Cerebrolysin as an Early Add-on to Reperfusion Therapy: Risk of Hemorrhagic Transformation after Ischemic Stroke (CEREHETIS). A Prospective, Randomized, Active-Control, Multicenter Pilot Study

Statistical Analysis Plan

Date of issue: 01 December 2020 (study's original report)

Editing:

- 01 May 2023: Added SAP for post hoc analysis (heterogeneous treatment effect analysis)
- 25 April 2024: Added SAP for secondary analysis (survival analysis)

I. Statistical Analysis Plan (Original Study)

1. Introduction

The CEREHETIS trial is a prospective, randomized, multicenter study designed to evaluate the efficacy and safety of Cerebrolysin as an adjunct to reperfusion therapy in patients with acute ischemic stroke (AIS). Hemorrhagic transformation (HT) remains a serious complication of intravenous thrombolysis (IVT) in AIS, contributing significantly to post-stroke morbidity and mortality. Cerebrolysin, a neuroprotective agent, has shown promise in preclinical studies for its potential to reduce blood-brain barrier permeability and mitigate neuroinflammation. This study aims to determine whether the addition of Cerebrolysin to standard IVT can reduce the incidence of HT and improve neurological recovery and functional outcomes in stroke patients.

The primary outcome focuses on the rate of any HT and symptomatic HT within 14 days post-intervention, while secondary outcomes include assessments of safety, neurological recovery, and long-term functional status. Advanced imaging analyses, including diffusion-tensor imaging (DTI) and computed tomography (CT) perfusion, will provide further insights into the impact of Cerebrolysin on brain recovery.

2. Objective and Endpoints

- **Primary Objective**: To assess whether Cerebrolysin reduces the incidence of any and symptomatic hemorrhagic transformation (HT) within 14 days in AIS patients undergoing IVT.
- **Secondary Objectives**:
    - To evaluate neurological recovery using the National Institutes of Health Stroke Scale (NIHSS) at day 1 and day 14.
    - To assess long-term functional outcomes using the modified Rankin Scale (mRS) at day 90.
    - To assess the safety of Cerebrolysin in combination with IVT by evaluating adverse event (AE) rates.
    - To explore changes in advanced imaging parameters, specifically DTI and CT perfusion metrics, as markers of Cerebrolysin's effect on blood-brain barrier integrity and infarct recovery.

3. Trial Design

The CEREHETIS trial is a prospective, randomized, open-label, multicenter, parallel-group, phase IIIb pilot study with an active control. Patients are randomized in a 1:2 ratio to receive either IVT alone or IVT with Cerebrolysin.

4. Sample Size Calculation

- **Primary Endpoint (HT Rate)**: Based on the assumption that 20% of control group patients will experience HT, with a target odds ratio (OR) of 0.5 in the Cerebrolysin group.
- **Significance Level**: 0.05
- **Power**: 80%
- **Dropout Rate**: 10%
- **Design**: 1:2 allocation ratio between the intervention and control groups to optimize power efficiency and minimize required sample size for this pilot study.

5. Randomization and Blinding

- **Randomization**: Simple randomization across centers using Bernoulli variates (probability parameter of 0.333), with sealed opaque envelopes for treatment allocation.
- **Blinding**: Due to the distinct yellow color of Cerebrolysin, treatment blinding was not feasible; however, imaging analyses are blinded.

6. Data Collection and Time Points

Data collection occurs at the following time points:

- **Baseline** (Day 0): Demographic data, NIHSS, CT, DTI baseline imaging
- **Day 1**: Follow-up imaging and NIHSS assessment
- **Day 7 and Day 14**: NIHSS score, follow-up CT, and DTI imaging on day 14
- **Day 90**: Modified Rankin Scale (mRS) assessment for functional outcome

7. Statistical Analysis

a. Descriptive Statistics

- **Continuous Variables**: Presented as median (M) and interquartile range (IQR) due to non-normal distributions.
- **Categorical Variables**: Presented as percentages and analyzed with χ^2-tests.

b. Comparative Analysis

- **Primary Analysis (HT Rates)**:
    - **Comparison of HT Rates**: Logistic regression will compare the rates of any and symptomatic HT between the Cerebrolysin and control groups. Odds ratios (OR) with 95% confidence intervals (CI) will be reported.
    - **Adjustment for Confounders**: Age, NIHSS, ASPECTS score, and baseline variables such as history of hypertension and previous stroke will be included in multivariate models to control for potential confounding.
- **Secondary Analysis**:
    - **Neurological Recovery (NIHSS)**: The Mann–Whitney U test will compare NIHSS scores between groups at day 1 and day 14.
    - **Functional Outcome (mRS)**: The mRS scores on day 90 will be assessed using a χ^2-test for favorable outcomes (mRS ≤ 2). Additionally, a shift analysis will be conducted to evaluate distributional differences in mRS scores between groups.

