study by the primary treating team with the consent of the patient. 4 patients were discharged against medical advice during or after the study period; life supports were withdrawn in 4 patients in the Vitamin C arm with family's consent. Three patients were later planned for non-escalation of care – 1 in Vitamin C arm and 2 in control arm.

5.4 BASELINE CHARACTERISTICS OF THE STUDY GROUPS

The general demographics and known prognostic factors of mortality in sepsis, were compared between the groups at baseline. The mean age of the study population was 50.43(SD: 17.23) years. The intervention group had a higher mean age as compared to the placebo group (54.41 vs 46.09 years).

Table 6: Baseline characteristics of the study population: Demographics and diagnosis

VARIABLE		VITAMIN C GROUP (n=24)	PLACEBO GROUP (n=22)	STUDY POPULATION (n=46)
Mean Age (in years)		54.41 ± 15.68	46.09 ± 18.15	50.43 ± 17.23
Admitted to				
	ICU	10 (41.7%)	13(59.1%)	23(50%)
	Ward	14(58.3%)	9(40.9%)	23(50%)
Gender				
	Males	14 (58.3%)	9(40.9%)	23(50%)
	Females	10(41.7%)	13(59.1%)	23(50%)
Diagnosis at enrolment				
	Pneumonia	11(45.8%)	7(31.8%)	18(39.1%)
	UTI	4(16.7%)	5(22.7%)	9(19.6%)
	CAUTI	0(0%)	1(4.5%)	1(2.2%)
	Cellulitis	3(12.5%)	1(4.5%)	4(8.7%)
	Meningitis	2(8.3%)	4(18.2%)	6(13%)
	IE	0(0%)	1(4.5%)	1(2.2%)
	VAP	1(4.2%)	1(4.5%)	2(4.3%)
	Liver Abscess	1(4.2%)	0	1(2.2%)
	AGE	2(8.3%)	2(9.1%)	48.7%)
Final Diagnosis				
	Pneumonia	10(41.7%)	6(27.3%)	16(34.8%)
	UTI	4(16.7%)	3(13.6%)	7(15.2%)
	CAUTI	0(0%)	1(4.5%)	1(2.2%)
	Cellulitis	3(12.5%)	1(4.5%)	4(8.7%)
	Meningitis	2(8.3%)	4(18.2%)	6(13%)
	IE	0(0%)	1(4.5%)	1(2.2%)
	SBP	0(0%)	1(4.5%)	1(2.2%)
	VAP	1(4.2%)	1(4.5%)	2(4.3%)
	Liver Abscess	1(4.2%)	0(0%)	
	Others +	0(0%)	1(4.5%)	2(4.3%)
	AGE	2(8.3%)	2(9.1%)	4(8.7%)
	Acute Cholecystitis	0(0%)	1(4.5%)	1(2.2%)
	Non-Infectious*	1(4.2%)	0(0%)	1(2.2%)
Hospital acquired infection		1(4.17%)	1(4.55%)	2(4.35)
Median days from symptom onset^		3	4	3
Median time from admission#		13	15	14

- *Values are expressed a mean(percentage)*
- *+ One patient in placebo group had viral myocarditis*
- ** One patient in intervention group was diagnosed to have Carcinoma lung*
- *^ Rounded off to the nearest whole number*
- *# In hours – rounded off to the nearest whole number*

The intervention group also had more male patients as compared to the placebo group (58.3% vs 40.9%). The most common diagnosis at enrolment as well as the final diagnosis was Pneumonia in both groups. However, the proportion of patients with pneumonia was more in the Intervention groups as compared to the control. One patient in each group had a hospital acquired infection (both ventilator associated pneumonia). Overall the baseline characteristics of age, gender and diagnosis were not very well matched.

Table 7: Baseline characteristics of the study population: Physiological variables

VARIABLE	VITAMIN C GROUP (n=24)	PLACEBO GROUP (n=22)	TOTAL POPULATION (n=46)
Mean Physiological Variable at recruitment			
Heart Rate	112.13 ± 16.163	113.50 ± 24.291	112.78±20.33
Systolic Blood Pressure	107.75 ± 15.607	110.36 ± 20.355	109.00±17.878
Diastolic Blood pressure	62.75 ± 8.502	63.55 + 9.028	63.13+8.668
MAP	74.96 ± 17.863	77.27 ± 9.161	76.07±14.269
Rectal Temp	100.467 ± 1.369	101.055 ± 1.276	100.748±1.344
Respiratory rate	26.38±6.612	29.14±7.173	46±6.950
On Invasive Ventilation at recruitment	7(29.2%)	8(36.4%)	15(32.6%)
On NIV at recruitment	4(16.75)	5(22.75)	9(19.56%)
On Inotropes at recruitment	9(37.5%)	10(45.5%)	19(41.3%)
Number of inotropes at recruitment			
1	6(25%)	6(27.3%)	12(26.08%)
2	2(8.3%)	2(9.1%)	4(8.69%)
3	1(4.2%)	2(9.1%)	3(6.52%)
Inotropes used at recruitment			
Noradrenaline	9(37.5%)	10(45.4%)	19(41.3%)
Adrenaline	3(12.5%)	4(18.2%)	7(15.2%)
Vasopressin	1(4.2%)	2(9.1%)	3(6.5%)
Dobutamine	0	1(4.5%)	1(2.1%)
Mean Inotrope doses at recruitment			
Noradrenaline(mcg/min)	17.11 ± 10.018	12.10±12.741)	
Adrenaline (mcg/min)	9.50±8.347	15.25±16.641	
Vasopressin (units/min)	1.5 ± 0.7071	2.6±0.849	
Dobutamine (mcg/min)	-	5	
Septic Shock at recruitment	9(37.5%)	10(45.5%)	19(41.3%)
ARDS at recruitment	19(19.2%)	12(54.5%)	31(67.3%)
AKI at recruitment	9(37.5%)	11(50%)	20(43.48%)
AKI Grade			
KDIGO1	5 (20.8%)	7(31.8%)	(26.09%)
KDIGO2	4(16.6%)	4(18.1%)	(17.4%)

- *Values are expressed as n (%) or mean ± SD*
- *mcg = microgram*

The physiological variables at baseline are shown in Table 6. Heart rate, systolic and diastolic blood pressures, and mean arterial pressures were well matched between both groups. The patients with septic shock and AKI were comparable between both groups, however the Intervention group had more patients with ARDS at enrollment.

The mean creatinine at recruitment was higher in the placebo group. Hemoglobin, platelet counts, and WBC counts were not significantly different between groups. The lab variables are shown in Table 7.

Table 8: Baseline characteristics of the study population: Laboratory parameters

VARIABLE		VITAMIN C GROUP (n=24)	PLACEBO GROUP (n=22)	TOTAL POPULATION (n=46)
Lab Variables at recruitment				
	Creatinine	1.31±0.74	1.54±0.82	1.42±0.78
	Total Bilirubin	1.52±2.6	1.05±0.73	1.29±1.57
	pH	7.38±0.11	7.36±0.19	7.37±0.15
	Lactates	4.01±4086	3.26±2.66	3.65±3.94
	Hemoglobin	11.16±2.57	11.87±1.97	11.49±2.30
	Platelets	2.31±1.06	2.17±1.15	2.24±1.09
	WBC Counts	19200±11393	17627±10173	18447±10737

- *Values are expressed as mean ± SD*

The most common co morbidities present among the groups were hypertension and diabetes. Prevalence of diabetes was comparable among both groups, whereas hypertension was more common in the placebo group. Placebo group also had higher prevalence of other co morbidities as described in Table 8. One patient in the placebo group was admitted with organo-phosphorus poisoning and developed a VAP in the hospital. The Charlson co-morbidity Index was higher in the intervention group as compared to the placebo group, but with overlapping 95% CIs.

Table 9 : Baseline characteristics of the study population: Co-morbidities

VARIABLE	VITAMIN C GROUP (n=24)	PLACEBO GROUP (n=22)	TOTAL POPULATION (n=46)
Diabetes Mellitus Type 2	12(50%)	17(77.3%)	29(63/04%)
Hypertension	14(58.3%)	17(77.3%)	31(67.39%)
Others	12(50%)	14(63.6%)	28(60.86%)
CVA	5	2	
Smoking	2	2	
Hypothyroidism	2	0	
Cancer*	1	1	
Hepatitis B	0	1	
COPD	2	2	
OP Poisoning	0	1	
CKD	2	1	
Depression	1	0	
Heart Failure	3	3	
Parkinsonism	0	1	
Type 1 Diabetes	0	1	
Paraplegia	0	1	
CLD	1	1	
Asthma	1	0	
Seizure	1	1	
Charlson Co Morbidity Index	2.63±2.58	1.82±2.44	2.24±2.523

- *Values are expressed as n (%) or mean ± SD*
- ** Both the patients had Multiple Myeloma*

The SOFA and APACHE 2 scores of both the groups at recruitment were similar. The predicted mortality by APACHE 2 score, of the entire study population was 31.76 % (SD: 19.178).

Table 10:Baseline Characteristics of the Study Population: Prognostic Scores

VARIABLE	VITAMIN C GROUP (n=24)	PLACEBO GROUP (n=22)	TOTAL POPULATION (n=46)
SOFA Score	6.75±3.365	6.95±3.273	6.85±3.286
APACHE-2 Score	18.46±7.644	17.82±6.919	18.15±7.233
Predicted mortality by APACHE-2(%)	32.17±20.708	31.32±17.834	31.76±19.178

- *Values are expressed as mean ± SD*

The number of participants with a body fluid positive for culture or PCR was 37.5 % and 45.5 % in the intervention and control groups respectively. Most cultures were positive in blood samples followed by sputum or ET aspirates. The most common

organism cultured were *Acinetobacter baumannii* followed by *Escherichia coli.* Concomitant influenza infection was present in 2 patients in the intervention and 4 in the control group (tested by PCR in Nasopharyngeal swab).

Table 11 : Baseline characteristics of the study groups: Culture positivity

VARIABLE		VITAMIN C GROUP (n=24)	PLACEBO GROUP (n=22)
Culture/PCR positivity		9(37.5%)	10(45.5%)
Organism cultured/detected			
	Escherichia coli	1(4.2%)	2(9.1%)
	Klebsiella pneumoniae	1(4.2%)	1(4.5%)
	Pseudomonas aeruginosa	1(4.2%)	1(4.5%)
	Acinetobacter baumannii	3(12.5%)	1(4.5%)
	Streptococcus pneumoniae	0	1(4.5%)
	Streptococcus pyogenes	0	0
	Staphylococcus aureus	1(4.2%)	1(4.5%)
	*Neisseria meningitides**	0	1(4.5%)
	Enterobacter spp.	0	1(4.5%)
	Haemophilus parainfluenza	1(4.2%)	0
	Citrobacter spp.	0	1(4.5%)
	*Entamoeba histolytica**	1(4.2%)	0
Body fluid that showed culture positivity			
	Blood	4(16.7%)	5(22.7%)
	Sputum/ETA	3(12.5%)	2(9.1%)
	CSF	0	1(4.5%)
	Pus	1(4.2%)	1(4.5%)
	Urine	1(4.2%)	1(4.5%)
Concomitant Influenza infection		2(8.4%)	4(18.2%)

- *Values are expressed as mean (%)*
- ** PCR positive*

Over all the baseline characteristics were not satisfactorily matched. Among the most important baseline characteristics that did not match were the age and Charlson comorbidity index. The Vitamin C group had a higher age and a higher comorbidity index at the baseline, which puts them at a higher risk of adverse outcome at baseline itself. However, the SOFA and APACHE 2 scores at baseline were well matched.

5.5 TREATMENT RECEIVED BY THE STUDY GROUPS

The patients in both the groups were treated according to the discretion of the ICU team and the primary treating team. All the doctors were encouraged to use the Sepsis 3 guidelines. The initial antibiotics were chosen based on the most likely site of infection and the local antibiotic resistance pattern, or based on a previous culture if available. The antibiotics were changed once culture reports were available, at the discretion of the treating team. Both groups received comparable initial antibiotics at the time of recruitment. However, more patients in the Placebo group were on meropenem as compared to intervention group. Meropenem was the most commonly used antibiotic.

More patients in the Intervention group received thiamine, glucocorticoids or both compared to the placebo group. 2 patients in the intervention group who were recruited from the ward were shifted to ICU during the study period.

