Statistical Analysis Plan of the ‘Early high-dose vitamin C in post-cardiac arrest syndrome (VITaCCA) trial’

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Section 1: Administrative information

Trial status

VITaCCA was approved by the ethics committee at Amsterdam UMC, location VUmc (Protocol Record METC-2018.120) and registered at ClinicalTrials.gov (NCT03509662) on April 26, 2018 https://clinicaltrials.gov/ct2/show/NCT03509662 and at European Clinical Trials Database (EudraCT) (2017-004318-25) on June 8, 2018. https://www.clinicaltrialsregister.eu/ctr-search/trial/2017-004318-25/NL

Enrollment of patients in the VITaCCA trial started at October 7, 2019 and finished at February 11, 2024. Last patient 180-day follow-up was at August 29, 2024.

Trial protocol and statistical analysis plan (SAP)

The study protocol has been published in *Trials* [1]. A detailed pre-specified statistical analysis plan (SAP) of the VITaCCA-trial is presented in this document (v1.0). The SAP has been written by S Rozemeijer, HJ de Grooth, AME de Man and JWR Twisk, the trial statistician/independent methodologist.

Section 2: Introduction

Background and rationale

The prognosis of patients suffering from cardiac arrest is still poor, with high morbidity and mortality. Once the circulation has recovered, reactive oxygen species (ROS) generated during the ischemia/reperfusion response further contribute to organ damage and death. Intravenous vitamin C may improve the clinical outcome of post-cardiac arrest patients as vitamin C directly scavenges free radicals, repairs oxidized scavengers and reduces the production of ROS. The 'Early high-dose vitamin C in post-cardiac arrest syndrome (VITaCCA) trial' will evaluate whether an early high-dose intravenous vitamin C improves organ function in patients after cardiac arrest [1].

Objectives

The primary objective of the trial is to determine whether early high-dose intravenous vitamin C can improve organ function in patients after cardiac arrest, and to explore the optimal dosing regimen for high dose intravenous vitamin C.

Section 3: Trial methods

Trial design

The VITaCCA trial is a double-blind, multicenter, randomized placebo-controlled trial with a three-arm comparative design. Patients are randomized to one of 3 treatment groups. Group 1 will be treated with placebo (0.9% NaCl), group 2 with a twice-daily intravenous bolus of 1.5g vitamin C and group 3 with a twice-daily intravenous bolus of 5g vitamin C for 4 days or until ICU discharge. In addition, all patients receive intravenous thiamine 200 mg every 12h for 4 days or until ICU discharge.

The clinical trial has been conducted in six Dutch ICUs: Amsterdam UMC, location VUmc (coordinating center), Erasmus MC, Amphia Hospital, OLVG, location East, Maasstad Hospital and Gelderse Vallei Hospital.

Randomization

Eligible patients will be randomized using a computer-generated randomization list in a 1:1:1 ratio to one of the three treatment groups. Randomization will be stratified by site and age (≤66 and >66 years) in blocks of 6. Patients were randomized only if early administration of the first dose (<5 hours after ROSC) was still feasible. Deferred informed consent was obtained <72 hours after inclusion/randomization.

Sample size

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 [1]. The study aimed to include 3x 90 patients to compensate for 10% loss after randomization for late outcomes.

Framework

A superiority framework will be used for primary and secondary outcomes.

Interim analysis

A blinded interim analysis focused on mortality will be caried out by the trial statistician after inclusion of 135 evaluable patients. Analysis will be group comparative. The data safety monitoring board (DSMB) will be blinded but may be unblinded by the trial statistician. The DSMB can advise to stop further recruitment in case of significantly higher mortality in the

vitamin C group(s) compared to placebo and after thorough examination of the clinical content.

Timing of final analysis

The final analysis for the primary and secondary endpoints is planned when the 180-day follow-up is completed for the last patient included.

