

This supplement contains the following items:

1. Summary of changes.
2. Original version of the trial protocol.
3. Final version of the trial protocol.

1. Summary of changes

Protocol version 6 is the first version of the protocol approved by the Medical Ethical Committee VUmc (before recruitment of the first patient).

Protocol version 7 includes that prehospital data can be obtained from the ambulance services (also described in the consent forms).

Protocol version 8 includes the new principle investigator at ErasmusMC.

Non-substantial changes in this amendment includes:

- The minimal reflection period for deferred consent of 2 hours was made advisory.
- The exact timing of obtaining the questionnaires was further explained, with a small window around days 28 and 180 to make it logistically feasible.
- That survival data was collected at day 28 instead of day 30, to enable the calculation of ventilator- and vasopressor free days at day 28.
- That SAEs were collected up to day 28 post-randomization for included patients. This collection period was not clarified earlier.

Protocol version 9 includes the new principle investigator at ErasmusMC, elaboration about collecting primary outcome data of ICU-transferred patients from the hospitals involved (also described in the consent forms), and information about non-invasive mitoPO2 measurements (part of new substudy OLVG)

Non-substantial changes in this amendment includes:

- When patients were transferred to another hospital <28 days, SAEs were collected up to transfer.

Protocol version 10 includes the new principle investigator at Maasstad Hospital, the definitions and protocol follow-up of the mITT and full analysis population, clarification that the first dose must have been administered within 5 hours after ROSC. At study start <4 hours was aimed for, with potential extension up to 5 hours. So practically, this clarification in the study protocol reflected the actual workflow since study start.

Early high-dose vitamin C in post-cardiac arrest syndrome.

PROTOCOL TITLE 'Early high-dose vitamin C in post-cardiac arrest syndrome'

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)
AE	Adverse Event
AR	Adverse Reaction
CA	Competent Authority
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CV	Curriculum Vitae
DSMB	Data Safety Monitoring Board
EU	European Union
EudraCT	European drug regulatory affairs Clinical Trials
GCP	Good Clinical Practice
IB	Investigator's Brochure
IC	Informed Consent
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
METC	Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)
(S)AE	(Serious) Adverse Event
SPC	Summary of Product Characteristics (in Dutch: officiële productinformatie IB1-tekst)
Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.
SUSAR	Suspected Unexpected Serious Adverse Reaction
Wbp	Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgegevens)
WMO	Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen)

SUMMARY

Background of the study:

Only half of the patients suffering from cardiac arrest arrive at the hospital alive. Of these survivors, more than 50% will still die or remain severely disabled. During cardiac arrest ischemia causes damage to the vital organs, especially the brain. When with return of spontaneous circulation oxygen is re-offered to the ischemic organs, massive amounts of reactive oxygen species (ROS) are produced. These ROS can further increase the damage to the myocardium and brain (reperfusion injury). Vitamin C is the primary circulating antioxidant. It scavenges free radicals and reduces the production of ROS. In a recent study we demonstrated that vitamin C plasma levels are deficient in ~60% of the patients after cardiac arrest, probably due to massive consumption. Vitamin C deficiency reduces the protection against oxidative stress. Intravenous supplementation is needed to restore deficiency and the antioxidative effect of vitamin C is much more potent if it is administered in a supraphysiological dose (≥ 3 g per day). Its strong antioxidative effect may reduce damage to the circulation and to brain, heart and other organs. Beneficial effects of high dose i.v. vitamin C after cardiac arrest have been demonstrated in preclinical studies, but not in patients.

Objectives:

- To determine whether an early high dose i.v. vitamin C can improve organ function, especially neurological outcome, in patients after cardiac arrest.
- To explore the optimal dosing regimen for high dose i.v. vitamin C.
- To investigate *in vitro* the difference in effect of plasma obtained from post cardiac arrest patients treated with placebo, 3 g/day or 10 g/day vitamin C on endothelial cell viability and underlying oxidative pathways.

Study design:

In this multicentre, placebo controlled double-blind randomized clinical trial patients will be recruited from the Shock Rooms/Intensive Care Units of 8 hospitals.

360 adult comatose patients suffering an out-of-hospital cardiac arrest with ventricular fibrillation/tachycardia as first registered cardiac rhythm and EMV-score ≤ 8 will be included (we aim for 270 evaluable patients).

As soon as possible patients will be randomly allocated to one of 3 treatment groups of 120 (90 evaluable) patients each and vitamin C or placebo will be started for 96 hours. Group 1 will be treated with placebo, group 2 with a twice daily a bolus of 1.5 g vitamin C and group 3 with a twice daily a bolus of 5 g vitamin C. All patients will receive thiamine 200 mg q 12 hourly for 4 days to limit the conversion of vitamin C to oxalate.

Primary study parameters:

We will determine organ failure at 96 hours measured by the delta (Δ) Sequential Organ Failure Assessment (SOFA) score. Δ SOFA score is defined as the difference between SOFA admission and SOFA at 96 hour.

Secondary study parameters :

As secondary outcomes we will investigate: neurological outcome, IC- and hospital stay, mortality, myocardial injury, vasopressor support, lung injury score, ventilation time, renal function, IC-acquired weakness, delirium, oxidative stress parameters and vitamin C plasma concentrations. In vitro we will determine the effect of plasma obtained from the trial patients on cultured human systemic microvascular endothelial cells with regard to endothelial cell viability, intracellular vitamin C concentrations and oxidative pathways.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness (if applicable):

A total of 100 ml blood will be taken during a period of four days from an arterial line which is routinely present for monitoring. The patient will not notice the blood sampling. He/she will not notice the collection of urine samples, because all patients have a bladder catheter.

The risk of potential side effects of high dose vitamin C is very small. There is a small risk of formation of oxalate crystals in the urine. Formation of kidney stones of oxalate crystals has only been observed after prolonged intake of high dose vitamin C. Vitamin C can exert pro-oxidative effects, predominantly in patients with hemochromatosis. Pro-oxidative effects are related to the formation of the ascorbyl radical (which poorly reacts with other molecules than itself), presence of free iron and the formation of H_2O_2 (which is rapidly scavenged by human erythrocytes and for which especially cancer cells are sensitive, not human cells). Pro-oxidative effects are hardly shown. In this study patients with hemochromatosis will be excluded. Sometimes high-dose i.v. vitamin C can lead to factitious hyperglycemia when measured with point-of-care devices. In our study we will measure blood glucoses during the period of vitamin C administration by blood gas analysis or in the central laboratory.

1. INTRODUCTION AND RATIONALE

1.1 Problem definition

In Europe, each day more than 1000 patients suffer from cardiac arrest¹. Despite improvement of medical technologies mortality is still very high, around 75 - 80%. Of the patients who initially survive to ICU admission, more than 50% still dies or remains severely disabled due to the post cardiac arrest syndrome (PCAS)². Crucial in this syndrome is the overwhelming oxidative stress, which is caused by systemic ischemia/reperfusion injury and leads to destruction of endothelial function³ with cardiovascular failure and brain damage⁴. Besides targeted temperature management, we have no effective therapy to improve prognosis. The levels of our primary circulating antioxidant, vitamin C, are markedly depressed after cardiac arrest⁵. Early, high dose intravenous (i.v.) vitamin C administration can boost the body's antioxidant defence, and could be a new promising therapeutic intervention to improve clinical outcome by limiting oxidative damage.

1.2 Rationale high dose vitamin C

Vitamin C administration is often wrongly considered as complementary or even alternative medicine, which does not do justice to the strong scientific base of the pleiotropic antioxidative effects of high-dose i.v. (not enteral!) vitamin C administration as demonstrated in multiple preclinical and clinical studies³. With enteral supplementation maximally tolerated dosages cannot achieve plasma levels of $> 250 \mu\text{mol/l}$ ⁶ due to limited absorption⁷. In critically ill patients, enteral supplementation even cannot restore deficiency due to the acutely increased requirements⁸. I.v. vitamin C administration generates much higher plasma levels, thus yielding more and more potent antioxidative effects. The underlying pathophysiological mechanisms are well elucidated. High plasma levels of vitamin C not only limit the generation of reactive oxygen species (ROS), repair other oxidized scavengers such as glutathione and modulate numerous enzyme

reactions, but can also act as a direct radical scavenger. In addition, vitamin C maintains nitric oxide mediated endothelial integrity and vasomotor control^{9,10}. Furthermore, vitamin C is a cofactor in several biosynthetic pathways, such as collagen, catecholamines and peptide hormones. Deficiency will decrease their formation. Vitamin C can thereby recover endogenous vasopressor synthesis¹¹ and improve wound healing.

Post cardiac arrest huge amounts of ROS are generated by various pathways. The main source of ROS are the mitochondria due to uncoupling of oxidative phosphorylation. In addition, ROS are produced by upregulated enzymes such as NADPH oxidase¹² or during oxidation of catecholamines¹³. When unopposed these ROS can damage virtually every biomolecule and cause severe endothelial dysfunction. This has been demonstrated *in vitro*: plasma derived from patients after cardiac arrest induced massive cell death of cultured endothelial cells due to pro-oxidant stress and deterioration of anti-oxidant defences¹⁴. Cell death was highest immediately after admission to the ICU.

1.3 Vitamin C depletion

This overpowering oxidative stress during PCAS can quickly exhaust body stores of vitamin C due to massive cellular consumption and reduced regeneration. We have shown that vitamin C plasma concentrations were decreased by more than 50% compared to healthy volunteers already on the first day after cardiac arrest. After 3 days plasma concentrations further declined and more than half the patients were deficient. Low vitamin C levels were associated with multiple organ dysfunction (higher SOFA scores) and mortality. Other studies, though investigating septic and not post cardiac arrest patients, also show markedly depressed vitamin C levels on the day of admittance (~ 10 and $6 \mu\text{mol/l}$)^{15,16} and an association between low vitamin C levels and multiple organ failure¹⁷.

However, these deficient vitamin C levels in critically ill patients often will go unnoticed. Due to the complexity and cost of its laboratory measurement plasma levels are not available in daily practice. In addition, the vitamin C content of enteral nutrition is assumed

to be sufficient. However, current nutrition protocols (even with immune enhanced nutrition) fail to normalize vitamin C levels^{8,18}. These low plasma levels are likely to reflect real deficiency, since they are accompanied by scorbutic intracellular leucocyte vitamin C levels as well¹⁹. Even with i.v. vitamin C dosages up to 1 g per day vitamin C depletion persists^{15,16}.

1.4 (Pre) clinical studies

Multiple preclinical experiments support the potential beneficial effect of high-dose i.v. vitamin C post cardiac arrest. In a rat cardiac arrest model vitamin C administration immediately after ROSC improved survival rate and neurological outcome and decreased myocardial damage²⁰. In organ-specific ischemia-reperfusion models of kidney, liver and skeletal muscle i.v. vitamin C ameliorated respectively renal structure and function²¹, bile flow and cholate secretion²² and muscle function²³.

Up to now no clinical study specifically addressed the post cardiac arrest population, but several controlled studies in critically ill patients showed favourable results³. In critically ill surgical patients 3 g i.v. vitamin C per day reduced pulmonary morbidity, new organ failure, duration of ICU/hospital stay and mortality^{24,25}. In burn patients very high dose i.v. vitamin C (66 mg/kg/hr) reduced fluid requirements, body weight gain and respiratory dysfunction. In a recent pilot trial of patients with severe sepsis vitamin C both 50 mg/kg/day and 200 mg/kg/day caused earlier recovery from organ failure with reduction of the pro-inflammatory biomarkers¹⁸. In a before and after study of patients with septic shock high dose i.v. vitamin C combined with i.v. thiamine and stress dose steroids substantially accelerated shock reversal and improved survival²⁶. Two studies in critically ill patients administering respectively 2.7 g/day and 1.5 g/day showed no clinical benefit. These different results might be explained by difference in timing (relatively late²⁷) and route of administration (enteral²⁸). None of the clinical studies reported negative results of vitamin C.

1.5 Safety of high dose vitamin C

Up to now, no adverse events due to high-dose vitamin C have been reported^{18,24,29}, even with extremely high dosing schedules²⁹. Theoretical risks comprise a paradoxal pro-oxidative effect in case of iron overload, oxalate kidney stones and factitious hyperglycemia. In critically ill patients with sepsis 200 mg/kg/day¹⁸ and in cancer patients even megadoses up to 1500 mg/kg i.v. vitamin C three times weekly³⁰ were tolerated without significant side effects. Vitamin C can reduce catalytic metals such as Fe^{2+} and Cu^{2+} with adverse, pro-oxidative effects in patients with hemochromatosis. These patients are excluded in most studies and will also be excluded in our study. High dose vitamin C increases urinary oxalate excretion. However, oxalate nephrocalcinosis and calcium oxalate stones take months to years to develop and none of the studies with short-term vitamin C administration reported kidney stone formation^{18,24,29}. Sometimes high-dose i.v. vitamin C can lead to factitious hyperglycemia when measured with point-of-care devices, mostly at dosages much higher than used in our study. We will measure blood glucoses during the period of vitamin C administration by blood gas analysis or in the central laboratory.

