SAHLGRENSKA ACADEMY

A pilot study on the role of intravenous Vitamin C, Thiamine and Steroids in the treatment of Sepsis in the ICU, Kathmandu, Nepal

Degree Project in Medicine

Author:

Jacob Kjellberg
Programme in Medicine
Sahlgrenska Academy, Gothenburg, Sweden

Supervisors:

Göran Kurlberg, MD, PhD
Associate Professor of Surgery
Sahlgrenska Academy, Gothenburg, Sweden

Dr. Subhash P. Acharya, MD
Consultant Intensivist & Clinical Coordinator ICU
TU Teaching Hospital, Kathmandu, Nepal

Sweden, 2020-01-19

Table of contents

Abbreviations

ARDS – Acute respiratory distress syndrome

ICU – Intensive care unit

IRC – Institutional Review Committee

NET – Neutrophil extracellular traps

IV – Intravenous

TUTH – Tribhuvan University Teaching Hospital

AFC – Alveolar fluid clearance

C&S – Culture & Sensitivity

Abstract

Degree Project, Programme in Medicine

"A pilot study on the role of intravenous vitamin C, thiamine and steroids in the treatment of sepsis in the ICU, Kathmandu, Nepal"

Author: Jacob Kjellberg, Student of Medicine, 2020

Gothenburg University, Sahlgrenska Academy

Supervisors: Göran Kurlberg, MD, PhD, Associate Professor of Surgery, Sahlgrenska Academy

Dr. Subhash P. Acharya, MD, Consultant Intensivist and Clinical Coordinator, TUTH

Background

Sepsis is estimated to cause 5 million deaths from 19 million cases each year making it one of the world's most deadly disease. In low-income countries, such as Nepal, the mortality rate can be as high as 49%. This pilot study was conducted in at Tribhuvan University Teaching Hospital, Kathmandu, Nepal. Current treatment for sepsis consists of aggressive fluid treatment and broad-spectrum antibiotics. However, research has shown that addition of Vitamin C, Thiamine and Steroids might have a positive effect on the outcome.

Objective

To evaluate if a treatment protocol consisting of Vitamin C, Thiamine and Steroids has a positive effect on the outcome for patients in the ICU with sepsis and/or septic shock.

Methods

Medical records of patients treated for sepsis and/or septic shock were obtained from the surgical and medical ICU as controls. Patients arriving to the ICU were given a treatment protocol consisting of Vitamin C, Thiamine and Steroids along the standard treatment. In order to compare outcomes, the data from patients receiving the protocol were to be compared with the historical controls treated conventionally.

Results

51 patients were included of whom four had received the treatment protocol. The mean age were 56.7 years. 52.9% of the patients admitted to the ICU were male. 51% of the patients were diagnosed with pneumonia. 43.1% of the patients did not survive.

Conclusion

Due to the limited amount of data in this study, no conclusions can be drawn on Vitamin C, Thiamine and Steroids effect on sepsis. However, previous studies have shown that early administration of Vitamin C, Thiamine and Steroids has a positive effect on the outcome for patients with sepsis and/or septic shock. For patients that has developed severe sepsis and acute respiratory distress syndrome, Vitamin C has no effect on SOFA score. This indicates that early administration of the protocol is necessary to obtain desired effect. More randomised controlled studies must be made to determine the role of Vitamin C in the treatment of sepsis.

Key Words: Sepsis, Vitamin C, Nepal

1. Introduction

Sepsis is defined as “a life-threatening organ dysfunction due to a dysregulated host response to infection” [1]. It is estimated that 19 million cases of sepsis each year cause 5 million deaths worldwide. The mortality of sepsis and septic shock in low-income countries is estimated to 49 percent, compared to high-income countries with a mortality of 25-30 percent [2]. This makes sepsis to one of the leading causes of death in the world.

1.1 Setting

This study was conducted at Tribhuvan University Teaching Hospital (TUTH) situated in Nepal, a country famous for its mountains and beautiful nature. Nepal is located in South Asia, bordering China to the north and India to the east, south and west. The country has a population of around 28.6 million and around 20 % of the population lives in urban areas [3, 4]. Nepal is one of the least developed countries in the world and in 2015 roughly a fifth (21.6%) of the population lived below the poverty line [5]. For a lot of people, particularly in western Nepal, there can be vast distances between their home and healthcare clinic [6]. People may have to walk several hours to get help, and while in labour or having a serious infection, this can be an impossible challenge.

The health spending in Nepal is financed largely by out of pocket spending (47.7%). This means the patients pay at or after the time of health care delivery. The second largest part is government health spending (28.6%). The third largest part comes from development assistance

for health (DAH, 17.8%) which comes from development agencies such as UNICEF. Lastly is prepaid private spending (5.9%), constituted by non-public programs funded prior to obtaining healthcare (such as private insurances).

TUTH is one of Nepal's University Hospitals and was established in 1983. It has 700 beds and takes care of more than 2000 patients in the outpatient department every day. The surgical ICU at TUTH, where this study takes place, has 11 beds and treats patients in need of critical care.

1.2 Host response to sepsis

As the definition of sepsis implies, the symptoms are caused by a dysregulated immune system response to an underlying infection. The organs of the host are affected negatively by this response and begin to lose their function.

The most common type of white blood cells in mammals are neutrophils, which constitutes an essential part of our defence against microorganisms [7]. However, neutrophils can cause serious damage to the organs of the body when they are activated out of proportion [8]. Neutrophils use several different mechanisms in the battle against microorganisms. They release antimicrobial peptides and lytic enzymes and produce reactive oxygen molecules before phagocyting the invader. Another mechanism is the formation of neutrophil extracellular traps (NET). These traps consist of neutrophil DNA, expelled from the nucleus, together with

histones and other proteins. NETs have the ability to bind and kill bacteria and fungi as some of the proteins including histones have bactericidal effects [7]. Unfortunately, the histones also have a concentration-dependent cytotoxic effect which can damage or kill the hosts epi- and endothelial cells [7, 8].

One of the main issues in sepsis is the loss of function in the epi- and endothelium [9]. The endothelium is a layer of cells that line the wall of our blood- and lymphatic vessels. It has many functions including regulation of the vasomotor tone and cell nutrient trafficking. When there is a small localized infection, the endothelial cells get stimulated and in response release pro-inflammatory substances, recruiting leukocytes among other things. One other important mechanism in the endothelium is the increased permeability in the presence of TNF-alfa [10]. This leads to oedema in the tissue, making it easier for the immune system to reach the site of infection. Normally, this does not give rise to any problem. However, when there is a larger, systemic response from the immune system as in sepsis, the endothelium gets over stimulated leading to loss of fluids to the interstitial space. This results in hypovolemia and drop in blood pressure [9].

The endothelium is not the only cell line suffering from impaired function. As mentioned previously, the epithelium is also affected. In the lungs, epithelium lines the alveolus creating a barrier between the air-filled alveolus and the capillaries. Between the epithelium of the alveolus and the endothelium of the capillaries lie the interstitial space. When a patient becomes septic, the inflammation causes protein-rich fluid to flow into the interstitial space because of

the dysfunctional endothelium. As the neutrophils enter the lungs and release their NETs, it damages or even destroys the epithelium, letting the protein-rich fluid into the alveolus. This in turn gives a perfusion-ventilation mismatch, arterial hypoxemia and reduced lung compliance. These changes result in a syndrome called Acute Respiratory Distress Syndrome (ARDS) [7, 9, 11]. ARDS is a serious complication with a high mortality rate. A study conducted in Germany compared mortality for ARDS patients in the ICU at university hospitals vs non-university hospitals. They showed a mortality at 39.3% for university hospitals vs 57.5% for non-university hospitals [12]. Please see table 1 below for diagnostic criteria regarding ARDS.

