Neuroprotect study

Statistical Analysis Plan

Final Version 1.0

Project Title: Neuroprotective goal-directed hemodynamic optimization in post-cardiac arrest patients: a randomized controlled trial (the NEUROPROTECT post-CA trial)

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This statistical analysis plan is approved by:

Principal Investigator:

Date	Professor Stefan Janssens, M.D., PhD

Principal Investigator:

Date	Koen Ameloot, M.D.

Principal Investigator

Date	Joseph Dens, M.D., PhD

Principal Investigator

Date	Professor Cathy De Deyne, M.D., PhD

Statistician:

Date	Ann Belmans, M.Sc.

Contents

1 Purpose

This document provides a detailed description of the statistical analyses prescribed in the final protocol (version 4, dated 20Jan2017), that will be performed for the evaluation of the primary safety, secondary efficacy, clinical and safety endpoints of the NEUROPROTECT study.

2 Study Objectives and Endpoints

2.1 Study Objective

The **primary objective** of the trial is to assess acute whether or not a new goal-directed hemodynamic optimization strategy can reduce cerebral ischaemia in post-cardiac arrest (CA) patients as quantified by diffusion weighted MRI (DW-MRI) to be performed at day 4-5 with quantification of the percentage of ischaemic voxels with an apparent diffusion coefficient (ADC) below 0.650 mm²/s.

2.2 Study Endpoints

2.2.1 Primary Target Variable

% of ischaemic voxels with an ADC<0.650 mm²/s on 4-5 day DW-MRI.

2.2.2 Secondary Efficacy Variables

% of patients with CPC 3-5 at discharge from ICU.

% of patients with CPC 3-5 at 180 days post-CA

SF-36 questionnaire at 180 days post-CA: total score and subscales

Neurocognitive testing at discharge from the hospital: total percentile scores of the Wechsel Memory Scale

Biomarkers – neuron-specific enolase on days 1 to 5.

Activities Daily Life (ADL) score at discharge from the hospital

6-minute walking distance (6MWD) [m] at discharge from the hospital

Renal function: creatinine [mg/dL] and urinary output [mL/24h] at days 1 to 5.

Length of ICU stay

Duration of mechanical ventilation

Placement of tracheostomy

2.2.3 Safety Variables

% of patients with life threatening arrhythmias during intervention period

% of patients with new onset atrial fibrillation during intervention period

% of patients with pulmonary congestion requiring diurectics during intervention period

3 Study Methods

3.1 Overall Study Design and Plan

This was a prospective, multicentre, interventional, controlled, randomized, open-label investigator-driven study.

Patients were recruited at 2 tertiary care hospitals in Belgium (UZ Leuven and ZOL-Genk) and were randomized in a 1:1 ratio to either goal-directed hemodynamic optimization or standard care. In patients randomized to receive the intervention, the target mixed venous oxygen saturation (SVO_2) was between 65-75% and the target mean arterial pressure (MAP) was between 85- 100mmHg. Fluid administration and hemodynamic support was adjusted according to a flow chart in case SVO_2 or MAP were below or above the suggested targets on an hourly basis. The intervention lasted up to 36 hours after admission to ICU.

Once admitted to ICU, the patients were assessed hourly up to 36 hours after admission and daily up to 5 days after admission. Further assessments were done at discharge from ICU, discharge from the hospital and at 180 days after the initial cardiac arrest.

3.2 Selection of Study Population

All patients presenting or referred to the investigational site after being successfully resuscitated from out-of-hospital cardiac arrest were screened for enrollment. All enrolled patients were expected to meet all inclusion and exclusion criteria.

3.3 Method of Treatment Assignment and Randomisation

Randomization was performed by site and was stratified according to the presence of shockable rhythm.

Randomization was available daily for 24 hours via a centralized telephone Interactive Voice Response System (IVRS) at the LCC.

3.4 Blinding of Study Treatment

Health care professionals caring for the patients were aware of the intervention assignment. However, physicians performing neurological prognostication, radiologists interpreting MRIs, study personnel assessing final outcome and the study

statistician were kept unaware of the randomized group up to the moment of the unblinding of the study after database lock.

4 Sequence of Planned Analyses

4.1 Interim Analyses

No interim analysis was planned.

A Data Monitoring Committee reviewed the data on a regular basis. The primary responsibility of the DSMB was to review the progress and conduct of the trial in order to maintain scientific rigor and to ensure the well being of patients participating in the trial.

