STATISTICAL ANALYSIS PLAN

Official Title

Comparison of Intraoperative Pain Scores Between Radiofrequency Ablation and Microwave Ablation for Lower Extremity Varicose Veins: A Prospective Single-Center Randomized Controlled Study

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SAP Version

Version 1.0 (based on the revised protocol)

The statistical analysis plan is extracted from, and consistent with, the protocol of the study cited above.

This document does not contain the names or identifying information of any research participants.

Sponsor / Study Site

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1 Introduction and Study Synopsis

This Statistical Analysis Plan (SAP) specifies the statistical analyses for the study "Comparison of Intraoperative Pain Scores Between Radiofrequency Ablation and Microwave Ablation for Lower Extremity Varicose Veins: A Prospective Single-Center Randomized Controlled Study." It is based on the revised version of the protocol, in which four primary intraoperative pain assessment timepoints are defined: immediately after venous access and sheath insertion (T1), immediately after tumescent local anesthesia injection (T2), during catheter ablation (T3), and at the end of surgery (T4). The analyses described below are consistent with the protocol; any deviation from this SAP will be documented and justified in the clinical study report.

The study is a prospective, single-center, open-label, randomized controlled trial (RCT) conducted at the Department of Vascular Surgery, the Affiliated Hospital of Chengdu University of Traditional Chinese Medicine. A total of 80 participants (40 per group) will be randomized in a 1:1 ratio to undergo great saphenous vein (GSV) trunk ablation with either radiofrequency ablation (RFA; ClosureFast, Medtronic) or microwave ablation (MWA; Nanjing ECO). Surgery is performed under local tumescent anesthesia (TLA) only, and patients remain awake throughout. The trial compares intraoperative pain, assessed with the Visual Analog Scale (VAS; 0 = no pain, 10 = worst imaginable pain) at four timepoints, together with TLA- and ablation-related parameters, postoperative pain, satisfaction, acceptance of surgery, and adverse events.

2 Objectives and Endpoints

2.1 Primary Endpoints (Co-Primary Endpoints)

The study has two co-primary endpoints:

(1) **VAS-T2:** the VAS score immediately after TLA injection. The TLA injection stage is defined as the entire period from the first puncture/injection to ultrasound-confirmed completion of injection and readiness to begin ablation; the patient reports the most pronounced pain experienced during this stage. The score is obtained immediately after completion of TLA injection (T2), provided verbally by the patient and recorded by an independent research assistant blinded to group assignment.

(2) **VAS-T3:** the VAS score during catheter ablation. The ablation stage is defined as the entire period from activation of the ablation device to completion of GSV trunk ablation and withdrawal of the ablation catheter/antenna; the patient reports the most pronounced pain experienced during this stage. The score is obtained immediately after completion of trunk ablation (T3), provided verbally by the patient and recorded by an independent research assistant blinded to group assignment.

2.2 Reference and Global Assessment Timepoints

VAS-T1 (immediately after venous access and sheath insertion): the puncture and sheath insertion procedure is identical in both groups, and no between-group difference is expected. This timepoint serves to (1) verify the baseline balance of pain sensitivity between groups, (2) provide a within-subject reference covariate for individual pain sensitivity used to adjust subsequent assessments, and (3) define the starting point of the intraoperative pain trajectory.

VAS-T4 (end of surgery): a retrospective global assessment of pain over the entire operation (including tributary treatment and compression bandaging), reflecting the patient's overall intraoperative pain experience and completing the pain trajectory together with T1–T3.

VAS-T0 (preoperative baseline, before puncture) is the resting baseline pain score of the affected limb and is used as a covariate.

2.3 Secondary Endpoints

- Intraoperative pain trajectory (VAS at T1–T4), analyzed as repeated measures including the group × time interaction;
- TLA injection-related parameters: total injected volume (mL), injection time (min), number of puncture sites;
- Ablation-related parameters: total ablation time, ablation length, ablation energy parameters (RFA: number of segments/temperature; MWA: power/pullback speed);
- VAS scores at 2 hours and 24 hours after surgery (at rest);
- Patient satisfaction score (0–10) and acceptance of surgery (willingness to undergo the same type of surgery again: yes/no/unsure) at 24 hours;
- Incidence of adverse events (see Section 2.4).

