

# Full English Translation of the Clinical Research Protocol and Statistical Analysis Plan

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Source document: Investigator-Initiated Interventional Clinical Research Protocol, Fudan University Zhongshan Hospital, Version V1.2, dated January 27, 2026.

Note: This is a faithful English translation of the protocol text provided by the user. The bibliographic references are retained in their original citation format.

## **Fudan University Zhongshan Hospital**

### **Investigator-Initiated Interventional Clinical Research Protocol**

Project Title (Chinese): Effectiveness of acupuncture for reducing recurrence after catheter ablation in patients with persistent atrial fibrillation: a prospective, open-label, randomized, blank-controlled study

Project Title (English): Effectiveness of acupuncture for atrial tachyarrhythmia prevention after catheter ablation in persistent atrial fibrillation patients: a prospective, open-label, randomized, blank-controlled trial

Study Site: Fudan University Zhongshan Hospital

Principal Investigator: Baozhen Qi

Sponsor: Fudan University Zhongshan Hospital

Version Number: V1.2

Version Date: January 27, 2026

## Version History / Revision History

Document	Version No.	Version Date	Reason for Revision and Summary of Changes
Protocol	V1.0	September 1, 2025	
Protocol	V1.1	December 5, 2025	In response to the review comment to “assess the accuracy of the sample size calculation,” the sample size calculation section was revised.
Protocol	V1.2	January 27, 2026	In accordance with the ethics pre-review comments, exploratory endpoints and EDC-related statements were removed.

## Investigator’s Statement

I will conscientiously fulfill the responsibilities of an investigator in accordance with Chinese GCP requirements, and will personally participate in or directly supervise this clinical study. I have read and confirmed this protocol and agree with its scientific validity and ethical acceptability. I will perform the relevant responsibilities required under Chinese laws and regulations, the Declaration of Helsinki, Chinese GCP, and this study protocol, and the study will not be implemented until approval has been obtained from the Academic Committee and the Ethics Committee. Unless measures must be taken to protect the safety, rights, and interests of the subjects, I will keep this study protocol confidential.

Study Site: Fudan University Zhongshan Hospital

Principal Investigator: Baozhen Qi

Principal Investigator (Signature): Baozhen Qi

Date of Signature: 2026.01.27

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## Protocol Summary

Item	Content
Protocol Title	Effectiveness of acupuncture for reducing recurrence after catheter ablation in patients with persistent atrial fibrillation: a prospective, open-label, randomized, blank-controlled study.
Study Objective	The primary objective is to evaluate the preventive effect of electroacupuncture on recurrence of atrial tachyarrhythmias (recurrence of atrial fibrillation/atrial flutter/atrial tachycardia) after catheter ablation in patients with persistent atrial fibrillation. Secondary objectives include improvement in left heart structure and function, atrial fibrillation burden, and quality of life.
Study Endpoints	Primary endpoint: recurrence rate of atrial tachyarrhythmias at Month 12. Secondary endpoints: changes at Month 12 in left atrial diameter, LVEDD, LVESD, LVEF, atrial fibrillation burden, and AFEQT score.
Overall Design	Prospective, single-center, open-label, randomized, blank-controlled study. Block randomization will be implemented through a central system, with a 12-month follow-up period.
Sample Size	The planned sample size is 120 subjects (60 per group), estimated based on the expected difference in recurrence rates (39.8% vs 16%) and a 15% dropout rate.
Study Groups	Simple block randomization will be used to allocate subjects 1:1 to the treatment group (electroacupuncture + standard medication) or the control group (standard medication). Block lengths will be random to avoid predictability.
Inclusion/Exclusion Criteria	Inclusion criteria: age 20–75 years, persistent atrial fibrillation, restoration of sinus rhythm after first catheter ablation, left atrial diameter <50 mm, and voluntary written informed consent. Exclusion criteria: cardiovascular events within the prior 3 months, severe valvular disease, hypotension, structural heart disease, malignancy, severe hepatic or renal dysfunction, pregnancy/lactation, or other diseases affecting compliance or safety.
Intervention	Starting on postoperative Day 2, the treatment group will receive electroacupuncture (Neiguan and Jianshi; low frequency 2 Hz; twice weekly for 5 weeks) plus standard medication; the control group will receive standard medication alone. Concomitant medications will be recorded and safety will be monitored.
Criteria for Withdrawal	Voluntary withdrawal of informed consent; medical judgment that continued participation is not in the patient's best interest; pregnancy or major protocol

	violations will lead to exclusion.
Statistical Analysis Plan	The primary analysis will be based on the FAS population (ITT principle), and the PPS will be used for sensitivity analysis. Endpoints will be analyzed using Kaplan–Meier curves, the log-rank test, and Cox models for recurrence; ANCOVA will be used to evaluate changes in echocardiographic parameters, atrial fibrillation burden, and QoL scores. P<0.05 will be considered statistically significant.