4.2 Final Analysis and Reporting

Upon final database lock, statistical analyses of the data will be performed according to the methods described in this document.

Any deviations will be documented.

The analysis populations were finalised at a Blinded Review Meeting on 5Mar2018 that was held by the Steering Committee upon inclusion of all patients into the study. The decisions made at this meeting were documented in the Analysis Sets document.

5 Sample Size Determination

Based on the results of a pilot study at Stanford University in which the standard deviation of the percentage ADC < 0.650 mm^2/s in comparable post-CA patients was 8.9%, it was estimated that 56 patients would be necessary in each study group to show a 40% reduction in the extent of cerebral ischemia with a power of 80% at a two-sided alpha level of 0.05.

6 Analysis Populations

6.1 Safety Set (SS)

The safety analysis set will include all patients who have received at least one dose of study medication.

The safety set will be used for the analysis of all safety data. Summaries will be done by actual rather than randomised treatment.

6.2 Full Analysis Set (FAS)

Because the Intention-to-Treat (ITT) principle is so difficult to achieve in practice, the ICH-E9 guidelines allow for a Full Analysis Set, which "is used to describe the

analysis set which is as complete as possible and as close as possible to the intention-to-treat ideal of including all randomised subjects".

For this study, the Full Analysis Set will include all randomized patients, but exclude patients who clearly did not have a cardiac cause for their cardiac arrest.

At the Blind Review Meeting held on 5Mar 2018 by the Steering Committee, it was decided to exclude 3 patients from the FAS because their cardiac arrest was due to asphyxia.

6.3 Per Protocol Set (PPS)

Patients from the FAS with major protocol deviations will be excluded from the per-protocol set (PPS).

The Per Protocol Set was reviewed and finalized at the Blind Review Meeting which took place on 5Mar2018 by the Steering Committee.

All major protocol deviations that lead to exclusion from the PPS are fully documented in Analysis Sets Document.

7 General Issues for Statistical Analysis

7.1 Analysis Software

All analyses will be performed using SAS software version 9.41 or higher for Windows 8 or higher1.

7.2 Summary Statistics

Continuous variables will be summarized by the number of non-missing data points, mean, standard deviation, median and interquartile range.

Categorical and ordinal variables will be summarized by treatment group by observed frequencies and percentages relative to the total number of non-missing items.

All summary statistics will be presented by treatment group and overall.

Data collected at several time points during the trial will be presented by planned visit, regardless of when the visit actually took place.

7.3 Statistical Comparisons between Groups

Since randomization was stratified by the presence of shockable rhythm, all comparisons between groups should account for this stratification.

So, an ANOVA using factors for randomized group and shockable rhythm will be used rather than a two-sample t-test for the comparison of continuous and normally distributed variables. Continuous variables showing serious deviations from normality will be compared using a Van Elteren test. This test will also be used for the comparison of ordinal data.

For binary and categorical data, a Cochran-Mantel-Haenszel test will be used.

For primary and secondary variables, the effect of treatment will be estimated by an appropriate statistic and presented together with its associated 95% confidence interval.

7.4 Methods for Withdrawals, Missing Data and Outliers

For the primary endpoint, multiple imputation methodology will be used to account for missing data. A total of 100 imputations will be done. The imputation model will include the following:

Age, gender, presence of bystander CPR, presence of initial shockable rhythm, neuron specific enolase at days 1-2-3-4-5, presence of a malign EEG pattern on Day 1 and allocated treatment and death within 7 days of randomisation.

7.5 Data Transformations

Since the main analyses are performed using general estimating equation (GEE) models, no transformation of the data will be necessary since these models are quite robust in the presence of deviations from the normality assumption.

For the analysis of the primary endpoint, data transformation using logtransformation can be considered if the model assumptions of normality and constant variance for the residuals are not met.

7.6 Multicentre Study

Although randomization was stratified by study site, the primary and secondary analyses will not take the stratification by study site into account.

7.7 Multiple Comparisons

This study has only one primary endpoint, so no multiplicity issues are present.

For the secondary endpoints, no adjustment will be made to the significance level to account for multiple testing.