We hypothesize that vitamin C reduces organ damage, especially cerebral injury, if administered for a short period as a high i.v. dose during the very early phase of reperfusion after cardiac arrest.

2. OBJECTIVES

Primary Objective:

- To determine whether an early high dose iv vitamin C improves organ function, especially neurological outcome, in patients after cardiac arrest.

Secondary Objectives:

- To explore the optimal dosing regimen for high dose i.v. vitamin C.
- To investigate *in vitro* the difference in effect of plasma obtained from post cardiac arrest patients treated with placebo, 3 g/day or 10 g/day vitamin C on endothelial cell viability and underlying oxidative pathways.

3. STUDY DESIGN

Design: double blind randomized, clinical multicenter trial

Duration: 3 – 4 years

Setting: Intensive Care Units of: VU University Medical Centre, Gelderse Vallei

Hospital, Franciscus Gasthuis & Vlietland, Tergooiziekenhuizen, Amphia Hospital, Erasmus

Medical Centre, Noordwest Ziekenhuisgroep, OLVG Oost and Maasstad Hospital.

4. STUDY POPULATION

4.1 Population (base)

The study population consists of adult patients admitted to the Intensive Care Units of: VU University Medical Centre, Gelderse Vallei Hospital, Franciscus Gasthuis & Vlietland, Tergooiziekenhuizen, Amphia Hospital, Erasmus Medical Centre, Noordwest Ziekenhuisgroep, OLVG Oost and Maasstad Hospital.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- An out-of-hospital cardiac arrest with return of spontaneous circulation;
- Ventricular fibrillation or ventricular tachycardia as first registered cardiac rhythm;
- EMV-score ≤ 8 .

4.3 Exclusion criteria

A potential subjects who meet any of the following criteria will be excluded from participation in this study:

- Patients with terminal renal insufficiency, i.e. receiving renal replacement therapy (RRT);
- Known glucose 6-phosphate dehydrogenase deficiency (risk of hemolysis);
- History of urolithiasis, oxalate nephropathy or hemochromatosis;
- Treatment limitations.

4.4 Sample size calculation

The Δ SOFA score at 96 hours has an estimated standard deviation of 3.5 points (highest estimate based on previous data in our hospital and our recent systematic review³¹). The study is powered to detect a clinically meaningful effect of 2.0 SOFA points difference between the trial arms. Eighty-two patients per group are needed to detect a treatment effect

of 2 SOFA points with 90% power and a two-sided alpha of 0.01. The type-I error rate is set at 0.01 instead of 0.05 to minimize the risk of false-positive results. With an estimated 10% loss after randomization for late outcomes, the study aims to include 3x90 patients. If we estimate that 50% of the 1500 potential study patients will not be eligible and that 50% of the patients will refuse consent, we expect to have more than enough safety margin to recruit 360 patients, of whom 270 will be evaluable, in 3 years.

We consider a 2-point difference in Δ SOFA score between the intervention- and control groups a meaningful effect. Firstly, a 2-point difference is sufficiently large to be clinically relevant and to impact future practice. For example, 2 SOFA points is the difference between mild neurologic deficit and coma, between moderate kidney dysfunction and dialysis-dependent kidney failure, or between moderate oxygenation disturbance and life-threatening respiratory failure. Secondly, the association between Δ SOFA score and outcomes is such that a 2-point difference can lead to real and measurable reductions in length-of-stay, mortality and costs when the intervention is broadly implemented: each 1 point increase in Δ SOFA score at 96 hours is associated with a 1.24 (95%CI 1.04-1.47) odds ratio of mortality^{5,32}.

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

Included patients receive a placebo, 1.5 g vitamin C b.i.d. or 5 g vitamin C b.i.d within 4 hours of hospital admission for 4 days. Vitamin C will be administered intravenously as ascorbic acid 1.5 per 50 ml NaCl 0.9% or 5 gr per 50 ml NaCl 0.9%. The study thereby compares 2 doses equivalent to those currently used in clinical studies in critically ill patients^{18,26,33}. The placebo will be administered intravenously and consists of 50 ml NaCl 0.9% .

Vitamin C is being used in accordance with the registered indication of vitamin C deficiency. The investigational aspect is the administration of higher dosages of vitamin C than registered in the summary of product characteristics (SPC). However, the use of much higher doses up to 66 mg/kg/h in critically ill burn patients has been reported (see paragraph 1.4).

5.2 Use of co-intervention (if applicable)

All patients will receive thiamine 200 mg q 12 hourly for 4 days to limit the conversion of vitamin C to oxalate²⁶.

5.3 Escape medication (if applicable)

Not applicable.

6. INVESTIGATIONAL PRODUCT

Name and description of investigational product(s)

Placebo will be administered intravenously and consists of 50 ml NaCl 0.9%.

Vitamin C will be administered intravenously 1.5 g per 50 ml NaCl 0.9% or 5 g per 50 ml NaCl 0.9% (ascorbic acid 100 mg/ml, Centrafarm, Etten-Leur, the Netherlands).

The LUMC pharmacy is currently developing ready-to-use study medication. When the production of this study medication has been finished, we will notify the METc and the competent authority with an substantial amendment.

6.1 Summary of findings from non-clinical studies

See 1.4, and as attachments our reviews in Crit Care 2014^{3,34}

6.2 Summary of findings from clinical studies

See 1.4, and as attachments our review in Crit Care 2014^{3,34}.

6.3 Summary of known and potential risks and benefits

Potential risks

Up to now, no adverse events of high-dose vitamin C has been reported, even with extremely high dosing schedules^{29,30}. Theoretical risks of high dose i.v. vitamin C are oxalate kidney stones, a paradoxical ROS generating effect in case of iron overload, toxicity of adjuvants and factitious hyperglycemia. Patients with kidney stones, iron overload and glucose 6-phosphate dehydrogenase deficiency (developing hemolysis with drugs) will be excluded from our study.

Several safety and tolerability studies have been done in cancer patients, cardiovascular

patients and in critically ill patients with sepsis^{18,26,30,35,36}. In cancer patients a megadose of 1500 mg/kg i.v. three times weekly was tolerated without important adverse effects³⁰. The JAMA published a controlled trial enrolling 1708 cardiovascular patients, who received infusions of 7 g vitamin C weekly for 40 weeks in addition to chelation therapy, or placebo. No difference was found in adverse events between groups³⁶. In critically ill septic patients, vital signs were monitored every 5 min during and after infusion of 200 mg vitamin C per kg/day by bedside IC nurses. No side effects were reported¹⁸.

Urinary oxalate crystallization.

High dose vitamin C can increase urinary oxalate excretion, since oxalate is a metabolic end product of vitamin C³⁷. In renal insufficiency, conversion to oxalate may be increased (or not). Worsening of renal function is therefore a concern with high dose vitamin C. However, in the very recently published before and after study in septic patients high-dose vitamin C for 4 days yielded renal benefit, no harm. The need of renal replacement therapy declined from 37% to 10% and renal function improved in all the patients with acute kidney injury²⁶. The high urine concentrations of vitamin C might protect renal tubular cells against oxidative stress. In this study patients thiamine was added to reduce oxalate excretion²⁶. Thiamine is cofactor for glyoxylate aminotransferase which promotes the oxidation of glyoxylate (a byproduct of the intermediary metabolism) to carbon dioxide (instead of oxalate). A higher conversion of vitamin C to oxalate is seen in thiamine deficiency and thiamine deficiency is common in septic patients.³⁸ Oxalate nephrocalcinosis and calcium oxalate stones develop over months to years in primary hyperoxaluria, a disease in which oxalic acid excretion exceeds 100 mg/day and can reach 400 mg/day³⁹. In susceptible patients, prolonged high dose therapy for weeks or months can indeed cause oxalate nephropathy⁴⁰⁻⁴². Generally *acute* oxalate nephropathy has been described after prolonged intake of massive doses (ten-times higher than in the present study), in combination with underlying renal disease or use of non-steroidal anti-inflammatory drugs⁴³. In our study vitamin C will be infused for four days only (as in the

other studies with vitamin C in critically ill patients) and patients with a history of renal stones, oxalate nephropathy or those with a terminal renal insufficiency will be excluded. In addition, we will also administer thiamine to our study patients. Renal function of all patients at our ICU is monitored routinely by daily determination of serum creatinine, estimated Glomerular Filtration Rate (eGFR) and fluid balance. Diuresis is monitored hourly. When indicated, an urine sediment analysis or echography of the kidneys will be carried out.

Pro-oxidant properties

Vitamin C has pro-oxidant properties as well. Pro-oxidative effects are related to the formation of the ascorbyl radical, which poorly reacts with other molecules than itself; presence of free iron and the formation of H_2O_2 , which is rapidly scavenged by human erythrocytes and for which especially cancer cells are sensitive, not human cells⁴⁴. It can generate very low levels of superoxide and reduce catalytic metals such as Fe^{3+} and Cu^{3+} , and thereby initiate radical chain oxidations. Vitamin C could therefore have pro-oxidative effects in case of iron overload. Consequently, in our trial patients with hemochromatosis will be excluded. However, in other settings pro-oxidative effects are not or only controversially shown⁴⁵.

Factitious hyperglycemia

Sometimes high-dose i.v. vitamin C can lead to factitious hyperglycemia when measured with point-of-care devices, mostly at dosages much higher than used in our study⁴⁶. We will measure blood glucoses during the period of vitamin C administration by blood gas analysis or in the central laboratory⁴⁷.

Potential benefits

See also the introduction. Of the yearly number of 6000 Dutch patients who suffer from an out-of-hospital cardiac arrest, 3000 survive to hospital. 50% of these post-cardiac arrest

IC patients also survive to hospital discharge². Each 1 point decrease in Δ SOFA score at 96 hours is associated with a 1.24 (95%CI 1.04-1.47) odds ratio of survival^{5,32}. Thus, at a 50% mortality rate, this would translate into an absolute survival effect of 5.5% per SOFA point (10% per 2 points). However, we overstretch the statistics if we directly translate this into a number of 'lives saved' for the at-risk population.

6.4 Description and justification of route of administration and dosage

With enteral supplementation maximally tolerated dosages cannot achieve plasma levels of $> 250 \mu\text{mol/l}$ ⁶ due to limited absorption⁷. Moreover, in critically ill patients, deficiency cannot be restored by the enteral route. I.v. vitamin C administration generates much higher plasma levels, thus yielding more and more potent antioxidative effects (see also 1.2). The optimal dose of vitamin C is not settled yet. In our trial we will compare two different dosages to placebo (group 1), one aiming at (high-) normal plasma concentrations (group 2) and one targeting supranormal concentrations (group 3).

I.v. vitamin C in a dose of 3 g/day appears to be the minimal daily dosage necessary to normalize vitamin C levels in critically ill patients and this dosage proved to be beneficial in 2 clinical studies^{24,25}. Our pharmacokinetic trial showed that a twice daily 1 g vitamin C is required to quickly normalize plasma levels and twice daily 5 g to achieve supra-normal plasma concentrations with peaks above 1mM, the concentration needed for direct superoxide scavenging⁴⁸. We chose for group 2 1.5 g vitamin C b.i.d (3 g/day) to normalize vitamin C plasma levels.

The choice for the dosage in group 3 was subject of intense discussion among co-investigators, because the high dosages used in clinical studies vary tremendously. In critically ill patients, high dosages varying from 3 up to even 126 g i.v. vitamin C/day were used. In a small study with septic patients 16 g/day resulted in better recovery from organ failure and more reduction of pro-inflammatory biomarkers compared to 4 g/day¹⁸. In septic patients the before and after trial with 6 g/day showed a huge effect on mortality²⁶.

The dosage of 126 g/day used in a small study with burn patients was much higher (10-fold)²⁹ than the dosage used in the other studies showing significant effects of vitamin C^{18,26}. Considering all these data, we chose for group 3 a dosage of 5 g vitamin C b.i.d. for 4 days (10 g/day).

6.5 Dosages, dosage modifications and method of administration

See also 6.4.

As soon as possible (<4 hours) after their arrival at the Emergency Department, patients will be randomly allocated to one of 3 treatment groups of 90 patients each and vitamin C or placebo will be started for 96 hours.

Group 1 will be treated with placebo, group 2 with 1.5 g vitamin C b.i.d. (3 g/day) and group 3 with 5 g vitamin C b.i.d. for 4 days (10 g/day). After these 4 days the overwhelming oxidative stress and associated organ damage will have settled down and body stores of vitamin C will be restored. Vitamin C administration will then be lowered to standard nutritional dose to allow the physiological signaling and repair function of low concentrations of ROS.