Table 1. The Berlin definition of ARDS.

Timing	Within one week of a known clinical insult or new or worsening respiratory syndrome.	
Chest imaging	Bilateral opacities – not fully explained by effusions, lobar/lung collapse, or nodules.	
Origin of oedema	Respiratory failure not fully explained by cardiac failure or fluid overload. Need objective assessment (e.g. echocardiography) to exclude hydrostatic oedema in no risk factor present.	
Oxygenation	Mild	200 mm Hg < PaO2/FiO2 ≤ 300 mmHg with PEEP or CPAP ≥ 5 cmH2O
	Moderate	100 mm Hg < PaO2/FiO2 ≤ 200 mmHg with PEEP or CPAP ≥ 5 cmH2O
	Severe	PaO2/FiO2 ≤ 100 mmHg with PEEP or CPAP ≥ 5 cmH2O

PEEP: *Positive End-Expiratory Pressure,* ***CPAP:*** *Continuous Positive Airway Pressure.*

Not only infections in the lungs can give rise to complications such as ARDS. Sepsis with focus from e.g. the gut can cause damage in the lungs [13]. Many more mechanisms on both organ,

tissue and cellular level plays a part in the development of sepsis and septic shock but has been left out in this limited study.

1.3 Current treatment

The current treatment for sepsis and septic shock initially focuses on stabilising respiration through supplemental oxygen or – if necessary – intubation, establishing a venous access for resuscitation to manage hypotension and initial investigation including patient history, physical examination, laboratory and bacterial culture [14, 15]. Broad spectrum antibiotics targeting the suspected source of infection should be administrated intravenously as soon as blood has been drawn for cultivation. When the culture and sensitivity results are ready, a smaller spectrum antibiotic targeting the microorganism responsible for the infection should be used to avoid development of antibiotic resistance [16]. The patient's vital parameters – for example oxygen saturation, pulse, blood pressure, GCS-score and temperature – is followed closely to evaluate the treatment and to determine of any more intervention is necessary.

To evaluate the extent of the sepsis and the level of organ failure a scoring system is used called Sequential Organ Failure Assessment (SOFA) score. It evaluates the respiratory-, nervous- and cardiovascular system as well as liver- and kidney function and coagulation. Every part is graded from 0 to +4 based on predetermined levels, giving a minimum score of zero and a maximum score of 24. The definition of sepsis includes the term organ dysfunction, which is characterised as a change of two points or more in SOFA score [17]. This approach to use

changes in score instead of just looking at the predetermined level makes it possible to evaluate patients that suffers from chronic organ dysfunction before the onset of the infection.

Table 2. SOFA-score [18]

SOFA score	0	1	2	3	4
Respiration					
PaO_2/FIO_2 (mmHg)	> 400	301–400	201–300	101–200	≤ 100
(kPa)	> 5.3)	(4.1–5.3)	(2.8–4.0)	(1.4–2.7)	≤ 1.3)
Coagulation					
Platelets ($x10^3/mm^3$)	> 150	101–150	51–100	21–50	≤ 20
Liver					
Bilirubin (mg/dl)	< 1.2	1.2–1.9	2.0–5.9	6.0–11.9	≥ 12.0
(μmol/l)	< 20)	(20–32)	(33–101)	(102–204)	≥ 204)
Cardiovascular					
Hypotension	No hypotension	MAP < 70 mmHg	Dopamine ≤ 5 or dobutamine (any dose)*	Dopamine > 5	Dopamine > 15
Central nervous system					
Glasgow coma score	15	13–14	10–12	6–9	< 6
Renal					
Creatinine (mg/dl)	< 1.2	1.2–1.9	2.0–3.4	3.5–4.9	> 5.0
(μmol/l)	< 110)	(110–170)	(171–299)	(300–440)	> 440)
or urine output				< 500 ml/day	< 200 ml/day

* adrenergic agents administered for at least 1 h (doses given are in μg/kg/min)

MAP: Mean Arterial Pressure

If a patient does not respond to the initial treatment despite adequate fluid administration and antimicrobial regimen, there may be a need for an addition of vasopressors. Numerous studies have shown that Norepinephrine should be the first line of choice [19, 20]. Additionally, the sepsis diagnosis and the suspected focus of the infection should be re-evaluated if the treatment is non effective. Studies have shown that delay in the initial treatment, especially delay with antibiotics, increases the mortality by 4% every hour. Thus it is of utmost importance that the treatment is started as soon as possible [21].

1.4 Vitamin C

Vitamin C, also known as ascorbic acid, is an essential vitamin for humans. Most animals can synthesize it by themselves, but humans have unfortunately lost this ability and therefore need it in their diet or as a supplement. For several hundreds of years, vitamin C deficiency constituted a problem for humans in environments lacking sources of vitamin C. For example, during long sea voyages where fresh fruit and vegetables were not available, sailors developed symptoms of scurvy after two to three months [22]. In 1753, James Lind showed that scurvy could be treated with citrus fruits [23]. One of the most common symptoms of scurvy is loss of teeth, poor wound healing and wound reopening. As vitamin C is a catalyst for the enzyme that converts procollagen to collagen, in its absence, the tissue becomes unstable and gives rise to the previous mentioned symptoms.

Vitamin C is an electron donor working as a reducing agent, capable of donating two electrons. It has many known functions in the body where its electron donating capability is responsible for all [24]. During infections as pneumonia and common cold, the level of Vitamin C in the bloodstream decreases [25]. It has been shown that leukocytes accumulate Vitamin C up to 50 to 100-fold the concentration in plasma [26]. Neutrophils exposed to pathogens produce oxidants to kill the bacteria. They also increase their intra cellular storage of Vitamin C 10-fold, although it is not known why. One theory is that they accumulate Vitamin C to protect them from the oxidants they produce [22].

Vitamin C is also crucial for production of catecholamines (Epinephrine, Norepinephrine & Dopamine) in the nervous system and adrenal gland. The catecholamines plays an important role as neurotransmitters and hormones in the body [24]. Its electron donating capabilities is also vital for fourteen mammal enzymes.

As mentioned earlier, one of the feared complications of sepsis is ARDS. Two of the mechanisms that plays a part in the development of ARDS is lowered fluid clearance in the alveolus and destruction of the lung epithelium by neutrophils. Two studies made on the subject has shown that in patients with sepsis, vitamin C both increases the alveolar fluid clearance (AFC) [13] as well as inhibits the neutrophils NET-formation [27]. Both these mechanisms are important in the development of ARDS, which gives a new possible treatment option.

1.5 Thiamine

Thiamine, also known as vitamin B1, is another essential vitamin. Different configurations of Thiamine are involved in several processes in the body. It is crucial in the development of the brain and its function because of its involvement in the generation of acetylcholine. It is a cofactor in the pyruvate dehydrogenase complex, making it important in the cellular respiration. In the immune system, Thiamine regulate and activate many different types of cells and proteins, both direct and indirect. For example, it protects neutrophils through antioxidation and works as an anti-inflammatory component regulating the expression of inflammatory agents. These are just some examples of the vast number of processes that Thiamine is involved in.

If there is a deficiency of Thiamine, one can develop several different conditions. As Thiamine is vital for the conversion from pyruvate to acetyl coenzyme A, the cells can no longer use the aerobic pathway in the cell respiration and is forced to use the anaerobic pathway. This is much less effective and releases lactic acid that lower the pH of the patients' blood and causes lactic acidosis [28].