Table 12: Treatment received by the study groups

VARIABLE	VITAMIN C GROUP (n=24)	PLACEBO GROUP (n=22)
Received Thiamine	16(66.7%)	12(54.5%)
Received Glucocorticoids	20(83.3%)	12(54.5%)
Hydrocortisone	18(75)	10(45.5)
Dexamethasone	2(8.3)	2(9.1)
Received both thiamine and glucocorticoids	15(62.5%)	8(36.4%)
Antibiotics at enrolment		
Azithromycin	9(37.5%)	8(36.4%)
Piperacillin - Tazobactam	5(20.8%)	6(27.3%)
Ceftriaxone	2(8.3%)	5(22.7%)
Meropenem	17(70.8%)	11(50%)
Colistin		1(4.5%)
Vancomycin	5(20.8%)	5(22.7%)
Metronidazole	2(8.3%)	
Doxycycline	1(4.2%)	1(4.5%)
Amoxicillin-Clavulanic Acid	1(4.2%)	1(4.5%)
Shifted from ward to ICU	2(8.3%)	0(0%)

- *Values are expressed as mean (%)*
- *Patients could have been on multiple antibiotics*

5.6 PRIMARY OUTCOME

As 3 patients who were included in the analysis were lost to follow up (Discharged against medical advice), for the intention-to-treat analysis, the worst-case scenario was assumed i.e. the DAMA patients from the Vitamin C group die and from the placebo group survive. Analyses were also conducted assuming the following scenarios

- Best-case scenario: Assuming the DAMA patients from Vitamin C group survive and Placebo group die
- Analyzing all DAMA patients as alive at hospital discharge
- Excluding the DAMA patients from analysis

A per protocol analysis was also performed as detailed above. The results are given in Table 12

Table 13: Primary Outcome: In-hospital mortality

	VITAMIN C	PLACEBO	RR	95% CI	PEARSON CHI SQUARE	p VALUE+
Intention-to-treat analysis*	13/24 (54.16%)	5/22 (22.72%)	2.383	1.015 – 5.598	4.763	0.038
Assuming best Case Scenario	11/24 (45.83%)	6/22 (27.27%)	1.681	0.748 – 3.775	1.697	0.233
DAMA assumed as alive at discharge	11/24 (45.83%)	5/22 (22.72%)	2.017	0.832-4.885	2.701	0.129
Excluding DAMA patients	11/22 (50%)	5/21 (23.81%)	2.100	0.878-5.021	3.154	0.116
Per protocol Analysis	6/17 (35.29%)	2/17 (11.76%)	3.000	0.702– 12.818	2.615	0.225

- *Values are expressed as ratio (percentage)*
- *+ Fisher's exact test*
- **Assuming worst-case scenario*
- *RR = Relative risk*
- *CI = Confidence interval*
- *DAMA = Discharged against medical advice*

In the intention-to-treat analysis (assuming worst case scenario), Vitamin C group had a higher in-hospital mortality (54.16%) as compared to the placebo group (22.72%), and the results were statistically significant (RR 2.38; 95% CI 1.015 – 5.598; p value 0.038) . In all other analyses and the per protocol analysis, there was a trend towards higher mortality with Vitamin C, but the results were not statistically significant.

The observed mortality was much higher than the mean predicted mortality by APACHE 2 scores (31.76% ± 19.178 in the study population) in the Vitamin C group (54.16%), but was lower in the Placebo group (22.72%). This indicates the fact that the harmful effect of Vitamin C seen in the study, might in-fact be a true observation.

5.7 SECONDARY OUTCOMES

The secondary outcomes analysis was performed only in the patients where the outcome was available. Most of the secondary outcomes analyzed were decided a priori, and a few chosen de novo.

There was higher incidence of new onset organ failure, new onset AKI and ARDS in the Vitamin C group, however the results were not statistically significant. Similarly, the placebo group had a trend to higher time till ventilator independence in patients on ventilator, with statistically insignificant results. The hospital length of stay and ICU length of stay were longer in the placebo group. None of the secondary outcomes analyzed showed statistically significant results.

Table 14: Secondary Outcomes

Outcome	Vitamin C	Placebo	RR/ ΔMean	95% CI	Pearson Chi Square	p value*
Dialysis	0/24	1/22 (4.5%)			1.115	0.478
New organ failure	8/24 (33.3%)	6/22 (27.3%)	1.222	0.504 to 2.966	1.99	0.754
New onset CVS failure	3/24 (12.5%)	4/22 (18.2%)	0.688	0.173 to 2.735	0.287	0.694
New onset AKI	4/24 (16.66%)	0/22			4.016	0.110
New onset ARDS	6/24 (25%)	2/22 (9%)	2.750	0.618 to 12.227	2.022	0.247
New onset CNS failure	0/24	1/22 (4.5%)			1.115	0.478
Time till Ventilator independence	6.67±4.16 (3)	8.50±12.61 (10)	-1.833	-12.140 to 8.474		0.710
Time till vasopressor independence	2.75±2.36 (4)	2±20 (10)	0.750	-1.953 to 3.453		0.573
ICU LOS	7±5.20 (3)	8.89±8.21 (9)	-1.889	-13.327 to 9.549		0.432
Hospital LOS	11.7±9.92 (10)	12.81±8.42 (16)	-1.113	-8.609 to 6.384		0.391
Days till death	8.08±5.57 (12)	11±11.68 (5)	-2.917	-17.168 to 11.335		0.959

- *Values are expressed as ratio(percentage) or mean ± SD(n)*
- ** Fisher's exact test*

5.8 SUBGROUP ANALYSIS

Subgroup analyses were performed within the subgroups decided a-priori. Few de novo analyses were also performed. In all the subgroups except Age 31-50, Vitamin C showed a higher trend to in-hospital mortality, however outcomes in none of the subgroups were statistically significant. Notably, even in the patients who were co administered thiamine, hydrocortisone or both, there was a higher trend to in-hospital mortality in the Vitamin C group.

A Cochrane metanalysis(155) had shown beneficial effect of Vitamin C in patients with pneumonia. In the subgroup of patients with a final diagnosis of pneumonia in this study, Vitamin C showed a trend towards harm.

Table 15 :Sub-group Analysis

Subgroup	Vitamin C	Placebo	Study population	RR	95% CI	Pearson Chi Square	P value
Patients initially admitted to ICU	7/10 (70%)	4/12 (33.33%)	11/22 (50%)	2.100	0.856-5.150	2.933	0.198
Age 18-30	1/2 (50%)	0/3 (0%)	1/5 (20%)			1.875	0.400
Age 31-50	2/6 (33.33%)	4/11 (36.36%)	6 (17)	0.917	0.232-3.627	0.016	1.000
Age >50	8/14 (57.14%)	1/7 (14.28%)	9/21 (42.86%)	4	0.616-25.964	3.500	0.159
Days from symptom onset <48h	3/9 (33.33%)	0/7 (0%)	3/16 (18.75%)			2.872	0.213
Days from symptom onset >48 hours	8/13 (61.53%)	5/14 (35.71%)	13/27 (48.15%)	1.723	0.756-3.927	1.801	0.257
Pneumonia	5/9 (55.55%)	2/6 (33.33%)	7/15 (46.66%)	1.667	0.466-5.956	0.714	0.608
Septic shock at Admission	6/9 (66.66%)	2/9 (22.22%)	8/18 (44.44%)	3	0.812-11.081`	3.600	0.153
ARDS at admission	9/18 (50%)	3/12 (25%)	12/30 (40%)	2	0.677-5.909	1.875	0.260
AKI at admission	3/9 (33.33%)	3/10 (30%)	6/19 (31.58%)	1.111	0.296-4.171	2.24	1
Received thiamine	9/15 (60%)	3/11 (27.27%)	12/26 (46.15%)	2.2	0.770-6.285	2.735	0.130
Received glucocorticoids	11/18 (61.11%)	5/11 (45.45%)	16/29 (55.17%)	1.344	0.638-2.832	0.677	0.466
Received thiamine and glucocorticoids	9/14 (64.28%)	3/7 (42.85%)	12/21 (57.14%)	1.500	0.586-3.841	0.875	0.397
Culture positive	4/9 (44.44%)	4/10 (40%)	8/19 (42.10%)	1.11	0.387-3.186	0.038	1
Blood Culture Positive	2/3 (66.67%)	3/5 (60%)	5/8 (62.5%)	1.11	0.380-3.251	0.36	1
Predicted mortality >50% by APACHE2	3/5 (60%)	2/4 (50%)	5/9 (55.55%)	1.2	0.357-4.038	0.90	1

5.9 ADVERSE EFFECTS AND SAFTEY

No immediate infusion related adverse effects were reported with study drug administration. As shown in Table 14, the incidence of new AKI and ARDS was higher in the Vitamin C group, however none of the patients developed new onset AKI / ARDS unexpectedly. In all these cases the AKI/ARDS were attributed to the severity of the disease. Study drug was not discontinued in any of these patients. The treating team discontinued the study drug in one patient after 2 days due to the anticipation of worsening AKI as the Creatinine clearance was markedly low. As this was a pragmatic trial, the same was allowed and discussed with the patient. The patient was however on a placebo.

Within the context of a pragmatic trial, data was collected only on adverse events that had been judged by the treating team to be related to the trial drug, and this judgement was not adjudicated by the investigators. This approach may weaken the inferences about adverse events

6. DISCUSSION

6.1 INTRODUCTION

Sepsis is a heterogenous syndrome which is very common in the day to day clinical practice of a general physician. Despite good understanding about its pathophysiology, the therapeutic armamentaria available is limited, and the mortality and morbidity remain high.

Vitamin C is an essential micronutrient that is known to have significant anti-oxidant properties. Its role in sepsis has been studied in detail in pre-clinical models. Recent human studies have shown promising results with therapeutic results of IV Vitamin C in sepsis. However, the generalizability of these studies was limited due to the poor methodology and confounders.

This Randomized clinical trial was undertaken to study the usefulness of high dose Vitamin C in sepsis, assessing in-hospital mortality as the primary outcome, and was an attempt at bridging the knowledge gap in this area. This being a randomized control trial would provide high quality evidence, easing the clinical decision making regarding its use. This pragmatic trial was designed with statistical power to detect a clinically plausible effect on in-hospital mortality. To reduce bias, a central randomization process was used and concealment and blinding of trial-group assignments were ensured. The statistical analysis was done before unblinding, further limiting the chance of bias.

We chose in-patient mortality as a patient-centered primary outcome, which is one of the most relevant outcome assessments in clinical management of sepsis. The inclusion criteria were based on the latest Sepsis 3 definition. The trial was successful in enrolling ~ 80% of the intended population. Most enrolled patients received the trial intervention as planned, and lost to follow-up was negligible. An intention-to-treat analysis was performed, only the patients who refused consent to be included in the analyses were excluded. The study protocol was approved by the institutional IRB and the trial was registered in CTRI before starting recruitment.

6.2 FINDINGS OF THE STUDY

This study found that administration of high dose Vitamin C was associated with an increase in the in-hospital mortality compared to placebo among patients with Sepsis. The trend towards harm was consistent in all the methods of analysis and among all the subgroups analyzed, making it likely that this is a true observation. In the ITT analysis (assuming the worst-case scenario), Vitamin C administration was associated with a 31.44 % increase in mortality (RR: 2.383. 95% 1.015 – 5.598), and the results were statistically significant. Even in subgroups who received thiamine, glucocorticoids or both, and in subgroup of patients with pneumonia, Vitamin C showed trend towards harm.

There were no significant between-group differences with respect to secondary outcomes of ICU length of stay. Hospital length of stay, time to ventilator and vasopressor independence, or new onset organ dysfunction. Patients who had been assigned to receive high dose Vitamin C had more incidence of new onset AKI and

ARDS as compared to placebo, but these were not statistically significant, and not attributed by the treating team to the intervention.

The recently published CITRUS-ALI trial (223) had failed to show any benefit of 96-hour infusion of Vitamin C compared with placebo in improving organ dysfunction scores or altering markers of inflammation and vascular injury. However, to the best of our knowledge, this is the first study showing harm of Vitamin C in sepsis. A phase 1 clinical trial had shown prompt reduction in SOFA score, reduction in CRP and Procalcitonin levels, and reduction in thrombomodulin levels as compared to placebo in patients with sepsis(142). In another small randomized trial that included 28 patients in septic shock intravenous ascorbic acid reduced mean dose and duration of vasopressors (primary outcome) and 28-day mortality (secondary outcome) as compared to the placebo(142).

This study revealed results contrary to the hypothesis. Few causes that could explain this were identified. First, the Vitamin C group in the trial had a higher Charlson co-morbidity index and higher mean age, which may have adversely affected the outcome. More patients in the Vitamin C group underwent withdrawal of life supports as compared to placebo group. The lack of effect of Vitamin C could be because it was given too late. In the study, the median number of days after symptom onset when the 1st dose of the study drug was administered was 3 days. One may assume that the biological pathways through which Vitamin C acts, may have undergone irreversible damage by then. However, this is usually the case in a real-world scenario.

Although the above factors could have affected the results of the study, the fact that the intention to treat, per protocol and all subgroup analyses shows a consistent trend towards harm, leads one to believe that the study findings are in fact true. Vitamin C showed trend towards harm in all major clinically relevant secondary outcomes as well. If these findings were to be believed, Vitamin C may be harmful in Sepsis.

6.3 STRENGTHS OF THE STUDY

This study has considerable strengths.

- This represents one of the first, few and larger randomized, placebo-controlled clinical trials of Vitamin C administration in patients with sepsis.
- The trial protocol was pre-decided and registered in CTRI. The protocol adherence was very good, with excellent follow-up.
- The enrolled patients appear representative of the target population. The baseline characteristics were comparable to that of the population of sepsis, described in various studies
- Considerable clinical and laboratory data were collected.
- The outcomes were representative of a real-world scenario and clinically meaningful. The ITT analysis showed a statistically significant outcome.
- The trial was pragmatic in nature, thus making the results more generalizable

6.4 LIMITATIONS OF THE STUDY

The study also comes with a number of limitations.

- Sample size calculation was based on the assumption that the mortality benefit of Vitamin C in sepsis is huge, resulting in a small sample size. Smaller benefits will not have been picked up. For the sake of sample size calculation, it was also

assumed that the mortality difference showed in a previous study was due to Vitamin C alone (74), whereas the intervention group in that study had received Vitamin C, hydrocortisone and thiamine . We know that these three agents interact with each other, and thiamine and hydrocortisone may have independent benefits in sepsis, making the above assumption false.

- This was a single centre trial, thus limiting its generalizability.
- The baseline characteristics of the two study groups were not perfectly matched. For example, the Charlson co-morbidity index was significantly high in the Vitamin C group as compared to the Placebo. This may have contributed to the higher mortality seen in the Vitamin C arm. Other clinical confounding factors may also be present. We did not do a stratified randomization to match the groups to known prognostic variables.
- There were 2 protocol violations as described earlier. However as discussed above, these are unlikely to have affected the study results.
- No interim analyses were performed, due to the small sample size
- The study protocol was amended after recruiting 20 participants to include patients from the ward as well. This may have led to the inclusion of a less sick cohort of patients in the trial. However, the other inclusion criteria were not altered.
- We did not measure the baseline Vitamin C concentrations in the study population. There may have been significant benefit of administering Vitamin C, in Septic patients with low Vitamin C levels.

