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Research Protocol

I. Research Background

Acute kidney injury (AKI) is one of the most common and severe complications following cardiac surgery, characterized by a rapid decline in renal function within hours to days [1-3]. Despite continuous advancements in cardiac surgical techniques, the incidence of postoperative AKI remains as high as 10.4% – 42%, with approximately 2% – 3% of patients requiring renal replacement therapy [2-7]. AKI not only significantly prolongs patients' time in the intensive care unit (ICU) and overall hospital stay but is also strongly associated with increased 30-day and long-term mortality rates, posing a major threat to patient prognosis [2,4]. Cardiopulmonary bypass (CPB), as a critical technical support for cardiac surgery, can induce ischemia-reperfusion injury, systemic inflammatory responses, and autonomic dysfunction due to non-physiological perfusion [8-11], leading to a further increase in the incidence of CPB-associated AKI to 20.8% – 50.8%. The risk is significantly positively correlated with CPB duration and is particularly prominent in complex surgeries [2-6,12-18].

Regarding the prevention of CPB-associated AKI, current clinical strategies primarily focus on perioperative hemodynamic optimization, volume management, and pharmacological interventions. However, these measures are predominantly supportive in nature and fail to fundamentally prevent the onset and progression of AKI [9,19,20]. Although previous studies have explored various pharmacological and blood purification interventions, there remains a lack of highly evidence-based specific therapies with robust high-level evidence [21-23]. This situation

highlights the fundamental limitations of current prevention strategies centered on systemic hemodynamic support and nonspecific anti-inflammatory measures, underscoring the urgent need for novel intervention strategies targeting new mechanisms and targets to overcome the challenges in AKI prevention.

Recent studies have demonstrated that excessive activation of the sympathetic nervous system and inhibition of the parasympathetic nervous system are key driving factors in the occurrence and progression of CPB-associated AKI, with inflammatory responses playing a central role [24-27]. Renal ischemia-reperfusion induced by extracorporeal circulation significantly enhances sympathetic nerve activity, disrupts renal vascular homeostasis by releasing catecholamines and activating the renin-angiotensin system, leading to vasoconstriction, inadequate perfusion, and inflammatory cell infiltration, thereby exacerbating tubular injury and creating a vicious cycle [25,26,28-31]. In this context, vagus nerve stimulation represents an emerging strategy that modulates immune and autonomic functions through electrical stimulation of the vagus nerve [25,32,33], which can mitigate inflammatory responses and improve circulatory function by restoring sympathetic-parasympathetic balance and activating the cholinergic anti-inflammatory pathway (CAP) [26,33-35].

Recent studies have revealed that after vagus nerve activation, signals are transmitted to CD4+ T cells in the spleen via the splenic nerve, promoting their release of acetylcholine. This subsequently activates macrophages expressing α 7 nicotinic acetylcholine receptors (α 7nAChR), inhibiting the release of pro-inflammatory factors and alleviating systemic and renal local inflammation [24,26,33,36,37]. Transcutaneous auricular vagus nerve stimulation (ta-VNS), as a non-invasive technique, can induce physiological effects similar to invasive stimulation by stimulating the auricular branch of the vagus nerve in the auricular region. It activates CAP and demonstrates organ-protective effects. Preclinical studies have confirmed its ability to improve renal function, reduce levels of renal injury markers, and inhibit the release of inflammatory factors and oxidative stress responses in CPB models [25,32,34,35,37-41].

Although ta-VNS has demonstrated potential value in preventing CPB-associated AKI, there remains a lack of rigorously designed randomized controlled trials providing high-level clinical evidence. To address this research gap, this study aims to systematically evaluate the efficacy and safety of ta-VNS in preventing AKI during cardiopulmonary bypass in cardiac surgery patients

through a meticulously designed randomized controlled clinical trial. The goal is to develop an innovative neuromodulation strategy for perioperative renal protection.

II. Research Objective

This study aims to evaluate the efficacy of perioperative transcutaneous auricular vagus nerve stimulation (ta-VNS) in reducing the incidence of postoperative acute kidney injury (AKI) in patients undergoing elective cardiopulmonary bypass surgery through a randomized controlled trial. The hypothesis posits that perioperative ta-VNS application significantly lowers the AKI incidence rate within 7 days postoperatively compared to sham stimulation. Additionally, this study will investigate the effects of ta-VNS on AKI severity, related renal function parameters, and biomarkers to validate its clinical value as a non-invasive neuromodulation approach for perioperative renal protection.

