Supplementary Online Content 2

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Statistical Analysis Plan (SAP)

Regional citrate versus systemic heparin anticoagulation for continuous renal replacement therapy in critically ill patients with acute kidney injury

Statistical Analysis Plan (SAP)

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A clinical trial within the Sepsis Competency Network (SepNet)

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Münster, 17.12.2019

Place, date

Signature

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Münster, 17.12.2019

Place, date

Signature

1 Background of the Study

1.1 Study objectives

Primary trial objective:
Anticoagulation of the extracorporeal circuit is required in continuous RRT (CRRT). To this date, it is not clear which anticoagulant should be used for CRRT. HYPOTHESIS: Regional citrate anticoagulation for CRRT in critically ill patients with AKI prolongs filter life span and reduces 90-day all cause mortality by approximately 8% (from 48% to 40%) compared to systemic heparin anticoagulation for CRRT.

Secondary trial objective:
Evaluation of the clinical impact of the intervention on

- Length of ICU and hospital stay / 1 year all cause mortality
- Renal replacement therapy
- Safety of the intervention
- Cost analysis of renal replacement therapy

1.2 General study design and plan

Multi-centre Clinical Trial
Two arms, randomised, open, controlled, parallel-group trial

2 Analysis populations

2.1 Full analysis set

The full analysis population contains all patients who were randomized with informed consent.

2.2 Per protocol population

The per protocol population contains all patients without major protocol deviations.
Major protocol deviations that lead to exclusion from the per protocol population are

- violation of inclusion/exclusion criteria
- start of continuous renal replacement therapy (CRRT) later than 24h after achievement of KDIGO stage 3 or after clinical indication for CRRT
- noncompliance to the randomized intervention regional citrate anticoagulation (RCA) or systemic heparin anticoagulation (SHA)

2.3 Safety population

The full analysis population contains all patients who were randomized with informed consent and CRRT was started.

3 Endpoints and variables

3.1 Primary endpoints

- Filter life span (hours)
- Overall survival in a 90-day follow-up period (90-day all cause mortality)

3.2 Secondary endpoints

- ICU length-of-stay and hospital length-of-stay
- Duration of renal replacement therapy
- Bleeding complications
- Transfusion requirement
- Rate of infection during primary ICU stay
- Major adverse kidney events at day 28, 60, 90 and after 1 year
- Complications of therapy during study treatment „ Recovery of renal function and requirement for hemodialysis after day 28, 60, 90 and 1 year
- SOFA Scores at day 1-14, 21 and 28
- 28-day, 60-day and 1-year all cause mortality
- Selected laboratory parameters
- Cost analysis of renal replacement therapy

3.3 Statistical Analyses

3.3.1 Baseline Characteristics

3.3.1.1 Number of randomized patients
3.3.1.2 Age, Weight, Height
3.3.1.3 Stratification factors: gender, SOFA Cardiovascular Organ Failure Score 0-2 versus 3-4, presence or absence of oliguria)
3.3.1.4 Baseline creatinine, eGFR, SOFA, APACHE
3.3.1.5 Comorbidities: hypertension, congestive heart failure, diabetes, COPD, chronic kidney disease, cardiac arrhythmia
3.3.1.6 Source of hospital admission: surgical / non-surgical
3.3.1.7 Reason for admission to ICU: sepsis, pneumonia/ARDS, cardiac surgery, vascular surgery, general surgery, trauma, others
3.3.1.8 Severe acute kidney injury (KDIGO 3-classification)
3.3.1.9 Critically illness with clinical indication for CRRT
3.3.1.10 Mechanical ventilation at start of CRRT
3.3.1.11 Vasopressors at start of CRRT
3.3.1.12 Creatinine at start of CRRT
3.3.1.13 Time from randomization to initiation of RRT

3.3.2 Clinical outcomes

3.3.2.1 Primary endpoint 1: Filter life span [hours]
3.3.2.2 Primary endpoint 2: Overall survival in a 90-day follow-up period (90-day all cause mortality)

3.3.2.3 RRT Duration
3.3.2.4 RRT free days through day 28
3.3.2.5 Infections
3.3.2.6 Hospital length of stay
3.3.2.7 ICU length of stay
3.3.2.8 28-day all cause mortality
3.3.2.9 Requirement of RRT on day 28
3.3.2.10 Persistent renal dysfunction (PRD) on day 28
3.3.2.11 Major adverse kidney events (MAKE) on day 28
3.3.2.12 Complete Renal Recovery on day 28
3.3.2.13 60-day all cause mortality
3.3.2.14 Requirement of RRT on day 60
3.3.2.15 PRD on day 60
3.3.2.16 MAKE on day 60
3.3.2.17 Complete Renal Recovery on day 60
3.3.2.18 Requirement of RRT on day 90
3.3.2.19 PRD on day 90
3.3.2.20 MAKE on day 90
3.3.2.21 Complete Renal Recovery on day 90
3.3.2.22 365-day all cause mortality
3.3.2.23 Requirement of RRT on day 365
3.3.2.24 PRD on day 365
3.3.2.25 MAKE on day 365
3.3.2.26 Complete Renal Recovery on day 365
3.3.2.27 Chronic kidney disease at one year
3.3.2.28 New onset of CKD at day 90
3.3.2.29 New onset of CKD at day 365
3.3.2.30 SOFA-Scores at Screening, Baseline, and days 21 and 28
3.3.2.31 Cumulative fluid balance at days 0+1+2
3.3.2.32 Bleeding complications and transfusion requirement
3.3.2.33 Total filter down time