Early timing. The first infusion of vitamin C will start within four hours after emergency room admission. Reason for this early start is that the oxidative damage of patient plasma to endothelial cells was highest early after resuscitation¹⁴. IC admission indicates the point where the pro/antioxidant balance is lost and the oxidative stress response becomes uncontrolled. Starting the infusion before this point seems crucial. We hypothesize that at this early timing, when the body's feedback mechanisms fail, high dose i.v. vitamin C can minimize further damage to endothelium and organs.

6.6 Preparation and labelling of Investigational Medicinal Product

The vitamin C ampoules of Centrafarm will be labelled in conformity with EU GMP Annex 13 by the VUmc pharmacy. Unblinded nurses will be trained to prepare the study medication in perfusor syringes according to a standard operating procedure:

- Placebo, 50 ml NaCl 0.9%
- 1,5 gram vitamin C in 50 ml NaCl 0.9%
- 5 gram vitamin C in 50 ml NaCl 0.9%

6.7 Drug accountability

- Production by Centrafarm B.V., Ettenleur, the Netherlands.
- The pharmacy of the VUmc will deliver the Annex 13 labeled Centrafarm vitamin C to the Intensive Care/Medium Care units of the VUmc and to the pharmacies of the other 8 participating hospitals.
- The transport from VUmc to the other centra will be carried out by a certified courier with temperature monitoring.
- Receipt of medication according to local procedures with QP release certificate and drug accountability.
- Destruction by all nine centra according to local procedures.

7. NON-INVESTIGATIONAL PRODUCT

7.1 Name and description of non-investigational product(s)

Thiamine will be administered intravenously (200 mg q 12 hourly for 4 days).

Thiamine is a cofactor for glyoxylate aminotransferase which promotes the oxidation of glyoxylate (a byproduct of the intermediary metabolism) to carbon dioxide (instead of oxalate).

7.2 Summary of findings from non-clinical studies

A study in which a thiamine-deficient diet was applied to rats for 4 weeks showed that more oxalate was excreted in the urine, causing hyperoxaluria⁴⁹.

7.3 Summary of findings from clinical studies

Because of the high prevalence of thiamine deficiency in critically ill patients, several clinical studies have been performed to investigate the potential benefit of thiamine administration. These studies do not show uniform results. Doses and timing differed substantially between studies.

Cardiac surgical patients

In two studies preventive administration of thiamine in cardiac surgical patients did not affect post-operative lactate levels or clinical outcome. However, cellular oxygen consumption was higher in the thiamine group⁵⁰.

Sepsis

In a small study in patients with septic shock⁵¹ thiamine did not reduce lactate levels or mortality in the overall group. However, in a subgroup of patients with thiamine deficiency at baseline, thiamine decreased lactate levels and lower mortality. Secondary analysis of this study showed lower creatinine levels and less progression to renal function requiring renal replacement therapy in the thiamine group (overall population). The previously mentioned before/after study in patients with severe sepsis or septic shock, thiamine in combination with vitamin C and hydrocortisone showed an huge reduction in organ dysfunction and mortality and improvement of renal function.²⁶

Burns

In a very small study of critically ill burned patients thiamine administration significantly reduced pyruvate and lactate levels ⁵².

Trauma

In a mixed population of patients admitted to the ICU after complicated cardiac surgery, subarachnoidal hemorrhage or major trauma intravenous administration of a mixture of selenium, zinc, vitamin C and thiamine (100 mg/day) did not improve organ function, but attenuated the inflammatory response in cardiac surgery/trauma patients and decreased hospital length-of-stay ²⁷.

7.4 Summary of known and potential risks and benefits

Potential risks

Enteral and intravenous administration of thiamine hardly causes any side effects. This is supported by animal toxicological data. The only risk of intravenous administration is the occurrence of an anaphylactic reaction. Doses of more than 400 mg may induce nausea, anorexia, and mild ataxia.

Potential benefits

The administration of thiamine to vitamin C may reduce the risk of oxalate nephropathy as thiamine promotes the conversion of glyoxylate to carbon dioxide instead of oxalate in case of thiamine deficiency. Furthermore it acts synergistically with vitamin C to attenuate oxidative stress, cellular apoptosis and restore organ function.

7.5 Description and justification of route of administration and dosage

The Recommended Daily Intake of thiamine is 1.1-1.2 mg/day for adults. Standard enteral nutrition contains 2-3 mg and parenteral nutrition 3 – 4 mg/day. The doses recommended for refeeding syndrome are 200 – 300 mg i.v./day, whereas for Wernicke's encephalopathy 200 - 500 mg i.v. 3 times daily. Intravenous suppletion can restore thiamine levels in critically ill patients within hours. In the clinical studies

in critically ill patients doses in the same range to those administered for refeeding and Wernicke's encephalopathy were used.

7.6 Dosages, dosage modifications and method of administration

Patients will receive 200 mg thiamine intravenously, every 12 hours during 4 days.

Thiamine will be administered together with vitamin C.

7.7 Preparation and labelling of Non Investigational Medicinal Product

The product will be delivered by the hospital pharmacy.

7.8 Drug accountability

n.a.

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter

We will determine organ failure at 96 hours measured by the delta (Δ) Sequential Organ Failure Assessment (SOFA) score. Δ SOFA score is defined as the difference between SOFA admission and SOFA at 96 hour. Death at 96-hours will be counted as the maximum SOFA score (24 points). The SOFA-score is more extensively described in the table below.

Table 1. Sequential Organ Failure Assessment Score (SOFA)					
	Score				
System	0	1	2	3	4
Respiration					
<i>PaO₂/FiO₂, mmHg (kPa)</i>	≥ 400 (53.3)	< 400 (53.3)	< 300 (40)	< 200 (26.7) with ventilation assistance	< 100 (13.3) with ventilation assistance
Coagulation					
Platelets, $\times 10^3/\mu\text{L}$	≥ 150	< 150	< 100	< 50	< 20
Liver					
<i>Bilirubin, mg/dL ($\mu\text{mol/L}$)</i>	< 1.2 (20)	1.2-1.9 (20-32)	2.0-5.9 (33-101)	6.0-11.9 (102-204)	≥ 12.0 (204)
Cardiovascular	MAP ≥ 70 mmHg	MAP < 70 mmHg	Dopamine < 5 of dobutamine	Dobutamine > 5.1 -15 of adrenaline ≤ 0.1 of noradrenaline ≤ 0.1	Dobutamine > 15 of adrenaline ≤ 0.1 of noradrenaline ≤ 0.1
Central nervous system					
<i>Glasgow Coma Scale score</i>	15	13-14	10-12	6-9	< 6
Renal					
<i>Creatinine, mg/dL ($\mu\text{mol/L}$)</i>	< 1.2 (110)	1.2-1.9 (110-170)	2.0-3.4 (171-299)	3.5-4.9 (300-440)	> 5.0 (440)
<i>Urine output, mL/d</i>				< 500	< 200
Abbreviations: PaO ₂ : Partial pressure of oxygen; FiO ₂ : Fraction of inspired oxygen; MAP: Mean arterial pressure					

8.1.2 Secondary study parameters

As secondary outcomes we will investigate: neurological outcome (Maximal Glasgow Coma Score at 96-h and at the end of the ICU-stay; Cerebral Performance Categories scale and Modified Rankin Scale at 30 and 180 days; HUI-3 questionnaire at 180 days;

neuron-specific enolase day 1, 2 and 3); clinical parameters (IC- and hospital stay, 30-day and 90-day mortality), duration of vasopressor support, organ injury (myocardial injury measured by troponin and CK-MB (maximum day 1), lung injury score, ventilation time, renal function, need of renal replacement therapy, IC-acquired weakness (Medical Research Council score), delirium (CAM-ICU score), inflammation (C-reactive protein), oxidative stress parameters (such as F₂-isoprostanes and oxidation reduction potential (ORP)), anti-oxidant capacity (AOC) and vitamin C plasma concentrations.

8.1.3. Other study parameters

To determine the different effect of 3 gr or 10 g/day vitamin C or placebo on the underlying oxidative pathways we will investigate how plasma obtained from the trial patients affects cultured human systemic microvascular endothelial cells with regard to endothelial cell viability, ROS production intracellular vitamin C concentrations, NADPH-oxidase expression, p47Phox expression, endothelial barrier function and mitochondrial respiratory chain function.

8.2 Randomisation, blinding and treatment allocation

Randomisation will be performed with the use of a randomisation list generated by VUmc Pharmacy, Amsterdam, the Netherlands. Randomisation will be stratified based on site and age. Age is categorised in 2 groups: ≤66 years old and >66 years old. Patients will be assigned by an unblinded ICU/MC nurse to a subsequent study number which is linked to one of the study arms. The unblinded nurses will be trained by the investigators for the randomization, blinding and treatment allocation procedure. This training will be logged. The nurse who is attending the patient is blinded for the treatment group and will administer the study medication. The prepared perfusor syringes with vitamin C and placebo are identical in appearance and have a maximum shelf life of 24 hours. 1.5 grams

vitamin C, 5 grams vitamin C and placebo will be administered as a 50 cc bolus. Patients, physicians, and outcome assessors will be kept blinded to the allocation at all time.

8.3 Study procedures

- 1) Deferred consent by patient representative (*for study*)
- 2) Routine clinical data (demographic characteristics, reason of admission, comorbidity, APACHE II) (*part of medical treatment*)
- 3) Treatment with vitamin C or placebo for 4 days (*for study*)
- 4) Blood sample collection (non-invasive by arterial line) daily for 4 days for determination of vitamin C levels and parameters of oxidative stress (*in total 100 ml extra for study*). Remaining blood material after analysis will be stored for additional analyses in the future (*for study*)
- 5) Collection of urine samples during the first 4 days (*for study*)
- 6) After 30 and 180 days a follow up with questionnaires will be used to estimate the neurological condition (*for study*)

8.4 Withdrawal of individual subjects

The legal representative of the patient can decide to withdraw consent at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

8.5 Replacement of individual subjects after withdrawal

In case of withdrawal of individual subjects, subjects will be replaced according to inclusion and exclusion criteria.

8.6 Follow-up of subjects withdrawn from treatment

After withdrawal of subjects from the study, no specific follow up will be carried out.

8.7 Premature termination of the study

A blinded interim analysis focused on mortality will be carried out blinded by the independent methodologist (Prof. dr. J.W.R. Twisk) after inclusion of 135 **evaluable** patients. Analysis will be group comparative. The DSMB will be blinded, but may be unblinded by the independent methodologist on request without any further clarification. This request will be granted in all cases. In case of a significant larger mortality in the vitamin C group compared to placebo and based on thorough examination of the clinical content, the DSMB can advise to stop the trial. The DSMB will assess the unblinded data before arriving at a material recommendation.

9. SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1. Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the investigational product.

All included patients are admitted to the Intensive Care with a life-threatening disease.

Any adverse development can be expected as part of the underlying disease. Therefore, it is not achievable to report all these developments individually as AEs.

9.2.2 Serious adverse events (SAEs)

All included patients are admitted to the Intensive Care with a life-threatening disease.

Any major adverse development can be expected as part of the underlying disease.

Therefore, it is not achievable to report all these developments individually as SAEs.

Every three months we will send a line-listing of all SAEs to the METC.

Events that can be considered SAE's within the context of this study population are:

- death;
- end-stage organ failure, specifically:
 - Start of renal replacement therapy;
 - Start of mechanical circulatory support (intra-aortic balloon pump or extracorporeal life support);
 - Start of prone position ventilation;
 - Bilirubin > 50 µmol/l;
 - Any event that is unexpected in the course of the underlying disease and that results in chronic and significant disability or incapacity.

9.2.3. Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see chapter 9.2.2);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
 - Summary of Product Characteristics (SPC) for an authorised medicinal product;
 - Investigator's Brochure for an unauthorised medicinal product.

The sponsor will report expedited the following SUSARs through the web portal *ToetsingOnline* to the METC:

- SUSARs that have arisen in the clinical trial that was assessed by the METC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern.

The expedited reporting of SUSARs through the web portal *ToetsingOnline* is sufficient as notification to the competent authority.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report. The investigators in the other participating centres will reports SUSARs as soon as possible (within maximal 7 days) to the coordinating investigator. The

coordinating investigator will report these SUSARs through the web portal ToetsingOnline not later than 15 days after first knowledge of the adverse reactions.

9.3 Annual safety report

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States.

This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

9.4 Follow-up of adverse events

All SAEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

9.5 Data Safety Monitoring Board (DSMB)

An independent DSMB will be established to evaluate safety during the trial and at 1 scheduled interim analysis. The DSMB can advise to continue, stop or pause the trial. The members of the DSMB are:

- Prof. dr. K.C.B. Roes, Department of Biostatistics and Research Support, University Medical Center Utrecht , div. Julius Centrum
- Prof. dr. A. Slooter, Department of Intensive Care, University Medical Center Utrecht
- Dr. R.W. Koster, Department of Cardiology, Academic Medical Center
- Prof. dr. J.W.R. Twisk, Department of Epidemiology & Biostatistics, VU University (trial statistician/independent methodologist).