c. Advanced Imaging Analysis (Exploratory)

- Evaluate DTI and CT perfusion parameters in middle cerebral artery (MCA) stroke patients, focusing on markers such as fractional anisotropy, axial, radial, and mean diffusivity, and permeability–surface area product (PS).
- **Statistical Methods**:
    - **Group Comparisons**: The Mann–Whitney U test will be used to compare continuous imaging metrics between the Cerebrolysin and control groups at each time point (day 1 and day 14).
    - **Laterality Index Analysis**: Calculate the laterality index to assess asymmetry in imaging metrics between the affected and contralateral sides, with paired t-tests or Wilcoxon signed-rank tests for within-group comparisons from day 1 to day 14.

- **Correlation Analysis**: Spearman's rank correlation coefficient will evaluate associations between imaging metrics (e.g., DTI metrics and PS values) and clinical outcomes, such as NIHSS scores and HT incidence.

8. Safety Analysis

- **Adverse Events (AEs)**: Safety will be assessed by tracking and comparing the incidence of AEs in each group, with specific attention to known Cerebrolysin side effects such as mild agitation and hypotension. Proportions will be compared using χ^2-tests or Fisher's exact test, as appropriate.

9. Software and Statistical Significance

- **Software**: STATA v.14.2 and IBM SPSS v.26.
- **Significance Level**: $\alpha = 0.05$ for primary and secondary endpoints, with multiple hypothesis correction for primary outcomes using Romano-Wolf adjustment.

This Statistical Analysis Plan (SAP) provides a detailed framework for assessing the impact of Cerebrolysin on hemorrhagic transformation, neurological recovery, functional outcomes, and safety in MCA stroke patients undergoing IVT. The exploratory imaging analysis aims to substantiate Cerebrolysin's neuroprotective role by examining advanced imaging biomarkers of blood-brain barrier stability and infarct recovery. This SAP is essential for ensuring that the study findings are robust, reproducible, and clinically meaningful.

II. Statistical Analysis Plan for Post Hoc Analysis of the CEREHETIS Trial (Heterogeneous Treatment Effect Analysis)

1. Introduction

The post hoc analysis of the CEREHETIS trial aims to evaluate the heterogeneous treatment effects (HTE) of Cerebrolysin as an early add-on to intravenous thrombolysis (IVT) in MCA stroke patients. Given the variability in hemorrhagic transformation (HT) risk, this analysis seeks to identify subgroups of patients who benefit most from Cerebrolysin by stratifying them according to HT risk at admission. Multiple predictive scores, including the DRAGON, SEDAN, and Hemorrhagic Transformation Index (HTI), will be used for this purpose. The primary endpoints include any and symptomatic HT and functional outcome at day 90, defined by the modified Rankin Scale (mRS).

2. Objectives and Endpoints

- **Primary Objective**: To evaluate the heterogeneous treatment effects (HTE) of Cerebrolysin on hemorrhagic transformation (HT) and functional outcomes based on HT risk levels.
- **Primary Endpoints**:
    - **Any HT** and **Symptomatic HT** rates within 14 days.
    - **Functional Outcome**: Modified Rankin Scale (mRS) score on day 90, with favorable functional outcome (FFO) defined as mRS ≤ 2.
- **Secondary Endpoints**:
    - Subgroup-specific treatment effects on symptomatic and any HT, as well as FFO, across risk levels determined by DRAGON, SEDAN, and HTI scores.
    - Evaluation of Cerebrolysin's effect on different levels of HT risk to guide potential stratification in future clinical practice.

3. Statistical Analysis

3.1 HT Risk Stratification

- **Scores Used**:
    - **DRAGON**: Combines clinical and imaging features such as the hyperdense MCA sign, pre-stroke mRS, age, glucose levels, onset-to-treatment time, and NIHSS score.

    - **SEDAN**: Uses predictors such as blood sugar, ASPECTS score, hyperdense MCA sign, age, and NIHSS score to predict symptomatic HT.
    - **HTI**: Predicts HT risk using ASPECTS, NIHSS, hyperdense MCA sign, and atrial fibrillation presence, specifically tailored to MCA strokes.
- **Model Selection**:
    - A regression analysis will be performed to evaluate each scoring tool's effectiveness in predicting HT and FFO. The score with the best predictive performance (expected to be HTI) will be selected as the primary risk stratification tool.