The full dataset will be assessed for correctness and completeness of the data by reviewing all individual records in Castor. Any errors, discrepant or missing data will be queried and resolved. Thereafter, the database will be locked and exported for statistical analyses. First, the analyses will be performed with blinded data, i.e. included patients will be assigned to intervention A, B or C. Conclusions about the primary outcome will be drawn according to the blinded results and reported in short (abstract) format. This blinded abstract will be included as supplementary material in the paper in which the main results will be presented.

Timing of outcome assessments

Detailed information about timing of the outcome assessments can be found in the methods section of the trial protocol paper [1].

When patients were discharged early, i.e. during the first 4 days of ICU-admission, data was also collected on the day of discharge except for diuresis and fluid balance as these do not reflect a full 24 h period of time. Data was not collected on the ward after ICU-discharge.

Section 4: Statistical principles

Confidence intervals

Effect estimates will be reported with confidence intervals. The confidence intervals will be 95% and two-sided.

Adherence and protocol deviations

Information regarding adherence to the study intervention is collected in Castor for each patient, most importantly 'first dose <5 hours after ROSC' and 'total doses of study medication administered'. All protocol deviations will be noted on a protocol deviation form and shared with the coordinating center. Analysis will be performed according to the intention-to-treat principle, see later.

The study procedures were terminated when a patient did not fulfill all inclusion criteria and/or when a patient suddenly fulfilled one of the 3 upper safety exclusion criteria (see section 5 for study criteria), when more information became available after the study had already been started (after at least one dose of study medication). Deferred consent was asked in all cases in order to follow-up every randomized and treated patient.

Definition of analysis population

The patient populations for the final analyses are defined as follows, with approval of the Data Safety Management Board and the ethics committee:

'Modified intention-to-treat analysis' (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.

For clarification: treated patients who met the inclusion criteria but suddenly fulfilled one of the 3 upper safety exclusion criteria were also included in the primary mITT analysis, but further administration of study medication was terminated.

‘Full analysis set’ (ITT)

- All patients who met the in- and exclusion criteria, for whom an early administration of the first dose (<5 hours after ROSC) was still feasible, were randomized and treated according to the protocol.
    - Including treated patients, who turned out not to meet all inclusion criteria.
    - Including treated patients, who suddenly fulfilled one of the 3 upper safety exclusion criteria.
- This analysis will contain >270 patients.

As can be understood from the information above, all randomized patients who received at least one dose (‘treated’) will be analyzed in the ITT-analysis, despite not fulfilling the inclusion criteria or fulfilling the exclusion criteria. These patients have not been subsequently excluded. As described earlier, further study treatment was terminated, but the patients were followed up according to the protocol.

Patients for whom deferred consent was not obtained <72 hours were excluded from the study and all analyses, except patients who died before consent could be obtained (<72 hours).

Section 5: Trial population

Screening data and eligibility

The inclusion and exclusion criteria [1] are presented below:

Table 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
An out-of-hospital cardiac arrest with return of spontaneous circulation (ROSC); Ventricular fibrillation (VF) or ventricular tachycardia (VT) as first registered cardiac rhythm[a]; Glasgow Coma Scale (GCS) ≤8[b].	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[c].

[a] If an automated external defibrillator advised to shock, then VF or VT was registered by the device.

[b] The last GCS-score before the start of sedatives will be used.

[c] The presence of treatment limitations will only be assessed at the moment of randomization.

The following screening data is collected:

- Number of OHCA patients screened
- Number of OHCA patients eligible for inclusion
- Number of eligible OHCA patients included in the VITaCCA-trial
- Reasons for non-eligibility
- Reasons for not including eligible patients

A flow diagram of all randomized patients is constructed, including:

- Number of randomized patients in whom the study was eventually not initiated, with reason
- Number of randomized patients in whom study treatment A, B or C was initiated
- Number of excluded patients, with reason
- Number of patients in each arm included in the ITT-analysis
- Number of patients in each arm included in the mITT-analysis
- Number of patients (in ITT- and mITT-analysis) in whom study treatment was discontinued, with reason

Follow-up

There are two main reasons for lost to follow up:

- Included patients who are transferred to an ICU or ward of another hospital. Primary endpoint data (to calculate SOFA T96 h) will be collected from the hospitals involved (with consent). Other secondary endpoints will be missing, except for the questionnaires at day 28 and day 180.
- Included patients or their relatives who are not reachable by phone for questionnaires at day 28 and day 180.