3.3.3 Advanced inclusion criteria for dialysis initiation

3.3.3.1 (Severe) sepsis or septic shock
3.3.3.2 High doses of vasopressors / catecholamines
3.3.3.3 (Refractory) fluid overload or worsening pulmonary edema
3.3.3.4 Number of advanced inclusion criteria

3.3.4 Characteristics of renal replacement therapy

3.3.4.1 Effectiveness of anticoagulation therapy (iCa^{2+}/PTT)
3.3.4.2 CRRT dose
3.3.4.3 Modality of CRRT (CVVH/CVVHD/CVVHDF)
3.3.4.4 Blood flow
3.3.4.5 RRT Duration
3.3.4.6 Change to intermittent technique
3.3.4.7 Catheter diameter
3.3.4.8 Catheter localization

3.3.5 Subgroup analysis: surgical patients
3.3.5.1 Number of Patients
3.3.5.2 Primary endpoint 1: Filter life span [hours]
3.3.5.3 Primary endpoint 2: Overall survival in a 90-day follow-up period (90-day all cause mortality)
3.3.5.4 Secondary outcomes
3.3.5.5 Bleeding complications and transfusion requirement
3.3.5.6 Total filter down time

3.3.6 Subgroup analysis: non-surgical patients

3.3.7 Subgroup analysis: Patients with CKD at Baseline
3.3.8 Subgroup analysis: Patients without CKD at Baseline

3.3.9 Subgroup analysis: Patients with Cardiovascular SOFA = 0/1/2
3.3.10 Subgroup analysis: Patients with Cardiovascular SOFA = 3/4

3.3.11 Subgroup analysis: Male patients
3.3.12 Subgroup analysis: Female patients

3.3.13 Per Protocol Analysis

3.3.14 Adverse events: Heparin induced thrombocytopenia
3.3.14.1 All patients
3.3.14.2 Surgical patients
3.3.14.3 Non-surgical patients

3.3.15 Adverse events: Bleeding Complications
3.3.16 Adverse events: Thrombotic, thromboembolic complications
3.3.17 Adverse events: Transfusion requirement
3.3.18 Adverse events: Hyperkalemia
3.3.19 Adverse events: Severe hypocalcemia
3.3.20 Adverse events: Severe alkalosis
3.3.21 Adverse events: Metabolic acidosis
3.3.22 Adverse events: Citrate accumulation
3.3.23 Adverse events: Hypophosphatemia
3.3.24 Adverse events: Fluid overload
3.3.25 Adverse events: Severe cardiac-rhythm disorders
3.3.26 Adverse events: Hypotensive episodes
3.3.27 Adverse events: Other cardiovascular complications
3.3.28 Adverse events: Respiratory complications
3.3.29 Adverse events: Gastrointestinal complications
3.3.30 Adverse events: Neurologic complications

4 Statistical Methods

Statistical analyses will be performed according to the principles of the ICH guideline E9 'Statistical Principles for Clinical Trials'.

4.1 Descriptive summary of study data

All continuous variables will be summarized using the following descriptive statistics: n (non-missing sample size), number of missing values, mean, standard deviation, median, first and third quartile, minimum, maximum, skewness.

For categorical variables the frequency and percentages of observed levels will be reported based on the non-missing sample size. All summary tables will be structured with a column for each treatment and will be annotated with the total population size relevant to that table/treatment, including any missing observations.

Censored variables will be summarized using Kaplan Meier estimation.

4.2 Inferential statistical summary of study data

Differences between treatment groups will be quantified as follows.

- Normally distributed continuous variables: Student's two-sample t test, absolute mean difference between groups
- Non-normally distributed continuous variables: Wilcoxon two-sample test (t approximation), Hodges-Lehmann estimator of location shift
- Categorical variables: Absolute difference of percentages, odds ratio
- Censored variables: Absolute difference of percentages, hazard ratio

The duration of RRT, hospital stay and ICU stay will be evaluated in two ways. If a patient dies or reaches his/her end of follow-up,

a) the duration of RRT / hospital stay / ICU stay is finished.
b) the duration of RRT / hospital stay / ICU stay is censored at the day of death or end of follow-up according whatever occurred first.