1.6 Steroids

Cortisol, also known as hydrocortisone, is a type of glucocorticoid produced in the adrenal cortex. It has a wide set of functions including helping the body resist stress and inflammation as well as the regulation of carbohydrate, fat and protein metabolism [29]. Cortisol works through several mechanisms that inhibit inflammation. One of the most important mechanisms is the stabilization of the membrane of lysosomes, a membrane bound organelle. This makes it harder for the lysosomes membrane to rupture, thus preventing pro-inflammatory enzymes to be released [29]. Cortisol has the ability to both prevent inflammation before onset as well as reduce ongoing inflammation. When cortisol is administered to a patient with an inflammatory process such as glomerulonephritis, the inflammation almost always starts to subside within 24 hours [29].

If the adrenal cortex gets damaged or undergoes atrophy and loses its ability to produce corticosteroids, it can lead to development of Addison's disease. As the adrenal gland can no longer produce the important glucocorticoids, the body is no longer capable to regulate the

metabolism making it impossible to maintain normal blood glucose level [29]. If a patient with Addison's disease gets an infection or other stress-inducing events they can develop an Addison crisis which can be fatal.

Studies conducted on the effect of steroids in sepsis showed that the administration of steroids has a positive effect on the 28-day mortality [30]. However, it did not have any effect on the transition from severe sepsis to septic shock [31].

2. Aim/Specific objective/Research question

Does the combination of Vitamin C, Thiamine and Steroids improve the outcome for patients with sepsis and septic shock, comparing mortality between patients receiving either the Vitamin C protocol or conventional treatment?

3. Material and methods

This prospective study with historical controls was conducted during eight weeks at Tribhuvan University Teaching Hospital, Kathmandu, Nepal. In total, 50 patients arriving to the ER with the diagnosis of sepsis and/or septic shock were planned to receive treatment with a Vitamin C protocol. Patients receiving the protocol were compared with controls previously treated with conventional treatment in the ICU. The protocol went on for 96 hours and included

administration of 1500 mg IV Vitamin C every 6th hour, 100 mg IV Thiamine every 6th hour and 50 mg IV Steroids every 6th hour. Data regarding gender, age, co-morbidity, length of hospitalisation, length of stay in ICU/medical ward, outcome (survivor, non-survivor, leave against medical advice, withdrawal), diagnose/pathogen, patient delay (time from onset of serious symptoms e.g. lowered degree of consciousness until arrival at the ER), doctor delay (time from ER to admittance to the ICU), use of vasopressors, fluids obtained in the ICU, use of antibiotics and if the patient were treated with the vitamin C protocol was collected and compiled in an Excel file.

Due to circumstances regarding availability of vitamin C and communication in the ICU, only a few patients received the treatment protocol – too few to be of use in the study. Therefore, previous studies will be used in the discussion regarding the effectiveness of the treatment protocol.

3.1 Data collection procedures

Data from previous patients treated for sepsis and septic shock in the ICU were collected during eight weeks at TUTH. To find historical controls, the admittance book in the ICU was used. Medical records of patients admitted with the diagnose sepsis and/or septic shock were obtained for respective patient. Data was collected according to a pre-determined pro forma.

3.2 Statistical methods

Because of the lack of data obtained, no statistical calculations could be done. The data regarding e.g. mortality, pathogens and patient delay are all presented in figures and tables.

4. Ethics

The study aligned to the principles of the Helsinki declaration. An application to the IRC were submitted and approved before the study began. Please see Appendix 1 for ethical clearance.

5. Results

5.1 Age & Gender

Figure 1. Age of patients admitted to the ICU at TUTH

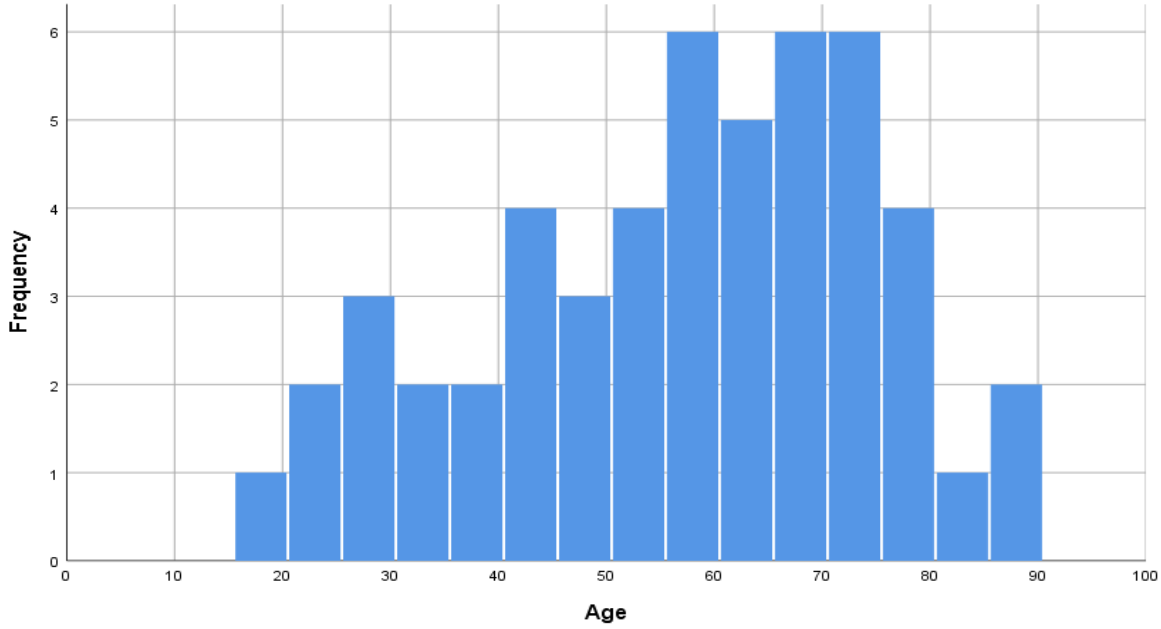


Figure 1 shows the age of the patients diagnosed with sepsis and/or septic shock that were admitted the ICU. As seen in the figure, the most common age was between 55-80 years. The mean age of the patients admitted were 56.7 years.

Figure 2. Gender of patients admitted to the ICU at TUTH

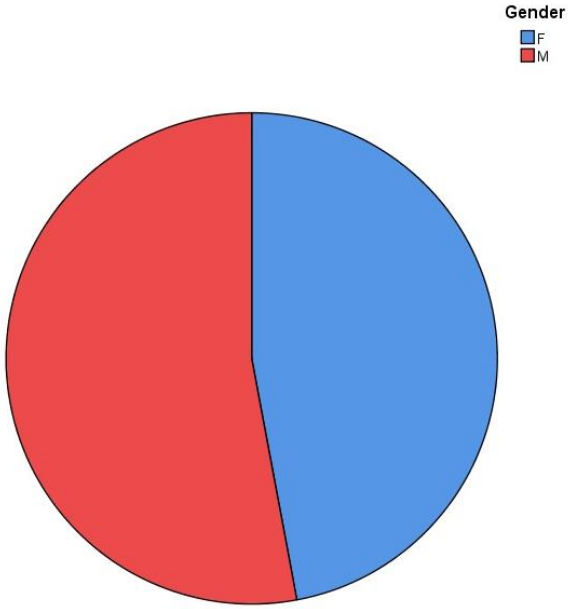


Figure 2 shows the gender distribution of patients diagnosed with sepsis and/or septic shock that were admitted to the ICU. 52.9% of the patients were male, 47.1% of the patients were female.

5.2 Mortality

As seen in table 3, 51 patients were included in the study. Roughly half of the patients survived (51.0%) and 43.1% of the patients did not survive. In 5.9% of the cases, the patient left the ICU against medical advice.