2.4 Safety Endpoints

Adverse events recorded intraoperatively and within 30 days after surgery: (1) thermal skin injury/burn; (2) paresthesia or numbness along the course of the GSV; (3) subcutaneous hematoma; (4) superficial thrombophlebitis; (5) deep vein thrombosis (DVT); (6) pulmonary embolism; (7) incision/puncture site infection; and (8) other surgery-related adverse events.

3 Analysis Populations

(1) **Full analysis set (FAS):** all randomized participants who underwent surgery, analyzed according to the intention-to-treat (ITT) principle;

(2) **Per-protocol set (PPS):** participants who completed all follow-up without major protocol violations;

(3) **Safety set (SS):** all participants who underwent surgery.

The primary analysis is based on the FAS. The PPS serves as a sensitivity analysis for the primary endpoints. Safety analyses are based on the SS.

4 Sample Size

The study has two co-primary endpoints (VAS-T2 and VAS-T3), both continuous variables; the sample size is calculated for the comparison of two group means. Because the research hypothesis requires both endpoints to reach statistical significance, a Bonferroni correction controls the family-wise type I error rate: the significance level for each endpoint is $\alpha' = 0.025$ (two-sided), with an overall $\alpha = 0.05$. Comparisons at T1 (reference timepoint) and T4 (global assessment timepoint) are treated as secondary analyses and are not included in the multiplicity correction framework for the primary endpoints.

Parameter	VAS-T2	VAS-T3	Rationale
Significance level (α')	0.025 (two-sided)	0.025 (two-sided)	Bonferroni correction; overall $\alpha = 0.05$
Power ($1 - \beta$)	0.80	0.80	Conventional setting
Expected mean VAS in the RFA group (μ_1)	3.5	2.5	Based on previous studies of intraoperative pain during RFA [9,10]

Parameter	VAS-T2	VAS-T3	Rationale
Expected mean VAS in the MWA group (μ_2)	5.0	4.0	MWA has greater thermal penetration; pain at both stages assumed higher than RFA
Expected between-group difference (δ)	1.5	1.5	A 1.5-point VAS difference is considered the minimal clinically important difference [14]
Pooled standard deviation (σ)	2.0	2.0	Based on comparable studies [9,10]
Attrition rate	15%	15%	Accounting for dropout and loss to follow-up

Table 1. Sample size parameters for the co-primary endpoints.

The sample size per group for the comparison of two means (two-sided test) is:

$$n \text{ per group} = 2 \times (Z_{\alpha'/2} + Z_{\beta})^2 \times \sigma^2 / \delta^2$$

With the Bonferroni-corrected $\alpha' = 0.025$: $Z_{\alpha'/2} = 2.24$; $Z_{\beta} = 0.84$ ($\beta = 0.20$, power = 80%); $\sigma = 2.0$; $\delta = 1.5$. Thus:

$$n \text{ per group} = 2 \times (2.24 + 0.84)^2 \times 2.0^2 / 1.5^2 \approx 33.7 \approx 34$$

Adjusted for a 15% attrition rate: n adjusted = 34 / 0.85 ≈ 40 per group. The two co-primary endpoints use identical parameter settings ($\sigma = 2.0$, $\delta = 1.5$) and therefore require the same sample size.

Conclusion: 40 participants per group; total sample size 80 (RFA group 40 + MWA group 40).

Blinded sample size reassessment: no formal interim efficacy analysis is planned. An assessment of the observed standard deviation is recommended after 10 participants per group have been enrolled; if the observed standard deviation deviates markedly from the assumed value of 2.0, the sample size will be recalculated using the observed parameters (sample size re-estimation based on pooled blinded variance).

