STATISTICAL ANALYSIS PLAN

Effects of Stress Ball Use for Patients Undergoing Local Anesthesia in Ambulatory Surgery: A Randomized Controlled Trial

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1. Background and Rationale

Outpatient surgery has expanded across surgical specialties and offers significant cost and efficiency advantages while maintaining acceptable short-term outcomes. In hand surgery, the wide-awake local anesthesia no tourniquet (WALANT) technique is increasingly used; patients remain fully conscious throughout the procedure without sedation or tourniquet, which may give rise to perioperative anxiety that is not always fully captured by self-report measures.

Heart rate variability (HRV) provides a continuous, objective, electrocardiography-derived index of autonomic regulation. The root mean square of successive differences (RMSSD) selectively reflects parasympathetic (vagally mediated) modulation and is well-suited to short-term clinical recordings. Stress ball use is a simple, low-cost, non-pharmacological strategy hypothesized to attenuate procedure-related stress through attentional distraction, active motor engagement, and tactile-proprioceptive input.

This trial evaluates whether intraoperative stress ball use enhances intraoperative parasympathetic modulation (indexed by lnRMSSD) and reduces perioperative anxiety and pain in patients undergoing outpatient hand surgery under local anesthesia without sedation. The trial was prospectively registered at ClinicalTrials.gov (NCT06742814) on December 15, 2024, prior to first-participant enrollment, and was approved by the Research Ethics Committee D of National Taiwan University Hospital (IRB No. 202408018RIND, approved August 2024).

2. Objectives and Hypotheses

2.1 Primary Objective

To evaluate the effect of intraoperative stress ball use on intraoperative HRV in patients undergoing outpatient hand surgery under local anesthesia without sedation, compared with standard perioperative care. (Prospectively specified in the ClinicalTrials.gov registration.)

2.2 Secondary Objectives

- To evaluate the effect of intraoperative stress ball use on postoperative state anxiety.
- To evaluate the effect of intraoperative stress ball use on postoperative pain intensity.

2.3 Hypotheses

Compared with the control group, the intervention group is hypothesized to demonstrate:

- H1 (Primary): Higher intraoperative lnRMSSD, reflecting enhanced parasympathetic modulation.
- H2 (Secondary): Lower postoperative state anxiety scores.
- H3 (Secondary): Lower postoperative pain intensity scores.

3. Study Design

This is a single-center, two-arm, parallel-group, statistician-blinded, randomized controlled trial reported in accordance with the CONSORT 2025 Statement (Hopewell et al., 2025). Participants are randomly allocated 1:1 to the intervention group (IG) or the control group (CG).

3.1 Eligibility Criteria

(Prospectively specified in the ClinicalTrials.gov registration and IRB-approved protocol.)

Inclusion Criteria

- Age ≥ 18 years and able to communicate in Mandarin or Taiwanese Hokkien.
- Scheduled to undergo outpatient hand surgery under local anesthesia.

Exclusion Criteria

- Use of anxiolytics or sedatives within one week prior to surgery.
- Concurrent bilateral hand surgery during the same operative session.
- Known cardiac arrhythmia or use of a cardiac pacemaker (rationale: ectopic beats and paced rhythms invalidate RMSSD-based HRV analysis).

3.2 Randomization and Blinding

Eligible participants are randomized 1:1 to IG or CG using a pre-generated computer randomization sequence (www.random.org). The allocation list is stored on a password-protected computer accessible only to the principal investigator. Participants are assigned strictly in the order in which they entered the study; no replacement or reassignment is permitted. Participant blinding is not feasible because of the nature of the intervention. Clinical staff present in the operating room are unaware of group assignments. Intraoperative HRV is recorded continuously and objectively by a portable ECG device and is therefore not subject to assessor interpretation. Statistical analyses are performed by an independent

statistician blinded to group allocation, with groups coded as “A” and “B” until the primary analysis is finalized.