The members of the DSMB have no conflict of interest with the study.

If there is disagreement between the DSMB and Trial Steering Committee (TSC) and the DSMB has serious problems or concerns with the TSC decision a meeting of these groups will be held. The information to be shown would depend upon the action proposed and the DSMB's concerns. Depending on the reason for the disagreement confidential data may have to be revealed to all those attending such a meeting. The meeting will be chaired by an external expert who is not directly involved with the trial. The VU University Management Board will be informed.

10. STATISTICAL ANALYSIS

10.1 Primary study parameter(s)

The difference in Δ SOFA score at 96 hours between intervention- and control groups will be compared on a univariate basis (ANOVA with unadjusted analysis and after adjustment for, among others, age, sex and prehospital parameters) or depending on normality the Kruskal-Wallis test).

10.2 Secondary study parameter(s)

Ordered categorical outcome variables (Glasgow Coma score, CPC scale, Modified Rankin Scale and HUI-3 questionnaire) will be compared using the Cochran-Armitage test for trend. Continuous variables will be compared using linear mixed models or Friedman test where appropriate. Mortality will be analyzed using Kaplan-Meier curves and a Cox proportional hazards model with relevant prognostic covariates (especially time to return of spontaneous circulation). All statistical analyses will be performed according to the intention-to-treat principle.

10.3 Other study parameters

n.a.

10.4 Interim analysis

A blinded interim analysis focused on mortality will be carried out by the independent methodologist after inclusion of 135 **evaluable** patients. Analysis will be group

comparative. The DSMB will be blinded, but may be unblinded by the independent methodologist on request without any further clarification. This request will be granted in all cases. In case of a significant larger mortality in the vitamin C group compared to placebo and based on thorough examination of the clinical content, the DSMB can advise to stop the trial. The DSMB will assess the unblinded data before arriving at a material recommendation.

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

This study will be conducted according the principles of the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO).

11.2 Recruitment and consent

For this study we ask for deferred consent and we appeal to the emergency procedure for consent in medical research as stated in article 6, paragraph 4 of the WMO because of the following reasons. To achieve the optimal effect of high-dose vitamin C, it is very important that it is administered as soon as possible and is not postponed. The patients we aim to include (post cardiac arrest with an EMV-score ≤ 8 who will be admitted to the Intensive Care Unit) are per definition incompetent to give informed consent. Obtaining informed consent from a legal representative takes time, even by an experienced research team. We propose to randomize each patient who meets the inclusion criteria ultimately within 4 hours after arrival in the hospital. Vitamin C or placebo will be administered after randomization. The legal representative will be informed and asked for informed consent ('deferred informed proxy consent') by the investigator or the treating physician as soon as possible, but not later than 72 hours after ICU-admission (similar as in other ICU-studies^{53,54}). A deferred consent procedure is allowed due to the possible therapeutic benefit the patient could have by participating in this trial. The legal representative has a period of reflection of 2 hours and will make his/her decision within 1 day. If informed consent cannot be obtained within 72 hours, the patient is excluded and data will no longer be used. If a legal representative denies participation, the patient is excluded and the collected data and (residual) bodily material will be stored and used unless the legal representative objects. Without informed consent, the administration of vitamin C/placebo will then be stopped immediately. Refusal to give consent to take part

in the study has no influence whatsoever on the treatment and care. After deferred informed proxy consent is obtained, we will undertake two serious attempts to inform the patient about his/her participation in this study. The patient is free to fill in an withdrawal form when he/she does not want to further participate in the study or when he/she wants his/her collected data and body material to be removed. Our procedure is more extensively described in our attached standard operating procedure (SOP).

Any questions regarding the participation of their relatives legal representatives can ask the investigator, treating physician or independent physician who is mentioned at the end of the patient information letter. This physician knows everything about the study, but is not involved in the conduct of the study.

The legal representative is one of the following persons, in descending order of importance: a person acting under the written authority of the patient, registered partner or life partner, parents, children, brothers/sisters and grandchildren.

11.3 Objection by minors or incapacitated subjects

It is not possible to perform this study in capacitated patients. The patients have potential benefit from the intervention and the risks are negligible.

11.4 Benefits and risks assessment, group relatedness

See also 6.3. It has been shown that vitamin C can have beneficial effects in circumstances of oxidative stress, which occur after cardiac arrest. Different studies with high doses vitamin C have shown no significant adverse events. In this study a maximum of 100 ml blood will be taken during a period of four days from an arterial line which is routinely present for monitoring. The patient will not notice the sampling and it will not impose any risk to the patient. Study parameters are being collected by non-invasive measurements causing minor discomfort, but no pain or risk for the patient.

11.5 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7, subsection 6 of the WMO.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO and the Measure regarding Compulsory Insurance for

Clinical Research in Humans of 23th June 2003). This insurance provides cover for damage to research subjects through injury or death caused by the study.

1. € 650.000,-- (i.e. four hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;
2. € 5.000.000,-- (i.e. three million five hundred thousand Euro) for death or injury for all subjects who participate in the Research;
3. € 7.500.000,-- (i.e. five million Euro) for the total damage incurred by the organisation for all damage disclosed by scientific research for the Sponsor as 'verrichter' in the meaning of said Act in each year of insurance coverage.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

11.6 Incentives

The patients will not receive any incentives for their participation in the study.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

Clinical data will be recorded in the Patient Data Management System (PDMS) of the hospital (every hospital has its own PDMS) and will be transported to the study database in Castor. Access to the study database will require a password. Patients will receive a unique study number consisting of 6 numbers. The first 3 numbers indicate the hospital followed by 3 personal numbers. These numbers are not compiled by patient-identifying data such as date of birth or hospital number. In a separate key file, the unique study number and name of the patient will be linked to the hospital number of the patient. After completion of the trial this separate key file, including the name, the hospital number and study number will be stored by the secretary of the intensive care unit.

The same unique study number with key file will be used for body materials that will be stored in the Biobank of the Intensive Care at the VUmc. Remaining body materials will be stored in the VUmc for 15 years.

12.2 Monitoring and Quality Assurance

An independent monitor (quality officer) will monitor the study data according to the regulations described under Good Clinical Practice (GCP) in VUmc and all the other participating hospitals. In a selection of study subjects the Informed Consents are controlled. Additionally, during onsite monitoring the officer will perform a Source Data verification of data described in the Case Report Forms to investigate the agreement between source data and study reports. The intensity of this verification is related to the study risk assessment. In particular, inclusion and exclusion criteria and the primary endpoints of the investigation are subject to monitoring. The monitor will evaluate whether SAE's and SUSAR's are adequately reported within the time frame as directed by the Dutch law. The monitoring plan will be enclosed in the Trial Master File.

12.3 Amendments

A 'substantial amendment' is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/serious adverse reactions, other problems, and amendments.

12.5 Temporary halt and (prematurely) end of study report

The sponsor will notify the accredited METC and the competent authority of the beginning and the end of the study within a period of 90 days after completion. The end of the study is defined as the last patient's last visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC and the competent authority within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC and the Competent Authority.

12.6 Public disclosure and publication policy

We are free to make a publication and have no restrictions made by a sponsor. Patient data will be published anonymously.

13. STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

a. Level of knowledge about mechanism of action

See also 1.3. Beyond simply preventing scurvy by the correction of vitamin C deficiency, supraphysiological vitamin C levels can exert more potent antioxidative effects⁵⁵. When vitamin C is administered in considerable larger dosage than the currently registered 0.5 – 2.0 g per day (at least 3 g i.v. vitamin C /day is necessary just to correct deficiency in critically ill patients), much higher plasma levels are generated which not only limit the generation of ROS, but also directly scavenge ROS, repair other oxidized scavengers such as glutathione, urate and vitamin E, modulate numerous enzyme reactions and recover endogenous vasopressor synthesis¹¹. In addition, especially relevant for post cardiac arrest patients, vitamin C may protect the brain. Neurons in the brain have high rates of oxidative metabolism and contain some of the highest concentrations of vitamin C. Intracellular vitamin C serves several functions in the brain, including antioxidant protection, peptide amidation, myelin formation, synaptic potentiation, catecholamine synthesis and protection against glutamate toxicity⁵⁶.

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism

See also 1.4 and our reviews in Crit Care ^{3,34}.

c. Can the primary or secondary mechanism be induced in animals and/or in ex-vivo human cell material?

See also 1.4 and our reviews in Crit Care ^{3,34}.

d. Selectivity of the mechanism to target tissue in animals and/or human beings

See also 1.4 and our reviews in Crit Care ^{3,34}.

e. Analysis of potential effect

Of the yearly number of 6000 Dutch patients who suffer from an out-of-hospital cardiac arrest, only 3000 survive to hospital. 50% of these post-cardiac arrest IC patients also survive to hospital discharge². Each 1 point decrease in Δ SOFA score at 96 hours is associated with a 1.24 (95%CI 1.04-1.47) odds ratio of survival^{5,32}. Thus, at a 50% mortality rate, this would translate into an absolute survival effect of 5.5% per SOFA point (10% per 2 points). However, we overstretch the statistics if we directly translate this into a number of 'lives saved' for the at-risk population.

f. Pharmacokinetic considerations

Enteral supplementation with maximally tolerated dosages cannot achieve plasma levels of $> 250 \mu\text{mol/l}$ due to limited absorption, especially so during critical illness where deficiency is even not correctable by the enteral route. Therefore, i.v. administration is necessary to achieve the optimal antioxidative effect.

Normal plasma levels are around $60 \mu\text{mol/L}$ (lower limit $40 \mu\text{mol/L}$). Without supplementation, baseline low plasma concentrations ($6\text{-}20 \mu\text{mol/L}$) further declined in ICU-patients⁸. Normal plasma concentrations were attained while supplementing 3 g/day intravenously¹⁶. Supra-normal plasma concentration were attained while infusing 50 or 200 mg/kg/day in septic patients¹⁸ and even 66 mg/kg per hour in burn patients²⁹.

Very recently, we finished a pharmacokinetic study investigating 2 intermittent ($2 \times 1 \text{ g}$ and $2 \times 5 \text{ g}$ per day) and 2 continuous i.v. dosing regimens (2 g and 10 g per day) in critically ill patients⁴⁸. The results suggest that a bolus is required to quickly attain supernormal plasma levels above 1 mM , and a linear dose-concentration relationship, e.g. a fivefold higher dosage resulted in fivefold higher plasma concentrations.

g. Study population

Comatose adult patients suffering an out-of-hospital cardiac arrest who are admitted to the intensive care are eligible for inclusion in this present study.

h. Interaction with other products

There is an interaction between thiamine and vitamin C. Thiamine is co-enzyme to glyoxylate aminotransferase which promotes the oxidation of glyoxylate (a byproduct of the intermediary metabolism) to carbon dioxide (instead of oxalate). A higher conversion of vitamin C to oxalate is seen in thiamine deficiency and thiamine deficiency is common in septic patients³⁸. In our study patients thiamine will be added to reduce oxalate excretion.

i. Can effects be managed?

There are no antidotes or antagonists. We do not anticipate major adverse events. In addition, the patients and all their vital functions are monitored continuously at the ICU.

13.2 Synthesis

It has been shown that vitamin C can have beneficial effects in circumstances of oxidative stress, which occurs after cardiac arrest. It is a promising, cheap and safe therapy which may improve outcome after cardiac arrest. Different studies with high doses vitamin C in

critically ill patients have shown that therapy with high-dose vitamin C is safe in this patient group. In addition, the vital functions of the patients are continuously monitored during their ICU-stay. We have established a DSMB and an interim analysis for safety will be performed after inclusion of 135 evaluable patients. In our opinion the potential benefits of high-dose vitamin C outweigh the negligible risks in our study.

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Early high-dose vitamin C in post-cardiac arrest syndrome.

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)
AE	Adverse Event
AR	Adverse Reaction
CA	Competent Authority
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CV	Curriculum Vitae
DSMB	Data Safety Monitoring Board
EU	European Union
EudraCT	European drug regulatory affairs Clinical Trials
GCP	Good Clinical Practice
IB	Investigator's Brochure
IC	Informed Consent
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
METC	Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)
(S)AE	(Serious) Adverse Event
SPC	Summary of Product Characteristics (in Dutch: officiële productinformatie IB1-tekst)
Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.
SUSAR	Suspected Unexpected Serious Adverse Reaction
Wbp	Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgegevens)
WMO	Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen)

SUMMARY

Background of the study:

Only half of the patients suffering from cardiac arrest arrive at the hospital alive. Of these survivors, more than 50% will still die or remain severely disabled. During cardiac arrest ischemia causes damage to the vital organs, especially the brain. When with return of spontaneous circulation oxygen is re-offered to the ischemic organs, massive amounts of reactive oxygen species (ROS) are produced. These ROS can further increase the damage to the myocardium and brain (reperfusion injury). Vitamin C is the primary circulating antioxidant. It scavenges free radicals and reduces the production of ROS. In a recent study we demonstrated that vitamin C plasma levels are deficient in ~60% of the patients after cardiac arrest, probably due to massive consumption. Vitamin C deficiency reduces the protection against oxidative stress. Intravenous supplementation is needed to restore deficiency and the antioxidative effect of vitamin C is much more potent if it is administered in a supraphysiological dose (≥ 3 g per day). Its strong antioxidative effect may reduce damage to the circulation and to brain, heart and other organs. Beneficial effects of high dose i.v. vitamin C after cardiac arrest have been demonstrated in preclinical studies, but not in patients.