5 General Statistical Considerations

- Statistical software: R (version 4.3.0 or later; packages lme4, nlme, interactions, and mediation) or SPSS 26.0;
- All statistical tests are two-sided; $P < 0.05$ is considered statistically significant, except for the co-primary endpoints, for which the Bonferroni-corrected significance level of $\alpha' = 0.025$ applies;
- Continuous variables are summarized as mean ± standard deviation (SD) or median (interquartile range, IQR), depending on the distribution;
- Categorical variables are summarized as frequency (percentage);
- Between-group comparisons of continuous data use the independent-samples t test (if normality and homogeneity of variance are satisfied) or the Mann–Whitney U test (otherwise); normality is assessed graphically (Q–Q plots) and with the Shapiro–Wilk test, and homogeneity of variance with Levene's test;
- Between-group comparisons of categorical data use the chi-square test or Fisher's exact test;
- Effect estimates are reported with two-sided 95% confidence intervals (CIs).

6 Analysis Methods

6.1 Baseline Characteristics

Baseline characteristics (demographics, CEAP classification, VCSS, GSV diameter, preoperative anxiety score [STAI-State], baseline VAS, etc.) are summarized descriptively by group and compared between groups using the tests specified in Section 5. Baseline comparisons are descriptive; no formal significance testing is required for balance assessment, but P values are reported for completeness.

6.2 Primary Endpoint Analysis (Co-Primary Endpoints)

Multiplicity: a Bonferroni correction is applied, with the significance level for each co-primary endpoint set at $\alpha' = 0.025$ (two-sided), so that the family-wise type I error rate is controlled at $\alpha = 0.05$. Only when both endpoints reach statistical significance ($P < 0.025$) can it be concluded that the intraoperative pain in the MWA group is overall higher than that in the RFA group.

For each co-primary endpoint (VAS-T2 and VAS-T3):

- Between-group comparison using the independent-samples t test or the Mann–Whitney U test, as appropriate;
- Reporting of the between-group mean difference with its 95% CI;
- Analysis of covariance (ANCOVA) adjusting for potential confounders (baseline VAS, VAS-T1, preoperative anxiety score, GSV diameter, TLA volume, ablation length, etc.), with VAS-T1 included as a covariate as the within-subject reference for individual pain sensitivity; adjusted mean differences with 95% CIs are reported.

Pain trajectory analysis (key supportive analysis):

- A linear mixed-effects model (LMM) is used for the repeated-measures analysis of VAS scores at T1–T4, with fixed effects for group, time (timepoint), and the group × time interaction, and a random intercept for the individual patient;
- A statistically significant group × time interaction indicates that the intraoperative pain trajectories of the two ablation modalities differ;
- Pain trajectory plots (T1–T4, mean ± standard error) are produced for both groups to visually present the pain contribution of each procedural stage.

Sensitivity analyses:

- If only one of the two co-primary endpoints reaches statistical significance, the result for that endpoint is reported and its clinical significance is discussed;
- The Hochberg step-up procedure is applied as a sensitivity analysis for multiplicity;
- ANCOVA with VAS-T1 as a covariate serves as a sensitivity analysis of the primary analysis, adjusting for individual differences in pain sensitivity;
- The primary analysis is repeated on the PPS.

6.3 Secondary Endpoint Analysis

- VAS scores at T1, T4, and 2 h and 24 h postoperatively: between-group comparisons using the independent-samples t test or the Mann–Whitney U test;
- TLA-related parameters (total volume, injection time, number of puncture sites) and ablation-related parameters (ablation time, ablation length, energy parameters): independent-samples t test or

Mann–Whitney U test;

- Patient satisfaction score: independent-samples t test or Mann–Whitney U test;
- Acceptance of surgery (yes/no/unsure) and other categorical variables: chi-square test or Fisher's exact test;
- VCSS at 30 days: between-group comparison and within-group change from baseline.