- We did not screen actively for renal oxalate stones, a known complication of High dose Vitamin C supplementation.
- The study attained only 85.32 % of the calculated 76.67% of the targeted sample size, due to time constraints.
- The appropriateness of antibiotic therapy was not adjudicated.

6.5 IMPLICATIONS IN CLINICAL PRACTICE

This study showed that Vitamin C administration was harmful in preventing in-hospital mortality in Sepsis. All the analyses showed a consistent trend towards harm. Although limited by a small sample size and possibility of confounders, based on this study, the use of Vitamin C in Sepsis should not be adopted to clinical practice. A lot of institutions worldwide are using IV Vitamin C in sepsis, with or without thiamine and hydrocortisone based on evidence from pre-clinical and retrospective studies. As this randomized clinical Trial and another recent RCT(223), provides a better quality of evidence against use of Vitamin C, this practice should be discouraged, till better evidence is available from large well conducted multi-centre randomized control trials.

6.6 IMPLICATIONS IN FUTURE RESEARCH

Despite this rigorous clinical trial, a lot of questions remain unanswered in the area of use of Vitamin C in Sepsis. The difference in mortality is tantalizing and likely to spur much debate. Due to conflicting evidence, and the fact that most of the human studies include only a small sample size of patients, large well conducted randomized control trials are the need of the hour to provide robust evidence regarding the use of Vitamin C in sepsis.

Co-administration of thiamine and hydrocortisone also should be explored due to the biological agonism and interactions of the three. A factorial design would be best suited for this.

The optimum dose of Vitamin C, also needs to be explored through a dose finding study. Whether Vitamin C administration is more beneficial in Vitamin C deficient septic patients also needs to be explored, by measuring baseline Vitamin C levels. Thus, in future studies, it would be helpful to reconsider optimal dosing and timing, as well as the likelihood that any potential benefits may only accrue to subsets of patients, given the underlying heterogeneity of sepsis. The search for an effective therapy for sepsis has thus far been elusive, and it's time we further strengthen our efforts to conquer this lethal entity.

7. CONCLUSION

- This single centre placebo-control randomized clinical trial conducted in a single centre in South India showed harmful effect of high dose intravenous Vitamin C administration in all cause in-hospital mortality as compared to placebo. Vitamin C administration also showed a trend towards harm in all sub groups analyzed.

- The trial also did not show any statistically significant benefit of Vitamin C administration on any of the secondary outcomes - time to ventilator independence, time to vasopressor independence, length of ICU stays, length of hospital stay and new onset organ dysfunction, in adult patients with Sepsis.

- The incidence of new onset Acute Kidney Injury and Acute Respiratory Distress Syndrome in patients with sepsis was more in patients who were administered high dose IV Vitamin C as compared to placebo, but this difference was not statistically significant.

- Overall, the findings of the study advice against the use of Vitamin C in sepsis. Large well conducted multi-centre clinical trials are required to further consolidate the evidence in this area.

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9. ANNEXURES

9.1 ABSTRACT

Department: Department of General Medicine
University Registration Number: 201711452
Degree and Subject: MD General Medicine
Institution: Christian Medical College, Vellore
Name of the Guide: Dr O C Abraham

Title of the abstract:

High dose intra-venous Vitamin C administration to prevent mortality in patients with sepsis - a randomized, double blind, placebo-controlled trial (The ViCS trial)

Background:

Sepsis is a syndrome of "life threatening organ dysfunction secondary to a dysregulated host response to infection". Despite a myriad of interventions that have been tried in its management, the mortality remains high. Vitamin C is a drug that has antioxidant properties and acts as a co-factor in synthesis of important biomolecules like catecholamines. Along with biological plausibility that is evident from biochemical and animal studies; multiple observational studies have shown potential benefit of this agent in Sepsis. The benefit of Vitamin C in Sepsis, however, has not been conclusively proven in randomized clinical trials.

Aim and objectives:

This study aimed at assessing the effect of high dose intravenous Vitamin C administration in patients with sepsis. The objectives included evaluation of the effect of High dose intravenous (IV) Vitamin C administration on all-cause in-hospital mortality, time to ventilator independence, time to vasopressor independence, length of ICU stay, length of hospital stay, and new onset organ dysfunction in adult patients with sepsis and to identify its adverse effects.

Methods:
This randomized, double-blind, placebo-controlled, parallel group, investigator initiated, pragmatic clinical trial was conducted in a single centre in South India. After informed consent, in-patients admitted with a diagnosis of sepsis were randomly assigned to receive IV Vitamin C 1.5g every 6 hours or a matching placebo for 4 days, along with the standard of care. The allocation was concealed and intervention was blinded. The primary outcome was in-hospital all-cause mortality. Data was collected by the primary investigator through structured questionnaire, entered through Microsoft Excel and analysed using SPSS 25.0.

Results:
Through 21.09.2018 to 13.10.2019, 224 patients were screened for eligibility and 51 patients were randomised according to the eligibility criteria. 24 patients in the intervention group and 22 in the control group were included in the intention-to-treat analysis. The baseline characteristics were imperfectly matched, but prognostic scores at baseline (SOFA and APACHE 2) were well matched. In the ITT analysis Vitamin C administration was associated with a 31.44 % increase in mortality (RR: 2.383, 95% 1.015 – 5.598, p=0.038), and the results were statistically significant. The per protocol and all subgroup analyses showed a trend towards harm, all though the results were not statistically significant. There were no unexpected, significant complications with Vitamin C administration.

Conclusions:
Among patients with sepsis, administration of high dose intra-venous Vitamin C was associated with an increased risk of in-hospital mortality, as compared to placebo.

Funding: Fluid research fund of Christian Medical College, Vellore
Trial Registration: CTRI/2018/05/013994 UTN: U1111-1207-5230
Conflicts of Interest: None declared

Key words: Vitamin C, Ascorbic Acid, Sepsis, Severe infection, Mortality

9.2 INSTITUTIONAL REVIEW BOARD(IRB) APPROVAL

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA

Ethics Committee Registration No: ECR/326/INST/TN/2013 Re Reg-2016 Issued under Rule 122D of the Drugs & Cosmetics Rules 1945, Govt. of India

April 28, 2018

Dr /
PG Registrar,
Department of Medicine - 4,
Christian Medical College,
Vellore - 632 002.

Sub: Fluid Research Grant: New Proposal:
High Dose Intra-Venous Vitamin C Administration to prevent Mortality in Patients with Sepsis –A Randomized, Double blind, Placebo controlled Trial
Dr , Employment Number: 29632PG Registrar, Department of General Medicine (Unit IV), Dr O.C Abraham, Employment Number: 05638, General Medicine and General Medicine Unit IV, Dr Binila Chacko, Employment Number: 28471, Critical Care, Dr Ravikar Ralph, Employment Number: 28852, General Medicine Unit 1, Dr Vignesh Kumar C, Employment Number: 33782, General Medicine Unit 2, Dr Mohammad Sadiq, Employment Number: 29104, General Medicine Unit 3, Dr Karthik G, Employment Number: 29074, General Medicine Unit 5, Mr. Bijesh Yadav, Employment Number:33244, Biostatistics.

Ref: IRB: 11125 (INTERVEN) dated: 24.01.2018

Dear Dr /

The Institutional Review Board **(Silver**, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project titled "High Dose Intra-Venous Vitamin C Administration to prevent Mortality in Patients with Sepsis –A Randomized, Double blind, Placebo controlled Trial" on January 24th 2018.

The Committee reviewed the following documents:

1. IRB Application Format
2. Clinical Research form
3. Permission Letter
4. GCP Certificate
5. Consent form and Information Sheet (English, Tamil)
6. CV's of Drs Binila, Bijesh, Karthik, Md. Sadiq, OC Abraham, Ravikar Ralph and Vignesh Kumar..
7. No. of Documents 1 - 6

The following Institutional Review Board (Silver, Research & Ethics Committee) members were present at the meeting held on January 24th 2017 at 9.45 am in the BRTC, Conference Room, Christian Medical College, Bagayam, Vellore 632002.

Name	Qualification	Designation	Affiliation
Dr. George Thomas	MBBS, D Ortho, PhD	Orthopaedic Surgeon, St. Isabella Hospital, Chennai, Chairperson, Ethics Committee, IRB, Chennai	External, Clinician
Rev. Dr. T. Arul Dhas	MSc, BD, DPC, PhD(Edin)	Chaplaincy Department, CMC, Vellore	Internal, Social Scientist
Dr. Biju George	MBBS, MD, DM	Professor, Haematology, Additional Vice Principal (Research), Deputy Chairperson (Research Committee), Member Secretary (Ethics Committee), IRB, CMC, Vellore.	Internal, Clinician
Dr. RV. Shaji	B.Sc, M.Sc, PhD	Professor, Heamatology, CMC, Vellore	Internal, Basic Medical Scientist
Prof. Keith Gomez	BSc, MA (S.W), M. Phil (Psychiatry Social Work)	Student counselor, Loyola College, Chennai, Deputy Chairperson, Ethics Committee, IRB	External, Lay Person & Social Scientist
Dr. P. Zachariah	MBBS, PhD	Retired Professor, Vellore	External, Clinician
Dr. Anuradha Bose	MBBS, DCH, MD,MRCP, FRCPCH	Professor of Paediatrics, Community Medicine, CMC, Vellore	Internal, Clinician
Dr. D. J. Christopher	BSc, MBBS, DTCD DNB, FRCP(Glasg), FCCP(USA)	Professor, Pulmonary Medicine, CMC, Vellore	Internal, Clinician
Dr. Ashish Goel	MBBS, MD, DM	Professor, Hepatology, CMC, Vellore	Internal, Clinician
Mr. Samuel Abraham	MA, PGDBA, PGDPM, M. Phil, BL.	Sr. Legal Officer, CMC, Vellore	Internal, Legal Expert
Mr. C. Sampath	BSc, BL	Advocate, Vellore	External, Legal Expert
Dr. Suresh Devasahayam	BE, MS, PhD	Professor of Bio-Engineering, CMC, Vellore	Internal, Basic Medical Scientist

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA

Ethics Committee Registration No: ECR/326/INST/TN/2013 Re Reg-2016 Issued under Rule 122D of the Drugs & Cosmetics Rules 1945, Govt. of India

Dr. Sathya Subramani	MD, PhD	Professor, Physiology, CMC, Vellore	Internal, Clinician
Mrs. Ruma Nayak	M Sc (Nursing)	Professor, Head of Paediatric Nursing & Deputy Nursing Superintendent, College of Nursing, CMC, Vellore	Internal, Nurse
Mrs. Pattabiraman	BSc, DSSA	Social Worker, Vellore	External, Lay Person

We approve the project to be conducted as presented.

Kindly provide the total number of patients enrolled in your study and the total number of withdrawals for the study entitled: "High Dose Intra-Venous Vitamin C Administration to prevent Mortality in Patients with Sepsis - A Randomized, Double blind, Placebo controlled Trial." on a monthly basis. Please send copies of this to the Research Office (research@cmcvellore.ac.in).

The Institutional Ethics Committee expects to be informed about the progress of the project, any **adverse events** occurring in the course of the project, any **amendments in the protocol and the patient information / informed consent**. On completion of the study you are expected to submit a copy of the **final report**. Respective forms can be downloaded from the following link: http://172.16.11.136/Research/IRB_Polices.html in the CMC Intranet and in the CMC website link address: http://www.cmch-vellore.edu/static/research/Index.html

Fluid Grant Allocation:
A sum of 94,080/- INR (Rupees Ninety four Thousand Eighty Only) will be granted for 2 years. 50,000/- INR (Rupees Fifty Thousand only) will be granted for 12 months as an Ist Installment. The rest of the 44,080/- INR (Rupees Forty four thousand eighty only)will be released at the end of the first year as 2 nd Installment.

Yours sincerely,

Dr. Biju George
Secretary (Ethics Committee)
Institutional Review Board

Dr. BIJU GEORGE
MBBS., MD., DM.
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

9.3 INSTITUTIONAL REVIEW BOARD(IRB) APPROVAL OF PROTOCOL AMENDEMENT

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA

Ethics Committee Registration No: ECR/326/INST/TN/2013 Re Reg-2016 Issued under Rule 122D of the Drugs & Cosmetics Rules 1945, Govt. of India

Ref: IRB – A28 – 28.08.2019

September 09, 2019.

Dr. [redacted]
Department of Medicine,
Christian Medical College,
Vellore 632 002

Ref: IRB Min. No. 11125 dated 24.01.2018

Dear Dr. [redacted]

The Institutional Review Board (Silver, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed the following amendment for the study titled "High Dose Intra-Venous Vitamin C Administration to prevent Mortality in Patients with Sepsis –A Randomized, Double blind, Placebo controlled Trial" on August 28th 2019.

1. In addition to the patients admitted to the Medical ICU / HDU, all patients admitted to the medical wards (I, E, C, MTS4, O6W, A Block) meeting the pre-specified eligibility criteria as submitted in the original protocol may be included in the study.

2. ICU Length of stay will be assessed as a secondary end point in those patients requiring ICU Care.

The following Institutional Review Board (Silver, Research & Ethics Committee) members were present at the meeting held on August 28th 2019 at 9.45 am in the New IRB Room, Christian Medical College, Bagayam, Vellore-632002.