III. Research Design and Methods

3.1 Study Design

This study is a randomized controlled, intervention-based clinical trial. Eligible patients were randomly assigned in a 1:1 ratio to either the ta-VNS group or the sham stimulation group. The primary endpoint was the incidence of acute kidney injury (AKI) within 7 days postoperatively. The objective of this study was to evaluate the efficacy and safety of continuous 6-day perioperative ta-VNS intervention in reducing the incidence of AKI following cardiac surgery under cardiopulmonary bypass (CPB).

3.2 Study Subjects

3.2.1 Inclusion Criteria

1) Age $\geqslant$18 years.

2) Scheduled elective CPB cardiac surgery to be performed under general anesthesia.

3) Baseline serum creatinine level for calculating eGFR $\geqslant$ 60 mL/min/1.73 m^2.

4) Classified as Grade I-III by the American Society of Anesthesiologists (ASA).

5) Cardiac function classification as Class I-III according to the New York Heart Association (NYHA) criteria.

6) Possess adequate comprehension and communication skills to collaborate with the

research process.

7) Voluntary participation in this study and signing of the informed consent form. If the subject is unable to read and sign the informed consent form due to lack of capacity for behavior or other reasons, their guardian must act as proxy for the informed consent process and sign the form. If the subject lacks the ability to read the informed consent form (e.g., illiterate subjects), a witness must observe the informed consent process and sign the form.

3.2.2 Exclusion Criteria

1) Unilateral kidney or post-renal transplantation (RRT) with preoperative AKI (KDIGO criteria) or preoperative RRT.

2) Contraindications for ta-VNS implantation include active infections, dermatitis, skin lesions, or severe psoriasis in the bilateral auricular stimulation areas; hypersensitivity to stimulator materials such as silicone or electrode gel; concurrent uncontrolled arrhythmias, symptomatic bradycardia, sick sinus syndrome, second-degree or higher atrioventricular block; existing cardiac pacemakers or defibrillators; carotid sinus hypersensitivity; uncontrolled epilepsy; or active peptic ulcers.

3) Received vagus nerve stimulation, auricular acupuncture, or transcutaneous electrical nerve stimulation treatment within 1 month prior to enrollment.

4) Drugs used long-term and unable to be safely discontinued preoperatively, which significantly affect autonomic nerve tone or renal blood flow.

5) Preoperative presence of active systemic infection or sepsis (based on Sepsis-3 criteria).

6) History of acute ischemic or hemorrhagic stroke or acute myocardial infarction within 3 months prior to enrollment.

7) Concurrent severe hepatic insufficiency (Child-Pugh class C), acute liver failure, or other serious comorbidities.

8) Vulnerable populations excluding the elderly/illiterates, including critically ill patients, psychiatric disorders, individuals with cognitive impairment, and pregnant women.

9) Other circumstances where the investigator deems the subject unsuitable for participation in the study.

3.2.3 Exit Criteria

1) The subject or their legal representative requests to withdraw from the study.

2) Development of intolerable ta-VNS-related adverse reactions (e.g., severe skin hypersensitivity, pain, dizziness, etc.).

3) Perioperative changes in condition (e.g., prolonged sedation, reoperation) render continued stimulation unfeasible.

4) The investigator determined that the subject was not suitable to continue participating in the study.

3.3 Research Content

3.3.1 Stratification, Blinding, and Intervention

After stratifying subjects according to the Cleveland Clinic scoring system for predicted dialysis-required AKI risk (high risk: $\geqslant$6 points vs. low-to-moderate risk: 1-5 points), independent statisticians generated computer-generated block random sequences (block size: 2 or 4) to assign participants at a 1:1 ratio to either the ta-VNS group or sham stimulation group. The grouping results were sealed in sequentially numbered opaque envelopes.

Blinding was implemented for both participants and outcome evaluators. After obtaining informed consent from participants, a non-blind study coordinator who would not be involved in subsequent clinical work publicly opened the envelope, recorded the grouping information, and handed it to a trained non-blind operator to execute the corresponding stimulation protocol. Other researchers (including those responsible for follow-up, data collection, testing, and statistical analysis) remained unaware of the grouping assignments.