Objectives:

- To determine whether an early high dose i.v. vitamin C can improve organ function, especially neurological outcome, in patients after cardiac arrest.
- To explore the optimal dosing regimen for high dose i.v. vitamin C.
- To investigate *in vitro* the difference in effect of plasma obtained from post cardiac arrest patients treated with placebo, 3 g/day or 10 g/day vitamin C on endothelial cell viability and underlying oxidative pathways.

Study design:

In this multicentre, placebo controlled double-blind randomized clinical trial patients will be recruited from the Shock Rooms/Intensive Care Units of 8 hospitals.

360 adult comatose patients suffering an out-of-hospital cardiac arrest with ventricular fibrillation/tachycardia as first registered cardiac rhythm and EMV-score ≤ 8 will be included (we aim for 270 evaluable patients).

As soon as possible patients will be randomly allocated to one of 3 treatment groups of 120 (90 evaluable) patients each and vitamin C or placebo will be started for 96 hours. Group 1 will be treated with placebo, group 2 with a twice daily a bolus of 1.5 g vitamin C and group 3 with a twice daily a bolus of 5 g vitamin C. All patients will receive thiamine 200 mg q 12 hourly for 4 days to limit the conversion of vitamin C to oxalate.

Primary study parameters:

We will determine organ failure at 96 hours measured by the delta (Δ) Sequential Organ Failure Assessment (SOFA) score. Δ SOFA score is defined as the difference between SOFA admission and SOFA at 96 hour.

Secondary study parameters :

As secondary outcomes we will investigate: neurological outcome, IC- and hospital stay, mortality, myocardial injury, vasopressor support, lung injury score, ventilation time, renal function, IC-acquired weakness, delirium, oxidative stress parameters and vitamin C plasma concentrations. In vitro we will determine the effect of plasma obtained from the trial patients on cultured human systemic microvascular endothelial cells with regard to endothelial cell viability, intracellular vitamin C concentrations and oxidative pathways.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness (if applicable):

A total of 100 ml blood will be taken during a period of four days from an arterial line which is routinely present for monitoring. The patient will not notice the blood sampling. He/she will not notice the collection of urine samples, because all patients have a bladder catheter.

The risk of potential side effects of high dose vitamin C is very small. There is a small risk of formation of oxalate crystals in the urine. Formation of kidney stones of oxalate crystals has only been observed after prolonged intake of high dose vitamin C. Vitamin C can exert pro-oxidative effects, predominantly in patients with hemochromatosis. Pro-oxidative effects are related to the formation of the ascorbyl radical (which poorly reacts with other molecules than itself), presence of free iron and the formation of H_2O_2 (which is rapidly scavenged by human erythrocytes and for which especially cancer cells are sensitive, not human cells). Pro-oxidative effects are hardly shown. In this study patients with hemochromatosis will be excluded. Sometimes high-dose i.v. vitamin C can lead to factitious hyperglycemia when measured with point-of-care devices. In our study we will measure blood glucoses during the period of vitamin C administration by blood gas analysis or in the central laboratory.

1. INTRODUCTION AND RATIONALE

1.1 Problem definition

In Europe, each day more than 1000 patients suffer from cardiac arrest¹. Despite improvement of medical technologies mortality is still very high, around 75 - 80%. Of the patients who initially survive to ICU admission, more than 50% still dies or remains severely disabled due to the post cardiac arrest syndrome (PCAS)². Crucial in this syndrome is the overwhelming oxidative stress, which is caused by systemic ischemia/reperfusion injury and leads to destruction of endothelial function³ with cardiovascular failure and brain damage⁴. Besides targeted temperature management, we have no effective therapy to improve prognosis. The levels of our primary circulating antioxidant, vitamin C, are markedly depressed after cardiac arrest⁵. Early, high dose intravenous (i.v.) vitamin C administration can boost the body's antioxidant defence, and could be a new promising therapeutic intervention to improve clinical outcome by limiting oxidative damage.

1.2 Rationale high dose vitamin C

Vitamin C administration is often wrongly considered as complementary or even alternative medicine, which does not do justice to the strong scientific base of the pleiotropic antioxidative effects of high-dose i.v. (not enteral!) vitamin C administration as demonstrated in multiple preclinical and clinical studies³. With enteral supplementation maximally tolerated dosages cannot achieve plasma levels of $> 250 \mu\text{mol/l}$ ⁶ due to limited absorption⁷. In critically ill patients, enteral supplementation even cannot restore deficiency due to the acutely increased requirements⁸. I.v. vitamin C administration generates much higher plasma levels, thus yielding more and more potent antioxidative effects. The underlying pathophysiological mechanisms are well elucidated. High plasma levels of vitamin C not only limit the generation of reactive oxygen species (ROS), repair other oxidized scavengers such as glutathione and modulate numerous enzyme

reactions, but can also act as a direct radical scavenger. In addition, vitamin C maintains nitric oxide mediated endothelial integrity and vasomotor control^{9,10}. Furthermore, vitamin C is a cofactor in several biosynthetic pathways, such as collagen, catecholamines and peptide hormones. Deficiency will decrease their formation. Vitamin C can thereby recover endogenous vasopressor synthesis¹¹ and improve wound healing.

Post cardiac arrest huge amounts of ROS are generated by various pathways. The main source of ROS are the mitochondria due to uncoupling of oxidative phosphorylation. In addition, ROS are produced by upregulated enzymes such as NADPH oxidase¹² or during oxidation of catecholamines¹³. When unopposed these ROS can damage virtually every biomolecule and cause severe endothelial dysfunction. This has been demonstrated *in vitro*: plasma derived from patients after cardiac arrest induced massive cell death of cultured endothelial cells due to pro-oxidant stress and deterioration of anti-oxidant defences¹⁴. Cell death was highest immediately after admission to the ICU.

1.3 Vitamin C depletion

This overpowering oxidative stress during PCAS can quickly exhaust body stores of vitamin C due to massive cellular consumption and reduced regeneration. We have shown that vitamin C plasma concentrations were decreased by more than 50% compared to healthy volunteers already on the first day after cardiac arrest. After 3 days plasma concentrations further declined and more than half the patients were deficient. Low vitamin C levels were associated with multiple organ dysfunction (higher SOFA scores) and mortality. Other studies, though investigating septic and not post cardiac arrest patients, also show markedly depressed vitamin C levels on the day of admittance (~ 10 and $6 \mu\text{mol/l}$)^{15,16} and an association between low vitamin C levels and multiple organ failure¹⁷.

However, these deficient vitamin C levels in critically ill patients often will go unnoticed. Due to the complexity and cost of its laboratory measurement plasma levels are not available in daily practice. In addition, the vitamin C content of enteral nutrition is assumed

to be sufficient. However, current nutrition protocols (even with immune enhanced nutrition) fail to normalize vitamin C levels^{8,18}. These low plasma levels are likely to reflect real deficiency, since they are accompanied by scorbutic intracellular leucocyte vitamin C levels as well¹⁹. Even with i.v. vitamin C dosages up to 1 g per day vitamin C depletion persists^{15,16}.

1.4 (Pre) clinical studies

Multiple preclinical experiments support the potential beneficial effect of high-dose i.v. vitamin C post cardiac arrest. In a rat cardiac arrest model vitamin C administration immediately after ROSC improved survival rate and neurological outcome and decreased myocardial damage²⁰. In organ-specific ischemia-reperfusion models of kidney, liver and skeletal muscle i.v. vitamin C ameliorated respectively renal structure and function²¹, bile flow and cholate secretion²² and muscle function²³.

Up to now no clinical study specifically addressed the post cardiac arrest population, but several controlled studies in critically ill patients showed favourable results³. In critically ill surgical patients 3 g i.v. vitamin C per day reduced pulmonary morbidity, new organ failure, duration of ICU/hospital stay and mortality^{24,25}. In burn patients very high dose i.v. vitamin C (66 mg/kg/hr) reduced fluid requirements, body weight gain and respiratory dysfunction. In a recent pilot trial of patients with severe sepsis vitamin C both 50 mg/kg/day and 200 mg/kg/day caused earlier recovery from organ failure with reduction of the pro-inflammatory biomarkers¹⁸. In a before and after study of patients with septic shock high dose i.v. vitamin C combined with i.v. thiamine and stress dose steroids substantially accelerated shock reversal and improved survival²⁶. Two studies in critically ill patients administering respectively 2.7 g/day and 1.5 g/day showed no clinical benefit. These different results might be explained by difference in timing (relatively late²⁷) and route of administration (enteral²⁸). None of the clinical studies reported negative results of vitamin C.

1.5 Safety of high dose vitamin C

Up to now, no adverse events due to high-dose vitamin C have been reported^{18,24,29}, even with extremely high dosing schedules²⁹. Theoretical risks comprise a paradoxal pro-oxidative effect in case of iron overload, oxalate kidney stones and factitious hyperglycemia. In critically ill patients with sepsis 200 mg/kg/day¹⁸ and in cancer patients even megadoses up to 1500 mg/kg i.v. vitamin C three times weekly³⁰ were tolerated without significant side effects. Vitamin C can reduce catalytic metals such as Fe^{2+} and Cu^{2+} with adverse, pro-oxidative effects in patients with hemochromatosis. These patients are excluded in most studies and will also be excluded in our study. High dose vitamin C increases urinary oxalate excretion. However, oxalate nephrocalcinosis and calcium oxalate stones take months to years to develop and none of the studies with short-term vitamin C administration reported kidney stone formation^{18,24,29}. Sometimes high-dose i.v. vitamin C can lead to factitious hyperglycemia when measured with point-of-care devices, mostly at dosages much higher than used in our study. We will measure blood glucoses during the period of vitamin C administration by blood gas analysis or in the central laboratory.

MitoPO2 measurements

Disturbances in mitochondrial respiratory chain function occur in patients after ischemia/reperfusion injury (1). Until recently bedside measurement of mitochondrial respiratory chain function was not possible. The recent development of the COMET device (CE-certified) enables measurement of mitochondrial oxygen tension (mitoPO2) using the protoporphyrin IX-triplet state lifetime technique (PpIX-TSLT). This technique requires an 5-aminolevulinic acid plaster (alacare) to be placed on the sternum of the patient. After a minimal induction time of 4 hours, the mitoPO2 can be measured by placing the light emitting and detecting sensor of the COMET device on the area of the plaster (for a duration of 1 minute).

As a result, this enables a non-invasive measurement of mitochondrial respiratory chain function at the bedside (2,3). The technique is currently being explored as a marker of resuscitation in critically ill patients in multiple observational studies at the OLVG hospital (clinical trial NCT03842722). The technique has been tested and validated by the OLVG group in human volunteers and approximately 100 anaemic and septic critically ill patients with no adverse effects being reported (4,5). In this study, we aim to investigate whether vitamin C therapy effects the mitochondrial function in post-cardiac arrest patients during the first day of ICU-admission. As only one COMET device is available, the mitoPO2 measurements will be done only in the OLVG hospital.

We hypothesize that vitamin C reduces organ damage, especially cerebral injury, if administered for a short period as a high i.v. dose during the very early phase of reperfusion after cardiac arrest.

2. OBJECTIVES

Primary Objective:

- To determine whether an early high dose iv vitamin C improves organ function, especially neurological outcome, in patients after cardiac arrest.

Secondary Objectives:

- To explore the optimal dosing regimen for high dose i.v. vitamin C.
- To investigate *in vitro* the difference in effect of plasma obtained from post cardiac arrest patients treated with placebo, 3 g/day or 10 g/day vitamin C on endothelial cell viability and underlying oxidative pathways.
- To determine the effect of vitamin C therapy on the mitochondrial respiratory chain function as measured by mitoPO2 and to correlate mitoPO2 with ROS in plasma samples.

3. STUDY DESIGN

Design: double blind randomized, clinical multicenter trial

Duration: 3 – 4 years

Setting: Intensive Care Units of: VU University Medical Centre, Gelderse Vallei Hospital, Franciscus Gasthuis & Vlietland, Tergooiziekenhuizen, Amphia Hospital, Erasmus Medical Centre, Noordwest Ziekenhuisgroep, OLVG Oost and Maasstad Hospital.