Name	Qualification	Designation	Affiliation
Prof. Keith Gomez	BSc, MA (S.W), M. Phil (Psychiatry Social Work)	Student counselor, Loyola College, Chennai, Deputy Chairperson, Ethics Committee, IRB	External, Lay Person & Social Scientist
Dr. Biju George	MBBS, MD, DM	Professor, Haematology, Additional Vice Principal (Research), Deputy Chairperson (Research Committee), Member Secretary (Ethics Committee), IRB, CMC, Vellore.	Internal, Clinician
Dr. P. Zachariah	MBBS, PhD	Retired Professor, Vellore	External, Clinician
Dr. Sathya Subramani	MD, PhD	Professor, Physiology, CMC, Vellore	Internal, Clinician

Dr. Prasanna Samuel	M. Sc, PhD	Lecturer, Biostatistics, CMC, Vellore	Internal, Statistician
Dr. Niranjan Thomas	MD (Paed) DCH, DNB	Professor, Paediatrics, CMC, Vellore	Internal, Clinician
Rev. Dr. T. Arul Dhas	MSc, BD, DPC, PhD(Edin)	Chaplaincy Department, CMC, Vellore	Internal, Social Scientist
Dr. Blessed Winston	MBBS., MD	Associate Professor, Clinical Pharmacology, CMC, Vellore	Internal, Pharmacologist
Dr. Suresh Devasahayam	BE, MS, PhD	Professor of Bio-Engineering, CMC, Vellore	Internal, Basic Medical Scientist
Mrs. Alice Sony	M.Sc. (Nursing)	Deputy Dean, College of Nursing, CMC, Vellore	Internal, Nurse
Dr. Shirley David	MSc, PhD	Professor, Head of Fundamentals Nursing Department, College of Nursing, CMC, Vellore	Internal, Nurse
Mr. Samuel Abraham	MA, PGDBA, PGDPM, M. Phil, BL.	Sr. Legal Officer, Vellore	External Legal Expert
Mr. C. Sampath	BSc, BL	Advocate, Vellore	External, Legal Expert
Mrs. Ilavarasi Jesudoss	M Sc (Nursing)	Deputy Nursing Superintendent, College of Nursing, CMC, Vellore	Internal, Nurse
Mrs. Pattabiraman	BSc, DSSA	Social Worker, Vellore	External, Lay person
Dr. Abhay Gahukamble	MS, D Ortho, DNB(Ortho)	Associate Professor, Paediatric Orthopaedics, CMC, Vellore	Internal, Clinician
Dr. Suceena Alexander	MBBS, MD, DM	Associate Professor, Nephrology, CMC, Vellore	Internal, Clinician

We approve the above amendment as presented.

Yours sincerely,

Dr. Biju George
Secretary (Ethics Committee)
Institutional Review Board.
IRB Min. No. 11125 dated 24.01.2018

Dr. BIJU GEORGE
MBBS., MD., DM.
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

9.4 FLUID RESEARCH FUND APPROVAL

Accounts Projects <accounts_projects@cmcvellore.ac.in> Wed, May 30, 2018 at 1:54 PM
To: [redacted]
Cc: Principal's Office - Research <research@cmcvellore.ac.in>, "Abraham O.C" <ocabraham@cmcvellore.ac.in>

CHRISTIAN MEDICAL COLLEGE
Office of the Treasurer

Date: 30-05-2018

Dear Dr.[redacted]

As requested by the Vice-Principal (Research) with the IRB Minute No. 11125 a new Fluid research account opened for your Project, the account no are as follows:-

22 Z 469

This is for your information

Thanking you.

Yours sincerely

P. BASKARAN
Sr. Manager (F&A)
Accounts

9.5 CTRI REGISTRATION

CLINICAL TRIALS REGISTRY - INDIA
ICMR - National Institute of Medical Statistics

PDF of Trial
CTRI Website URL - http://ctri.nic.in

Clinical Trial Details (PDF Generation Date :- Thu, 07 Nov 2019 07:20:06 GMT)

CTRI Number	CTRI/2018/05/013994 [Registered on: 21/05/2018] - **Trial Registered Prospectively**
Last Modified On	13/09/2018
Post Graduate Thesis	Yes
Type of Trial	Interventional
Type of Study	Drug
Study Design	Randomized, Parallel Group, Placebo Controlled Trial
Public Title of Study	A Study to find out usefulness of Vitamin C Injections in decreasing deaths due to Sepsis(A serious form of Infection)
Scientific Title of Study	High Dose Intra-Venous Vitamin C Administration to prevent Mortality in Patients with Sepsis – A Randomized, Double blind, Placebo controlled Trial

Secondary IDs if Any

Secondary ID	Identifier
U1111-1207-5230	UTN

Details of Principal Investigator or overall Trial Coordinator (multi-center study)

Details of Principal Investigator	
Name	
Designation	PG Registrar
Affiliation	Christian Medical College & Hospital,Vellore
Address	Department of General Medicine Unit 4, Christian Medical College and Hospital, Ida Scuddder Road, Vellore Vellore TAMIL NADU 632004 India
Phone	
Fax	
Email	@gmail.com

Details Contact Person (Scientific Query)

Details Contact Person (Scientific Query)	
Name	O C Abraham
Designation	Professor
Affiliation	Christian Medical College & Hospital,Vellore
Address	Department of General Medicine Unit 4, Christian Medical College and Hospital, Ida Scuddder Road, Vellore Vellore TAMIL NADU 632004 India
Phone	9994068771
Fax	
Email	o.cherianabraham@gmail.com

Details Contact Person (Public Query)

Details Contact Person (Public Query)	
Name	
Designation	PG Registrar
Affiliation	Christian Medical College & Hospital,Vellore
Address	Department of General Medicine Unit 4, Christian Medical College and Hospital, Ida Scuddder Road, Vellore Vellore TAMIL NADU 632004 India

9.6 CLINICAL RESEARCH FORM

Christian Medical College, Vellore, Department of General Medicine

High Dose Intra-Venous Vitamin C Administration to prevent Mortality in Patients with Sepsis – A Randomized, Double blind, Placebo controlled Trial

CLINICAL RESEARCH FORM

Phone No:

Patient Demographics:

Name of the Subject:

Hospital Number:

Age:

Sex:

State:

ALLOCATION GROUP		ALLOCATION NUMBER
A(0)	B(1)	

Date of onset of symptoms	
Date and Time of Triage	
Date and Time of ICU Admission	
Date and time of 1st dose of drug	
Time between symptom duration & 1st Dose(in days) (0 – for less than 24 hours, 1 – 24 to 48 hours etc)	days
Time between ICU admission & 1st dose (in hours, round to whole numbers)	hours

Baseline Characteristics:

Diagnosis at enrolment (if 10 specify):

Final diagnosis of the event (if 10, specify):

1.	Pneumonia
2.	UTI
3.	CAUTI
4.	Cellulitis
5.	Meningitis
6.	IE
7.	SBP
8.	VAP
9.	HAP
10.	Others

AT ENROLMENT	
Heart Rate (in beats per minute)	
Systolic BP (in mm Hg)	
Diastolic BP (in mm Hg)	
MAP (in mm Hg)	
Rectal Temperature(in ˚)	
Respiratory rate (in breaths per minute)	
GCS at enrolment (out of 15)	
Requiring Invasive ventilation 0 – No 1- Yes	

If Yes 0 – Assisted mode, 1 – Controlled mode	
Requiring NIV 0 – No 1- Yes	
On Inotropes 0- No 1 - Yes	
No of Inotropes at enrolment	
Doses of Inotropes at enrolment	1.Noradrenaline 2.Adrenaline 3.Vasopressin 4.Dopamine 5.

LAB VARIABLE	VALUE
Creatinine	
Total Bilirubin	
Direct Bilirubin	
Albumin	
Sodium	
Potassium	
Arterial pH	
HCO3 -	
Lactates	
Haemoglobin	
Haematocrit	
Total WBC Counts	
FiO2	
A-aO2	
PaO2	

COMORBIDITY (use APACHE 2 criteria)	YES	NO
Diabetes	1	0
Hypertension	1	0
Liver insufficiency	1	0
Heart Failure	1	0
If yes, NYHA Class 4	1	0
Severe COPD	1	0
CKD	1	0
If yes, on regular dialysis	1	0
Immunosuppression If yes, Circle which:	1	0

1	AIDS
2.	Chemotherapy
3.	Radiation

SOFA Score at admission :

APACHE 2 Score at admission:

Predicted Mortality based on APACHE 2 Score:

Charlson CO-Morbidity Index:

Parameter	Yes	No
Septic shock at admission	1	0
ARDS at admission	1	0
Received Thiamine during treatment course?	1	0
Received Hydrocortisone during treatment course?	1	0
Culture positivity	1	0

1	E.coli	
2	Klebsiella sp	
3	Pseudomonas	
4	Acinetobacter	
5	Streptococcus pneumonia	
6	Streptococcus pyogenes	
7	Staphylococcus	
8		

1	Blood	
2	Pleural Fluid	
3	Sputum/ETA	
4	Ascitic Fluid	
5	CSF	
6	Pus	
7		

	Antibiotics	Duration	Days
1	Piptaz		
2	Ceftriaxone		
3	Meropenem		
4	Ertapenem		
5	Colistin		
6	Azithromycin		
7	Vancomycin		
8	Tigecycline		

	Inotropes	Duration	Days
1	Noradrenaline		
2	Adrenaline		
3	Vasopressin		
4	Dopamine		

Comments:

OUTCOMES

	Yes	No
Alive at hospital Discharge	1	0
Discharged against medical advice ?	1	0
Withdrawal of care?	1	0
Non Escalation/ De escalation of care ?	1	0
Date and Time of Discharge		
Date and Time of Death		
Required Dialysis	1	0
ICU Days when dialysis was done		
Days alive and free from Dialysis		
Days alive and free from inotropes/Vasopressor		
Days alive and free of Ventilator		
Time till vasopressor independence (in survivors)		
ICU Length of Stay		
Hospital Length of stay		
NEW ONSET ORGAN FAILURE	1	0
Cardiovascular system failure	1	0
Renal failure	1	0
Respiratory failure	1	0
Hematologic dysfunction	1	0
Liver dysfunction	1	0
Central nervous system failure	1	0

ADR & SAFETY

Completed the trial: 1.Yes 0.No

If Yes, Reason for withdrawal:

Evidence of Renal Oxalate stones: 1.Yes 0.No

Any other adverse events identified	Attributed to the drug	Not attributed to the drug
	1	0
	1	0
	1	0
	1	0

9.7 PATIENT INFORMATION SHEET – ENGLISH

Christian Medical College, Vellore
Department of General Medicine

High Dose Intra-Venous Vitamin C Administration to prevent Mortality in Patients with Sepsis – A Randomized, Double blind, Placebo controlled Trial

Information sheet

You (The patient whom you represent) are being requested to participate in a study to see if injections of a Vitamin called Vitamin C can help to increase the chance of survival in the disease called Sepsis, that you (your patient) are(is) diagnosed to have. Sepsis is a dangerous condition produced by an infection and is associated with significant chance of death and failure of organs. The treatment of this condition currently consists of Antibiotics (medications to kill bacteria that cause infection), IV Fluids (injections of large amount of solutions to help keep the blood pressure up) and other supportive measures like intubation and ventilation (putting a tube through the wind pipe into the lung and connecting to a machine that will give breaths in case the lungs are not able to take enough breaths). Few studies done in other countries have shown that Vitamin C Injections can help in decreasing the number of deaths due to sepsis by up to 30% and help in other similar conditions like pancreatitis and burns. We hope to include about 60 people from this hospital in this study.

What is Vitamin C and what does it do?

Vitamin C is a natural compound which we consume with food every day and is required by the body in tiny amounts for its proper functioning. For example it is present in high quantitiy in fruits like oranges and lemons. Our body cannot produce Vitamin C and it needs to be taken in diet. In sepsis, the level of Vitamin C is very low. Giving high dose injection will build up the levels of Vitamin C and decrease the intensity of chemical reactions in the body that causes sepsis. Vitamin C thereby helps in decreasing deaths in sepsis, decrease the amount of injury to organs like kidneys and helps patients with sepsis recover faster and get discharged from the hospital earlier. But, we are not fully sure about these facts and want to use Vitamin C in more patients with sepsis and study the effects.

Does Vitamin C have any side effects?

Worldwide Vitamin C injections have been used for sepsis and multiple other conditions – including cancers and burns. From our information regarding their use in these conditions, Vitamin C is largely safe and does not cause any major side effects commonly. Side effects like kidney stone formation and allergy has been reported in a very few patients.

In the ICU of CMC, Vellore also some patients with Sepsis are being administered Vitamin C injections with no major side effects identified. Hence Vitamin C can be considered largely safe, although very minimal risk of side effects is present.

If you take part what will you have to do?

The patients who agree to participate in this study will be given either 4 Injections of Vitamin C daily 6 hours apart or an identical looking Injection that does not contain Vitamin C, for a total period of 4 days. Using thus dummy Injection will help us be sure that any improvement in the

condition is actually due to Vitamin C and not due to co-incidence. Neither the patients, relatives nor the doctors will have any choice in whether the patient will get Vitamin C or the dummy injection as this will be decided by a computer program; this is like tossing a coin and you have an equal chance of getting either injection. Also, neither the patients, relatives nor your doctor will know which injection you are getting till the study is over.

All other treatments for the illness will be given according to the treating doctors decision and in accordance with standard guidelines. Before giving the injection, the patient's details like name, and hospital number, blood pressure, pulse rate etc. and some lab investigations will be noted. Details of the medications given and course in hospital will be monitored till discharge. Any new onset of organ damage, the patient's outcome and number of days spent in ICU and in hospital will be noted. No additional procedures or blood tests will be conducted routinely for this study.

If at any time the patients experience a problem, it can be reported to the doctor.

Can you withdraw from this study after it starts?