3.3 Intervention

All participants receive standard perioperative nursing care under local anesthesia using a mixture of 5 mL of 2% lidocaine, 5 mL of normal saline, and 0.1 mL of epinephrine (1 mg/mL), yielding an approximate final epinephrine concentration of 1:100,000, without tourniquet.

Following completion of all baseline assessments (T1), participants in the IG receive a commercially available soft foam stress ball (6 cm in diameter, moderate resistance) in their non-operative hand. A trained research assistant delivers standardized verbal instructions, asking participants to squeeze the ball rhythmically at a rate of once every three seconds while focusing attention on the ball, with the opportunity to practice before entering the operating room. The intervention commences at the initiation of surgery and continues until completion. Participants in the CG rest the non-operative hand naturally on the arm board without any object.

4. Sample Size Justification

(Prospectively specified in the IRB-approved protocol.)

Sample size was determined a priori using G*Power v3.1.9 (α = 0.05 two-tailed; power = 0.80). Because no prior randomized controlled trial had examined the effect of stress ball use on HRV-based outcomes at the time of trial design, an effect size of Cohen's d = 0.71, derived from post-procedure pain scores in a randomized controlled trial of stress ball use during unsedated endoscopy (Karatas & Gezginci, 2023), was used as the reference effect size. This study was selected as the closest available proxy given its use of the same intervention in a comparable invasive procedural context.

The calculation indicated that 64 participants (32 per group) would be required to detect the reference effect. Allowing for an anticipated 10% attrition rate, the target enrollment was set at 74 participants (37 per group). The acknowledged limitation that the reference effect size derives from a subjective pain outcome rather than an HRV-specific endpoint is discussed in the limitations section of the primary publication.

5. Analysis Populations

The following analysis populations are defined for this analysis:

Population	Definition
Full Analysis Set / ITT	All randomized participants, analyzed according to the group to which they were randomized regardless of intervention received. The FAS is the primary analysis population for all efficacy endpoints.
Per-Protocol Set	Participants in the FAS who: (a) received the allocated intervention as specified, (b) had no major protocol deviations, and (c) had complete primary outcome data with intraoperative HRV recordings meeting the R-wave validity criterion (≥ 75% valid beats). Used in supportive sensitivity

	analyses.
Safety Set	All randomized participants who underwent the surgical procedure, analyzed by the intervention actually received. Used for descriptive safety reporting only.

Major protocol deviations include: receipt of a non-allocated intervention, conversion to general or regional anesthesia, unplanned use of anxiolytic or sedative medication during the perioperative window, and HRV recording with < 75% valid R-waves.

6. Endpoints

6.1 Primary Endpoint

(Prospectively specified in the ClinicalTrials.gov registration as “Heart Rate Variability”.)

Intraoperative HRV, operationalized as the natural log–transformed root mean square of successive differences (lnRMSSD), recorded continuously during the surgical procedure (T2) with the participant in the supine position.

HRV Acquisition and Preprocessing

- Device: portable ECG recorder (Amor HRV Guard, model 8z05; Leadtek Research Inc., Taiwan).
- Sampling rate: 500 Hz.
- Recording position: supine throughout the surgical procedure.
- Validity criterion: a minimum R-wave validity of 75% is required for inclusion in the primary analysis. RMSSD remains valid when up to 36% of beat-to-beat intervals are removed (Sheridan et al., 2020); the 75% threshold provides a conservative margin appropriate to the intraoperative environment.
- Transformation: because raw RMSSD values are typically right-skewed, a natural logarithmic transformation is applied prior to all analyses, in accordance with Laborde et al. (2017). The transformed variable, lnRMSSD, is used in all subsequent analyses.

6.2 Secondary Endpoints

(Prospectively specified in the ClinicalTrials.gov registration.)

- Postoperative state anxiety, assessed by the Mandarin-Chinese version of the State–Trait Anxiety Inventory–State subscale (STAI-State; Ma et al., 2013), administered at T3 (immediately postoperative).
- Postoperative pain intensity at the surgical site, assessed by a 10-cm Visual Analogue Scale (VAS; Myles et al., 2017), administered at T3.