Table 3. Number of surviving patients admitted to the ICU at TUTH with sepsis and/or septic shock

	Frequency	Percent
S[a]	26	51.0
NS[b]	22	43.1
LAMA[c]	3	5.9
Total	**51**	**100.0**

a. Survivor

b. Non-Survivor

c. Leave against medical advice

5.3 Vitamin C

Figure 3. Outcome by Vitamin C protocol of patients admitted to the ICU at TUTH

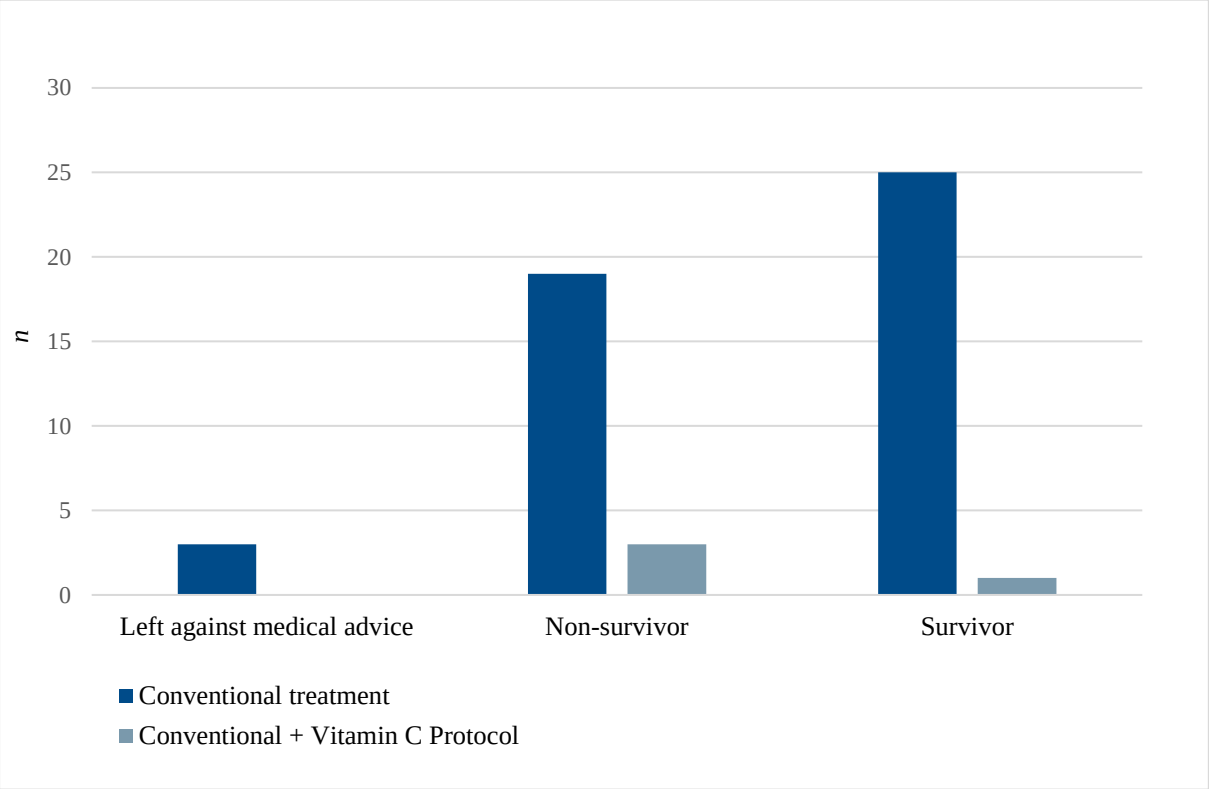


Figure 3 shows outcome with regards to if the patient received the Vitamin C protocol or not. As mentioned previously, only four patients received the Vitamin C protocol where one survived and three died. Among the patients receiving the conventional treatment 19 died and 25 survived. Three patients left the ICU against medical advice, making it not possible to know the outcome.

5.4 Panorama of infections

Figure 4. Diagnoses of patients admitted to the ICU at TUTH

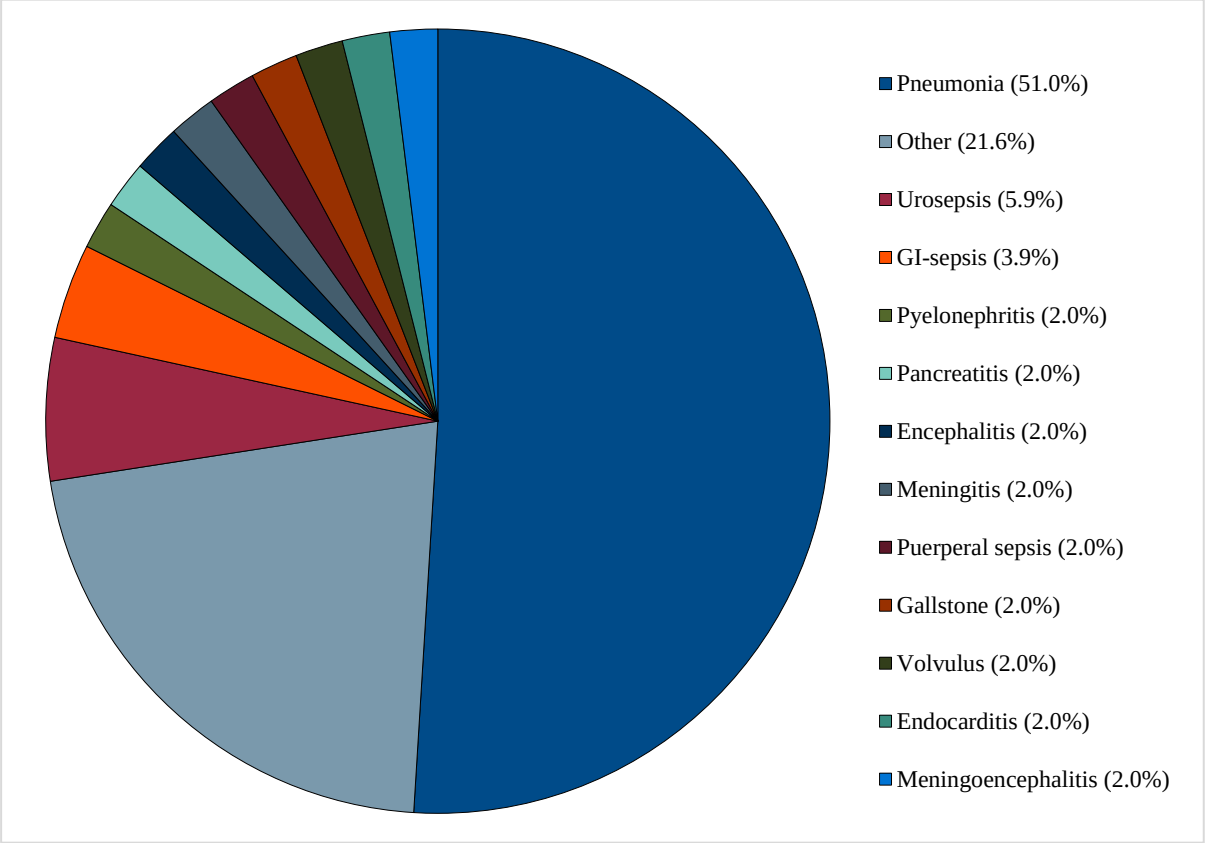
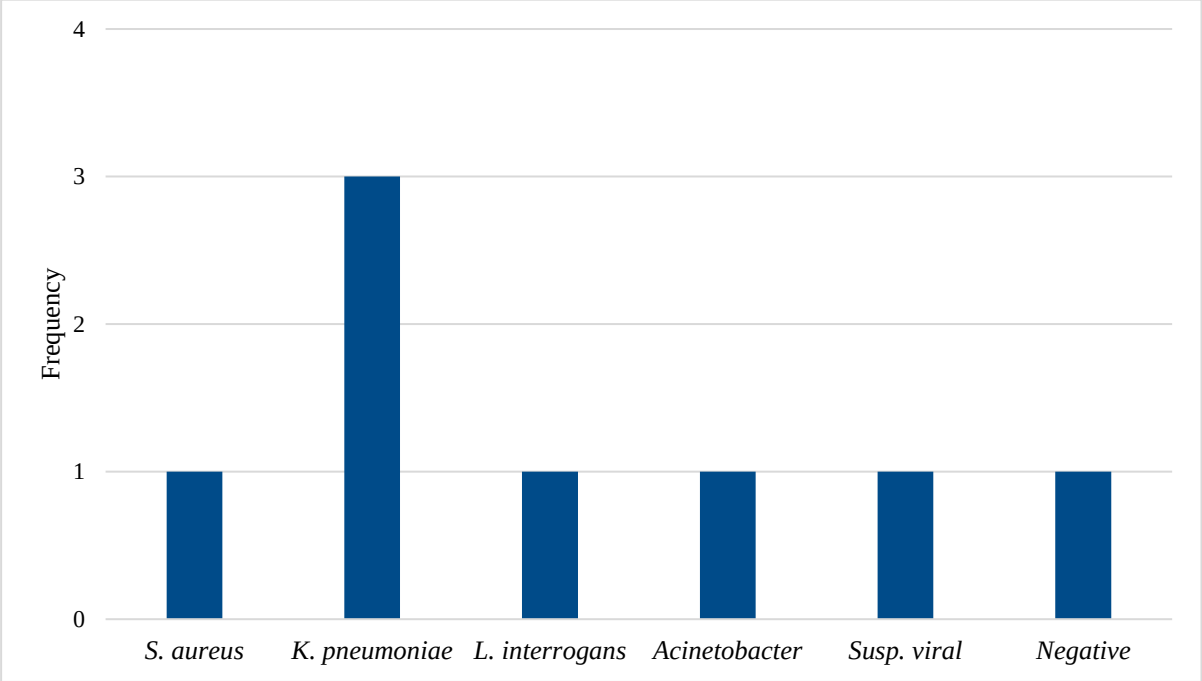