The ta-VNS group received electrical stimulation via a transcutaneous auricular vagus nerve stimulator (tVNS501) applied to the left auricular cartilage. The parameters were set as follows: frequency 25Hz, pulse width 250μs, with a stimulation-interval cycle of 30 seconds stimulation followed by 30 seconds rest. The stimulation intensity was incrementally increased starting from 0.4V in 0.4V increments until the subject experienced pain, then adjusted downward to just below the pain threshold to determine the optimal stimulation intensity. The stimulation coverage spanned preoperative, intraoperative, and postoperative phases: beginning on the day of surgery upon entering the preoperative preparation room and continuing until the patient was transferred to the ICU, followed by 6 hours of stimulation. From postoperative days 1 to 5, the stimulation regimen was restored to twice daily (at 9:00 AM and 2:00 PM) with each session lasting 120 minutes [42-44].

In the sham-stimulation group, electrodes were placed at the same site and within the same cycle period with optimal intensity determined, but stimulation output was turned off without administering actual electrical stimulation.

All equipment was dedicated to specific individuals and labeled for identification. Participants were informed that the stimulation process might induce or fail to induce any discomfort. Heart rate and blood pressure were continuously monitored during the intervention. If persistent heart rate <50 beats per minute, significant hemodynamic instability, or other severe adverse events occurred, stimulation was immediately discontinued and recorded.

The tympanic vagus nerve stimulator (tVNS501) involved in this study exhibits off-label use (off-label indication). The tympanic vagus nerve stimulator (tVNS501) (as specified in the package insert) may be used as adjunctive therapy for sleep disorders, fatigue, anorexia, and anxiety symptoms; when combined with medications, it is applicable for adjunctive treatment of diabetes mellitus. In this study, the tympanic vagus nerve stimulator (tVNS501) was employed to prevent AKI following cardiac surgery under CPB, constituting off-label use. Only the left ear unit was utilized in this study, as the device's built-in metal electrodes enable precise stimulation of the cochlear cone. Although the right ear unit was available, it lacked stimulation functionality and was neither worn nor activated during the study.

3.3.2 Observation indicators

3.3.2.1 Primary Outcomes

Incidence of AKI within 7 days postoperatively

Percentage of subjects who developed new-onset AKI within 7 days postoperatively. AKI diagnosis was determined by clinicians who remained blinded to group assignments, based on the criteria established by the Global Outcomes in Kidney Disease (KDIGO) standards, as specified below (meeting any one criterion suffices):

1) Serum creatinine elevation $\geqslant 26.5$ μmol/L within 48 hours; 2) Increase $\geqslant 1.5$-fold from baseline within 7 days; 3) Urine output reduced to <0.5 mL/kg/h for 6 consecutive hours.

Baseline creatinine was defined as the most recent preoperative serum creatinine measurement.

After baseline creatinine levels were obtained, serum creatinine and urine output were measured daily for seven consecutive days postoperatively.

3.3.2.2 Secondary Outcomes

1) Severity of AKI

Within 7 days postoperatively, the postoperative AKI classification was assessed according to KDIGO criteria based on AKI severity (KDIGO staging: Stage 1/2/3):

Stage 1: Serum creatinine elevation ≥0.3 mg/dl or 1.5 – 1.9 times the baseline value, or urine output <0.5 mL/(kg・h) for 6 – 12 hours;

Stage 2: Serum creatinine levels increased 2.0 – 2.9-fold, or urine output <0.5 mL/(kg・h) for ≥12 hours;

Stage 3: Serum creatinine elevation ≥3.0 mg/dl, or ≥3 times the baseline value, or urine output <0.3 mL/(kg・h) for ≥24 hours, or requiring RRT.

The AKI severity grading was determined by the highest KDIGO stage achieved within 7 days postoperatively.

2) Renal replacement therapy

The proportion and duration of acute RRT required during hospitalization.

3) Renal function recovery

In patients with AKI, those who still meet AKI criteria on postoperative day 7 were defined as having persistent AKI. The time from diagnosis to the first recovery of serum creatinine to within 1.5 times the baseline level without the need for RRT was calculated as the creatinine recovery time.

4) Long-term renal function

Serum creatinine and eGFR at 30 days postoperatively.

5) Autonomic nervous function

5-minute electrocardiogram (ECG) signals were collected preoperatively and at 24 hours, 48 hours, and 72 hours postoperatively, with heart rate variability (HRV) analyzed using Kubios HRV software.

