

STATISTICAL ANALYSIS PLAN

Randomized 2×2 Factorial Clinical Trial of Acupuncture-Based Interventions for Post-Acute Ischemic Stroke

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CONFIDENTIALITY / DOCUMENT STATUS

This Statistical Analysis Plan (SAP) specifies the planned statistical analyses for the clinical trial identified above. It is intended to be finalized and approved before database lock and before unblinded analysis of the primary outcomes. Any substantive deviation from this SAP will be documented, justified, dated, and reported in the final study report/publication.

IMPORTANT NOTE ON FINALIZATION

The source protocol/SPIRIT document identifies a 2×2 factorial design, two proposed primary outcomes (Fugl-Meyer Assessment and GUSS), and a planned sample size of 440 participants (110 per group), but explicitly notes that the primary outcome, multiplicity strategy, interaction handling, software, and some missing-data specifications require confirmation. The present SAP converts the available protocol information into a coherent analysis framework. The Principal Investigator and study statistician should approve the highlighted methodological choices before this document is uploaded as the final public SAP.

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1. Study Overview

The study is a randomized, parallel-group, four-arm, 2×2 factorial clinical trial with a 1:1:1:1 allocation ratio. The first treatment factor is acupuncture technique (electro-field acupuncture versus electro-acupuncture). The second treatment factor is acupoint injection regimen (Cerebrolysin versus vitamin B regimen). All four groups receive acupressure massage as background therapy.

Participants are adults with post-acute ischemic stroke, hemiplegia/hemiparesis and dysphagia who meet the protocol eligibility criteria. The intervention period is 30 days, with outcome assessments planned at baseline (D0), Day 15 (D15), and Day 30 (D30). A post-study follow-up at Day 90 (D90) is planned, subject to confirmation of the outcomes and method of assessment.

2. Objectives and Estimands

The protocol's principal objectives are to evaluate: (1) the effect of electro-field acupuncture compared with electro-acupuncture on motor recovery; and (2) the effect of Cerebrolysin acupoint injection compared with the vitamin B acupoint injection regimen on motor recovery and swallowing function.

The primary estimands are the adjusted between-factor differences in change from baseline to Day 30 for the total Fugl-Meyer Assessment (FMA) score and the Gugging Swallowing Screen (GUSS) score, together with the interaction between acupuncture technique and injection regimen. The primary analysis population is the intention-to-treat (ITT) population.

3. Study Design and Treatment Factors

Factor	Level 1	Level 2
Acupuncture technique	Electro-field acupuncture	Electro-acupuncture
Acupoint injection	Cerebrolysin	Vitamin B regimen

Treatment combinations:

- Group 1: electro-field acupuncture + Cerebrolysin acupoint injection + acupressure massage.
- Group 2: electro-field acupuncture + vitamin B acupoint injection + acupressure massage.
- Group 3: electro-acupuncture + Cerebrolysin acupoint injection + acupressure massage.
- Group 4: electro-acupuncture + vitamin B acupoint injection + acupressure massage.

Randomization is specified in the SPIRIT document as restricted block randomization, block size 8, with allocation 1:1:1:1. The randomization list is generated by an independent statistician and concealed until allocation.

4. Analysis Populations

4.1 Intention-to-treat (ITT) population

All randomized participants will be analyzed according to the treatment allocation assigned at randomization, irrespective of treatment adherence, protocol deviations, withdrawal, or subsequent treatment changes, provided that outcome data are available or can be appropriately handled under the prespecified missing-data approach.

4.2 Per-protocol (PP) population

The PP population will comprise randomized participants who receive the assigned intervention sufficiently according to the finalized protocol and have no major protocol deviation judged to affect the primary

efficacy assessment. The minimum adherence threshold will be defined before database lock in accordance with the final protocol; if no threshold is specified in the approved protocol, PP analyses will be considered supportive/sensitivity analyses rather than confirmatory analyses.

4.3 Safety population

All participants who receive at least one session of any randomized intervention will be included in the safety population and analyzed according to the treatment actually received for safety summaries.