3.2 Descriptive and Comparative Analysis

- **Descriptive Statistics**:
    - Continuous variables will be presented as medians with interquartile ranges (IQR) and compared using the Mann–Whitney U test.
    - Categorical variables will be presented as percentages and compared using Pearson's χ^2-test.
- **Logistic Regression for Score-Adjusted Treatment Effects**:
    - Logistic regression models will assess the impact of Cerebrolysin on symptomatic HT and FFO, adjusting for each risk score (HTI, DRAGON, and SEDAN) individually and in combination.
    - Odds ratios (ORs) with 95% confidence intervals (CI) will be calculated.

3.3 Conditional Marginal Effects and Propensity Score Matching

- **Conditional Marginal Effects**:
    - For each HT risk level, conditional marginal effects will be estimated to quantify the Cerebrolysin effect on the likelihood of symptomatic HT and achieving FFO.
- **Propensity Score Matching**:
    - Propensity scores will be calculated for each patient based on baseline covariates, such as age, NIHSS, and comorbidities, to balance baseline differences between treated and untreated patients.
    - Treatment effects will be estimated for matched cohorts using:
        - **Average Treatment Effect (ATE)**: Overall effect across the population.
        - **Average Treatment Effect on the Treated (ATT)** and **Average Treatment Effect on the Untreated (ATC)**.

3.4 Heterogeneous Treatment Effect (HTE) Analysis with Matching-Smoothing

- **Matching-Smoothing Method**:
    - A matching-smoothing method will be applied to assess HTEs. This approach will use propensity scores to match patients and apply local polynomial smoothing to evaluate Cerebrolysin's impact across different HT risk levels.
- **Kernel and Smoothing Parameters**: The Epanechnikov kernel function will be used for smoothing. Bootstrapping (1,000 samples) will be performed to generate robust confidence intervals for the HTE estimates.
- **Output**: Local polynomial smoothed estimates of treatment effects across risk levels with 95% CIs, visually represented to show how Cerebrolysin's impact varies by HT risk level.

3.5 Generalized Ordered Logistic Regression

- **Model Application**:
    - Generalized ordered logistic regression with proportional and partial proportional odds assumptions will be applied to assess the association between mRS outcomes and HT risk.
    - **Brant Test**: The parallel odds assumption will be evaluated using the Brant test; if this assumption is violated, partial proportional odds models will be used.
    - **Output**: Estimated probabilities of mRS outcomes by HT risk and treatment group, with confidence intervals.

3.6 Meta-Analysis for Subgroup Heterogeneity

- **Meta-analytic Techniques**:
    - Subgroups defined by HT risk scores (e.g., low, moderate, high HTI scores) will be treated as individual "studies" in a meta-analysis framework.
    - **Effect Size and Heterogeneity Assessment**:
        - Fixed-effects Mantel–Haenszel models will assess binary outcomes, while random-effects models will evaluate heterogeneity using I^2 and H^2 statistics.
        - Small-study effects will be tested with the Egger test.
    - **Visualization**: A forest plot will display effect sizes across subgroups.

4. Software and Statistical Significance

- **Software**: Stata v.14.2 and Stata v.17.0.
- **Significance Level**: $\alpha = 0.05$ for all primary and secondary outcomes, with corrections for multiple comparisons using the Romano-Wolf adjustment.

5. Interpretation and Reporting

- **Heterogeneity and Interpretation of HTEs**:
    - Results will highlight subgroups where Cerebrolysin significantly reduces HT or improves functional outcomes. Any significant HTE findings will guide further investigation and inform potential stratification for future clinical recommendations.
- **Limitations**:
    - Post hoc analyses bear an inherent risk of finding spurious associations. To address this, all models and outcomes will be rigorously tested and reported regardless of significance. Additionally, findings will be discussed within the context of generalizability and the need for prespecified subgroup analyses in future trials.

This SAP for the post hoc analysis of the CEREHETIS trial outlines a robust, stratified approach to identify subgroups with potential benefits from Cerebrolysin. Advanced statistical methods for matching, smoothing, and meta-analysis will help address treatment heterogeneity, contributing valuable insights into personalized treatment strategies in ischemic stroke.

III. Statistical Analysis Plan for Secondary Data Analysis of the CEREHETIS Trial (Survival Analysis)

1. Introduction

This analysis aims to evaluate the timing of anticoagulation resumption in MCA stroke patients treated with Cerebrolysin, stratified by hemorrhagic transformation (HT) risk. The primary focus is on determining Cerebrolysin's effect on the incidence and hazard of HT over time and establishing the optimal timing for anticoagulation resumption using advanced survival and hazard-based methods. Patients will be categorized into low-risk (HTI = 0) and high-risk (HTI > 0) groups based on the Hemorrhagic Transformation Index (HTI) scores, and outcomes will be analyzed for both symptomatic and any HT within 14 days.