6) Early recovery quality

Postoperative early recovery quality was systematically evaluated from multiple dimensions. Mechanical ventilation duration was defined as the time from the first successful extubation after surgery. The vasoactive drug index was calculated daily within 7 days postoperatively, with peak values and mean values recorded separately. Delirium incidence was assessed daily using either

the Confusion Assessment Measure (CAM) or the ICU Confusion Assessment Measure (CAM-ICU) for both preoperative and postoperative periods (over 7 consecutive days). Early cognitive function was screened using the Mini-Mental State Examination (MMSE) on preoperative day 1, postoperative day 3, and postoperative day 7. Sleep quality was evaluated using the Richards-Campbell Sleep Questionnaire (RCSQ) on preoperative day 1, postoperative day 1 morning, and postoperative day 3 morning to assess prior night sleep status. Recovery quality was scored using the 15-item Quality of Recovery Scale (QoR-15) on preoperative day 1, postoperative day 1, postoperative day 3, and postoperative day 7. Pain and analgesic demand were recorded as baseline pain levels on preoperative day 1 and pain numerical scores (NRS) within 48 hours postoperatively (recorded at least once daily), along with total opioid consumption within 48 hours postoperatively (converted to oral morphine equivalents).

7) Perioperative complications and mortality rate

The incidence rates of cardiovascular complications (including new-onset atrial fibrillation and other arrhythmias, acute myocardial infarction, postoperative myocardial injury, and low cardiac output syndrome), stroke, pulmonary embolism, pulmonary infection, deep vein thrombosis, surgical site deep infection, and all-cause mortality within 30 days postoperatively.

8) Resource consumption

Length of hospital stay, ICU stay duration, and re-admission rate due to complications.

9) Biomarkers

To comprehensively evaluate patient status and explore the pathogenesis of AKI, this study detected the following biomarkers:

a) Routine testing: Collect daily routine laboratory data before surgery and for 7 days postoperatively, including C-reactive protein (CRP), neutrophil-to-lymphocyte ratio (NLR), cardiac troponin (cTn), creatine kinase isoenzyme MB (CK-MB), N-terminal pro-brain natriuretic peptide (NT-proBNP), and serum cystatin C.

b) Specific inflammatory markers and renal injury indicators: Peripheral blood samples were collected at 24h,48h, and 72h postoperatively and placed in EDTA anticoagulation tubes. Plasma interleukin-6 (IL-6) and neutrophil gelatinase-associated lipocalin (NGAL) concentrations were detected in batches using the ELISA method.

c) Peripheral blood immune cell phenotype analysis: Peripheral blood samples were collected at 24h,48h, and 72h postoperatively and placed in EDTA anticoagulation tubes. Peripheral blood mononuclear cells (PBMCs) were isolated within 4 hours. Multicolor flow cytometry was employed to detect the expression level of α7 nAChR on the surface of CD14+ monocytes and the proportion of intracellular IL-6+, as well as the proportions of Th17 (IL-17A+) and Treg (CD25+CD127low/-) subsets among CD4+ T cells, with the Th17/Treg ratio calculated. The analysis was performed using a CytoFLEX flow cytometer and FlowJo software.

Note: Items b and c represent exploratory additional testing mechanisms. A total of 40 patients were selected from the overall sample using stratified random sampling for additional diagnostic evaluation items. The stratification factors included group assignment and Cleveland Clinic score-predicted risk level of dialysis-dependent AKI.

All samples were processed and analyzed using a blinded method. All blood samples were aliquoted into low adsorption cryovials (0.5 mL/vial) to avoid repeated freeze-thaw cycles, and immediately stored in an ultra-low temperature freezer at-80 ° C. Complete sample numbering and time records were established. Upon study completion, the samples were disposed of in accordance with medical waste disposal standards.

3.3.2.3 Basic Clinical Indicators

Prior to randomization of patients, trained investigators collected the following baseline data. All measurements were based on the most recent test conducted after hospital admission and prior to surgery, and must be completed before randomization.

Demography and anthropometry: age, gender, height, weight, body mass index (BMI), ASA classification.

Cardiac function and risk scoring: NYHA cardiac function classification, left ventricular ejection fraction, EuroSCORE II score, Cleveland Clinic score, history of cardiac surgery, recent myocardial infarction (MI) history (<3 months).

Clinical history and comorbidities: history of hypertension, diabetes mellitus, chronic kidney disease, cerebrovascular disease, peripheral vascular disease, chronic obstructive pulmonary disease, hepatic insufficiency (Child-Pugh classification), smoking history, and alcohol consumption history.