5. General Statistical Principles

- All statistical tests will be two-sided.
- The nominal significance level will be $\alpha=0.05$ unless otherwise specified in the multiplicity strategy.
- Continuous variables will generally be summarized using mean, standard deviation, median, interquartile range, minimum and maximum as appropriate.
- Categorical variables will be summarized using counts and percentages.
- Effect estimates will be presented with 95% confidence intervals (CIs). P-values will be reported to three decimal places; values below 0.001 will be reported as $p<0.001$.
- Baseline characteristics will be descriptive. No formal significance testing of baseline differences will be used to determine comparability of randomized groups.
- All analyses will be based on the prespecified analysis datasets and will be performed after database lock unless an analysis is explicitly identified as blinded or safety-related.

6. Baseline Characteristics

Baseline variables will include age, sex, occupation, time since stroke, BMI, stroke risk factors, MRI lesion side, number of previous strokes, YHCT disease pattern, baseline NIHSS, MMSE, MMT, Ashworth, Barthel Index, Rankin, Orgogozo, FMA, MASA, GUSS, and other protocol-specified clinical or laboratory variables.

Continuous variables will be summarized by treatment group using mean (SD) and median (IQR), with ranges when useful. Categorical variables will be summarized using n (%). No baseline p-values will be used as evidence of successful randomization.

7. Primary Efficacy Analyses

7.1 Primary outcomes

- Primary outcome 1: change in total Fugl-Meyer Assessment (FMA) score from D0 to D30.
- Primary outcome 2: change in Gugging Swallowing Screen (GUSS) score from D0 to D30.

The direction of change will be defined as D30 minus D0; positive values therefore represent improvement for scales in which higher scores indicate better function.

7.2 Primary model

For each continuous primary outcome, the primary analysis will use a linear mixed-effects model (LMM) using all available repeated measurements at D0, D15 and D30. The model will include fixed effects for acupuncture technique, injection regimen, time, acupuncture technique $\times$ injection regimen, and treatment-factor $\times$ time interactions as appropriate, with the baseline value included as a covariate where the modeling parameterization requires it. A participant-level random intercept will account for within-participant correlation. The primary contrast will be the adjusted between-factor difference at D30.

The main effects will estimate: (a) electro-field acupuncture versus electro-acupuncture; and (b) Cerebrolysin versus vitamin B regimen. The interaction term will assess whether the effect of one factor depends on the level of the other factor.

If the interaction is clinically/statistically important, treatment combinations will be interpreted separately and the main-effect interpretation will be qualified. If the interaction is not important, the main effects will be emphasized.

7.3 Model assumptions

- Residual distributions will be examined using diagnostic plots.
- Homoscedasticity and influential observations will be assessed.
- If substantial departures from model assumptions are identified, an appropriate transformation or robust/non-parametric sensitivity analysis will be considered and documented.
- The covariance structure for repeated measures will be selected based on clinical/statistical plausibility and model fit; the selected structure will be prespecified before unblinding the treatment results.

7.4 Alternative analysis of change scores

As a supportive analysis, D30 change scores may be compared using an analysis of covariance (ANCOVA) with D30 outcome as the dependent variable, baseline outcome as a covariate, and the two randomized treatment factors plus their interaction as predictors. This analysis will be considered supportive to the repeated-measures primary analysis.

8. Secondary Efficacy Analyses

Secondary outcomes specified in the protocol include motor function, swallowing function, clinical outcomes, laboratory/physiological measures, and YHCT outcomes.

For continuous or approximately continuous repeated outcomes measured at D0, D15 and D30 (including MMT-derived scores where appropriate, Ashworth score, NIHSS, Orgogozo, FMA-UE, FMA-LE, MASA and GUSS), mixed-effects models appropriate to the scale and distribution will be used. For outcomes assessed only at baseline and Day 30, ANCOVA or an appropriate regression model will be used, adjusting for baseline value where applicable.