4. STUDY POPULATION

4.1 Population (base)

The study population consists of adult patients admitted to the Intensive Care Units of: VU University Medical Centre, Gelderse Vallei Hospital, Franciscus Gasthuis & Vlietland, Tergooiziekenhuizen, Amphia Hospital, Erasmus Medical Centre, Noordwest Ziekenhuisgroep, OLVG Oost and Maasstad Hospital.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- An out-of-hospital cardiac arrest with return of spontaneous circulation;
- Ventricular fibrillation or ventricular tachycardia as first registered cardiac rhythm;
- EMV-score ≤ 8 .

4.3 Exclusion criteria

A potential subjects who meet any of the following criteria will be excluded from participation in this study:

- Patients with terminal renal insufficiency, i.e. receiving renal replacement therapy (RRT);
- Known glucose 6-phosphate dehydrogenase deficiency (risk of hemolysis);
- History of urolithiasis, oxalate nephropathy or hemochromatosis;
- Treatment limitations.

4.4 Sample size calculation

The Δ SOFA score at 96 hours has an estimated standard deviation of 3.5 points (highest estimate based on previous data in our hospital and our recent systematic review³¹). The study is powered to detect a clinically meaningful effect of 2.0 SOFA points difference between the trial arms. Eighty-two patients per group are needed to detect a treatment effect

of 2 SOFA points with 90% power and a two-sided alpha of 0.01. The type-I error rate is set at 0.01 instead of 0.05 to minimize the risk of false-positive results. With an estimated 10% loss after randomization for late outcomes, the study aims to include 3x90 patients. If we estimate that 50% of the 1500 potential study patients will not be eligible and that 50% of the patients will refuse consent, we expect to have more than enough safety margin to recruit 360 patients, of whom 270 will be evaluable, in 3 years.

We consider a 2-point difference in Δ SOFA score between the intervention- and control groups a meaningful effect. Firstly, a 2-point difference is sufficiently large to be clinically relevant and to impact future practice. For example, 2 SOFA points is the difference between mild neurologic deficit and coma, between moderate kidney dysfunction and dialysis-dependent kidney failure, or between moderate oxygenation disturbance and life-threatening respiratory failure. Secondly, the association between Δ SOFA score and outcomes is such that a 2-point difference can lead to real and measurable reductions in length-of-stay, mortality and costs when the intervention is broadly implemented: each 1 point increase in Δ SOFA score at 96 hours is associated with a 1.24 (95%CI 1.04-1.47) odds ratio of mortality^{5,32}.

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

Included patients receive a placebo, 1.5 g vitamin C b.i.d. or 5 g vitamin C b.i.d for 4 days. It is aimed to administer the first dose within 5 hours after ROSC. The treatment will be continued if the first dose is administered too late. Vitamin C will be administered intravenously as ascorbic acid 1.5 per 50 ml NaCl 0.9% or 5 gr per 50 ml NaCl 0.9%. The study thereby compares 2 doses equivalent to those currently used in clinical studies in critically ill patients^{18,26,33}. The placebo will be administered intravenously and consists of 50 ml NaCl 0.9% .

Vitamin C is being used in accordance with the registered indication of vitamin C deficiency. The investigational aspect is the administration of higher dosages of vitamin C than registered in the summary of product characteristics (SPC). However, the use of much higher doses up to 66 mg/kg/h in critically ill burn patients has been reported (see paragraph 1.4).

5.2 Use of co-intervention (if applicable)

All patients will receive thiamine 200 mg q 12 hourly for 4 days to limit the conversion of vitamin C to oxalate²⁶.

5.3 Escape medication (if applicable)

Not applicable.

6. INVESTIGATIONAL PRODUCT

Name and description of investigational product(s)

Placebo will be administered intravenously and consists of 50 ml NaCl 0.9%.

Vitamin C will be administered intravenously 1.5 g per 50 ml NaCl 0.9% or 5 g per 50 ml NaCl 0.9% (ascorbic acid 100 mg/ml, Centrafarm, Etten-Leur, the Netherlands).

The LUMC pharmacy is currently developing ready-to-use study medication. When the production of this study medication has been finished, we will notify the METc and the competent authority with a substantial amendment.

6.1 Summary of findings from non-clinical studies

See 1.4, and as attachments our reviews in Crit Care 2014^{3,34}

6.2 Summary of findings from clinical studies

See 1.4, and as attachments our review in Crit Care 2014^{3,34}.

6.3 Summary of known and potential risks and benefits

Potential risks

Up to now, no adverse events of high-dose vitamin C have been reported, even with extremely high dosing schedules^{29,30}. Theoretical risks of high dose i.v. vitamin C are oxalate kidney stones, a paradoxical ROS generating effect in case of iron overload, toxicity of adjuvants and factitious hyperglycemia. Patients with kidney stones, iron overload and glucose 6-phosphate dehydrogenase deficiency (developing hemolysis with drugs) will be excluded from our study.

Several safety and tolerability studies have been done in cancer patients, cardiovascular patients and in critically ill patients with sepsis^{18,26,30,35,36}. In cancer patients a megadose of 1500 mg/kg i.v. three times weekly was tolerated without important adverse effects³⁰. The JAMA published a controlled trial enrolling 1708 cardiovascular patients, who received infusions of 7 g vitamin C weekly for 40 weeks in addition to chelation therapy, or placebo. No difference was found in adverse events between groups³⁶. In critically ill septic patients, vital signs were monitored every 5 min during and after infusion of 200 mg vitamin C per kg/day by bedside IC nurses. No side effects were reported¹⁸.

Urinary oxalate crystallization.

High dose vitamin C can increase urinary oxalate excretion, since oxalate is a metabolic end product of vitamin C³⁷. In renal insufficiency, conversion to oxalate may be increased (or not). Worsening of renal function is therefore a concern with high dose vitamin C. However, in the very recently published before and after study in septic patients high-dose vitamin C for 4 days yielded renal benefit, no harm. The need of renal replacement therapy declined from 37% to 10% and renal function improved in all the patients with acute kidney injury²⁶. The high urine concentrations of vitamin C might protect renal tubular cells against oxidative stress. In this study patients thiamine was added to reduce oxalate excretion²⁶. Thiamine is cofactor for glyoxylate aminotransferase which promotes the oxidation of glyoxylate (a byproduct of the intermediary metabolism) to carbon dioxide (instead of oxalate). A higher conversion of vitamin C to oxalate is seen in thiamine deficiency and thiamine deficiency is common in septic patients.³⁸ Oxalate nephrocalcinosis and calcium oxalate stones develop over months to years in primary hyperoxaluria, a disease in which oxalic acid excretion exceeds 100 mg/day and can reach 400 mg/day³⁹. In susceptible patients, prolonged high dose therapy for weeks or months can indeed cause oxalate nephropathy⁴⁰⁻⁴². Generally *acute* oxalate nephropathy has been described after prolonged intake of massive doses (ten-times higher than in the

present study), in combination with underlying renal disease or use of non-steroidal anti-inflammatory drugs⁴³. In our study vitamin C will be infused for four days only (as in the other studies with vitamin C in critically ill patients) and patients with a history of renal stones, oxalate nephropathy or those with a terminal renal insufficiency will be excluded. In addition, we will also administer thiamine to our study patients. Renal function of all patients at our ICU is monitored routinely by daily determination of serum creatinine, estimated Glomerular Filtration Rate (eGFR) and fluid balance. Diuresis is monitored hourly. When indicated, an urine sediment analysis or echography of the kidneys will be carried out.

Pro-oxidant properties

Vitamin C has pro-oxidant properties as well. Pro-oxidative effects are related to the formation of the ascorbyl radical, which poorly reacts with other molecules than itself; presence of free iron and the formation of H_2O_2 , which is rapidly scavenged by human erythrocytes and for which especially cancer cells are sensitive, not human cells⁴⁴. It can generate very low levels of superoxide and reduce catalytic metals such as Fe^{3+} and Cu^{3+} , and thereby initiate radical chain oxidations. Vitamin C could therefore have pro-oxidative effects in case of iron overload. Consequently, in our trial patients with hemochromatosis will be excluded. However, in other settings pro-oxidative effects are not or only controversially shown⁴⁵.

Factitious hyperglycemia

Sometimes high-dose i.v. vitamin C can lead to factitious hyperglycemia when measured with point-of-care devices, mostly at dosages much higher than used in our study⁴⁶. We will measure blood glucoses during the period of vitamin C administration by blood gas analysis or in the central laboratory⁴⁷.

Potential benefits

See also the introduction. Of the yearly number of 6000 Dutch patients who suffer from an out-of-hospital cardiac arrest, 3000 survive to hospital. 50% of these post-cardiac arrest IC patients also survive to hospital discharge². Each 1 point decrease in Δ SOFA score at 96 hours is associated with a 1.24 (95%CI 1.04-1.47) odds ratio of survival^{5,32}. Thus, at a 50% mortality rate, this would translate into an absolute survival effect of 5.5% per SOFA point (10% per 2 points). However, we overstretch the statistics if we directly translate this into a number of 'lives saved' for the at-risk population.

6.4 Description and justification of route of administration and dosage

With enteral supplementation maximally tolerated dosages cannot achieve plasma levels of $> 250 \mu\text{mol/l}$ ⁶ due to limited absorption⁷. Moreover, in critically ill patients, deficiency cannot be restored by the enteral route. I.v. vitamin C administration generates much higher plasma levels, thus yielding more and more potent antioxidative effects (see also 1.2). The optimal dose of vitamin C is not settled yet. In our trial we will compare two different dosages to placebo (group 1), one aiming at (high-) normal plasma concentrations (group 2) and one targeting supranormal concentrations (group 3). I.v. vitamin C in a dose of 3 g/day appears to be the minimal daily dosage necessary to normalize vitamin C levels in critically ill patients and this dosage proved to be beneficial in 2 clinical studies^{24,25}. Our pharmacokinetic trial showed that a twice daily 1 g vitamin C is required to quickly normalize plasma levels and twice daily 5 g to achieve supra-normal plasma concentrations with peaks above 1mM, the concentration needed for direct superoxide scavenging⁴⁸. We chose for group 2 1.5 g vitamin C b.i.d (3 g/day) to normalize vitamin C plasma levels.

The choice for the dosage in group 3 was subject of intense discussion among co-investigators, because the high dosages used in clinical studies vary tremendously. In critically ill patients, high dosages varying from 3 up to even 126 g i.v. vitamin C/day were used. In a small study with septic patients 16 g/day resulted in better recovery from organ

failure and more reduction of pro-inflammatory biomarkers compared to 4 g/day¹⁸. In septic patients the before and after trial with 6 g/day showed a huge effect on mortality²⁶. The dosage of 126 g/day used in a small study with burn patients was much higher (10-fold)²⁹ than the dosage used in the other studies showing significant effects of vitamin C^{18,26}. Considering all these data, we chose for group 3 a dosage of 5 g vitamin C b.i.d. for 4 days (10 g/day).

6.5 Dosages, dosage modifications and method of administration

See also 6.4.

As soon as possible (ultimately < 5 hours after ROSC) after their arrival at the Emergency Department, patients will be randomly allocated to one of 3 treatment groups of 90 patients each and vitamin C or placebo will be started for 96 hours.

Group 1 will be treated with placebo, group 2 with 1.5 g vitamin C b.i.d.(3 g/day) and group 3 with 5 g vitamin C b.i.d. for 4 days (10 g/day). After these 4 days the overwhelming oxidative stress and associated organ damage will have settled down and body stores of vitamin C will be restored. Vitamin C administration will then be lowered to standard nutritional dose to allow the physiological signaling and repair function of low concentrations of ROS.

Early timing. It is aimed to start the first infusion of vitamin C within 5 hours after ROSC. Reason for this early start is that the oxidative damage of patient plasma to endothelial cells was highest early after resuscitation¹⁴. IC admission indicates the point where the pro/antioxidant balance is lost and the oxidative stress response becomes uncontrolled. Starting the infusion before this point seems crucial. We hypothesize that at this early timing, when the body's feedback mechanisms fail, high dose i.v. vitamin C can minimize further damage to endothelium and organs.

6.6 Preparation and labelling of Investigational Medicinal Product

The vitamin C ampoules of Centrafarm will be labelled in conformity with EU GMP Annex 13 by the VUmc pharmacy. Unblinded nurses will be trained to prepare the study medication in perfusor syringes according to a standard operating procedure:

:

- Placebo, 50 ml NaCl 0.9%
- 1,5 gram vitamin C in 50 ml NaCl 0.9%
- 5 gram vitamin C in 50 ml NaCl 0.9%

6.7 Drug accountability

- Production by Centrafarm B.V., Ettenleur, the Netherlands.
-
- The pharmacy of the VUmc will deliver the Annex 13 labeled Centrafarm vitamin C to the Intensive Care/Medium Care units of the VUmc and to the pharmacies of the other 8 participating hospitals.
- The transport from VUmc to the other centra will be carried out by a certified courier with temperature monitoring.
- Receipt of medication according to local procedures with QP release certificate and drug accountability.
- Destruction by all nine centra according to local procedures.