Preoperative medication history: Record medications used within 24 hours prior to randomization that cannot be safely discontinued, including RAS inhibitors, β -blockers, calcium channel blockers, diuretics, statins, antiplatelet agents, oral hypoglycemic agents or insulin, and nonsteroidal anti-inflammatory drugs (within 48 hours before surgery).

Baseline laboratory tests: serum creatinine, eGFR (CKD-EPI formula), blood urea nitrogen, hemoglobin, platelet count, albumin, myocardial troponin I, NT-proBNP, and high-sensitivity C-reactive protein.

Surgical parameters: Type of surgery, surgical urgency, extracorporeal circulation time, aortic cross-clamping duration, minimum nasopharyngeal temperature, intraoperative red blood cell transfusion volume, intraoperative urine output, and use of vasoactive drugs during surgery.

Previous intervention history: Whether the participant received vagus nerve stimulation, auricular acupuncture, transcutaneous electrical stimulation therapy, or participated in other clinical studies within 1 month prior to enrollment.

3.3.3 Statistical Analysis Plan

All data in this study were processed using SPSS 26.0 and R 4.3.0 software.

For continuous variables, the Shapiro-Wilk normality test is first performed. Variables conforming to normal distribution are expressed as "mean ± standard deviation," with intergroup comparisons conducted using the independent samples t-test. Variables not conforming to normal distribution are represented by median values (25th and 75th percentiles), and intergroup comparisons are performed using the Mann-Whitney U rank-sum test. Categorical variables are presented as frequencies (percentages), with intergroup comparisons analyzed using the χ^2 test. If theoretical frequencies do not meet the test requirements, the Fisher exact probability method must be employed.

The primary endpoint of this study was the incidence of AKI within 7 days postoperatively, analyzed based on the intention-to-treat principle. Kaplan-Meier curves were constructed to plot the cumulative incidence of AKI within 7 days postoperatively for both groups, with intergroup comparisons performed using the log-rank test. The Cox proportional hazards regression model was employed to estimate the hazard ratio (HR) and its 95% confidence interval for the ta-VNS group versus the sham stimulation group. A single-factor COX regression model incorporating only grouping variables was first fitted to estimate the crude effect, followed by a multivariate

COX regression model that adjusted for preoperative eGFR (<90 vs. ⩾90 mL/min/1.73 m²), diabetes history (present vs. absent), left ventricular ejection fraction (<35% vs. ⩾35%), and surgical type (high-risk group [valve and coronary artery combined/aortic surgery] vs. low-risk group [valve and septal repair]) as covariates. Under the random missingness assumption, imputation models were constructed based on covariates related to outcomes and missingness states, generating 20 imputed datasets for analysis, with results combined according to the Rubin rule. Under the non-random missingness assumption (all missing values in the ta-VNS group were AKI, while all missing values in the sham stimulation group were non-AKI), intergroup comparisons were re-evaluated.

The severity of AKI was analyzed using multivariate ordered logistic regression, adjusting for the same covariates as in the primary analysis and testing the proportional hazards assumption. Results were reported as odds ratios (OR) and their 95% confidence intervals (CI).

The proportion of patients requiring RRT during hospitalization was compared between groups using χ^2 test or Fisher's exact probability method; the duration of RRT was compared between groups based on its distribution characteristics using independent samples t-test or Mann-Whitney U test.

The proportion of patients with persistent AKI was compared between groups using the χ^2 test; the time to renal function recovery was plotted as cumulative recovery curves using the Kaplan-Meier method, with intergroup comparisons performed via the log-rank test. Patients who did not recover during the observation period were treated as censored data.

Serum creatinine and eGFR at 30 days postoperatively were compared between groups using independent samples t-test or Mann-Whitney U test based on their distribution characteristics.

HRV was analyzed using generalized estimating equations, with grouping, time points, and their interaction terms included as fixed effects. The optimal working-related matrix structure was selected based on the quasi-likelihood independence criterion. Significant group × time interaction terms indicated statistically distinct temporal trends in HRV metrics between the two groups.

The duration of mechanical ventilation, peak/mean values of vasoactive drug index, peak/mean values of postoperative 48-hour pain NRS scores, and total opioid consumption were compared between groups using independent samples t-test or Mann-Whitney U test based on

their distribution characteristics. The incidence of delirium was compared between groups using χ^2 test. MMSE, QoR-15, and RCSQ were analyzed using generalized estimating equations (GEE), with model specifications identical to those used for HRV analysis.