Ordinal outcomes such as Rankin will be analyzed using an ordinal regression model when proportional-odds assumptions are reasonable; otherwise an appropriate alternative or non-parametric analysis will be used.

Binary outcomes, when prespecified (for example, achievement of a predefined clinically meaningful swallowing or functional status), will be analyzed using logistic regression or generalized linear mixed models as appropriate.

Electromyography, electroencephalography, cerebral rheography, electrocardiography, hematological, biochemical, liver, renal, urinalysis and vital-sign variables will be analyzed descriptively and, where prespecified as efficacy/supportive outcomes, using models appropriate to their distribution and measurement schedule.

All secondary analyses are supportive unless explicitly designated as confirmatory in the final approved protocol/SAP.

9. Safety Analyses

Adverse events (AEs) and serious adverse events (SAEs) will be summarized from first exposure through the end of the prespecified safety follow-up.

The following will be summarized by treatment group: number and percentage of participants with any AE; number and percentage with treatment-related AEs; severity; seriousness; action taken; outcome; and relationship to each intervention when available.

Protocol-specified acupuncture/injection-related events include fainting during acupuncture, bleeding at the puncture site, broken or bent needle, drug reactions, anaphylaxis, and infection at the puncture site.

Participants with multiple occurrences of the same AE will generally be counted once for participant-level incidence summaries, using the highest severity/most serious classification where applicable. Event-level listings will retain all occurrences.

10. Missing Data, Protocol Deviations and Sensitivity Analyses

10.1 Missing data

The extent, timing, and reasons for missing outcome data will be summarized by treatment group. The primary mixed-effects analysis will use likelihood-based estimation under a missing-at-random assumption, assuming the model is correctly specified.

Multiple imputation may be used as a sensitivity analysis when the amount or pattern of missingness could materially affect conclusions. The final approved SAP should specify the imputation model, variables included, number of imputations, and combination rules before unblinding.

Single-value imputation such as last observation carried forward will not be used as the primary method.

10.2 Protocol deviations

Major protocol deviations will be identified and classified before database lock and before unblinded efficacy analysis. A listing of deviations will be provided. ITT will remain the primary analysis population; PP analyses will assess robustness.

10.3 Sensitivity analyses

- PP analysis of primary outcomes.
- Alternative covariance structures for the repeated-measures model, if relevant.
- Multiple-imputation analysis for missing primary outcomes, when justified.
- Alternative analysis excluding influential observations, if prespecified criteria identify such observations.
- If clinically relevant, analysis under alternative assumptions about treatment-by-factor interaction.

11. Subgroup and Exploratory Analyses

The protocol identifies the following potential subgroup/exploratory variables: YHCT disease pattern, degree of motor impairment, upper-limb involvement, lower-limb involvement, and other prespecified prognostic factors.

Subgroup analyses will estimate treatment-factor effects within clinically meaningful strata and will include treatment-factor × subgroup interaction terms where statistically appropriate. These analyses will be considered exploratory unless adequate power and a formal interaction hypothesis were prespecified.

Unplanned subgroup analyses identified after database lock will be clearly labeled exploratory.

12. Multiplicity and Interpretation

Because the protocol proposes two primary outcomes and a 2×2 factorial design with main effects and an interaction, multiplicity must be explicitly controlled in the final approved SAP.

Recommended confirmatory hierarchy for approval: first, the prespecified primary motor outcome (FMA) and primary swallowing outcome (GUSS) should be handled under a declared multiplicity procedure (for example, hierarchical testing or an appropriate alpha-allocation strategy). The final choice must be approved before database lock and should be consistent with the sample-size justification.

The factorial interaction should be interpreted cautiously unless the study was powered specifically to detect interaction. Main-effect estimates should not be interpreted as independent of interaction when a clinically important interaction is present.

Secondary outcomes and exploratory subgroup analyses will not be interpreted as confirmatory evidence unless a multiplicity-adjusted testing strategy has been prespecified.