Figure 4 shows what diagnoses were the most common in the enrolled patients. Pneumonia was by far the leading cause of sepsis (51%), followed by the “Other/Unknown” category (21.6%), which contains cases with unknown diagnosis or whom only received a diagnose that were not responsible for the sepsis, e.g. acidosis. Urosepsis was the second most common diagnose (5.9%).

5.5 Bacteriology

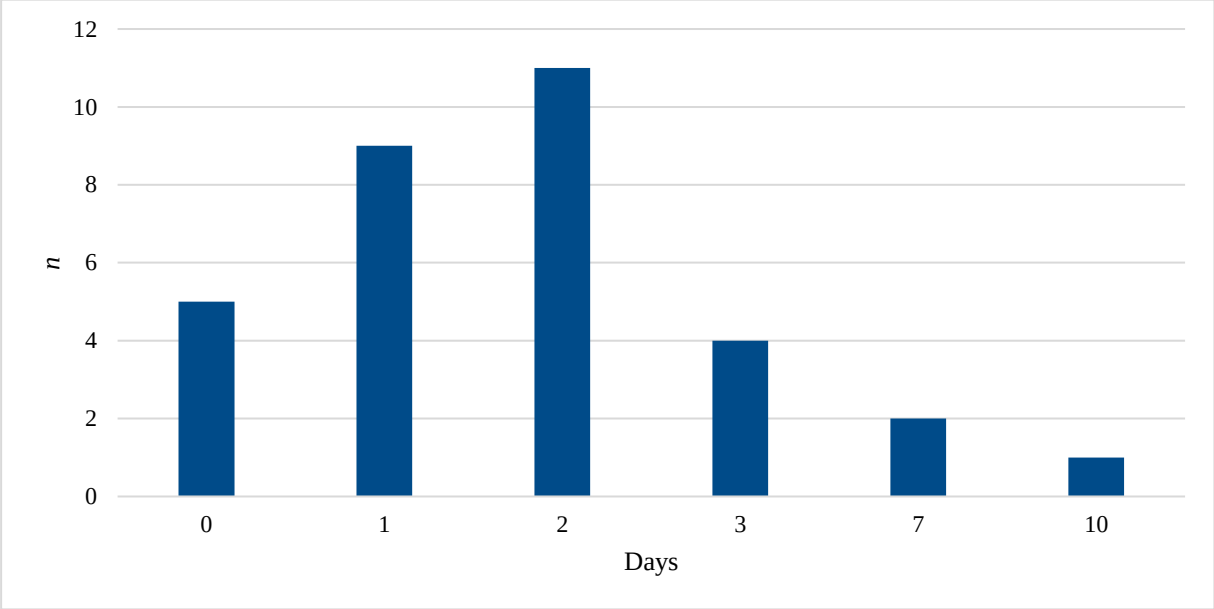
Figure 5. Pathogens of patients admitted to the ICU at TUTH



Only eight cases had any information regarding blood culture (C&S) and pathogens responsible for the infection. The bars represent how many cases contained data and what information was to be found. *K. pneumoniae* was the most common pathogen and was found in three cases. The rest of the categories had one patient each.

5.6 Patient delay

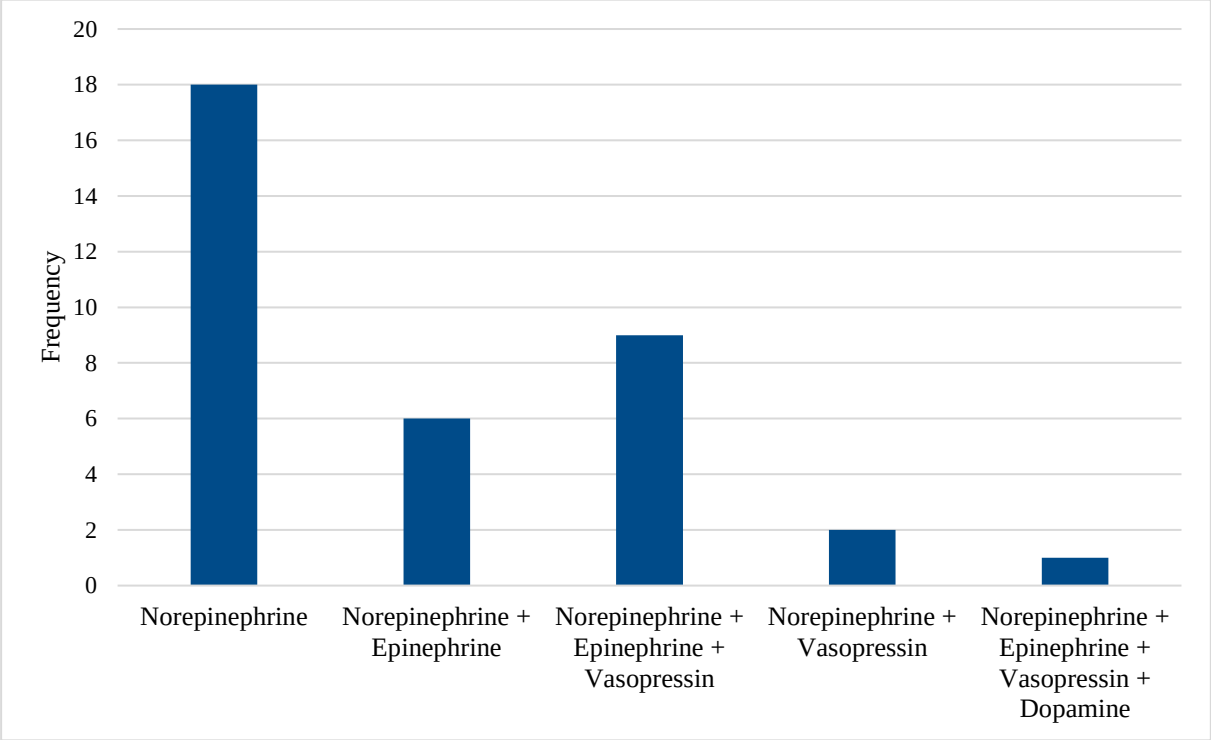
Figure 6. Patient delay of patients admitted to the ICU at TUTH



Number of days patients delayed before visiting the ER. In 32 cases there were information regarding delay. For the rest, the information was missing. The numbers were estimated from onset of more serious symptoms; e.g. lowered consciousness, anuria and vomiting. The most common were two days of delay (11 patients) followed by one day (nine patients).