The amount and timing of patients lost to follow up will be mentioned in the manuscript or supplementary file.

Description of baseline characteristics

Patients will be described with respect to age, gender, body mass index, medical history of hypertension, diabetes, myocardial infarction, heart failure, COPD and stroke, Charlson Comorbidity Index (CCI), whether the arrest was witnessed, whether bystander-CPR was performed, AED-usage, amount of shocks administered, first monitored rhythm, time from cardiac arrest to initiation of CPR, time from cardiac arrest to sustained ROSC (>20 minutes), time from ROSC to first dose of study medication, and clinical characteristics on admission (first measured pH, first lactate level, ST-segment elevation myocardial infarction, Glasgow Coma Scale).

Categorial variables will be presented as numbers with percentages. Continuous variables will be presented as mean with standard deviation or as median with interquartile range when appropriate. Q-Q plots will be assessed visually to check if a given variable follows a normal distribution.

Section 6: Analysis

Primary outcome and analyses

The primary outcome is the delta 96 h resuscitation-sequential organ failure assessment score (ΔR-SOFA$_{96\text{-baseline}}$): The R-SOFA score at 96 h after randomization minus the score after ROSC (baseline R-SOFA score). Our primary outcome has been described in detail in our published protocol [1]. All relevant data for the primary outcome was collected for all included patients according to the protocol. When included patients were transferred to another ICU prior to T96 h, data was retrieved from the involved hospitals (with consent).

The difference in ΔR-SOFA$_{96\text{-baseline}}$ score between intervention and control groups will be analyzed using linear mixed model analyses, with a random intercept on site level. The analysis will be adjusted for the baseline SOFA-score. When the residuals are not normally distributed the outcome will be transformed before analysis.

Analysis will be performed crude and adjusting for *age* (stratification variable). This age adjusted analysis will be the primary analysis.

In additional analyses, the model will be adjusted for relevant covariates such as delay (time of no flow), total duration of CPR (time of low flow) and first measured pH [2]. Moreover, vitamin C groups will be combined and compared to placebo as well to estimate the overall effect of vitamin C.

Secondary outcomes and analyses

The secondary outcomes are summarized in a SPIRIT figure [1].

Dichotomous secondary outcomes include:

- Delirium
    - CAM-ICU score, ICDSC-score or DOSS-score (yes or no)
- ICU-acquired weakness
    - Medical Research Council sum score (yes: <48, no: 48-60)
- Need for renal replacement therapy (yes or no)
- At day-28 and day-180:
    - Survival (yes or no)

Dichotomous variables will be presented as numbers with percentages. For dichotomous variables logistic regression analyses will be carried out. Results will be presented with odds ratios and 95% confidence intervals.

Ordinal secondary outcomes include:

- Renal
    - Calcium oxalate crystals in urine (negative, few or many)
- At day-28 and day-180:
    - Functional outcome (mRS scale 0-6)
    - Neurological outcome (CPC scale 0-5)

Ordinal variables will be presented as numbers with percentages. For ordinal variables a multinomial logistic regression analysis will be carried out. Results will be presented with odds ratios and 95% confidence intervals.