The incidence rates of new-onset atrial fibrillation, pulmonary infection, stroke, and 30-day all-cause mortality were compared between groups using χ^2 test or Fisher's exact probability method. If the number of events was sufficient, Cox proportional hazards regression model could be employed for analysis, with adjustment for covariates when necessary. Hazard ratios (HR) and their 95% CI were reported.

Hospitalization duration and ICU stay duration were treated as continuous variables, which were expected to follow a skewed distribution. Between-group comparisons were performed using the Mann-Whitney U test. The re-admission rate due to complications was compared using the χ^2 test.

GEE were employed to analyze differences in the temporal variation trajectories of routine laboratory parameters and plasma IL-6/NGAL levels between two groups. The model included grouping, time, and their interaction as fixed effects, with subjects as random effects. Appropriate work-related matrices and distribution families were selected to assess the significance of the group $\times$ time interaction. Independent samples t-test or Mann-Whitney U test was used to compare postoperative monocyte α7nAChR expression levels, intracellular IL-6+ proportion, and Th17/Treg ratio at various time points between the two groups. GEE analysis was also conducted to evaluate overall trends and intergroup differences. Spearman correlation analysis was employed to investigate associations between immune cell markers (α7nAChR, IL-6+, Th17/Treg), plasma biomarkers (IL-6, NGAL), and clinical outcomes (AKI incidence rate, peak serum creatinine level).

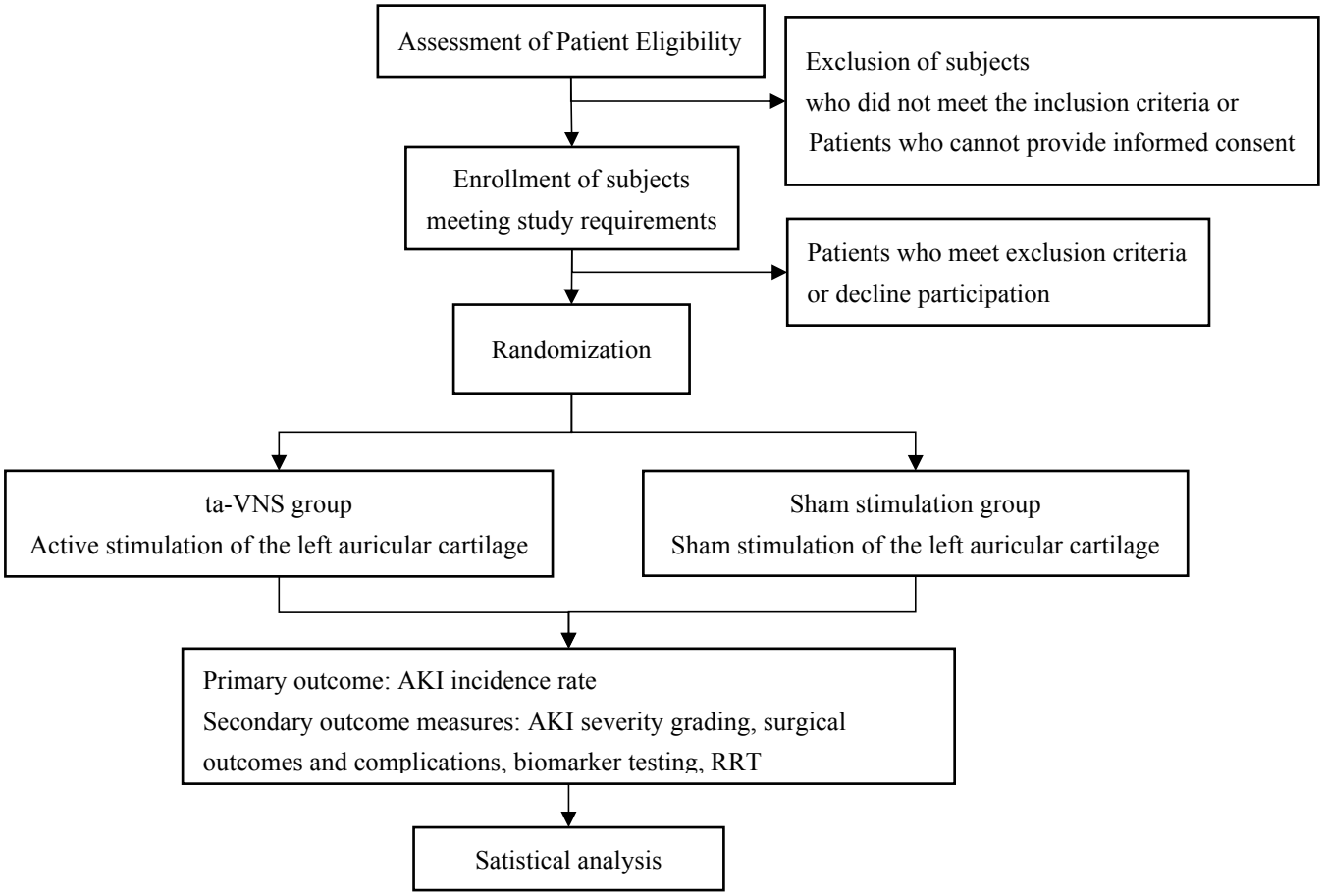
To determine the minimum number of stimulation days required to achieve clinical efficacy, the completion of stimulation was defined as when the actual daily stimulation duration reached $\geqslant$80% of the planned duration. Based on the total number of days (0-6 days) during which subjects completed stimulation, a multivariate logistic regression analysis was performed to examine the dose-response relationship between cumulative stimulation days and the incidence of AKI, thereby identifying the critical number of days when the protective effect tends to plateau.