5.7 Vasopressors

Figure 7. Vasopressors of patients admitted to the ICU at TUTH



This figure describes the use of vasopressors in cases with sepsis and/or septic shock in the ICU at TUTH. All patients that were treated with vasopressors received Norepinephrine either alone or in combination with another drug. Most common was Norepinephrine (Noradrenaline) alone (18 patients) followed by a combination of Norepinephrine, Epinephrine and Vasopressin (9 patients).

6. Discussion

Key findings of the present trial were that sepsis has a high mortality, 43% of the patients did not survive. Pneumonia is responsible for most infections, 51% of the patients admitted to the ICU at TUTH were diagnosed with pneumonia. Norepinephrine is by far the most common used vasopressor.

6.1 Findings

Due to problems regarding the availability of Vitamin C and communications in the ICU, no patients received treatment and could be enrolled to the study. 51 cases were collected from medical records of patients previously treated in the ICU, where four had received the Vitamin C protocol (table 3). Therefore, it not possible to make any conclusions about the effectiveness of the protocol and Vitamin Cs place in the treatment of sepsis.

The most common diagnose among the patients admitted to the ICU at TUTH were pneumonia responsible for 51% of the cases (figure 4). This was followed by Urosepsis who accounted for 5.9%, making pneumonia the single largest diagnose. Data were also collected on what pathogens (figure 5) were responsible for the sepsis. It shows that *Klebsiella Pneumoniae* were the most common pathogen. However, because of the low number of available cultivations, no conclusions can be drawn.

Patient delay showed some interesting patterns (figure 6). The delay was estimated from the onset of more serious symptoms, e.g. lowered consciousness, anuria and vomiting. The data showed that most patients delayed one or two days before visiting the ER. One interesting aspect would be to analyse patterns of patient delay and wherever the patient lives inside or outside Kathmandu Valley. There may exist a pattern between time before visiting the ER and wherever the patient lives in rural or urban environment.

The most common used vasopressor was Norepinephrine (figure 7). Every patient included in the study that received vasopressor had Norepinephrine either alone or in combination with another drug. As mentioned in the introduction, numerous studies have shown that Norepinephrine should be the first choice when treating a patient with vasopressors.

6.2 Comparison

One of the studies that has shown the most promising results was conducted by Marik et. al. in 2017 [32]. They gave patients a Vitamin C protocol consisting of Vitamin C (1500 mg every 6^{th} hour for 96 hours or until discharge from ICU), Thiamine (200 mg every 12^{th} hour for 96 hours or until discharge from ICU) and Steroids (50mg every 6^{th} hour for 168 hours or until discharge from ICU, followed by a taper over 72 hours). The protocol was initiated within 24 hours after admittance to the ICU. 47 patients were enrolled to each group where both groups received antibiotics, one group received the protocol and the other placebo. The groups had no difference in baseline characteristic. The results showed a difference in mortality between the

groups where the protocol group had a mortality of 8.5% and placebo 40.4% ($P < 0.001$). The patients who died in the protocol group died of complications from their underlying disease, e.g. diabetes and severe heart failure. They also showed a decrease in SOFA-score after 72 hours where the protocol group got a mean decrease of 4.8 and the placebo 0.9 ($P < 0.001$).

Another study named CITRIS-ALI made in October 2019, had a different approach [33]. In this study, 167 patients were enrolled with 83 assigned to placebo and 84 received only Vitamin C 50mg/kg every 6^{th} hour for 96 hours. In a patient weighing 70kg, this would give a dose of 14g/24 hours. This dose is significant higher compared to the one used by Marik et. al. Also note the lack of Thiamine and Steroids. To be included in the study, the patients had to meet certain criteria very different from the study mentioned above. They were only included if they met the criteria for ARDS (please see 1.2 for exact criteria) and the treatment could be started within 48 hours of the ARDS diagnosis. One of the primary outcomes was a modified SOFA-score (bilirubin eliminated) to evaluate if Vitamin C had any effect on organ failure. The results showed no significant difference between the two groups. However, a secondary outcome measured the 28-day mortality where a significant difference was found between the groups. The Vitamin C protocol group had 29.8% mortality compared to the placebo group which had 46.3% ($P = 0.01$). Interestingly, when looking at mortality and ICU graduation after 96 hours when the protocol was done, 19 patients in the placebo group had died compared to 4 in the Vitamin C group. In the Vitamin C group, 9 patients had graduated from the ICU after 96 hours, compared to only 1 in the placebo group. This is a remarkable finding that calls for more research regarding Vitamin Cs role in sepsis.

One mechanism that could support the results in the CITRIS-ALI study is the increased AFC showed in mice models by Fisher et. al. [13]. This study showed a decrease in AFC compared with controls when mice were injected intra peritoneal with faeces, inducing septic peritonitis. This lowered the expression of Aqp5, one of the key proteins in fluid transport. When the septic mice were injected with Vitamin C, the expression of Aqp5 increased as well as the AFC. This might explain the results in the CITRIL-ALI study where the patients who received Vitamin C had a higher number of ICU and hospital free days as they cleared out their pulmonary oedema quicker.

Another mechanism behind the development of ARDS is the neutrophils NET formation and the following destruction of the epithelium in the alveolus. There is a possibility that early administration of Vitamin C can prevent the neutrophils from destroying the epithelium in the alveolus protecting the lungs from development of ARDS. This might explain the results obtained by Malik et. al. where they showed a significant difference in mortality between the groups that received Vitamin C or placebo. Indeed, there are more mechanisms involved in the development of ARDS that might play a bigger part.

More randomized studies are on the way, one called Ascorbic Acid, Corticosteroids, and Thiamine in Sepsis (ACTS) [34]. This study is trying to replicate the results obtained by Malik et. al. and aims to be complete in February 2020.

According to Dr Patak, a resident in the ICU at TUTH, one of the biggest concerns when treating patients with sepsis is the time between the arrival to the ER and admittance to the ICU. Patients can wait for more than 48 hours before there is a free bed in the ICU. This makes a major concern for the Vitamin C aspect as it seems that early administration of the drug is important. More studies need to be made to evaluate the patient flow in the ER and to find out if there is a possibility to start the treatment protocol in the ER while the patient waits for a spot in the ICU.

6.3 Authors Thoughts

There seem to be a positive effect regarding Vitamin Cs effect on the outcome on sepsis. One of the most important aspects is the time of administration. The studies that showed positive results included patients that were early in the course of the sepsis. As mentioned earlier, Marik et al administered the Vitamin C protocol within 24 hours of the sepsis diagnosis, opposed to other studies that waited until patients were almost on the brink of death and then gave the protocol. The results from these studies could indicate that Vitamin C is best used to slow the course of the disease, preventing the patient from developing ARDS or other complications.