2. Objectives and Endpoints

- **Primary Objective**: To evaluate the effect of Cerebrolysin on the timing of safe anticoagulation resumption in stroke patients with varying HT risk levels.
- **Primary Endpoints**:
    - Incidence of **any HT** and **symptomatic HT** over 14 days.
    - **Timing of Anticoagulation Resumption**: Defined by inception points based on hazard deceleration (HD) curves.
- **Secondary Endpoints**:
    - Survival probabilities for HT stratified by HT risk groups (HTI = 0 vs. HTI > 0).
    - Hazard and risk reduction metrics, including hazard ratios (HR), absolute risk reduction (ARR), number needed to treat (NNT), and restricted mean survival time (RMST) differences for symptomatic and any HT.

3. Statistical Analysis

3.1 Descriptive and Group Comparisons

- **Descriptive Statistics**:
    - Continuous variables will be summarized as medians and interquartile ranges (IQR) for non-normally distributed data.
    - Categorical variables will be expressed as percentages.
- **Group Comparisons**:

- Continuous variables: Mann–Whitney U test.
- Categorical variables: Pearson's χ^2-test.

3.2 Survival Analysis

- **Nonparametric Analysis**:
  - Kaplan–Meier survival curves will be generated for symptomatic and any HT, stratified by HT risk group and treatment arm. Log-rank tests will compare survival distributions.
  - The proportional hazard (PH) assumption will be tested using graphical diagnostics and statistical methods, with alternative combined permutation tests if the assumption is violated.
- **Semiparametric Analysis**:
  - Cox proportional hazards models will estimate hazard ratios (HRs) for symptomatic and any HT, adjusting for HTI category and treatment group.
  - If the PH assumption is violated, time-dependent Cox models with time-varying covariates will be employed.

3.3 Parametric Analysis and Model Selection

- **Parametric Survival Models**:
  - Candidate models include Gompertz, Weibull, exponential, log-logistic, lognormal, and Royston–Parmar flexible parametric models. Model fit will be assessed using the Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), and Cox–Snell residual plots.
  - The model providing the best fit will be used to estimate hazard rates, survival probabilities, and restricted mean survival times.
- **Hazard Deceleration (HD) Analysis**:
  - HD curves will be derived from the parametric hazard function, capturing changes in hazard rates over time.
  - Inception points for anticoagulation resumption will be identified based on HD values falling below 5% of the initial hazard peak, indicating stabilization of HT risk.

3.4 Risk Reduction Metrics

- **Absolute Risk Reduction (ARR)** and **Number Needed to Treat (NNT)**:
    - ARR will be calculated from survival curves to quantify the benefit of Cerebrolysin in reducing HT risk.
    - NNT will be derived from ARR to estimate the number of patients needing treatment to prevent one HT event.
- **Restricted Mean Survival Time (RMST)**:
    - RMST differences between treatment groups will be calculated, providing an alternative to HRs for assessing survival benefit.

3.5 Subgroup and Sensitivity Analyses

- **Subgroup Analysis**:
    - Separate analyses for low-risk (HTI = 0) and high-risk (HTI > 0) groups will examine Cerebrolysin's effects across HT risk strata.
- **Sensitivity Analysis**:
    - Parametric and semiparametric models will be compared using the Hausman specification test to ensure model consistency and robustness.

4. Software and Statistical Significance

- **Software**: Stata/SE v.17.0.
- **Significance Level**: $\alpha = 0.05$ for primary and secondary analyses, with multiple hypothesis correction using the Romano-Wolf adjustment.

5. Interpretation and Reporting

- **Clinical Implications**:
    - Findings will inform optimal anticoagulation timing in stroke patients treated with Cerebrolysin, stratified by HT risk.
- **Reporting**:
    - Results will be reported with corresponding HRs, ARR, NNT, and RMST differences, stratified by HTI categories. Visualizations will include Kaplan–Meier curves, hazard plots, and survival curves for clarity.

6. Limitations

- This analysis relies on post hoc stratification, which may introduce selection bias. Results should be interpreted cautiously and validated in prospective studies.

This SAP outlines a robust framework for analyzing the impact of Cerebrolysin on HT incidence and anticoagulation timing in ischemic stroke patients, ensuring methodological rigor and clinical relevance.