7. NON-INVESTIGATIONAL PRODUCT

7.1 Name and description of non-investigational product(s)

Thiamine will be administered intravenously (200 mg q 12 hourly for 4 days).

Thiamine is a cofactor for glyoxylate aminotransferase which promotes the oxidation of glyoxylate (a byproduct of the intermediary metabolism) to carbon dioxide (instead of oxalate).

7.2 Summary of findings from non-clinical studies

A study in which a thiamine-deficient diet was applied to rats for 4 weeks showed that more oxalate was excreted in the urine, causing hyperoxaluria⁴⁹.

7.3 Summary of findings from clinical studies

Because of the high prevalence of thiamine deficiency in critically ill patients, several clinical studies have been performed to investigate the potential benefit of thiamine administration. These studies do not show uniform results. Doses and timing differed substantially between studies.

Cardiac surgical patients

In two studies preventive administration of thiamine in cardiac surgical patients did not affect post-operative lactate levels or clinical outcome. However, cellular oxygen consumption was higher in the thiamine group⁵⁰.

Sepsis

In a small study in patients with septic shock ⁵¹ thiamine did not reduce lactate levels or mortality in the overall group. However, in a subgroup of patients with thiamine deficiency at baseline, thiamine decreased lactate levels and lower mortality. Secondary analysis of this study showed lower creatinine levels and less progression to renal function requiring renal replacement therapy in the thiamine group (overall population). The previously mentioned before/after study in patients with severe sepsis or septic shock, thiamine in combination with vitamin C and hydrocortisone showed a huge reduction in organ dysfunction and mortality and improvement of renal function.²⁶

Burns

In a very small study of critically ill burned patients thiamine administration significantly reduced pyruvate and lactate levels ⁵².

Trauma

In a mixed population of patients admitted to the ICU after complicated cardiac surgery, subarachnoidal hemorrhage or major trauma intravenous administration of a mixture of selenium, zinc, vitamin C and thiamine (100 mg/day) did not improve organ function, but attenuated the inflammatory response in cardiac surgery/trauma patients and decreased hospital length-of-stay ²⁷.

7.4 Summary of known and potential risks and benefits

Potential risks

Enteral and intravenous administration of thiamine hardly causes any side effects. This is supported by animal toxicological data. The only risk of intravenous administration is the occurrence of an anaphylactic reaction. Doses of more than 400 mg may induce nausea, anorexia, and mild ataxia.

Potential benefits

The administration of thiamine to vitamin C may reduce the risk of oxalate nephropathy as thiamine promotes the conversion of glyoxylate to carbon dioxide instead of oxalate in case of thiamine deficiency. Furthermore it acts synergistically with vitamin C to attenuate oxidative stress, cellular apoptosis and restore organ function.

7.5 Description and justification of route of administration and dosage

The Recommended Daily Intake of thiamine is 1.1-1.2 mg/day for adults. Standard enteral nutrition contains 2-3 mg and parenteral nutrition 3 – 4 mg/day. The doses recommended for refeeding syndrome are 200 – 300 mg i.v./day, whereas for Wernicke's encephalopathy 200 - 500 mg i.v. 3 times daily. Intravenous suppletion can restore thiamine levels in critically ill patients within hours. In the clinical studies

in critically ill patients doses in the same range to those administered for refeeding and Wernicke's encephalopathy were used.

7.6 Dosages, dosage modifications and method of administration

Patients will receive 200 mg thiamine intravenously, every 12 hours during 4 days. Thiamine will be administered together with vitamin C.

7.7 Preparation and labelling of Non Investigational Medicinal Product

The product will be delivered by the hospital pharmacy.

7.8 Drug accountability

n.a.

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter

We will determine organ failure at 96 hours measured by the delta (Δ) Sequential Organ Failure Assessment (SOFA) score. Δ SOFA score is defined as the difference between SOFA admission and SOFA at 96 hour. Death at 96-hours will be counted as the maximum SOFA score (24 points). The SOFA-score is more extensively described in the table below.

Table 1. Sequential Organ Failure Assessment Score (SOFA)

System	Score				
	0	1	2	3	4
Respiration					
<i>PaO₂/FiO₂, mmHg (kPa)</i>	≥ 400 (53.3)	< 400 (53.3)	< 300 (40)	< 200 (26.7) with ventilation assistance	< 100 (13.3) with ventilation assistance
Coagulation					
Platelets, $\times 10^3/\mu\text{L}$	≥ 150	< 150	< 100	< 50	< 20
Liver					
<i>Bilirubin, mg/dL ($\mu\text{mol/L}$)</i>	< 1.2 (20)	1.2-1.9 (20-32)	2.0-5.9 (33-101)	6.0-11.9 (102-204)	≥ 12.0 (204)
Cardiovascular	<i>MAP ≥ 70 mmHg</i>	<i>MAP < 70 mmHg</i>	<i>Dopamine < 5 of dobutamine</i>	<i>Dobutamine > 5.1-15 of adrenaline ≤ 0.1 of noradrenaline ≤ 0.1</i>	<i>Dobutamine > 15 of adrenaline ≤ 0.1 of noradrenaline ≤ 0.1</i>
Central nervous system					
<i>Glasgow Coma Scale score</i>	15	13-14	10-12	6-9	< 6
Renal					
<i>Creatinine, mg/dL ($\mu\text{mol/L}$)</i>	< 1.2 (110)	1.2-1.9 (110-170)	2.0-3.4 (171-299)	3.5-4.9 (300-440)	> 5.0 (440)
<i>Urine output, mL/d</i>				< 500	< 200

Abbreviations: PaO₂: Partial pressure of oxygen; FiO₂: Fraction of inspired oxygen; MAP: Mean arterial pressure

8.1.2 Secondary study parameters

As secondary outcomes we will investigate: neurological outcome (Maximal Glasgow Coma Score at 96-h and at the end of the ICU-stay; Cerebral Performance Categories scale and Modified Rankin Scale at 28 and 180 days; HUI-3 questionnaire at 180 days;

neuron-specific enolase day 1, 2 and 3); clinical parameters (IC- and hospital stay, 28-day and 180-day mortality), duration of vasopressor support, organ injury (myocardial injury measured by troponin and CK-MB (maximum day 1), lung injury score, ventilation time, renal function, need of renal replacement therapy, IC-acquired weakness (Medical Research Council score), delirium (CAM-ICU score), inflammation (C-reactive protein), oxidative stress parameters (such as F₂-isoprostanes and oxidation reduction potential (ORP)), anti-oxidant capacity (AOC) and vitamin C plasma concentrations.

MitoPO₂, mitoVO₂ on day 1 measured with the COMET system (in OLVG only).

8.1.3. Other study parameters

Demographics of the patient will be collected, including age, sex, body-mass index (weight/height²), any relevant medical history and medication use. Furthermore, prehospital data will be obtained from the ambulance service.

To determine the different effect of 3 gr or 10 g/day vitamin C or placebo on the underlying oxidative pathways we will investigate how plasma obtained from the trial patients affects cultured human systemic microvascular endothelial cells with regard to endothelial cell viability, ROS production intracellular vitamin C concentrations, NADPH-oxidase expression, p47Phox expression, endothelial barrier function and mitochondrial respiratory chain function.

8.2 Randomisation, blinding and treatment allocation

Randomisation will be performed with the use of a randomisation list generated by VUmc Pharmacy, Amsterdam, the Netherlands. Randomisation will be stratified based on site and age. Age is categorised in 2 groups: ≤66 years old and >66 years old. Patients will be assigned by an unblinded ICU/MC nurse to a subsequent study number which is linked to one of the study arms. The unblinded nurses will be trained by the investigators for the

randomization, blinding and treatment allocation procedure. This training will be logged.

The nurse who is attending the patient is blinded for the treatment group and will administer the study medication. The prepared perfusor syringes with vitamin C and placebo are identical in appearance and have a maximum shelf life of 24 hours. 1.5 grams vitamin C, 5 grams vitamin C and placebo will be administered as a 50 cc bolus. ...

Patients, physicians, and outcome assessors will be kept blinded to the allocation at all time.

8.3 Study procedures

- 1) Deferred consent by patient representative (*for study*)
- 2) Routine clinical data (demographic characteristics, reason of admission, comorbidity, APACHE II) (*part of medical treatment*)
- 3) Treatment with vitamin C or placebo for 4 days (*for study*)
- 4) Blood sample collection (non-invasive by arterial line) daily for 4 days for determination of vitamin C levels and parameters of oxidative stress (*in total 100 ml extra for study*). Remaining blood material after analysis will be stored for additional analyses in the future (*for study*)
- 5) Collection of urine samples during the first 4 days (*for study*)
- 6) After 28 and 180 days (with a time window of 2 weeks around these days) a follow up with questionnaires will be used to estimate the neurological condition (*for study*).

The study procedures will be terminated when a patient does not fulfil all inclusion criteria after randomization, and/or when a patient suddenly fulfil one of the 3 upper exclusion criteria. Deferred consent will be asked in all cases to follow every randomized patient.

8.4 Withdrawal of individual subjects

The legal representative of the patient can decide to withdraw consent at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

8.5 Replacement of individual subjects after withdrawal

In case of withdrawal of individual subjects, subjects will be replaced according to inclusion and exclusion criteria.

8.6 Follow-up of subjects withdrawn from treatment

After withdrawal of subjects from the study, no specific follow up will be carried out.

8.7 Premature termination of the study

A blinded interim analysis focused on mortality will be carried out blinded by the independent methodologist (Prof. dr. J.W.R. Twisk) after inclusion of 135 **evaluable** patients. Analysis will be group comparative. The DSMB will be blinded, but may be unblinded by the independent methodologist on request without any further clarification. This request will be granted in all cases. In case of a significant larger mortality in the vitamin C group compared to placebo and based on thorough examination of the clinical content, the DSMB can advise to stop the trial. The DSMB will assess the unblinded data before arriving at a material recommendation.

9. SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1. Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the investigational product.

All included patients are admitted to the Intensive Care with a life-threatening disease.

Any adverse development can be expected as part of the underlying disease. Therefore, it is not achievable to report all these developments individually as AEs.

9.2.2 Serious adverse events (SAEs)

All included patients are admitted to the Intensive Care with a life-threatening disease.

Any major adverse development can be expected as part of the underlying disease.

Therefore, it is not achievable to report all these developments individually as SAEs.

Every three months we will send a line-listing of all SAEs to the METC.

Events that can be considered SAE's within the context of this study population are:

- death;
- end-stage organ failure, specifically:
 - Start of renal replacement therapy;
 - Start of mechanical circulatory support (intra-aortic balloon pump or extracorporeal life support);
 - Start of prone position ventilation;
 - Bilirubin > 50 µmol/l;
 - Any event that is unexpected in the course of the underlying disease and that results in chronic and significant disability or incapacity.

SAEs will be collected until 1 month after inclusion for included patients. For excluded patients, SAE's will be collected during the participation time in the study (from inclusion until

time of exclusion). This has been described in our SOP 'Consent vragen op de ICV versie 7.1 dd 10-08-2020'.

When included patients are transferred to another hospital within 1 month after inclusion, SAE's will be collected until the moment of transfer.

9.2.3. Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see chapter 9.2.2);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
 - Summary of Product Characteristics (SPC) for an authorised medicinal product;
 - Investigator's Brochure for an unauthorised medicinal product.

The sponsor will report expedited the following SUSARs through the web portal *ToetsingOnline* to the METC:

- SUSARs that have arisen in the clinical trial that was assessed by the METC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern.

The expedited reporting of SUSARs through the web portal *ToetsingOnline* is sufficient as notification to the competent authority.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report. The investigators in the other participating centres will report SUSARs as soon as possible (within maximal 7 days) to the coordinating investigator. The coordinating investigator will report these SUSARs through the web portal ToetsingOnline not later than 15 days after first knowledge of the adverse reactions.

9.3 Annual safety report

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States.

This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

9.4 Follow-up of adverse events

All SAEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

9.5 Data Safety Monitoring Board (DSMB)

An independent DSMB will be established to evaluate safety during the trial and at 1 scheduled interim analysis. The DSMB can advise to continue, stop or pause the trial. The members of the DSMB are:

- Prof. dr. K.C.B. Roes, Department of Biostatistics and Research Support, University Medical Center Utrecht , div. Julius Centrum
- Prof. dr. A. Slooter, Department of Intensive Care, University Medical Center Utrecht
- Dr. R.W. Koster, Department of Cardiology, Academic Medical Center

- Prof. dr. J.W.R. Twisk, Department of Epidemiology & Biostatistics, VU University (trial statistician/independent methodologist).