I personally believe that Vitamin C could change the treatment foundation for sepsis and will follow the ACTS study with great interest.

7. Populärvetenskaplig sammanfattning

Sepsis, tidigare kallad blodförgiftning, är en allvarlig sjukdom som drabbar 19 miljoner människor och orsakar fem miljoner dödsfall världen över varje år. Sepsis beror på att kroppen reagerar överdrivet på en infektion vilket innebär att immunförsvaret orsakar skada på de egna organen. Detta kan leda till att patientens blodtryck sjunker på grund av att vätska tränger ut från blodkärlen till vävnaden, och även till lungorna, vilket kan vara dödligt. Idag finns ingen definitiv bot för sepsis, istället ges vätskebehandling direkt i blodet för att höja blodtrycket i kombination med antibiotika som täcker ett stort antal bakterier, så kallad bredspektrumantibiotika. Innan antibiotika ges tas prover och blododlingar för att försöka ta reda på vilken bakterie som ligger bakom infektionen och vilken antibiotika den är känslig för. När provsvaren kommit sätts en antibiotika med smalare spektrum in i syfte att undvika resistensutveckling.

Idag pågår intensiv forskning om nya behandlingar för sepsis. På senaste tiden har C-vitamin fångat forskarnas intresse. Forskning har visat att C-vitamin kan dämpa den kraftiga reaktionen från immunförsvaret och hjälpa till att få undan vätskan ifrån lungorna. De flesta djur kan bilda C-vitamin själva, människan har dock förlorat denna funktion till följd av en förändring i vårt DNA. Detta gör att C-vitamin är en så kallad essentiell vitamin vilket innebär den måste tillföras via kosten.

I studien har C-vitamin getts till patienter med sepsis eller septisk chock (en allvarlig form av sepsis med bland annat väldigt lågt blodtryck) tillsammans med Tiamin (även kallat vitamin B1) och steroider (även kallat kortison). Tiamin är, precis som C-vitamin, en essentiell vitamin som är viktig för vår ämnesomsättning samt för utvecklingen av fostrets nervsystem. Steroider har en kraftfull dämpande effekt på immunförsvaret och har visats ha en positiv effekt på utkomsten vid sepsis.

Tyvärr fanns inget C-vitamin tillgängligt när studien genomfördes på intensivvårdsavdelningen i Kathmandu, Nepal. Data från patienter som tidigare behandlats för sepsis samlades in och jämfördes med andra studier som gjorts på området. Det har visats att C-vitamin har en positiv effekt på dödligheten i sepsis om det ges tidigt i sjukdomsförloppet och vidare under minst fyra dagar. Mer forskning behövs inom området och flera studier genomförs idag som kommer undersöka C-vitaminets roll i behandlingen för sepsis.

8. Acknowledgements

Firstly, I would like to thank Professor Göran Kurlberg for the support during this project, both in Sweden and in Nepal. I also wish to express my gratitude to Dr. Sture Blomberg for his input and thoughts during the process. I want to thank Prof. Dr. Yogendra M. Shakya, Ajeli Shakya and the rest of the family at Hotel Metropolitan Kantipur for their support and warm hospitality. Thank you to Dr. Subhash P. Acharya for letting me do my research in the ICU. Lastly, I would like to express my deepest gratitude to Thérèse Henriksson for her company and endless support during our eight weeks in Kathmandu.

9. References

1. Singer, M., et al., *The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3).* Jama, 2016. **315**(8): p. 801-10.

2. Cohen, J., et al., *Sepsis: a roadmap for future research.* The Lancet Infectious Diseases, 2015. **15**(5): p. 581-614.

3. *World Urbanization Prospects: The 2018 Revision*. 2018, United Nations, Department of Economic and Social Affairs, Population Division: New York: United Nations.

4. *World Population Prospects 2019*. 2019, United Nations, Department of Economic and Social Affairs, Population Division.

5. *UNDP Nepal Annual Report 2018*. 2018, United Nations Developement Programme (UNDP).

6. Maru, D., J. Pierson, and C. King, *Himalayan Health: Improving Hospitals and Clinics in Western Nepal*. Vol. 4. 2013. 59-65.

7. Saffarzadeh, M., et al., *Neutrophil extracellular traps directly induce epithelial and endothelial cell death: a predominant role of histones.* PloS one, 2012. **7**(2): p. e32366-e32366.

8. Xu, Z., et al., *Sepsis and ARDS: The Dark Side of Histones.* Mediators of inflammation, 2015. **2015**: p. 205054-205054.

9. Gotts, J.E. and M.A. Matthay, *Sepsis: pathophysiology and clinical management.* BMJ, 2016. **353**: p. i1585.

10. Aird, W.C., *The role of the endothelium in severe sepsis and multiple organ dysfunction syndrome.* Blood, 2003. **101**(10): p. 3765-3777.

11. Chawla, L.S., et al., *The Epithelium as a Target in Sepsis.* Shock, 2016. **45**(3): p. 249-258.

12. Raymondos, K., et al., *Outcome of acute respiratory distress syndrome in university and non-university hospitals in Germany.* Critical Care, 2017. **21**(1): p. 122.

13. Fisher, B.J., et al., *Mechanisms of attenuation of abdominal sepsis induced acute lung injury by ascorbic acid.* American Journal of Physiology-Lung Cellular and Molecular Physiology, 2012. **303**(1): p. L20-L32.

14. Howell, M.D. and A.M. Davis, *Management of Sepsis and Septic Shock.* JAMA, 2017. **317**(8): p. 847-848.

15. Rhodes, A., et al., *Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016.* Intensive Care Medicine, 2017. **43**(3): p. 304-377.

16. De Waele, J.J., et al., *Antimicrobial resistance and antibiotic stewardship programs in the ICU: insistence and persistence in the fight against resistance. A position statement from ESICM/ESCMID/WAAAR round table on multi-drug resistance.* Intensive Care Medicine, 2018. **44**(2): p. 189-196.

17. Lambden, S., et al., *The SOFA score-development, utility and challenges of accurate assessment in clinical trials.* Critical care (London, England), 2019. **23**(1): p. 374-374.

18. Moreno, R., *Acute renal failure in the ICU: Risk factors and outcome evaluated by the SOFA score*. ResearchGate. p. Table over SOFA-score.

19. De Backer, D., et al., *Comparison of Dopamine and Norepinephrine in the Treatment of Shock.* New England Journal of Medicine, 2010. **362**(9): p. 779-789.

20. De Backer, D.M., PhD, et al., *Dopamine versus norepinephrine in the treatment of septic shock: A meta-analysis*.* Critical Care Medicine, 2012. **40**(March): p. 725-730.

21. Seymour, C.W., et al., *Time to Treatment and Mortality during Mandated Emergency Care for Sepsis.* New England Journal of Medicine, 2017. **376**(23): p. 2235-2244.

22. Padayatty, S.J. and M. Levine, *Vitamin C: the known and the unknown and Goldilocks.* Oral diseases, 2016. **22**(6): p. 463-493.

23. Bartholomew, M., *James Lind's Treatise of the Scurvy (1753).* Postgraduate Medical Journal, 2002. **78**(925): p. 695.

24. Amano, A., et al., *Effect of ascorbic acid deficiency on catecholamine synthesis in adrenal glands of SMP30/GNL knockout mice.* European Journal of Nutrition, 2014. **53**(1): p. 177-185.

25. Hemilä, H., *Vitamin C and Infections.* Nutrients, 2017. **9**(4): p. 339.

26. Carr, A.C. and S. Maggini, *Vitamin C and Immune Function.* Nutrients, 2017. **9**(11).

27. Mohammed, B.M., et al., *Vitamin C: a novel regulator of neutrophil extracellular trap formation.* Nutrients, 2013. **5**(8): p. 3131-3151.