6.3 Baseline (T1) Measurements

Baseline (T1) measurements include a 5-minute resting HRV recording (seated), STAI-State, and VAS, all collected on the day of surgery prior to the procedure. T1 values for each outcome are used as covariates in the corresponding analyses.

6.4 Other Variables

Demographic and clinical characteristics collected at baseline include age, sex, BMI, education level, marital status, type of surgical procedure, and relevant medical history (hypertension, diabetes, kidney disease, depression, anxiety, cancer).

7. Estimand Framework (ICH E9(R1))

The estimand for the primary endpoint is defined as follows:

Attribute	Specification
Treatment	Intraoperative rhythmic stress-ball squeezing throughout the surgical procedure (IG) versus standard perioperative nursing care without stress ball (CG).
Population	Adult patients undergoing outpatient hand surgery under local anesthesia without sedation who satisfy the eligibility criteria.
Variable	Intraoperative lnRMSSD measured continuously during the surgical procedure.
Intercurrent Events	(a) Conversion to general or regional anesthesia — treatment-policy strategy. (b) Unplanned anxiolytic or sedative use — treatment-policy strategy. (c) Premature termination of the procedure — while-on-treatment strategy. (d) HRV signal artifact — handled by the 75% R-wave validity criterion (see Section 10).
Population-level Summary	Conditional on the homogeneity-of-slopes test (see Section 9.3): if the assumption holds, the adjusted between-group difference in mean intraoperative lnRMSSD with 95% CI; if violated, the regions of significance from the Johnson–Neyman procedure across baseline lnRMSSD values.

8. General Statistical Considerations

8.1 Software

All analyses are performed using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, NY, USA). The Johnson–Neyman procedure (when triggered) is implemented using the PROCESS macro Version 4.2 (Model 1; Hayes, 2022).

8.2 Significance Level and Confidence Intervals

All hypothesis tests are two-sided with a nominal significance level of $\alpha = 0.05$. Confidence intervals are reported at the 95% level.

8.3 Reporting Conventions

- Continuous variables: mean (SD) for normally distributed data; median (IQR) for non-normal data.
- Categorical variables: frequency (%).
- Effect sizes: partial eta squared (ηp^2; small = 0.01, medium = 0.06, large = 0.14) for ANCOVA models; ΔR^2 for moderation models.
- P-values reported to three decimal places (values < 0.001 reported as "< .001").

9. Statistical Methods

9.1 Participant Disposition and Baseline Characteristics

Participant flow is summarized using a CONSORT 2025 flow diagram. Baseline demographic and clinical characteristics are summarized descriptively by group. Between-group comparability is assessed using independent-samples t-tests for continuous variables and chi-square (or Fisher's exact, as appropriate) tests for categorical variables, presented descriptively without inferential interpretation as the basis for primary inference.

9.2 Distributional Assumptions

Normality of continuous variables is assessed using the Shapiro–Wilk test together with visual inspection of histograms and Q–Q plots. Because raw RMSSD values are expected to be right-skewed, a natural log transformation is applied. Homogeneity of variance is assessed using Levene's test.

9.3 Primary Analysis (Conditional on Homogeneity-of-Slopes Test)

The primary analysis examines intraoperative lnRMSSD using a regression model that includes group, baseline lnRMSSD, the Group × Baseline lnRMSSD interaction term, age, and sex. The homogeneity of regression slopes assumption is evaluated by the significance of the Group × Baseline lnRMSSD interaction term. The analysis path is conditional on this test:

Path A — Interaction not significant (p ≥ .05)

If the homogeneity-of-slopes assumption holds, the interaction term is removed and a standard ANCOVA is fitted:

$$\text{lnRMSSD\_T2} = \beta_0 + \beta_1 \times \text{Group} + \beta_2 \times \text{lnRMSSD\_T1} + \beta_3 \times \text{Age} + \beta_4 \times \text{Sex} + \varepsilon$$

Results are reported as adjusted means (SE) per group, the adjusted between-group mean difference (IG − CG) with 95% CI, the F-statistic with degrees of freedom, p-value, and partial eta squared (ηp^2).