The members of the DSMB have no conflict of interest with the study.

If there is disagreement between the DSMB and Trial Steering Committee (TSC) and the DSMB has serious problems or concerns with the TSC decision a meeting of these groups will be held. The information to be shown would depend upon the action proposed and the DSMB's concerns. Depending on the reason for the disagreement confidential data may have to be revealed to all those attending such a meeting. The meeting will be chaired by an external expert who is not directly involved with the trial. The VU University Management Board will be informed.

10. STATISTICAL ANALYSIS

10.1 Primary study parameter(s)

The difference in Δ SOFA score at 96 hours between intervention- and control groups will be compared on a univariate basis (ANOVA with unadjusted analysis and after adjustment for, among others, age, sex and prehospital parameters) or depending on normality the Kruskal-Wallis test).

10.2 Secondary study parameter(s)

Ordered categorical outcome variables (Glasgow Coma score, CPC scale, Modified Rankin Scale and HUI-3 questionnaire) will be compared using the Cochran-Armitage test for trend. Continuous variables will be compared using linear mixed models or Friedman test where appropriate. Mortality will be analyzed using Kaplan-Meier curves and a Cox proportional hazards model with relevant prognostic covariates (especially time to return of spontaneous circulation). All statistical analyses will be performed according to the intention-to-treat principle.

MitoPO2 will be compared between intervention- and control groups and assessed for statistical significant difference using an ANOVA adjusted for sex, BMI, age (to correct for

systemic oxygen consumption/metabolic rate). MitoPO2 will be compared to other ROS variables using linear mixed models. MitoPO2 and oxidative stress in blood samples will be investigated using Pearson's correlation analysis.

Defining patient populations for final analyses

'Full analysis set' (ITT)

- All patients who meet the in- and exclusion criteria, where early administration of the first dose (<5 uur after ROSC) is still feasible, will be randomized and treated according to the protocol.
- All randomized patients, where study medication is terminated because of not meeting all inclusion criteria.
- All randomized patients, where study medication is terminated because of fulfilling one of the 3 upper exclusion criteria.
- This analysis will contain >270 patients.

'Modified intention-to-treat analyse' (mITT) – Primary analysis

- All randomized and treated patients
 - Without patients who received the first dose with a delay (>5 hours after ROSC).
 - Without patients who turned out not to be eligible (not fulfilling all inclusion criteria)
- For this analysis the trial is powered: 270 patients, 90 in each arm.

Included patients without informed deferred consent <72 hours will be excluded from all analyses.

10.3 Other study parameters

n.a.

10.4 Interim analysis

A blinded interim analysis focused on mortality will be carried out by the independent methodologist after inclusion of 135 **evaluable** patients. Analysis will be group comparative. The DSMB will be blinded, but may be unblinded by the independent methodologist on request without any further clarification. This request will be granted in all cases. In case of a significant larger mortality in the vitamin C group compared to placebo and based on thorough examination of the clinical content, the DSMB can advise to stop the trial. The DSMB will assess the unblinded data before arriving at a material recommendation.

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

This study will be conducted according the principles of the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO).

11.2 Recruitment and consent

For this study we ask for deferred consent and we appeal to the emergency procedure for consent in medical research as stated in article 6, paragraph 4 of the WMO because of the following reasons. To achieve the optimal effect of high-dose vitamin C, it is very important that it is administered as soon as possible and is not postponed. The patients we aim to include (post cardiac arrest with an EMV-score ≤ 8 who will be admitted to the Intensive Care Unit) are per definition incompetent to give informed consent. Obtaining informed consent from a legal representative takes time, even by an experienced research team. We propose to randomize each patient who meets the inclusion criteria ultimately within 5 hours after ROSC. Vitamin C or placebo will be administered after randomization. The legal representative will be informed and asked for informed consent ('deferred informed proxy consent') by the investigator or the treating physician as soon as possible, but not later than 72 hours after ICU-admission (similar as in other ICU-studies^{53,54}). A deferred consent procedure is allowed due to the possible therapeutic benefit the patient could have by participating in this trial. We will offer the legal representative a period of reflection of 2 hours.. If informed consent cannot be obtained within 72 hours, the patient is excluded and data will no longer be used. If a legal

representative denies participation, the patient is excluded and the collected data and (residual) bodily material will be stored and used unless the legal representative objects. Without informed consent, the administration of vitamin C/placebo will then be stopped immediately. Refusal to give consent to take part in the study has no influence whatsoever on the treatment and care. After deferred informed proxy consent is obtained, we will undertake two serious attempts to inform the patient about his/her participation in this study. The patient is free to fill in an withdrawal form when he/she does not want to further participate in the study or when he/she wants his/her collected data and body material to be removed. Our procedure is more extensively described in our attached standard operating procedure (SOP).

Any questions regarding the participation of their relatives legal representatives can ask the investigator, treating physician or independent physician who is mentioned at the end of the patient information letter. This physician knows everything about the study, but is not involved in the conduct of the study.

The legal representative is one of the following persons, in descending order of importance: a person acting under the written authority of the patient, registered partner or life partner, parents, children, brothers/sisters and grandchildren.

11.3 Objection by minors or incapacitated subjects

It is not possible to perform this study in capacitated patients. The patients have potential benefit from the intervention and the risks are negligible.

11.4 Benefits and risks assessment, group relatedness

See also 6.3. It has been shown that vitamin C can have beneficial effects in circumstances of oxidative stress, which occur after cardiac arrest. Different studies with high doses vitamin C have shown no significant adverse events. In this study a maximum of 100 ml blood will be taken during a period of four days from an arterial line which is routinely present for monitoring. The patient will not notice the sampling and it will not impose any risk to the patient. Study parameters are being collected by non-invasive measurements causing minor discomfort, but no pain or risk for the patient.

11.5 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7, subsection 6 of the WMO.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO and the Measure regarding Compulsory Insurance for Clinical Research in Humans of 23th June 2003). This insurance provides cover for damage to research subjects through injury or death caused by the study.

1. € 650.000,-- (i.e. four hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;
2. € 5.000.000,-- (i.e. three million five hundred thousand Euro) for death or injury for all subjects who participate in the Research;
3. € 7.500.000,-- (i.e. five million Euro) for the total damage incurred by the organisation for all damage disclosed by scientific research for the Sponsor as 'verrichter' in the meaning of said Act in each year of insurance coverage.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

11.6 Incentives

The patients will not receive any incentives for their participation in the study.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

Clinical data will be recorded in the Patient Data Management System (PDMS) of the hospital (every hospital has its own PDMS) and will be transported to the study database in Castor. Access to the study database will require a password. Patients will receive a unique study number consisting of 6 numbers. The first 3 numbers indicate the hospital followed by 3 personal numbers. These numbers are not compiled by patient-identifying data such as date of birth or hospital number. In a separate key file, the unique study number and name of the patient will be linked to the hospital number of the patient. After completion of the trial this separate key file, including the name, the hospital number and study number will be stored by the secretary of the intensive care unit.

The same unique study number with key file will be used for body materials that will be stored in the Biobank of the Intensive Care at the VUmc. Remaining body materials will be stored in the VUmc for 15 years.

12.2 Monitoring and Quality Assurance

An independent monitor (quality officer) will monitor the study data according to the regulations described under Good Clinical Practice (GCP) in VUmc and all the other participating hospitals. In a selection of study subjects the Informed Consents are controlled. Additionally, during onsite monitoring the officer will perform a Source Data verification of data described in the Case Report Forms to investigation the agreement between source data and study reports. The intensity of this verification is related to the study risk assessment. In particular, inclusion and exclusion criteria and the primary endpoints of the investigation are subject to monitoring. The monitor will evaluate whether SAE's and SUSAR's are adequately reported within the time frame as directed by the Dutch law. The monitoring plan will be enclosed in the Trial Master File.

12.3 Amendments

A 'substantial amendment' is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/serious adverse reactions, other problems, and amendments.

12.5 Temporary halt and (prematurely) end of study report

The sponsor will notify the accredited METC and the competent authority of the beginning and the end of the study within a period of 90 days after completion. The end of the study is defined as the last patient's last visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC and the competent authority within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC and the Competent Authority.

12.6 Public disclosure and publication policy

We are free to make a publication and have no restrictions made by a sponsor. Patient data will be published anonymously.

13. STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

a. Level of knowledge about mechanism of action

See also 1.3. Beyond simply preventing scurvy by the correction of vitamin C deficiency, supraphysiological vitamin C levels can exert more potent antioxidative effects⁵⁵. When vitamin C is administered in considerable larger dosage than the currently registered 0.5 – 2.0 g per day (at least 3 g i.v. vitamin C /day is necessary just to correct deficiency in critically ill patients), much higher plasma levels are generated which not only limit the generation of ROS, but also directly scavenge ROS, repair other oxidized scavengers such as glutathione, urate and vitamin E, modulate numerous enzyme reactions and recover endogenous vasopressor synthesis¹¹. In addition, especially relevant for post cardiac arrest patients, vitamin C may protect the brain. Neurons in the brain have high rates of oxidative metabolism and contain some of the highest concentrations of vitamin C. Intracellular vitamin C serves several functions in the brain, including antioxidant protection, peptide amidation, myelin formation, synaptic potentiation, catecholamine synthesis and protection against glutamate toxicity⁵⁶.

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism

See also 1.4 and our reviews in Crit Care ^{3,34}.

c. Can the primary or secondary mechanism be induced in animals and/or in ex-vivo human cell material?

See also 1.4 and our reviews in Crit Care ^{3,34}.

d. Selectivity of the mechanism to target tissue in animals and/or human beings

See also 1.4 and our reviews in Crit Care ^{3,34}.

e. Analysis of potential effect

Of the yearly number of 6000 Dutch patients who suffer from an out-of-hospital cardiac arrest, only 3000 survive to hospital. 50% of these post-cardiac arrest IC patients also survive to hospital discharge². Each 1 point decrease in Δ SOFA score at 96 hours is associated with a 1.24 (95%CI 1.04-1.47) odds ratio of survival^{5,32}. Thus, at a 50% mortality rate, this would translate into an absolute survival effect of 5.5% per SOFA point (10% per 2 points). However, we overstretch the statistics if we directly translate this into a number of 'lives saved' for the at-risk population.

f. Pharmacokinetic considerations

Enteral supplementation with maximally tolerated dosages cannot achieve plasma levels of $> 250 \mu\text{mol/l}$ due to limited absorption, especially so during critical illness where deficiency is even not correctable by the enteral route. Therefore, i.v. administration is necessary to achieve the optimal antioxidative effect.

Normal plasma levels are around $60 \mu\text{mol/L}$ (lower limit $40 \mu\text{mol/L}$). Without supplementation, baseline low plasma concentrations ($6\text{--}20 \mu\text{mol/L}$) further declined in ICU-patients⁸. Normal plasma concentrations were attained while supplementing 3 g/day intravenously¹⁶. Supra-normal plasma concentration were attained while infusing 50 or 200 mg/kg/day in septic patients¹⁸ and even 66 mg/kg per hour in burn patients²⁹.

Very recently, we finished a pharmacokinetic study investigating 2 intermittent ($2 \times 1 \text{ g}$ and $2 \times 5 \text{ g}$ per day) and 2 continuous i.v. dosing regimens (2 g and 10 g per day) in critically ill patients⁴⁸. The results suggest that a bolus is required to quickly attain supernormal plasma levels above 1 mM , and a linear dose-concentration relationship, e.g. a fivefold higher dosage resulted in fivefold higher plasma concentrations.

g. Study population

Comatose adult patients suffering an out-of-hospital cardiac arrest who are admitted to the intensive care are eligible for inclusion in this present study.

h. Interaction with other products

There is an interaction between thiamine and vitamin C. Thiamine is co-enzyme to glyoxylate aminotransferase which promotes the oxidation of glyoxylate (a byproduct of the intermediary metabolism) to carbon dioxide (instead of oxalate). A higher conversion of vitamin C to oxalate is seen in thiamine deficiency and thiamine deficiency is common in septic patients³⁸. In our study patients thiamine will be added to reduce oxalate excretion.

i. Can effects be managed?

There are no antidotes or antagonists. We do not anticipate major adverse events. In addition, the patients and all their vital functions are monitored continuously at the ICU.

13.2 Synthesis

It has been shown that vitamin C can have beneficial effects in circumstances of oxidative stress, which occurs after cardiac arrest. It is a promising, cheap and safe therapy which may improve outcome after cardiac arrest. Different studies with high doses vitamin C in

critically ill patients have shown that therapy with high-dose vitamin C is safe in this patient group. In addition, the vital functions of the patients are continuously monitored during their ICU-stay. We have established a DSMB and an interim analysis for safety will be performed after inclusion of 135 evaluable patients. In our opinion the potential benefits of high-dose vitamin C outweigh the negligible risks in our study.

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