7.8 Planned Subgroups, Interactions and Covariates

The following exploratory subgroup analyses of the primary endpoint are planned in the FAS:

- Age at hospital admission: <65 years; ≥ 65 years
- Gender: male; female
- Time to ROSC: < 25min; ≥ 25 min
- Initial rhythm: shockable; not shockable
- Bystander CPR: yes; no
- Presence of chronic hypertension: yes; no
- Study site: UZ Leuven; ZOL-Genk

Summary statistics per treatment and estimated treatment differences, obtained using ANOVA, will be presented for each subgroup. In addition, the interaction between the above subgroups and randomized treatment will be tested to assess whether the treatment effect differs according to subgroup.

8 Study Subjects

8.1 Disposition of Subjects and Withdrawals

A summary by treatment group will be provided for the following:

- Number of randomized subjects,
- Number in Full Analysis Set (FAS);
- Number of subjects included in the Per Protocol Set & Reasons for Exclusion
- Number who died in ICU
- Number discharged from ICU
- Number who died in-hospital (after ICU)
- Number discharged from hospital
- Number with Day 180 assessment
- Number who died prior to Day 180 assessment
- Number lost to follow-up prior to Day 180 assessment

8.2 Protocol Violations and Deviations

Important protocol violations and deviations that can impact the results of the statistical analyses will be fully documented prior to database lock in the Analysis Sets Specification Document.

9 Demographics and Other Baseline Characteristics

All data recorded at baseline will be summarized by treatment group.

Because this was an open-label study, formal statistical comparisons between groups will be done using the methods described above in section 7.3.

Summaries will be presented for FAS and PPS separately.

The following baseline information will be presented:

- Demographic and clinical characteristics
- Medical history
- Prior chronic medications
- Cardiac arrest characteristics (including cause of arrest)
- Admission characteristics
- PCI
- Sofa score

10 Primary and Secondary Endpoints

10.1 Primary Endpoint

The primary endpoint will be summarised by randomised treatment group.

Since it is expected that a substantial proportion will have missing data for the primary endpoint (due to early mortality or other reasons), a statistical analysis that does not take account of this missingness is very likely to be subject to considerable bias.

Therefore, missing primary endpoint data will be imputed using the imputation model described in section 7.4.

In each imputation sample, the data will be analysed using an ANOVA that includes a factor for randomised treatment and the stratification factor of shockable rhythm. The results of the analyses will then be combined with standard methodology, using SAS PROC MIANALYZE.

The effect of randomised group will be estimated by the difference between groups and presented along with its associated 95% confidence interval.

The statistical analysis of the primary endpoint will be performed for the FAS and PPS.

10.2 Secondary Endpoints

The statistical analysis of all secondary endpoints will be performed for the FAS and PPS.

10.2.1 **CPC 3-5 at Discharge**

Since patients who died in-hospital will have a CPC score of 5, it is expected that the number of patients with missing data will be negligible.

Therefore, the %patients with CPC 3-5 at discharge will be analysed using a Cochran-Mantel-Haenszel test with shockable rhythm as stratification factor.

The effect of randomised group will be estimated by the stratified odds ratio between the two groups and presented along with its 95% confidence interval.

10.2.2 **CPC 3-5 at 180 Days**

The %patients with CPC 3-5 at Day 180 will be analysed using the same methodology as used for the analysis of the CPC scores at discharge (see section 10.2.1) whereby all patients who did not have CPC scores due to death are given a score of 5.

A sensitivity analysis will be performed whereby all patients who did not die and do not have CPC data will be given a score of 5.

10.2.3 **SF-36 Questionnaire at Day 180**

Of main interest is the total score obtained from the SF-36 questionnaire. In addition, the subscales will be analysed using the same methodology.

It should be kept in mind that a considerable proportion of patients will have died prior to Day 180 and that therefore, the SF-36 results in these patients are not defined. A comparison of the scores in the survivors will yield biased estimates of the treatment effect, since the element of randomisation is lost in such comparison if survival depends on the assigned treatment group.

Therefore, the effect of randomised group will be evaluated by the "survivor average causal effect" (SACE), defined as the effect of randomised treatment on the outcome among the subgroup of patients that would have survived under either treatment arm. The SACE will be calculated as explained by Hayden et al by weighing the result of each survivor by the estimated survival probability in the other group. The confidence interval will be obtained by bootstrapping (500 samples).

The estimated survival probabilities will be obtained from a logistic regression using survival as dependent variable and the baseline covariates given in Section 7.4 as independent variables.