13. Tables, Listings and Figures

The final statistical report will include, at minimum:

- Participant disposition: screened, eligible, randomized, treated, completed, withdrawn/lost to follow-up, and analyzed.
- Baseline characteristics by the four randomized groups and by the two factorial factors.
- Primary FMA outcome: adjusted factor effects, interaction, treatment-by-time effects, estimated marginal means/differences and 95% CIs.
- Primary GUSS outcome: corresponding estimates.
- Secondary efficacy outcomes by prespecified analysis model.
- Adverse events and serious adverse events.
- Protocol deviations and missing-data summaries.
- Relevant model diagnostics and sensitivity analyses.

Participant-level listings will be generated for randomized treatment allocation, protocol deviations, withdrawals, and serious adverse events as appropriate. Figures may include participant flow, longitudinal estimated means with 95% CIs, and clinically relevant safety displays.

14. Software and Reproducibility

The source protocol states that the original statistical analysis used SPSS 20.0. The finalized SAP should specify the software and version used for the confirmatory analysis before database lock. Recommended implementation is R or another validated statistical package capable of mixed-effects modeling; if SPSS is used, the exact version and procedures should be documented.

All analysis programs, derived datasets, analysis datasets, output logs and final tables/figures will be version-controlled and retained with the trial master documentation. The randomization list and any

treatment-unblinding information will be stored securely and separately from blinded analysis datasets until the prespecified unblinding point.

15. Amendments to the SAP

Any change to this SAP after approval and before unblinded analysis will be dated and version-controlled, with the reason for the change and its potential impact on the analysis documented. Changes made after unblinding will not be used to alter confirmatory analyses without explicit disclosure.

Appendix A. Prespecified Outcome Summary

Outcome	Role	Assessment time	Planned analysis
FMA total score	Primary	D0, D15, D30	LMM; factorial main effects + interaction
GUSS score	Primary	D0, D15, D30	LMM; factorial main effects + interaction
MMT	Secondary	D0, D15, D30	Appropriate repeated-measures model
Ashworth	Secondary	D0, D15, D30	Ordinal/appropriate repeated-measures model
NIHSS	Secondary	D0, D15, D30	Appropriate repeated-measures model
Barthel Index	Secondary	D0, D30	ANCOVA/regression as appropriate
Rankin	Secondary	D0, D30	Ordinal regression as appropriate
Orgogozo	Secondary	D0, D15, D30	Appropriate repeated-measures model
FMA-UE / FMA-LE	Secondary	D0, D15, D30	Repeated-measures model
MASA	Secondary	D0, D15, D30	Repeated-measures model
Clinical dysphagia symptoms	Secondary	D0, D15, D30	Appropriate categorical/ordinal model
Laboratory/physiological measures	Supportive	Per protocol schedule	Distribution-appropriate model
AE/SAE	Safety	D0 through follow-up	Descriptive incidence and event summaries

Appendix B. Items Requiring Final PI/Statistician Approval Before Upload

- Confirm that the trial is formally a 2×2 factorial superiority trial.
- Confirm the two primary outcomes (FMA and GUSS) and the multiplicity-control strategy.
- Confirm the final sample size of 440 (110/group) and ensure the sample-size calculation matches the factorial primary analysis.
- Confirm the exact minimum adherence threshold for the PP population.
- Confirm the exact statistical software and version.
- Specify the covariance structure for repeated-measures models.
- Specify the multiple-imputation model and number of imputations, if used.
- Define the rules for clinically meaningful protocol deviations and influential observations.

- Confirm which outcomes, if any, will be evaluated at D90.

Source basis: Protocol Version 3 (26 November 2025), Protocol Identifier KC.10.01/21-30, and SPIRIT 2025 checklist document dated 24 August 2026. The source documents specify the four treatment combinations, 2×2 factorial framework, 440-participant sample, restricted block randomization (block size 8), ITT/PP principles, mixed-effects modeling preference, and the proposed FMA and GUSS primary outcomes. Items explicitly marked as requiring confirmation in the source documents are retained as finalization points in this SAP.