4.3 Primary analysis

An adaptive design with one interim analysis and optional sample size recalculation based on a group sequential plan with alpha spending function according to O'Brien/Fleming was established.
The interim analysis was conducted at the time when 470 patients were recruited in total across both treatment groups and primary outcome data was available (information rate 0.5). Regarding the first coprimary outcome, no futility stop was admitted. Regarding the second coprimary outcome overall survival, a non-binding futility stop was mentioned in the study protocol, if in the interim analysis the local p value of favourable survival in the RCA group is 0.5 or larger, or if stochastic curtailment shows a conditional power of the final statistical analysis with 1450 patients that is lower than 50%.
The treatment effect on the first coprimary outcome filter life span will be evaluated using a (two-sided) inverse normal Likelihood Ratio test based on a multivariable linear mixed model. The treatment effect on the second coprimary outcome overall survival will be evaluated using a (two-sided) inverse normal Likelihood Ratio test based on a multivariable Cox regression model. Both Likelihood Ratio tests will be performed by building a null model with the factors study center, cardiovascular SOFA Score (0-2 vs 3-4), presence or absence of oliguria, and gender. An additional factor in the null model accounts for the changes of inclusion/ exclusion criteria that were implemented via amendment 1. The first factor level indicates patients that were recruited before amendment 1 has been implemented and the second factor level

indicates patients that were recruited after implementation of amendment 1. The linear mixed model of the first coprimary outcome filter life span additionally includes a subject-specific random effect. The Likelihood Ratio tests are performed by comparing the null model to a model that additionally includes a treatment effect (RCA vs SHA).
The multiple (two-sided) significance level is set to alpha=0.05. In order to account for multiplicity due to the definition of two coprimary outcomes, a multiple testing procedure with fixed a priori ordered hypotheses is applied (hierarchical testing), that controls the familywise type I error in the strong sense. First, the null hypothesis of equal filter life span in both treatment groups is tested on a (two-sided) significance level alpha=0.05. If and only if this null hypothesis is rejected, subsequently the null hypothesis of equal overall survival is tested on a (two-sided) significance level alpha=0.05. Each of the above two-sided hypotheses is decomposed into two one-sided hypotheses on significance level alpha=0.025, respectively. The primary effectiveness analysis provides confirmatory statistical evidence.
If the applied (two-sided) inverse normal Likelihood Ratio test shows a significant treatment effect on overall survival, the treatment effect will be estimated by means of the 90-day all-cause mortality rate in both treatment groups.
The primary effectiveness analysis will be performed according to the intention-to-treat principle (ITT) using the full analysis set of all randomized patients. Beyond the primary ITT analysis of the primary outcomes, sensitivity analyses will be performed, including per-protocol analyses.

5 Power calculation

Power calculations were performed based on the two coprimary outcomes filter life span and overall survival in a 90-day follow-up period. The primary effectiveness analysis was intended to show a superiority of RCA versus SHA for CRRT in intensive care patients with AKI. The multiple (two-sided) significance level was set to alpha=0.05. The mean difference of filter life span between the treatment groups based on published data is expected to be at least 5 hours in favour of the RCA group (±27 hours SD within each group). Overall survival is expected to follow an exponential distribution. The expected 90-day mortality rate in the SHA group is 48% based on recently published multicentre trials investigating the same patient population. Differences between treatment groups are considered to be clinically meaningful, if the 90-day mortality rate in the RCA group is 40% or lower. During a 90-day follow-up period, 10% of living patients are expected to be lost to follow-up. The corresponding process is expected to follow an exponential distribution. The required power regarding the first and second primary outcome was set to 90% and 80%, respectively. This corresponds to a 70% power that both coprimary outcomes reach a significant result. The time points of the interim and final statistical analysis are determined from the first primary outcome. Resulting from these considerations, the interim analysis was planned to be performed when 400 patients have been recruited in total across both treatment groups and primary outcome data are available. The final analysis was intended to be performed when 1260 patients have been recruited. In the interim analysis, the sample size of the final analysis might have been recalculated under the restriction of a maximal total number of 1450 patients.
Power calculations were performed using the ADDPLAN software.

6 Randomization and Blinding

Randomization was performed centrally by the Clinical Trials Centre Leipzig in a 1:1 proportion using a minimization method with random component. Randomization was stratified by gender, cardiovascular sequential organ failure assessment (SOFA) score (0-2 versus 3-4) and by the presence or absence of oliguria. Patients were randomized to receive either regional anticoagulation with citrate or systemic anticoagulation with heparin.

7 Treatment of missing values

Missing values that may arise in effectiveness or safety parameters will not be replaced by any kind of statistical imputation.

8 Subgroup analyses

8.1 Surgical and non-surgical patients

8.2 Patients with and without CKD at Baseline

8.3 Patients with Cardiovascular SOFA = 0/1/2 and 3/4

8.4 Male and female patients

9 Software

Statistical analyses will be performed using the statistical software SAS.