Path B — Interaction significant (p < .05)

If the homogeneity-of-slopes assumption is violated, the conditional treatment effect across the range of baseline lnRMSSD values is examined using the Johnson–Neyman (J–N) procedure (Hayes, 2022; Ji, 2016) — the standard methodological response to violation of the homogeneity-of-slopes assumption when the moderating variable is continuous. The full moderation model is retained:

$$\text{lnRMSSD\_T2} = \beta_0 + \beta_1 \times \text{Group} + \beta_2 \times \text{lnRMSSD\_T1} + \beta_3 \times (\text{Group} \times \text{lnRMSSD\_T1}) + \beta_4 \times \text{Age} + \beta_5 \times \text{Sex} + \varepsilon$$

Results are reported as: (i) the overall model F-statistic and R^2; (ii) the Group × Baseline lnRMSSD interaction F-statistic, p-value, and ΔR^2; (iii) the J–N region(s) of significance; and (iv) the proportion of the analyzed sample that falls within each region. Conditional simple effects are plotted across the observed range of baseline lnRMSSD.

The primary analysis is performed in the FAS (ITT).

9.4 Secondary Analyses

Postoperative STAI-State and postoperative VAS are each analyzed using ANCOVA with the following model:

$$\text{Outcome\_T3} = \beta_0 + \beta_1 \times \text{Group} + \beta_2 \times \text{Baseline} + \beta_3 \times \text{Age} + \beta_4 \times \text{Sex} + \varepsilon$$

Where Baseline = STAI-State_T1 or VAS_T1, respectively. The homogeneity-of-slopes assumption is evaluated prior to each ANCOVA by testing the Group × Baseline interaction. If violated for a secondary endpoint, the J–N procedure is applied analogously to the primary analysis. ANCOVA is selected for its robustness to deviations from normality given the sample size (n > 30 per group); where Shapiro–Wilk indicates substantial departure from normality, a non-parametric supportive analysis (Mann–Whitney U on change scores or rank-based ANCOVA) is reported.

9.5 Sensitivity Analyses

- Per-Protocol analysis: the primary analysis (Path A or Path B) is repeated in the PP set.
- Unadjusted analysis: independent-samples t-test (or Mann–Whitney U) on T2 lnRMSSD without baseline or covariate adjustment.
- Covariate-minimal model: Path A or Path B fitted without age and sex, to assess covariate contribution.
- Influence diagnostics: standardized residuals from the primary model are inspected; analyses are repeated excluding observations with |standardized residual| > 3.

9.6 Subgroup Analyses (Exploratory)

Exploratory subgroup analyses examine consistency across: sex (male/female), age (< 60 vs. ≥ 60 years), and surgical procedure type. Results are exploratory and are interpreted descriptively without multiplicity adjustment.

10. Handling of Missing Data

Missing data are not anticipated for the primary endpoint, given that intraoperative HRV is recorded continuously throughout the surgical procedure. Should missing or invalid data occur, the following rules apply:

- HRV signal validity: epochs containing artifact are excluded prior to RMSSD computation. Participants must have ≥ 75% valid R-waves throughout the intraoperative recording to be included in the primary analysis. Participants below this threshold are treated as having missing primary outcome data.

- Missing post-test STAI or VAS: participants with missing T3 values for a secondary endpoint are excluded from the analysis of that endpoint only, with missingness reported by group.
- Primary endpoint missingness ≤ 5%: complete-case analysis is reported as the primary analysis.
- Primary endpoint missingness > 5%: multiple imputation (m = 50) under a missing-at-random (MAR) assumption is used as the primary approach, with complete-case analysis reported as a sensitivity analysis. A tipping-point sensitivity analysis is additionally reported.