6.4 Analysis of Influencing Factors and Their Interactions

For the exploratory objective of identifying the interacting factors underlying the difference in intraoperative pain between the two catheter-based ablation modalities:

(1) **Univariate analysis:** with VAS-T1, VAS-T2, VAS-T3, and VAS-T4 as the dependent variables, respectively, and sex, age, BMI, GSV diameter, GSV depth (distance from the skin), vein segment (thigh/calf), total TLA volume, TLA injection time, number of TLA puncture sites, ablation length, total ablation time, ablation energy parameters, preoperative anxiety score (STAI-State), and baseline VAS as independent variables, Pearson/Spearman correlation analysis or univariate regression is performed to screen potential influencing factors;

(2) **Multivariable linear regression:** with VAS-T2 and VAS-T3 as the main dependent variables, the variables with $P < 0.10$ in the univariate analysis together with the prespecified core variables (group, age, BMI, GSV diameter, TLA volume, preoperative anxiety score) are entered, and stepwise regression is used to identify the independent influencing factors of pain at each timepoint; regression coefficients β with 95% CIs are reported;

(3) **Group interaction analysis:** "group × factor" interaction terms (e.g., group × TLA volume, group × GSV diameter, group × sex, group × age group) are introduced into the multivariable regression to test whether the effect of each factor on pain differs by ablation modality (RFA/MWA), thereby identifying effect modifiers of the pain difference between the two modalities;

(4) **Mediation analysis (exploratory):** if the TLA volume differs between the two groups, mediation analysis is used to test the indirect pathway "ablation modality → TLA volume → injection pain";

(5) **Subgroup analyses:** stratified analyses are performed by sex, age (<60 vs ≥60 years), BMI (<28 vs ≥28 kg/m²), GSV diameter (<8 mm vs ≥8 mm), and preoperative anxiety level (split at the STAI median) to test the consistency of the between-group pain difference across subgroups, with interaction P values used to assess subgroup effects.

Multiplicity note: all influencing-factor analyses are exploratory; no multiplicity correction is applied. Results are presented as point estimates with 95% CIs, serve to generate hypotheses, and do not support confirmatory conclusions.

6.5 Safety Analysis

- Adverse events (AEs) are recorded from the signing of informed consent until the 30-day postoperative follow-up and are summarized by group as frequency (percentage) based on the safety set;
- Between-group comparisons of AE incidence use the chi-square test or Fisher's exact test;
- Serious adverse events (SAEs) are listed individually with their outcome;
- No formal hypothesis testing is applied to safety data; P values, where reported, are descriptive.

6.6 Missing Data

- Multiple imputation is used to handle missing data for the co-primary endpoints;
- The repeated-measures analysis (LMM) uses maximum likelihood estimation, which naturally handles timepoint data that are missing at random;
- Last observation carried forward (LOCF) is used as a sensitivity analysis;
- The number and pattern of missing data are summarized by group and timepoint.

7 Planned Outputs

- Table: participant disposition (screened, randomized, treated, completed follow-up, analyzed in FAS/PPS/SS);
- Table: baseline characteristics by group;
- Table: primary endpoints (VAS-T2, VAS-T3) — descriptive statistics by group, mean difference with 95% CI, unadjusted and adjusted (ANCOVA) analyses;
- Figure: intraoperative pain trajectory (T1–T4, mean ± SE) by group;
- Table: secondary endpoints (VAS at T1, T4, 2 h, 24 h; TLA and ablation parameters; satisfaction; acceptance);
- Table: influencing-factor analyses — univariate screening, multivariable regression coefficients, interaction terms, mediation analysis;
- Figure: subgroup analyses of the primary endpoints with interaction P values;
- Table: adverse events by group.

8 References Cited in This SAP

[9] Aherne TM, Ryan EJ, Boland MR, et al. A systematic review and meta-analysis of the safety and efficacy of endovenous ablation for varicose veins under local anesthesia. J Vasc Surg Venous Lymphat Disord. 2022;10(2):444-455.

[10] Lawaetz M, Serup J, Lawaetz B, et al. Comparison of endovenous ablation techniques, foam sclerotherapy and surgical stripping for great saphenous varicose veins. Extended 5-year follow-up of a RCT. Int Angiol. 2017;36(3):281-288.

[14] Bijur PE, Silver W, Gallagher EJ. Reliability of the visual analog scale for measurement of acute pain. Acad Emerg Med. 2003;10(4):390-392.