Continuous secondary outcomes include:

- Organ failure:
    - SOFA-score
- Neurological:
    - Glasgow Coma Scale
    - Neuron-specific enolase (NSE) – ng/mL
    - The Health Utilities Index-3
- Cardiac/hemodynamics:
    - Troponin – ng/L
    - CK – U/L
    - CK-MB – µg/L
    - Vasopressor duration – hours
    - Vasopressor-free days
- Lung:
    - Lung injury score
    - Ventilation duration – days
    - Ventilator-free days
- Renal
    - Creatinine clearance – µmol/L
    - Oxalic acid (urine) – mmol/24h

- Inflammation
    - C-reactive protein – mg/L
- Oxidative stress
    - Oxidation-reduction potential (ORP) – mV
    - Antioxidant capacity (AOC) – µC
- Pharmacokinetics
    - Plasma vitamin C concentration – µmol/L
- Other clinical outcomes
    - ICU-stay – days
    - Hospital stay – days

Continuous variables will be presented as mean with standard deviation or as median with interquartile range when appropriate. Q-Q plots will be assessed visually to check if a given variable follows a normal distribution. For continuous variables linear regression analyses or linear mixed model analyses will be carried out when appropriate. Results will be presented with effect sizes estimates and 95% confidence intervals. When the residuals are not normality distributed the outcome will be transformed before analysis. Analyses will be adjusted for baseline values if applicable.

Secondary analyses will be performed crude. In an additional analysis, vitamin C groups will be combined and compared to placebo as well to estimate the overall effect of vitamin C.

Planned sensitivity analyses

Two sensitivity analyses will be carried out:

- Excluding randomized patients who died <96 hours because death at 96h was counted as the maximum R-SOFA score of 24 points. The choice to give 24 points to deceased patients may have a large impact on the primary analysis.
- Excluding randomized patients from whom the study treatment was terminated prematurely (<96 hours). For instance, this applies to patients transferred from ICU to ICU during the study period and patients fulfilling one of the 3 safety exclusion criteria.

Subgroup analyses

In order to evaluate possible effect modification, in additional analyses, age and first measured pH will be added to the model including the interaction with the intervention variable. When the interaction term is statistically significant, results will be reported for relevant subgroups.

Missing data

No missing data is expected for the primary outcome. Missing covariates used for the adjusted analysis will be imputed.

For the secondary outcomes daily and follow-up data is missing from the moment included patients are transferred to an ICU or ward of another hospital. However, questionnaires were still carried out at day 28 and 180, thereby limiting missing data on the outcome mortality. In case patients or their relative could not be reached by phone, follow up data at day 28 and day 180 was missing. Some secondary endpoints are not routinely performed in every ICU, such as the MRC-sum score, CK-MB or NSE.

The number of participants with missing data for baseline variables and other variables used in the analyses will be reported in the manuscript.

Safety outcomes

Serious adverse events (‘SAEs’) were collected up to day 28 after inclusion. When a patient was excluded or transferred to another hospital, SAEs were only collected during the study period or up to the transfer, respectively. The following SAEs were collected and reported to the Ethical Board of the coordinating center: death, IHCA, start of renal replacement therapy, start of mechanical circulatory support, mechanical ventilation in prone position, new myocardial infarction, new serious liver failure, new cerebrovascular accident or other SAE (unexpected event resulting in a chronic and significant burden to the patient).

The number of SAEs and SUSARs will be reported for the three intervention groups separately.

Statistical software

The statistical analysis will be carried out in SPSS version 28 and RStudio.

References

1. Rozemeijer S, de Grooth HJ, Elbers PWG, Girbes ARJ, den Uil CA, Dubois EA, Wils EJ, Rettig TCD, van Zanten ARH, Vink R *et al*: **Early high-dose vitamin C in post-cardiac arrest syndrome (VITaCCA): study protocol for a randomized, double-blind, multi-center, placebo-controlled trial**. *Trials* 2021, **22**(1):546.
2. Martinell L, Nielsen N, Herlitz J, Karlsson T, Horn J, Wise MP, Unden J, Rylander C: **Early predictors of poor outcome after out-of-hospital cardiac arrest**. *Crit Care* 2017, **21**(1):96.