Patients participation in this study is entirely voluntary and each participant is free to decide to withdraw permission to participate in this study at any point. If one does so, this will not affect the usual treatment at this hospital in any way. In addition, if one experience any serious side effects or the condition worsens, the study injections will be stopped and the they may be given additional treatment.

What will happen if you develop any study related injury?

We do not expect any injury to happen to the participants but if one does develop any side effects or problems due to the study, these will be treated at no cost. We are unable to provide any monetary compensation, however.

Will you have to pay for the study injections?

Both Vitamin C and dummy injections will be given free for a total period of 4 days (Total of 16 doses).

Any other treatment that you otherwise require will continue and the expenses for the same will have to be borne by the patients or from other source as per your arrangement with the hospital.

What happens after the study is over?

The patients may or may not benefit from the study injection that they are given. Once the study is over, the treatment for the condition will continue.

Will your personal details be kept confidential?

The results of this study will be published in a medical journal, but participants will not be identified by name in any publication or presentation of results. However, the medical notes may be reviewed by people associated with the study, without any additional permission.

If you have any further questions, please contact [redacted] or send an E-Mail to thevicstrial@gmail.com. You are also free to contact us anytime during the trial for any clarification related to the trial or if you wish to withdraw from the study.

9.8 PATIENT INFORMATION SHEET - TAMIL

கிருஸ்துவ மருத்துவக் கல்லூரி, வேலூர்

பொது மருத்துவத் துறை

சீழ்பிடிப்பு காரணத்தால் ஏற்படும் மரணத்தைத் தடுக்க உயர் அளவு நரம்பு வழி வைட்டமின் சி நிர்வாகம்

[redacted]

தகவல் தாள்:

வைட்டமின் சி–யினை அதிக அளவில் நரம்பு வழியாக கொடுப்பதனால் சீழ்பிடிப்பினால் ஏற்படும் மரணம் குறைகிறதா என்ற இந்த ஆய்வில் உங்களை (அல்லது உங்களது நோயாளியை) பங்கேற்குமாறு தாழ்மையுடன் கேட்டுக்கொள்கிறேன். சீழ்பிடிப்பு என்பது கிருமிகளால் தொற்றும் உயிர்க்கு ஆபத்து விளைவிக்கும் ஒரு நிலை ஆகும். அதனால் உடல் உறுப்புகள் செயல் இழக்கவும், மரணம் நிகழவும் வாய்ப்புள்ளது. இந்த சீழ்பிடிப்பிற்கான சிகிச்சை என்பது நுண்ணுயிர் எதிர்ப்பிகள் (நோய்த்தொற்றை ஏற்படுத்தும் பாக்டீரியாக்களைக் கொல்லும் மருந்துகள்), நரம்பு வழி திரவங்கள் (இரத்த அழுத்தத்தை உயர்த்த அதிக அளவில் செலுத்தப்படும் திரவங்கள்), சுவாசத்திற்கு ஆதரவான நடவடிக்கைகள் (நுரையீரல்கள் சரியாக சுவாசத்தை எடுக்காவிட்டால், ஒரு குழாயினை நுரையீரலில் காற்றுக் குழாய் மூலம் செலுத்தி, அதனை சுவாசிக்கக்கூடிய ஒரு இயந்திரத்துடன் இணைத்தல்), பிற நாடுகளில் விட்டமின் சி தொடர்பாக மேற்கொள்ளப்பட்ட ஆய்வுகளின் முடிவு படி, இது 30% வரை சீழ்பிடிப்பு காரணத்தினால் இறப்பவர்களின் எண்ணிக்கையைக் குறைக்கின்றது என்றும், கணையம் தொடர்பான பிரச்சினைகளிலிருந்தும், தீக்காயத்தினால் ஏற்படும் விளைவுகளிலிருந்தும் விடுபட உதவுகின்றது எனவும் கண்டுபிடிக்கப்பட்டுள்ளது. இந்த ஆய்வில் சீழ்பிடிப்பு நோயினால் பாதிக்கப்பட்ட 60 நபர்களை நமது மருத்துவமனையிலிருந்து சேர்க்க விரும்புகிறேன்.

வைட்டமின் சி என்றால் என்ன, அதன் பயன்பாடு என்ன?

வைட்டமின் சி என்பது ஒரு இயற்கையான கலவை ஆகும். இது நாம் தினமும் உண்ணும் உணவிலிருந்து உருவாகி, சீரான உடல் செயல்பாட்டிற்கு சிறிய அளவில் தேவைப்படுகிறது. நமது உடலால் வைட்டமின் சி–யினை உருவாக்க இயலாது. அதனால், அது உணவில் எடுத்துக்கொள்ளப்பட வேண்டும். உதாரணமாக, ஆரஞ்சு மற்றும் எலுமிச்சை பழங்களில் அதிக அளவில் வைட்டமின் சி உள்ளது. சீழ்பிடிப்பு நிலையில் உடலில் உள்ள வைட்டமின் சி–யின் அளவு அதிக அளவில் குறைந்து காணப்படுகிறது. நரம்பு வழி வைட்டமின் –சி அதிக அளவில் செலுத்தப்பட்டால் உடலில் இதன் அளவு அதிகரிக்கின்றது. இதனால் உடலில் ஏற்படும் இரசாயன எதிர்வினை மாற்றங்கள் தீவிரமடைவதைத் தடுக்க இயலும், உடலுறுப்புகளுக்கு முக்கியமாக சிறுநீரகத்திற்கு ஏற்படும் பாதிப்புகளையும் குறைக்க உதவும். இதனால் நோயாளி விரைவில் குணமடைந்து வீட்டிற்கு செல்ல உதவும். ஆனால், இந்த உண்மைகள் பற்றி நாம் உறுதியாக கூற இயலாததால், அதிக அளவில் நோயாளிகளுக்கு வைட்டமின் சி செலுத்தி ஆய்வு மேற்கொள்ள வேண்டும்.

வைட்டமின் சி பயன்படுத்துவதால் ஏதேனும் பக்க விளைவுகள் உள்ளனவா?

உலகளவில் வைட்டமின் சி ஊசிகள் சீழ்பிடிப்பு மற்றும் பிற நிலைமைகளுக்கும் – புற்றுநோய் மற்றும் தீக்காயங்களுக்கும் அதிக அளவில் பயன்படுத்தப்படுகிறது. இது வரை கிடைக்கப்பெற்ற தகவல்களின் படி வைட்டமின் – சி கொடுப்பதனால் எந்த பெரிய பக்க விளைவுகளும் ஏற்பட வாய்ப்பில்லை எனவும் உறுதி செய்யப்பட்டுள்ளது. ஒரு சில நோயாளிகளில் சிறுநீரக கல் ஏற்பட்டதாகவும் ஒவ்வாமை காணப்பட்டதாகவும் பதிவு செய்யப்பட்டுள்ளது.

நமது வேலூர் கிறிஸ்துவ மருத்துவமனையில் தீவிர சிகிச்சைப் பிரிவில் சீழ்பிடிப்பு நோயினால் அனுமதிக்கப்பட்ட சிலருக்கு வைட்டமின் சி ஊசி பயன்படுத்தப்பட்டு எந்த பக்க விளைவுகளும் ஏற்படவில்லை. ஆகையால் வைட்டமின் சி மிகவும் பாதுகாப்பானதாக கருதலாம். எனினும் பக்க விளைவுகள் மிகக் குறைந்த அளவில் ஏற்பட வாய்ப்புள்ளது.

நீங்கள் இந்த ஆய்வில் பங்கு கொண்டால், என்ன செய்ய வேண்டும்?

இந்த ஆய்வில் பங்குக்கொள்ளும் நோயாளிகளுக்கு தினசரி நான்கு வைட்டமின் சி ஊசிகள், ஆறு மணி நேரத்திற்கு ஒரு முறை வழங்கப்படும் அல்லது வைட்டமின் சி போன்ற போலியான ஊசி வழங்கப்படும். இவ்வாறு நான்கு நாட்களுக்கு கொடுக்க வேண்டும். இவ்வாறு போலியான மருந்து பயன்படுத்தக்காரணம்,உடல்நிலையின் ஏற்படும் முன்னேற்றம் வைட்டமின் சி மூலம் என்பதைத் தெளிவுபடுத்தவும், அது கூட்டு நிகழ்வு காரணமாக ஏற்படவில்லை என்பதை உறுதிப்படுத்துவதற்காகவும் ஆகும். நோயாளிக்கோ, மருத்துவருக்கோ, நோயாளியின் உறவினருக்கோ எந்த மருந்து (வைட்டமின் சி அல்லது போலியான மருந்து) செலுத்தப்படுகிறது என்பதைப் பற்றிய தெரிவு இருக்காது. அவை கணினி நிரல் மூலம் முடிவு செய்யப்படுகிறது. இது ஒரு நாணயத்தை தூக்கிப் போடுவது போல, அனைவருக்கும் வைட்டமின் சி மருந்தையோ போலியான மருந்தையோ பெற சமமான வாய்ப்பு இருக்கிறது. இதன் விளக்கம் ஆய்வு முடியும் வரை யாவருக்கும் தெரிய வாய்ப்பில்லை.

நோய்க்கான மற்ற சிகிச்சைகள் அனைத்தும் சிகிச்சை அளிக்கும் மருத்துவரின் முடிவு மற்றும் நெறிமுறைகளுக்கு ஏற்ப வழங்கப்படும் ஊசி போடுவதற்கு முன் நோயாளியின் பெயர், மருத்துவமனை எண், இரத்த அழுத்தம், இருதய துடிப்பு விகிதம், மற்றும் சில ஆய்வக விசாரணைகள் குறித்தக் கொள்ளப்படும். மருத்துவமனையில் அளிக்கப்படும் சிகிச்சை விவரம் மற்றும் நோயாளியின் தினசரி உடல்நிலை குறித்த தகவல்களை தினமும் சேகரிக்கப்படும். ஏதேனும் புதிதாக ஏற்படும் உடலுறுப்பு பாதிப்புகளும், நோயாளியின் உடல்நிலையும், தீவிர சிகிச்சைப் பிரிவில் செலவிடப்பட்ட நாட்களும் குறித்தக் கொள்ளப்படும் கூடுதல் சோதனைகள் மற்றும் இரத்தப் பரிசோதனைகள் இந்த ஆய்வில் பயன்படுத்தப்படமாட்டாது.

எந்த நேரத்திலும், நோயாளி ஒரு சிக்கலைச் சந்தித்தால் அதை மருத்துவரிடம் தெரிவிக்கலாம்.

இந்த ஆராய்ச்சியில் பங்குக் கொண்டு, பிறகு நீங்கள் பின்வாங்கலாமா?

இந்த ஆய்வில் நோயாளிகள் பங்கேற்பது முற்றிலும் தன்னார்வ் விருப்பமாகும், மேலும் அவர்கள் எந்த நிலையிலும் இந்த ஆய்விலிருந்து விலகிக் கொள்ளலாம். ஒருவர் அவ்வாறு செய்தால், அது எந்த வகையிலும் இந்த மருத்துவமனையில் பெறும் வழக்கமான சிகிச்சையைப் பாதிக்காது. ஏதேனும் வைட்டமின் சி ஊசியினால் பக்க விளைவு ஏற்பட்டாலோ, உடல் நிலை மோசமடைந்தாலோ வைட்டமின் சி ஊசி நிறுத்தப்பட்டு கூடுதல் சிகிச்சை அளிக்கப்படும்.

இந்த ஆய்வினால் ஏதேனும் காயமோ, பாதிப்போ உங்களுக்கு ஏற்பட்டால் என்ன நடக்கும்?

பங்கேற்பாளர்களுக்கு எந்த காயமும் ஏற்படும் என எதிர்பார்க்கப்படவில்லை. ஏனினும் ஏதேனும் பக்க விளைவுகளோ, சிக்கல்களோ ஏற்படுமெனில் அவை கட்டணமின்றி சிகிச்சை செய்யப்படும் எந்த ஒரு பண இழப்பீடும் வழங்கப்படமாட்டாது.

நீங்கள் ஆய்வு ஊசிக்கு கட்டணம் செலுத்த வேண்டுமா?

வைட்டமின் சி மற்றும் போலியான ஊசிகள் நான்கு நாட்களுக்கு ஒரு நாளைக்கு நான்கு ஊசிகள் விகிதம் (மொத்தமாக 16 ஊசிகள்) இலவசமாக வழங்கப்படும்.

இதற்கு மேல் வேறு சிகிச்சைக் கூடுதலாக தேவைப்பட்டால் அதற்கான செலவுகளை நோயாளிகள் செலுத்த வேண்டும்.

ஆய்வின் முடிவில் என்ன நடக்கும்?

ஆய்வு ஊசியினால் நோயாளி பயன்பெறலாம் பெறாமலும் இருக்கலாம். இந்த ஆய்வின் பிறகும், நோயாளியின் நோய்க்கான சிகிச்சைத் தொடரும்.

உங்கள் தனிப்பட்ட விவரங்கள் இரகசியமாக வைக்கப்படுமா?

இந்த ஆய்வின் முடிவுகள் ஒரு மருத்துவ இதழில் வெளியிடப்படும், ஆனால் எந்தவொரு வெளியீட்டிலும், முடிவுகளின் விளக்கக் காட்சியிலும், பங்கேற்பாளரின் பெயர் அடையாளம் காண இயலாது. ஏனினும், மருத்துவ விபரங்கள் எந்த கூடுதல் அனுமதியுமின்றி இந்த ஆய்வில் தொடர்புடைய நபர்களால் மதிப்பாய்வு செய்யப்படலாம்.