28. Manzetti, S., J. Zhang, and D. van der Spoel, *Thiamin Function, Metabolism, Uptake, and Transport.* Biochemistry, 2014. **53**(5): p. 821-835.

29. Hall, J.E., *Guyton and Hall textbook of medical physiology*. Vol. 13th. 2016. 1168.

30. Annane, D., et al., *Corticosteroids for treating sepsis.* The Cochrane database of systematic reviews, 2015. **2015**(12): p. CD002243-CD002243.

31. Keh, D., et al., *Effect of Hydrocortisone on Development of Shock Among Patients With Severe Sepsis: The HYPRESS Randomized Clinical Trial.* JAMA, 2016. **316**(17): p. 1775-1785.

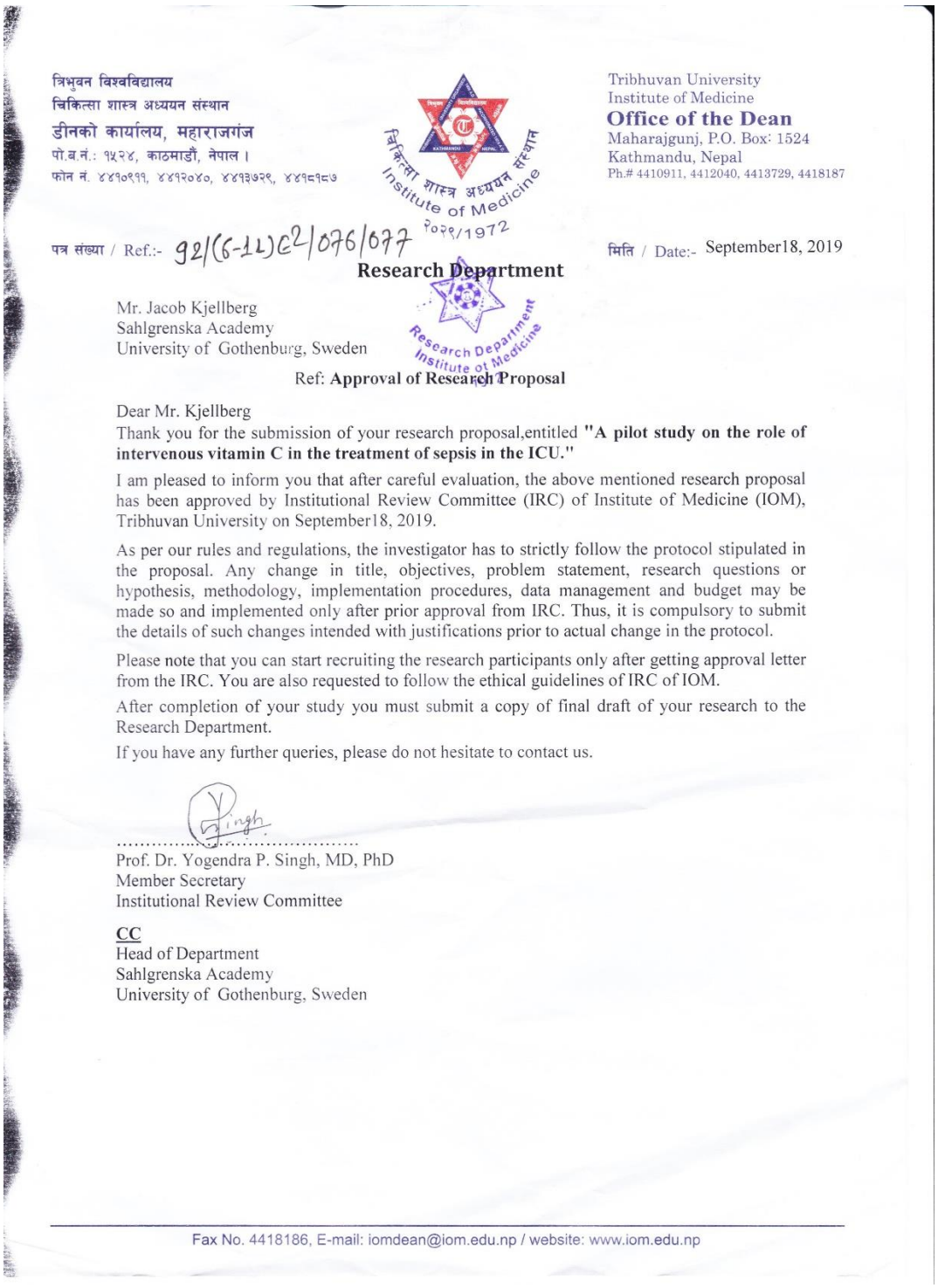
32. Marik, P.E., et al., *Hydrocortisone, Vitamin C, and Thiamine for the Treatment of Severe Sepsis and Septic Shock: A Retrospective Before-After Study.* Chest, 2017. **151**(6): p. 1229-1238.

33. Fowler, A.A., III, et al., *Effect of Vitamin C Infusion on Organ Failure and Biomarkers of Inflammation and Vascular Injury in Patients With Sepsis and Severe Acute Respiratory Failure: The CITRIS-ALI Randomized Clinical Trial.* JAMA, 2019. **322**(13): p. 1261-1270.

34. Moskowitz, A., et al., *Ascorbic Acid, Corticosteroids and Thiamine in Sepsis (ACTS) protocol and statistical analysis plan: a prospective, multicentre, double-blind, randomised, placebo-controlled clinical trial.* BMJ Open, 2019. **9**(12): p. e034406.

10. Tables, Figures and Appendices

10.1 Ethical agreement



त्रिभुवन विश्वविद्यालय
चिकित्सा शास्त्र अध्ययन संस्थान
डीनको कार्यालय, महाराजगंज
पो.ब.नं.: १५२४, काठमाडौं, नेपाल ।
फोन नं. ४४१०९११, ४४१२०४०, ४४१३७२९, ४४१८१८७

Tribhuvan University
Institute of Medicine
Office of the Dean
Maharajgunj, P.O. Box: 1524
Kathmandu, Nepal
Ph.# 4410911, 4412040, 4413729, 4418187

पत्र संख्या / Ref.:- 92/(6-11)E2/076/077

मिति / Date:- September18, 2019

Research Department

Mr. Jacob Kjellberg
Sahlgrenska Academy
University of Gothenburg, Sweden

Ref: **Approval of Research Proposal**

Dear Mr. Kjellberg

Thank you for the submission of your research proposal,entitled **"A pilot study on the role of intervenous vitamin C in the treatment of sepsis in the ICU."**

I am pleased to inform you that after careful evaluation, the above mentioned research proposal has been approved by Institutional Review Committee (IRC) of Institute of Medicine (IOM), Tribhuvan University on September18, 2019.

As per our rules and regulations, the investigator has to strictly follow the protocol stipulated in the proposal. Any change in title, objectives, problem statement, research questions or hypothesis, methodology, implementation procedures, data management and budget may be made so and implemented only after prior approval from IRC. Thus, it is compulsory to submit the details of such changes intended with justifications prior to actual change in the protocol.

Please note that you can start recruiting the research participants only after getting approval letter from the IRC. You are also requested to follow the ethical guidelines of IRC of IOM.

After completion of your study you must submit a copy of final draft of your research to the Research Department.

If you have any further queries, please do not hesitate to contact us.

Prof. Dr. Yogendra P. Singh, MD, PhD
Member Secretary
Institutional Review Committee

CC
Head of Department
Sahlgrenska Academy
University of Gothenburg, Sweden

Fax No. 4418186, E-mail: iomdean@iom.edu.np / website: www.iom.edu.np