11. Handling of Multiplicity

There is a single primary endpoint (intraoperative lnRMSSD), tested at α = 0.05 (two-sided). No multiplicity adjustment is required for the confirmatory inference.

The two secondary endpoints (postoperative STAI-State and postoperative VAS) are interpreted as supportive, with each tested at the nominal α = 0.05 without multiplicity adjustment. Conclusions regarding secondary endpoints are framed accordingly. No formal hierarchical or gatekeeping testing procedure is applied.

12. Interim Analyses and Data Monitoring

No formal interim analyses involving statistical testing of the study hypotheses are planned. No formal stopping rules are prespecified. A Data Monitoring Committee is not established for this low-risk, non-pharmacological behavioral intervention.

13. Safety Considerations

This trial evaluates a low-risk, non-pharmacological intervention. No drug-related safety endpoints are prespecified. Adverse events potentially related to the surgical procedure or the intervention are recorded descriptively in the Safety Set. No inferential testing of safety outcomes is planned.

14. Specification Status of Analytical Elements

In the interest of full transparency, this section identifies the prospective specification status of each analytical element described in this SAP. Elements are categorized into three groups:

Analytical Element	Specification Status
Primary outcome (intraoperative HRV)	Prospectively specified in the ClinicalTrials.gov registration on December 15, 2024, prior to first-participant enrollment.
Secondary outcomes (STAI-State, VAS)	Prospectively specified in the ClinicalTrials.gov registration.
Directional hypothesis (IG > CG on lnRMSSD)	Prospectively specified.
Eligibility criteria	Prospectively specified in the IRB-approved protocol and trial registration.
Randomization 1:1 with statistician blinding	Prospectively specified.
Sample size justification (n = 74)	Prospectively specified in the IRB-approved protocol.

ANCOVA as base analytic framework	Prospectively specified in the IRB-approved protocol.
Natural log transformation of RMSSD	Standard methodological convention (Laborde et al., 2017); applied prior to outcome modeling.
Inclusion of age and sex as covariates	Methodological refinement during the analysis phase, in line with standard recommendations for HRV research (Quigley et al., 2024). Applied uniformly to primary and secondary analyses prior to outcome modeling.
Johnson–Neyman procedure (Path B)	Methodological response to observed violation of the homogeneity-of-regression-slopes assumption (Hayes, 2022; Ji, 2016). The Johnson–Neyman procedure is the recognized standard analytical follow-up when the moderator is continuous and ANCOVA's homogeneity-of-slopes assumption is violated.
75% R-wave validity threshold	Methodological standard derived from Sheridan et al. (2020); applied uniformly to all participants.
Subgroup and sensitivity analyses	Exploratory; documented in this SAP.
Allocation concealment description	Clarified during manuscript preparation; reflects the actual procedure implemented since trial commencement, with no change in practice.

Critically, the primary outcome, the directional hypothesis, the analytic intent, and the eligibility criteria are unchanged from the prospectively registered ClinicalTrials.gov record (NCT06742814) and the IRB-approved protocol (202408018RIND). The methodological refinements documented above (inclusion of age and sex as covariates; application of the Johnson–Neyman procedure following observed assumption violation) do not constitute changes to the primary outcome or to the directional hypothesis. They represent transparent, methodologically grounded responses applied uniformly to the analysis.

15. Reporting

The trial is reported in accordance with the CONSORT 2025 Statement (Hopewell et al., 2025) and the TIDieR checklist. The primary publication includes: (i) a CONSORT 2025 flow diagram; (ii) a baseline characteristics table by group; (iii) the primary analysis results, with explicit indication of which analysis path (A or B) was followed and the homogeneity-of-slopes test outcome; (iv) secondary endpoint results in the same format; (v) sensitivity and subgroup analyses; and (vi) safety summary. The publication will further document the specification status of each analytical element consistent with Section 14 of this SAP.

16. References

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