உங்களுக்கு ஏதேனும் சந்தேகங்கள் இருந்தால், தயவுசெய்து [redacted] தொடர்பு கொள்ளலாம் அல்லது (E-mail:thevicstrial@gmail.com)க்கு மின்னஞ்சல் செலுத்தலாம். இந்த ஆய்வு குறித்த விசாரணைக்காவோ அல்லது இந்த ஆய்வை விட்டு விலக எண்ணினாலோ, எங்களை எந்த நேரத்திலும் தொடர்பு கொள்ளலாம்.

9.9 PATIENT INFORMATION SHEET - HINDI

क्रिश्चियन मेडिकल कॉलेज,वेल्लोर

जनरल मेडिसिन विभाग

पूति/सेप्सिस में मृत्यु दर को रोकने के लिए उच्च खुराक इंट्रा शिरापरक विटामिन सी प्रशासन

एक यादृच्छिक, डबल अंधा, प्लेसबो - नियंत्रित परीक्षण (Vics परीक्षण)

सूचना पत्रक

आप (रोगी जिसका आप प्रतिनिधित्व करते हैं) को एक अध्ययन में भाग लेने के लिए अनुरोध किया जा रहा है। अध्ययन यह देखने के लिए है कि क्या विटामिन सी नामक एक विटामिन का इंजेक्शन पूति/सेप्सिस रोग (जो निदान आपको / मरीज़ को दिया गया है) नामक एक बीमारी में जीवित रहने की संभावना को बढ़ाने में मदद कर सकता है । पूति/सेप्सिस एक खतरनाक स्थिति एक संक्रमण से उत्पन्न होत होती है और होती है और मृत्यु और अंगों के नुकसान के बड़े खतरे से जुड़ी है। इस हालत का उपचार वर्तमान में एंटीबायोटिक (संक्रमण करने वाले बैक्टीरिया को नष्ट करने के लिए) और इंट्रा-शिरापरक तरल पदार्थ (ब्लड प्रेशर को उचित रखने के लिए) और अन्य सहायक उपाय जैसे वेंटिलेशन (एक मशीन जो जो हवा पाइप के अंदर एक ट्यूब के द्वारा फेफड़ों को श्वास प्रदान करती है, जब फेफड़े अपने आप श्वास लेने के लिए असक्षम हो जाते हैं)। कुछ अन्य देशों में किया अध्ययन दर्शाते हैं कि विटामिन सी इंजेक्शन पूति/सेप्सिस के कारण मौतों की संख्या को 30% तक कम करने में और पैन्क्रीअटाइटिस, जलन जैसी स्थिति में मदद कर सकता है। हमें इस अध्ययन में इस अस्पताल से लगभग 60 लोगों को शामिल करने की उम्मीद है।

विटामिन सी क्या है और यह क्या करता है?

विटामिन सी एक प्राकृतिक यौगिक है जिसका हम भोजन के साथ उपभोग करते हैं और शरीर के लिए उसके समुचित कार्य के लिए छोटे मात्रा में आवश्यक है। उदाहरण के लिए यह संतरे और नींबू की तरह फल में

उच्च मात्रा में मौजूद है। हमारा शरीर विटामिन सी का उत्पादन नहीं कर सकता है और भोजन में होना चाहिए। पूति/सेप्सिस में, विटामिन सी का स्तर बहुत कम होता है। उच्च खुराक इंजेक्शन विटामिन सी का स्तर बहडेगा और वह रासायनिक प्रतिक्रियाएं जो शरीर में सेप्सिस का कारण बनती हैं - उनकी तीव्रता कम होगी

विटामिन सी इस तरह पूति/सेप्सिस में होने वाली मौतों को कम करने में मदद करता है, अंगों को चोट काम करता है और पूति/सेप्सिस के रोगियों को शीघ्र ठीक होकर अस्पताल से जल्दी छुट्टी पाने में मदद करता है।

लेकिन, हम इन तथ्यों के बारे में पूरी तरह से सुनिश्चित नहीं हैं, और पूति/सेप्सिस के रोगियों में विटामिन सी का उपयोग कर उसके प्रभाव का अध्ययन करना चाहते हैं।

क्या विटामिन सी के कोई साइड इफेक्ट हैं?

दुनिया भर में विटामिन सी का इंजेक्शन इस्तेमाल कैंसर और जलने सहित पूति/सेप्सिस और कई अन्य स्थितियों के लिए किया गया है। इन परिस्थितियों में उपयोग से मिली जानकारी से, विटामिन सी काफ़ी हद तक सुरक्षित है और आम तौर पर किसी भी बड़े दुष्प्रभाव का कारण नहीं है।

गुर्दे की पथरी के गठन और एलर्जी की तरह साइड इफेक्ट एक बहुत थोड़े रोगियों में बताया गया है।

CMC Vellore के ICU में भी पूति/सेप्सिस के कुछ रोगियों को विटामिन सी इंजेक्शन प्रशासित किया जा रहा है, और अब तक किसी बड़े दुष्प्रभावों की पहचान नहीं हुई है। इसलिए विटामिन सी, बड़े हद तक सुरक्षित माना जा सकता है, हालाँकि दुष्प्रभाव का बहुत छोटा जोखिम मौजूद है।

भाग लेने पर आपको क्या करना होगा?

जो मरीज़ इस अध्ययन में भाग लेने के लिए सहमत होंगे, उन्हें दिन में हर 6 घंटे इस तरह विटामिन सी के 4 इंजेक्शन या फिर बिलकुल समान दिखने वाला विटामिन सी हित 'डमी' इंजेक्शन, दिए जाएंगे। इस तरह बगैर विटामिन सी इंजेक्शन देने से हमें पता चलेगा की इंजेक्शन का प्रभाव विटामिन सी की वजह से है, न की संयोग से। न तो मरीज़ों, रिश्तेदारों और न ही डॉक्टरों को यह विकल्प होगा की इंजेक्शन में विटामिन सी है या नहीं - एक कंप्यूटर प्रोग्राम यह निर्णय लेगा; यह एक सिक्का उछालने की तरह है और किसी भी इंजेक्शन के चुनाव का बराबर मौका रहेगा। इसके अलावा ना मरीज़ों, रिश्तेदारों ना डॉक्टर को अध्ययन समाप्त होने तक यह जानकारी होगी की कौन सा इंजेक्शन दिया गया था ।

बाकी पूरा इलाज डॉक्टर के निर्णय से और मानक दिशानिर्देश का पालन करके दिया जाएगा। इंजेक्शन देने के पहले मरीज़ का नाम, अस्पताल क्रमांक, रक्त चाप, पल्स दर और खून जाँच परिणाम आदि देखा जाएगा ।

प्रदान की गयी दवाइयाँ और मरीज़ की हालत अस्पताल से छुट्टी होने तक देखी जाएगी।

कोई नई अंग क्षति, मरीज़ की परिस्थिति और अस्पताल और ICU में बिताये कुल दिनों की संख्या दर्ज़ किये जाएंगे। सिर्फ इस अध्ययन के लिए कोई अतिरिक्त जाँच या प्रक्रिया नहीं की जाएगी।

कोई भी समस्या होने पर डॉक्टर को बताया जा सकता है।

क्या अध्ययन शुरू होने के बाद आप इसमें से पीछे हट सकते हैं?

इस अध्ययन में भाग पूरी तरह स्वैच्छिक है और हर मरीज़ किसी भी समय पर अपनी सहमति वापस ले सकते हैं

ऐसा करने से अस्पताल में हो रहे सामान्य इलाज पर कोई प्रभाव नहीं पड़ेगा

इसके अतिरिक्त, अगर कोई दुष्प्रभाव हो या हालत बिघड जाये, तो इंजेक्शंस को बंद कर उचित इलाज दिया जाएगा।

अध्ययन से सम्बंधित कोई चोट होने पर क्या किया जाएगा?

हमें अपेक्षा नहीं है की कोई चोट होगी पर अगर अध्ययन की वजह से किसी को कोई दुष्प्रभाव होता है तो उसका इलाज निःशुल्क किया जाएगा। नगद मुआवज़ा संभव नहीं है।

क्या हमें अध्ययन के इंजेक्शंस खरीदने पड़ेंगे?

विटामिन सी और विटामिन सी हित 'डमी ' इंजेक्शन (16 खुराक) निःशुल्क दिए जाएंगे।

बाकि सारे इलाज का खर्च मरीज़ को या बीमा कंपनी को उठाना होगा - जो भी व्यवस्था भर्ती होने के समय निश्चित की गयी हो।

अध्ययन समाप्त होने पर क्या होगा?

अध्ययन के इन्जेक्शन्स से मरीज़ को शायद फायदा हो, शायद न हो। उसके बाद पूर्व समान इलाज जारी रहेगा।

क्या हमारी व्यक्तिगत जानकारी गुप्त रखी जाएगी?

अध्ययन का परिणाम चिकित्सायी पत्रिका में छापा जाएगा, लेकिन किसी भी मरीज़ का नाम की पहचान किसी भी प्रकाशन या परिणामों के प्रदर्शन में नहीं की जाएगी।

चिकित्सयी टिप्पणी की समीक्षा अध्ययन से जुड़े व्यक्ति करेंगे

अगर आपको कोई और प्रश्न हो, तो कृपया डॉ. [redacted] ई-मेल भेजें thevicstrial@gmail.com

9.10 INFORMED CONSENT FORM – ENGLISH

Informed Consent Form to Participate In Research Study

Study Title: High Dose Intra-Venous Vitamin C Administration to Prevent Mortality in Sepsis
A Randomized, Double Blind, Placebo Controlled Trial (The ViCS Trial)

Study Number: _____ **Subject's Hospital Number:** _____

Subject's Full Name: _____

Date of Birth / Age of the Subject: _____

1. I confirm that I have read and understood the information sheet for the above ☐
2. I have had the opportunity to ask questions and clarify my doubts about the study ☐
3. I understand that the participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
4. I understand that the Investigators of the trial, the Ethics Committee and the regulatory authorities will not need my permission to look at my (my patient's) health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published. ☐
5. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s). ☐
6. I am aware about the possible harms of participating in the study ☐
7. I agree to take part/ to include my patient in the above study ☐

1. **Signature/Thumb Impression of the Subject or Legally Acceptable Representative**

Signatory's Name: _____
Relation to the Subject: _____
Date: _____

Signature/Thumb Impression: Subject/Representative

2. **Signature/Thumb Impression of the Witness**

Witness's Name: _____
Date: _____

Signature/Thumb Impression: Witness

3. **Signature of the Investigator Obtaining the Consent**

Investigator's Name: _____
Date: _____

Signature: Investigator

9.11 INFORMED CONSENT FORM – TAMIL

ஆராய்ச்சி படிப்பில் பங்கேற்பதற்கான ஒப்புதல் படிவம்

படிப்புப் பெயர்: சீழ்பிடிப்பு காரணக்கால் ஏற்படும் மரணக்கைத் தடுக்க உயர் அளவு நரம்பு வழி வைட்டமின் சி நிர்வாகம்

ஆய்வு எண்: _____

பங்கேற்பாளர் மருத்துவமனை எண்: _____

பங்கேற்பாளர் முழு பெயர்:_____

பிறந்த தேதி / வயது : _____

1. மேற்கூறப்பட்ட தகவல் தாள்களை நான் படித்து புரிந்துகொண்டுள்ளேன் என்பதை உறுதிப்படுத்துகிறேன். ☐
2. ஆய்வு குறித்த கேள்விகளைக் கேட்கவும், சந்தேகங்களை தெளிவுபடுத்தவும் எனக்கு வாய்ப்பு கிடைத்தது. ☐
3. ஆய்வில் பங்கெடுப்பது தன்னார்வமான ஒன்று என்பதையும் நான் எப்போது வேண்டுமானாலும் எந்த காரணமும் இல்லாமல், என் மருத்துவ கவனிப்பு அல்லது சட்ட உரிமைகள் பாதிக்கப்படாமல் விலகலாம் என்றும் புரிந்து கொள்கிறேன். ☐
4. எனது மருத்துவ பதிவுகளை நடப்பு ஆய்வு மற்றும் தொடர்புடைய எந்தவொரு ஆராய்ச்சியையும் பரிசீலிப்பவர்களின் விசாரணைகள், நெறிமுறைகள் குழு மற்றும் ஒழுங்குமுறை அதிகாரிகள் பயன்படுத்த என் அனுமதி தேவையில்லை என்று புரிந்துகொள்கிறேன். நான் விசாரணையில் இருந்து விலகி இருந்தாலும் கூட இது பொருந்தும் என்று அறிவேன். இருப்பினும், என் குறித்த தகவல்களை மூன்றாம் தரப்பினருக்கு வெளியிடப்பட முடியாது என்பதை நான் புரிந்து கொள்கிறேன். ☐
5. இந்த ஆய்வுகளிலிருந்து எழும் முடிவுகளை விஞ்ஞான நோக்கத்திற்கு மட்டுமே பயன்படுமெனில் நான் அதனை கட்டப்படுத்துவதில்லை என உறுதி அளிக்கிறேன். ☐
6. ஆய்வில் பங்கு பெறுவதால் சாத்தியமான காயங்கள் பற்றி எனக்குத் தெரியும். ☐
7. மேலே உள்ள படிப்பில் என் நோயாளியை சேர்க்க / நான் பங்கேற்க ஒப்புக்கொள்கிறேன் ☐

1. பங்கேற்பாளர் கையொப்பம் / கட்டைவிரல் தாக்கம் அல்லது
சட்டப்பூர்வமாக ஏற்கத்தக்க பிரதிநிதி
கையொப்பமிட்டவரின் பெயர்: _____
பங்கேற்பாளருடனான தொடர்பு: _____
தேதி: _____