10.2.4 **Neurocognitive Testing at Discharge**

The total percentile scores of the Wechsel Memory Scale will be analysed using the same methodology as for the SF-36 questionnaire (see Section 10.2.3).

10.2.5 Neuron-Specific Enolase on Days 1 – 5

Enolase data collected on Days 1 to 5 will be analysed using a GEE2 (generalized estimating equations) model with identity link and normally distributed residuals. The model will include factors for shockable rhythm, randomised treatment, day and the interaction between treatment and day. An unstructured variance-covariance matrix will be used to account for interdependencies between the visits.

An overall Wald test of the interaction and the main effect of treatment will be used to assess whether there is any effect of treatment. The interaction will be tested using a Wald test to assess whether the shape of the evolution over the 5 days differs between the two groups.

In addition, treatment differences will be calculated and assessed at each day. To account for multiple testing, a stepwise Bonferroni correction will be used for both the calculation of the p-values and the confidence intervals. If the interaction is not statistically significant, an average treatment difference will be calculated by removing the interaction from the GEE model.

10.2.6 ADL Scores at Discharge

ADL scores will be analysed using the same methodology as for the SF-36 questionnaire (see Section 10.2.3).

10.2.7 6-Minute Walking Distance [m]

The 6-minute walking distance will be analysed using the same methodology as for the SF-36 questionnaire (see Section 10.2.3).

10.2.8 Creatinine and Urinary Output at Days 1 to 5

Creatinine and urinary output collected on Days 1 to 5 will be analysed using the same methodology as for neuron-specific enolase (see Section 10.2.5).

10.2.9 Length of ICU Stay

Length of ICU will be compared using a Van Elteren test.

The effect of randomised treatment will be estimated by the shift in location using the Hodges-Lehmann estimator and will be presented together with its associated 95% confidence interval.

It is expected that a substantial proportion will have died in ICU. Therefore, to take account of ICU mortality, a competing risk analysis will be performed for time to ICU discharge, considering ICU death as a competing risk. Cumulative incidence functions (CIF) will be calculated and presented together with their associated 95% confidence interval. Comparison of the curves will be done using Gray's test. The median time to discharge will be estimated for both randomised groups.

In addition, stacked CIF curves will be presented for both randomised groups of time to ICU discharge and time to ICU death.

10.2.10 Duration of Mechanical Ventilation

The duration of mechanical ventilation will be analysed a Van Elteren test.

The effect of randomised treatment will be estimated by the shift in location using the Hodges-Lehmann estimator and will be presented together with its associated 95% confidence interval.

10.2.11 Placement of Tracheostomy

The %patients with tracheostomy will be analysed using a Cochran-Mantel-Haenszel test with shockable rhythm as stratification factor.

The effect of randomised group will be estimated by the stratified odds ratio between the two groups and presented along with its 95% confidence interval.

A sensitivity analysis will be performed whereby patients with missing data will be imputed according to a worst-case scenario and will be assumed to have had a tracheostomy.

11 Other Data

11.1 Hourly Data

All hourly data will be summarized by randomized treatment and timepoint. In addition, means with standard deviations or medians with interquartile ranges, as appropriate, will be plotted versus time by randomized treatment.

Summaries will be presented for the FAS.

11.2 Daily Data

All daily data will be summarized by randomized treatment and timepoint. In addition, means with standard deviations or medians with interquartile ranges, as appropriate, will be plotted versus time by randomized treatment.

Summaries will be presented for the FAS.

11.3 Neurological Prognostication Data

Neurological Prognostication data will be summarized by randomized treatment group for the FAS using appropriate summary statistics.

11.4 MRI Data

All remaining MRI data will be summarized by randomized treatment group for the FAS using appropriate summary statistics.

11.5 Neuropsychological Testing

The percentile scores for the subscales and indices will be summarized by randomized group for the FAS using appropriate summary statistics.

11.6 Safety Outcomes

The number (%) of patients with safety outcomes (i.e. incidence of life-threatening arrhythmias, limb ischaemia, new onset atrial fibrillation, pulmonary congestion requiring diuretics) will be presented by actual group for the SS.

12 References

1. SAS software, version 9.4 of the SAS System for Windows. Copyright © 2002 SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA
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3. Hayden D, Pauler DK, Schoenfeld D, An Estimator for Treatment Comparisons Among Survivors in Randomized Trials, *Biometrics* 2005; 61, 305-310.