Subgroup analysis was conducted based on the following prespecified factors: gender, left ventricular ejection fraction (<35% vs. ⩾35%), age (<65 years vs. ⩾65 years), preoperative eGFR (<90 vs. ⩾90 mL/min/1.73 m²), CPB duration (<120 min vs. ⩾120 min), and surgical type (high-risk group [valve and coronary artery combined/aortic surgery] vs. low-to-moderate risk group [valve and septal repair]). Subgroup analysis was implemented in single-factor logistic regression models by introducing interaction terms between grouping and subgroup variables, with OR values and 95% CI for each subgroup visually presented in forest plots.

Safety analysis was based on the safety dataset (all subjects who received at least one stimulation). The incidence rates of all adverse events and serious adverse events were compared between groups using the χ^2 test or Fisher's exact probability method.

Unless otherwise specified, all tests were conducted as two-tailed tests with a significance level set at α=0.05, meaning that differences were considered statistically significant when P<0.05.

3.4 Research Flowchart



IV. Sample Size Calculation

The sample size for this study was estimated based on preliminary experimental results from our research center. In the pilot study, the incidence rates of CPB-associated AKI in the ta-VNS group and sham stimulation group were 17.1% and 37.9%, respectively. With a significance level of $\alpha = 0.05$ (bilateral) and a power of $1 - \beta = 0.80$, calculated using G*Power software (version 3.1.9.7),68 patients were required in each group. Considering a 10% dropout rate, a total of 152 patients were enrolled in this study, with 76 patients in each group.

V. Data Management and Confidentiality

Only investigators authorized by the principal investigator are permitted to access or analyze data. Computer systems used for data management must be password-protected, and paper documentation must be locked.

All information related to the subject's identity is kept confidential, and such information shall not be disclosed beyond the scope permitted by applicable laws and/or regulations.

VI. Informed Consent

Prior to enrollment in this study, the investigator responsible for obtaining informed consent must provide each participant with a comprehensive written explanation regarding the study's objectives, nature, procedures, potential benefits, and risks. Participants should be informed of their right to withdraw from the study at any time. Before enrollment, each participant must be fully informed and given sufficient time to consider participation. Enrollment is permitted only after voluntary participation and signing of the informed consent form.

The vulnerable groups potentially involved in this study include elderly individuals and illiterate persons. If the subject is unable to read or sign the informed consent form due to lack of capacity for action, their guardian must act as proxy for the informed consent process and sign the document. If the subject lacks the ability to read the informed consent form (e.g., illiteracy), a witness must observe the informed consent process and sign the consent form.

VII. Adverse Events and Related Management Measures

ta-VNS is a selective, non-invasive, and low-risk effective VNS strategy. The primary

adverse events include pain, influenza-like symptoms, and local skin discomfort, which resolve immediately after cessation of stimulation. To date, no literature has reported serious adverse events associated with this procedure.

In the event of injury, the research team will provide prompt medical treatment to the subjects.

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