2. சாட்சியின் கையொப்பம் / கட்டைவிரல் தாக்கம்
சாட்சியின் பெயர்: _____
தேதி: _____

3. ஆராய்ச்சியாளரின் கையொப்பம்
ஆராய்ச்சியாளரின் பெயர்: _____
நாள்: _____

9.12 INFORMED CONSENT FORM - HINDI

अनुसंधान अध्ययन में भाग लेने के लिए सूचित सहमति फॉर्म

अध्ययन शीर्षक : पूति/सेप्सिस में मृत्यु दर को रोकने के लिए उच्च खुराक इंट्रा शिरापरक विटामिन सी प्रशासन एक यादृच्छिक, डबल अंधा, प्लेसबो - नियंत्रित परीक्षण **(Vics** परीक्षण)

अध्ययन संख्या : _____
मरीज़ के अस्पताल संख्या:_____

मरीज़ का पूरा नाम: _____

मरीज़ का जन्म तिथि/ आयु: _____

1. मैं पुष्टि करता/ करती हूँ कि मैंने उपरोक्त के लिए सूचना पत्र को पढ़ा है और समझ लिया है ☐
2. मुझे अध्ययन के बारे में सवाल पूछने और संदेह को दूर करने का अवसर मिला है ☐
3. मैं समझता/ समझती हूँ कि अध्ययन में मेरी भागीदारी स्वैच्छिक है और मैं अपनी चिकित्सीय देखभाल या कानूनी अधिकार प्रभावित किये बिना, बिना कोई कारण बताए किसी भी समय सहमति वापस ले सकता/ सकती हूँ ☐
4. मैं समझता/ समझती हूँ कि परीक्षण के खोजकर्ता, आचार समिति, और नियामक अधिकारी को इस अध्ययन और इस के संबंध में आगे आयोजित किये अनुसंधान के संबंध में मेरे (मेरे मरीज़ के) स्वास्थ्य रिकॉर्ड को देखने के लिए मेरी अनुमति की जरूरत नहीं होगी, भले ही मैं परीक्षण से वापसी ले लूँ। मैं इस उपयोग के लिए सहमत हूँ। हालाँकि, मैं समझता हूँ कि मेरी पहचान का खुलासा तीसरे पक्ष को जारी, या किसी भी प्रकाशित जानकारी में नहीं किया जाएगा। ☐
5. मैं इस अध्ययन से उत्पन्न किसी भी डेटा या परिणाम के अप्रतिबंधित उपयोग के लिए सहमत हूँ, जब तक यह इस्तेमाल केवल वैज्ञानिक प्रयोजनों के लिए हो। ☐
6. मुझे अध्ययन में भाग लेने के संभावित नुकसान के बारे में जानकारी है।☐
7. मैं उपरोक्त अध्ययन में भाग लेने के लिए / मेरे मरीज़ शामिल को करने के लिए सहमत हूँ।☐

1. **मरीज़ या कानूनी तौर पर स्वीकृत प्रतिनिधि का हस्ताक्षर / अंगूठे का निशान**

हस्ताक्षरकर्ता का नाम:_____
मरीज़ से संबंध: _____
दिनांक: _____

2. **गवाह का हस्ताक्षर / अंगूठे का निशान**

गवाह का नाम:_____
दिनांक: _____

3. **खोजकर्ता हस्ताक्षर**

खोजकर्ता का नाम:_____
दिनांक: _____

Signature/Thumb Impression: Subject/Representative

Signature/Thumb Impression: Witness

Signature: Investigator

9.13 CERTIFICATE OF ANALYSIS (COA) OF THE STUDY DRUG

pharmafabrikon

UNIT II, 91-1,Sivagangai Road, Vilathur,Madurai-625 020
Phone: 0452- 2429416, email : qc.fabrikon@gmail.com

CERTIFICATE OF ANALYSIS

Name of the Sample	: **LIVOCEE 25% w/v INJECTION (Ascorbic acid injection IP 25%w/v) 20ml**		
Name of the Mfr	: M/s .Pharmafabrikon Unit II	Report No.	: **PFI/2671**
Batch No	: **17VJ160**	Mfg.Lic.No.	: TN00002699
Date of Sampling	: 31.10.2017	Date of Report	: 14.11.2017
Qty Manufactured	: 210 Liters	Manufacturing Date	: Oct'2017
Qty of Sample drawn	: 30 x 20ml	Expiry Date	: Mar'2019

S.NO	TEST	SPECIFICATION	RESULT
1.	Description	A yellow coloured Clear solution.	A yellow coloured Clear solution.
2.	pH	Limit : 5.5 - 8.0	7.11
3.	Weight per ml	Limit : 1.12 – 1.18gm/ml	1.1607 gm/ml
4.	Extractable Volume	20 ml	20.8 ml
5.	Identification Ascorbic acid	As per IP	Passes
6.	Sterility test	As per IP	Passes
7.	Test for Oxalic acid	As per IP	Passes
8.	Particulate matter	As per IP	Passes
9.	Bacterial Endotoxins test	As per IP	Passes
10.	ASSAY Each ml contains: Ascorbic acid IP	250 mg (Limit : 95% - 115%)	270.01 mg ie., 108%

Observation : The Sample complies as per the label claim in above respects.

REPORT :

The sample referred above confirms that the sample is of **Standard Quality**/ ~~Not of Standard quality~~.

TESTED BY	CHECKED BY	APPROVED BY
QC Officer	QC Executive	QC Manager

9.14 DATA SHEET

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	ALLOCA	ALLOCAT	ICU/WARD	WARD	ICU SHIFT	AGE	GENDER	DAYS FROM SYMPTOM ON	HOURS FROM ADM	HAS	INITIAL DIAG	FINAL DIAG	HR
2	1	A	ICU	ICU	9	65	M	3	11	0	1	15	90
3	2	B	ICU	ICU	9	35	M	3	8	0	5	5	80
4	3	B	ICU	ICU	9	35	M	13	10	0	1	1	154
5	4	B	ICU	ICU	9	45	F	2	24	0	4	4	108
6	5	A	ICU	ICU	9	62	F	0	9	1	8	8	90
7	6	A	ICU	ICU	9	49	M	3	24	0	1	1	76
8	7	B	ICU	ICU	9	55	F	5	12	0	2	2	96
9	8	B	ICU	ICU	9	33	M	7	1	0	1	1	156
10	9	A	ICU	ICU	9	54	M	10	23	0	1	1	126
11	10	A	ICU	ICU	9	58	F	2	20	0	11	11	96
12	11	A	ICU	ICU	9	24	M	3	5	0	1	1	98
13	12	B	ICU	ICU	9	38	M	1	23	1	8	8	94
14	13	A	ICU	ICU	9	77	F	1	19	0	4	4	138
15	14	B	ICU	ICU	9	50	M	60	23	0	6	6	100
16	15	B	ICU	ICU	9	42	F	6	8	0	5	5	102
17	16	A	ICU	ICU	9	55	F	3	9	0	2	2	131
18	17	B	ICU	ICU	9	22	F	5	24	0	5	5	96
19	18	A	ICU	ICU	9	67	M	9	24	0	4	4	118
20	19	B	ICU	ICU	9	36	M	2	23	0	1	1	92
21	20	B	ICU	ICU	9	25	F	7	14	0	11	11	94
22	21	A	Ward	I	0	40	M	3	13	0	1	1	102
23	22	A	Ward	I	0	22	M	7	15	0	1	1	112
24	23	A	Ward	I	0	55	F	6	9	0	5	5	100
25	24	B	ICU	ICU	9	24	F	7	4	0	1	10	141
26	25	A	Ward	I	0	63	M	11	23	0	1	1	106
27	26	A	Ward	C	0	67	M	1	4	0	4	4	120
28	27	B	Ward	E	0	66	F	2	16	0	2	2	112
29	28	B	Ward	E	0	66	F	4	22	0	2	2	112
30	29	A	Ward	E	0	50	F	3	24	0	2	2	138
31	30	B	ICU	ICU	9	20	F	2	5	0	1	1	176
32	31	B	Ward	C	0	60	M	4	5	0	3	3	126
33	32	B	Ward	E	0	86	F	1	11	0	2	7	116
34	33	A	Ward	MTS	1	67	M	3	24	0	1	1	130
35	34	A	Ward	E	0	32	M	1	14	0	10	10	116
36	35	A	Ward	I	1	34	F	8	3	0	5	5	118
37	36	B	Ward	Withdrew consent for inclusion in analysis									
38	37	B	Ward	MTS	0	74	M	1	16	0	1	1	100
39	38	A	Ward	MTS	0	59	M	1	10	0	1	1	116
40	39	B	Ward	Withdrew consent for inclusion in analysis									
41	40	A	ICU	Withdrew consent for inclusion in analysis									
42	41	A	ICU	ICU	9	69	M	1	2	0	11	11	116
43	42	B	Ward	Withdrew consent for inclusion in analysis									
44	43	A	Ward	I	0	84	F	16	20	0	1	1	130
45	44	B	Ward	Iso	0	35	M	16	23	0	11	11	114
46	45	A	Ward	Withdrew consent for inclusion in analysis									
47	46	A	Ward	C	0	46	F	8	23	0	1	1	112
48	47	B	Ward	E	0	49	F	3	4	0	2	12	116
49	48	B	Ward	MTS	0	69	F	5	23	0	5	5	122
50	49	B	Ward	MTS	0	49	F	3	22	0	1	1	90
51	50	A	Ward	C	0	47	M	2	10	0	2	2	96
52	51	A	Ward	C	0	60	F	2	3	0	2	2	116

	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
1	SBP	DBP	MAP	TEMP	RR	INVASIVE VE	NIV	INOTROPES	NUMBER (	NORADRENALINE	ADRENALINE	VASOPRESSIN	DOBUTAMINE
2	100	70	86	100	18	1	0	1	1	1	0	0	0
3	106	70	82	100	22	1	0	0	0	0	0	0	0
4	90	54	66	104	28	1	0	0	0	0	0	0	0
5	122	80	90	100	24	0	0	0	0	0	0	0	0
6	144	68	98	100	24	1	0	0	0	0	0	0	0
7	106	54	72	99.6	16	1	0	1	1	1	0	0	0
8	106	60	80	101	24	0	0	1	1	1	0	0	0
9	72	58	62	102	32	1	0	1	3	1	1	1	0
10	124	60	77	102	18	1	0	0	0	0	0	0	0
11	116	60	80	98	26	0	0	1	2	1	1	0	0
12	130	74	92	99	18	1	0	1	1	1	0	0	0
13	132	88	98	102	20	1	0	0	0	0	0	0	0
14	106	80	86	99	18	0	0	0	0	0	0	0	0
15	144	50	80	99.8	48	0	1	0	0	0	0	0	0
16	168	74	96	100.6	20	1	0	1	3	1	1	1	0
17	78	42	52	103	34	1	0	1	3	1	1	1	0
18	120	70	84	102	30	1	0	0	0	0	0	0	0
19	148	78	104	100	22	0	0	0	0	0	0	0	0
20	122	64	76	101	22	1	0	1	2	1	1	0	0
21	94	54	64	101	24	0	0	1	1	1	0	0	0
22	100	60	72	100	26	0	0	0	0	0	0	0	0
23	100	60	72	99.6	32	0	0	0	0	0	0	0	0
24	110	60	77	99	24	0	0	0	0	0	0	0	0
25	114	70	79	100	36	0	1	1	1	1	0	0	1
26	100	80	87	104	26	0	1	0	0	0	0	0	0
27	100	60	73	101	23	0	0	0	0	0	0	0	0
28	100	60	73	101	30	0	0	0	0	0	0	0	0
29	100	60	72	101	26	0	0	1	1	1	0	0	0
30	120	60	80	101	28	0	1	0	0	0	0	0	0
31	118	56	80	100	26	1	0	1	2	1	1	0	0
32	90	60	70	100	24	0	0	1	1	1	0	0	0
33	100	60	72	100	30	0	1	0	0	0	0	0	0
34	100	60	72	101	36	1	0	1	1	1	0	0	0
35	100	60	72	101	38	0	0	0	0	0	0	0	0
36	100	60	72	100	26	0	0	0	0	0	0	0	0
37													
38	110	70	77	99	43	0	1	0	0	0	0		0
39	100	60	72	99	32	0	0	0	0	0	0	0	0
40													
41													
42	104	60	77	101	24	0	1	1	2	1	1	0	0
43													
44	100	60	73	101	40	0	1	1	1	1	0	0	0
45	120	60	80	104	32	0	1	1	1	1	0	0	0
46													
47	100	60	73	100	24	0	0	0	0	0	0	0	0
48	100	60	73	101.8	34	0	0	0	0	0	0	0	0
49	100	60	73	101	36	0	0	0	0	0	0	0	0
50	100	60	73	102	30	0	0	0	0	0	0	0	0
51	100	60	73	101	30	0	0	0	0	0	0	0	0
52	100	60	7	102	30	0	0	1	1	1	0	0	0

	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ	AK
1	NORADRENALINE DOSE	ADRENALINE DOSE	VASOPRESSIN DOSE	DOBUT DOSE	CREATININE	TB	PH	LACTATES	HB	PLATELET	WBC C
2	8				0.9	1.4	7.41	4.4	12.6	3.26	10900
3					1.12	0.7	7.35	1.5	12.5	0.72	24800
4					0.8	0.7	7.51	1.2	11.4	2.59	40300
5					1.21	1.8	7.36	4.2	14.8	1.11	31300
6					0.33	2.8	7.51	2.9	7	0.45	1300
7	6				1	1.6	7.46	1.8	14.4	1.87	23700
8	3				2.47	2.4	7.33	4.5	13.1	2.13	16900
9	40	40	3.2		2.91	2	7.18	5.7	11.8	3.18	35500
10					0.88	0.9	7.36	2.2	12.7	1.78	41900
11	20	12			1.1	0.3	7.27	15.5	10.4	2.72	23100
12	8				0.78	0.5	7.42	1.7	12.9	1.8	4600
13					0.76	1.6	7.47	1.6	14.1	2.47	9700
14					1.97	0.4	7.33	1.4	7.2	3.55	18800
15					1.45	0.5	7.53	2.3	9.3	3.56	11000
16	14	6	2		1.37	1.8	7.65	4	12	4.16	27900
17	32	20	2		1.28	2.3	7.2	18.7	7.5	1.36	14600
18					0.72	0.5	7.52	0.9	12.1	3	11100
19					2.3	0.3	7.28	4	6.9	1.11	7700
20	6	10			1.58	1.6	7.2	10.6	16.8	2.2	30300
21	5				1.24	2	7.3	1.9	8.6	0.17	8500
22					1.18	0.4	7.4	1	7.6	1.58	9400
23					1.24	10	7.58	2.7	13	2.91	25600
24					1.08	1.4	7.39	1.3	12.4	0.8	11000
25	10			5	0.84	0.9	7.46	2.5	11	2.1	11500
26					0.92	1.1	7.49	1.5	13.9	2.21	8600
27					2.37	2	7.46	2	12.9	2.63	27300
28					3.9	0.5	7.31	4.7	9.5	1.9	5500
29	5				0.89	0.5	7.48	3	9.8	0.92	8700
30					1.94	0.9	7.37	2.3	11	0.73	9300
31	30	5			1.1	0.3	6.8	1.5	13.9	3.49	12700
32	5				1.08	0.2	7.19	9.2	12.3	2.41	11100
33					2	0.9	7.5	1.2	10.7	1.02	11200
34	25	1	1	1	0.86	0.6	7.2	6.4	12.7	2.4	14700
35					1.29	1.8	7.46	1.1	12.9	2.65	22300
36					0.62	0.2	7.47	0.8	11.7	1.83	11100
37											
38					2.88	0.6	7.21	5.8	10.5	3.35	23600
39					1.48	0.6	7.48	2.9	15.6	4.47	29100
40											
41											
42	30	5			2.53	0.7	7.19	13.9	7.8	4.12	45700
43											
44	15				0.39	1.1	7.21	2.5	11.5	3.66	35500
45	3				1.35	0.8	7.52	1.3	10.1	0.45	6900
46											
47					0.97	4.1	7.46	1.1	9.7	1.91	23200
48					1.56	0.2	7.11	0.7	12.9	2.36	9400
49					1.18	2.3	7.46	2.4	12.8	0.93	19400
50					1.44	0.3	7.47	1.1	11.1	3.59	20500
51					0.7	0.4	7.38	1.5	12.3	2.84	17800
52	10				3.32	0.6	7.31	2.8	10.7	2.79	23600

	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX
1	DM	SHTN	OTHERS	SOFA	APACHE 2	PREDICTED MORTALITY	CHARLSON	SEPTIC SHOCK	ARDS	AKI	AKI STAGE	THIAMINE	STEROIDS
2	1	1	1	10	20	40	6	1	1	0	9	1	1
3	0	0	0	7	16	25	0	0	1	0	9	1	1
4	0	0	0	8	28	55	0	0	1	0	9	0	1
5	0	0	0	4	5	8	0	0	0	0	9	1	0
6	0	1	1	8	20	40	3	0	1	0	9	1	1
7	0	0	0	11	19	25	0	1	1	0	9	1	1
8	1	1	0	9	12	15	2	1	0	1	2	1	1
9	0	0	0	13	30	73	0	1	0	1	2	1	1
10	1	0	0	6	24	40	1	0	1	0	9	1	1
11	1	1	0	7	18	25	2	1	1	0	9	1	1
12	0	0	0	11	17	25	0	1	1	0	9	1	1
13	0	0	1	7	16	25	0	0	0	0	9	1	0
14	1	1	1	5	27	55	4	0	0	1	1	1	1
15	0	0	0	3	11	15	1	0	1	1	1	1	1
16	0	0	0	12	22	40	0	1	0	1	1	1	1
17	0	0	1	15	36	85	1	1	1	1	1	1	1
18	0	0	0	3	15	25	0	0	0	0	9	1	1
19	1	1	1	6	25	55	5	0	1	1	2	1	1
20	0	0	1	10	26	55	1	1	1	1	1	1	0
21	0	0	0	11	10	15	0	1	0	1	1	1	1
22	0	0	0	2	10	15	0	0	1	0	9	0	0
23	0	0	0	6	11	15	0	0	1	1	1	1	1
24	0	0	0	5	9	8	1	0	0	0	9	0	1
25	0	0	0	4	11	15	0	1	1	0	9	1	1
26	1	1	1	4	13	15	5	0	1	0	9	0	1
27	1	0	0	3	19	25	3	0	0	1	2	0	0
28	1	1	0	4	26	55	3	0	1	1	2	0	0
29	0	0	1	6	15	25	2	1	0	0	9	0	0
30	1	0	0	7	19	25	3	0	1	1	1	0	0
31	0	0	1	12	26	55	1	1	1	0	9	1	1
32	0	0	1	5	19	25	3	1	0	0	9	0	0
33	0	1	1	4	23	40	8	0	1	1	2	0	0
34	1	1	1	9	20	40	9	1	1	0	9	1	1
35	0	0	1	3	14	15	1	0	0	1	1	1	0
36	0	0	0	2	10	15	0	0	0	0	9	0	1
37													
38	1	1	1	4	23	40	7	0	1	0	9	0	1
39	0	1	1	6	23	40	4	0	1	0	9	0	1
40													
41													
42	0	0	0	9	26	55	2	1	1	1	2	1	1
43													
44	1	0	1	11	33	73	8	1	1	0	9	0	1
45	0	0	0	11	8	8	0	1	1	1	1	0	0
46													
47	0	0	0	4	8	8	0	0	1	0	9	1	1
48	1	0	0	6	18	25	2	0	0	1	1	0	0
49	0	0	1	6	15	25	4	0	1	0	9	0	1
50	1	1	0	4	17	25	6	0	1	1	1	0	0
51	1	1	1	3	7	8	1	0	1	0	9	1	1
52	1	1	1	9	15	25	4	1	1	1	2	1	1

	AY	AZ	BA	BB	BC	BD	BE	BF	BG	BH
1	WHICH STEROID	CULTURE POSITIVITY	ORGANISM	SITE	PIPTAZ	CEFTRIAXONE	MEROPENEM	COLISTIN	AZITHROMICIN	VANCOMYCIN
2	1	0			0	0	1	0	1	0
3	2	1	8	5	0	1	0	0	0	1
4	1	1	3	3	0	0	1	0	1	0
5	9	1	10	6	0	0	0	0	0	0
6	1	1	2	1	0	0	1	0	0	1
7	1	1	11	1	0	0	1	0	1	0
8	1	0			0	0	1	0	0	0
9	1	0			0	0	1	0	1	0
10	1	1	4	3	0	0	1	0	0	1
11	1	0			0	0	1	0	0	0
12	1	1	7	3	0	0	1	0	1	0
13	9	1	7	3	1	0	0	0	0	0
14	1	0			0	0	1	0	0	0
15	1	1	2	1	0	1	0	0	0	1
16	1	1	4	1	0	0	1	1	1	0
17	1	1	4	1	0	0	1	0	0	0
18	1	0			0	1	0	0	1	1
19	1	0			0	0	1	0	0	0
20	9	0			1	0	0	0	0	0
21	1	0			1	0	0	0	1	0
22	9	0			1	0	0	0	0	0
23	1	0			1	0	0	0	1	0
24	2	0			0	1	0	0	0	1
25	1	0			0	0	1	0	0	0
26	1	0			1	0	0	0	0	0
27	9	0			0	0	1	0	0	0
28	9	1	1	1	0	0	1	0	0	0
29	9	1	1	1	0	0	1	0	0	0
30	9	1	1	1	0	0	1	0	0	0
31	1	0			0	0	3	0	0	1
32	9	1	12	7	0	0	1	0	0	0
33	9	0			1	0	0	0	0	0
34	1	0			1	0	0	0	1	0
35	9	1	13	6	0	0	1	0	0	0
36	2	0			0	1	0	0	0	1
37										
38	1	0			1	0	0	0	1	0
39	1	1	4	3	1	0	0	0	0	0
40										
41										
42	1	0			0	0	1	0	1	1
43										
44	1	0			0	0	1	0	1	0
45	9	0			0	0	1	0	1	0
46										
47	1	0			0	0	1	0	1	0
48	9	0			0	1	1	0	0	0
49	2	1	5	1	0	1	0	0	0	1
50	0	0			1	0	0	0	1	0
51	1	1	3	7	0	0	1	0	1	0
52	1	0			0	0	1	0	0	0

	BI	BJ	BK	BL	BM	BN	BO	BP	BQ
1	METRONIDAZOLE	DOXY	AMOX-CLAC	ALIVE AT HOSPITAL DISCHARGE	DAMA	WITHDRAWAL	DEESCALATION	DIALYSIS	VENTLATOR INDEPE
2	0	0	0	0	0	0	1	0	
3	0	0	0	1	0	0	0	0	2
4	0	0	0	0	0	0	1	0	
5	0	0	1	1	0	0	0	0	0
6	0	0	0	0	0	0	0	0	
7	0	0	0	0	0	0	0	0	
8	0	0	0	1	0	0	0	0	
9	0	0	0	0	0	0	0	0	
10	0	0	0	0	0	0	0	0	
11	0	0	0	0	0	1	0	0	
12	0	0	0	1	0	0	0	0	10
13	0	0	0	1	0	0	0	0	23
14	0	0	0	0	0	1	0	0	
15	0	0	0	0	0	0	0	0	
16	0	0	0	0	0	0	1	0	
17	0	0	0	0	0	0	0	0	
18	0	0	0	1	0	0	0	0	8
19	0	0	0	1	0	0	0	0	
20	0	0	0	1	0	0	0	0	3
21	0	0	0	9	1	0	0	0	
22	0	0	0	1	0	0	0	0	
23	0	0	0	0	0	0	0	0	
24	0	0	0	9	1	0	0	0	
25	0	0	0	1	0	0	0	0	3
26	0	0	0	1	0	0	0	0	8
27	0	0	0	1	0	0	0	0	
28	0	0	0	1	0	0	0	0	
29	0	0	0	1	0	0	0	0	
30	0	0	0	1	0	0	0	0	
31	0	0	0	1	0	0	0	1	39
32	0	0	0	1	0	0	0	0	
33	0	0	0	1	0	0	0	0	3
34	0	0	0	0	0	1	0	0	
35	1	0	0	1	0	0	0	0	
36	0	0	0	0	0	1	0	0	
37		0							
38	0	0	0	1	0	0	0	0	2
39	0	0	0	1	0	0	0	0	
40		0							
41		0							
42	0	0	0	1	0	0	0	0	2
43		0							
44	1	0	1	0	0	0	0	0	
45	0	0	0	1	0	0	0	0	2
46		0							
47	0	0	0	2	1	0	0	0	
48	0	1	0	1	0	0	0	0	
49	0	0	0	0	0	0	1	0	
50	0	0	0	1	0	0	0	0	
51	0	0	0	1	0	0	0	0	
52	0	1	0	1	0	0	0	0	

	BR	BS	BT	BU	BV	BW	BX	BY	BZ	CA	CB
1	VASOPRESSOR INDE	ICU LOS	HOSPITAL LOS	DAYS ALIVE	NEW ORGAN FAILURE	CVS	AKI	ARDS	HAEMAT	LIVER	CNS
2				6	1	0	1	0	0	0	0
3	0	2	7		0	0	0	0	0	0	0
4				27	1	1	0	1	0	0	0
5	0	2	8		0	0	0	0	0	0	0
6				19	1	0	0	1	0	0	0
7				13	0	0	0	0	0	0	0
8	2	7	11		1	0	0	1	0	0	0
9				2	0	0	0	0	0	0	0
10				3	1	1	1	1	0	0	0
11				5	1	0	1	0	0	0	0
12	1	13	24		0	0	0	0	0	0	0
13		21	27		1	1	0	0	0	0	0
14				15	1	0	0	1	0	0	0
15				20	1	1	0	0	0	0	1
16				3	0	0	0	0	0	0	0
17				3	0	0	0	0	0	0	0
18		10	14		0	0	0	0	0	0	0
19		4	5		0	0	0	0	0	0	0
20	3	6	10		0	0	0	0	0	0	0
21	1	2			0	0	0	0	0	0	0
22			7		0	0	0	0	0	0	0
23				4	1	1	0	1	0	0	0
24					1	1	0	1	0	0	0
25	2	6	16		0	0	0	0	0	0	0
26			11		0	0	0	0	0	0	0
27			7		0	0	0	0	0	0	0
28			7		0	0	0	0	0	0	0
29	2		12		0	0	0	0	0	0	0
30	6		10		0	0	0	0	0	0	0
31	7	24	37		0	0	0	0	0	0	0
32	1		5		0	0	0	0	0	0	0
33			10		0	0	0	0	0	0	0
34				7	0	0	0	0	0	0	0
35			35		0	0	0	0	0	0	0
36				12	1	0	1	1	0	0	0
37											
38			17		0	0	0	0	0	0	0
39				9	0	0	0	0	0	0	0
40											
41											
42	1	4	6		0	0	0	0	0	0	0
43											
44				1	0	0	0	0	0	0	0
45	2		7		0	0	0	0	0	0	0
46											
47					0	0	0	0	0	0	0
48			10		1	0	0	0	0	0	0
49				3	1	1	0	0	0	0	0
50			7		0	0	0	0	0	0	0
51			5		0	0		0	0	0	0
52	3		7		0	0	0	0	0	0	0