## List of Abbreviations

Abbreviation	Full Term
AF	Atrial Fibrillation
AFEQT	Atrial Fibrillation Effect on Quality of Life
ANCOVA	Analysis of Covariance
CRF	Case Report Form
CRP	C-reactive Protein
FAS	Full Analysis Set
GCP	Good Clinical Practice
HR	Hazard Ratio
IL-6	Interleukin-6
ITT	Intention-To-Treat
LVEF	Left Ventricular Ejection Fraction
LVEDD	Left Ventricular End-Diastolic Diameter
LVESD	Left Ventricular End-Systolic Diameter
MMP-2	Matrix Metalloproteinase-2
NT-proBNP	N-terminal pro-B-type Natriuretic Peptide
PPS	Per Protocol Set
QoL	Quality of Life
RR	Relative Risk
SAS	Statistical Analysis System
SAE	Serious Adverse Event
TGF-β1	Transforming Growth Factor Beta 1

## I. Research Background

Atrial fibrillation (AF) is the most common tachyarrhythmia in clinical practice, and its prevalence increases with age. Between 2014 and 2016, the prevalence of AF among the Chinese population aged over 45 years was 1.8%. Based on the data from China's Seventh National Population Census in 2020, it is estimated that approximately 12 million individuals in China have AF [1]. AF significantly increases the risks of death, heart failure, stroke, cognitive impairment, and dementia, and severely affects patients' quality of life [2,3]. Over the past two decades, the management of AF has improved substantially with the development of new drugs and advances in catheter ablation techniques; however, progress in understanding its mechanisms has stagnated, treatment efficacy remains limited, adverse effects persist, and the success rate of catheter ablation for persistent AF is only 50%–60% [4].

In traditional Chinese medicine, AF falls within the category of palpitations. Its pathogenesis is mainly attributed to imbalance of qi, blood, yin, and yang, resulting in qi stagnation, blood stasis, and phlegm turbidity obstructing the heart vessels. The sympathetic and parasympathetic nervous systems are considered concrete manifestations of yin and yang in traditional Chinese medicine [5]. Acupuncture is a simple, economical, and effective therapy. Through mechanisms such as improving the balance of autonomic nervous activity, it may be used to treat AF, effectively restore and maintain sinus rhythm, reduce recurrence after electrical cardioversion and catheter ablation, and is considered safe without obvious adverse reactions [6–8].

However, most current clinical studies of acupuncture for AF have small sample sizes, uncertainty regarding acupoint selection and needle retention time, and non-uniform evaluation indicators. Higher-quality evidence-based medical evidence is needed in the future to explore different acupuncture methods and acupoint selections so as to formulate the optimal acupuncture regimen for AF. Because the pathogenesis of AF remains incompletely understood, catheter ablation is expensive, the recurrence rate after surgery for persistent AF is high, and the medical burden on patients is substantial, while acupuncture may reduce recurrence after catheter ablation, further exploration and optimization of acupuncture regimens are warranted to prevent recurrence after catheter ablation for AF.

Therefore, we plan to screen hospitalized patients undergoing catheter ablation for AF and randomly assign patients with persistent AF to receive acupuncture treatment, in order to observe its effect on recurrence of atrial tachyarrhythmias. Because this population is prone to recurrence of atrial tachyarrhythmias, these patients are considered suitable for participation in this study after catheter ablation, and the anticipated benefits are expected to outweigh the risks.

## II. Study Objectives

The purpose of this trial is to evaluate the effectiveness of acupuncture treatment after catheter ablation in patients with persistent atrial fibrillation.

### 2.1 Primary Objective

To evaluate the preventive effect of acupuncture treatment on recurrence of atrial tachyarrhythmias (recurrence of atrial fibrillation/atrial flutter/atrial tachycardia) after catheter ablation in patients with persistent atrial fibrillation.

### 2.2 Secondary Objective

To explore the effects of acupuncture on improvement in left atrial diameter, left ventricular end-diastolic diameter, left ventricular end-systolic diameter, left ventricular ejection fraction, atrial fibrillation burden, and quality of life (QoL) after catheter ablation in patients with persistent atrial fibrillation.

## III. Study Overview

### 3.1 Overall Study Design and Plan

This is a prospective, single-center, open-label, randomized, blank-controlled study.

### 3.2 Sample Size and Grouping Method

The planned sample size is 120 cases. A simple block randomization method will be used. An independent biostatistician will generate the random number sequence according to the total sample size and block size using SAS 9.4 software and allocate subjects 1:1 to the treatment group and the control group, i.e., a randomization allocation list with 60 subjects in each group. The randomization process and concealment will be completed by an independent statistical unit.

Treatment allocation: After written informed consent has been obtained, all screening procedures and evaluations have been completed, and patient eligibility has been confirmed, the study center will obtain the subject identification number and treatment allocation from the randomization implementation unit. Starting on the second day after surgery, the treatment group will receive electroacupuncture (Neiguan and Jianshi; low frequency, 2 Hz; twice weekly for 5 weeks) and standard medication therapy, while the control group will receive standard medication therapy. Detailed information regarding treatment administration will be recorded in the case report form (CRF).

All concomitant medications must be reported to the investigators and recorded in the concomitant medication section of the CRF.

Patients' safety will be closely monitored throughout the study period.

### 3.3 Randomization and Blinding

This study will use a simple block randomization method. An independent biostatistician will generate a random number sequence using SAS 9.4 software and assign subjects to the treatment group and control group at a ratio of 1:1. The block size will be randomly varied and kept confidential by the statistical team in order to avoid predictable allocation. The randomization allocation sequence will be maintained and implemented by an independent statistical unit. After subjects sign informed consent, complete screening, and are confirmed to meet the inclusion criteria, investigators will obtain group allocation information through the central randomization system.

This study is an open-label design and will not be blinded. Electroacupuncture, as the intervention in the treatment group, is an adjunctive therapeutic approach. Although there is evidence supporting its safety, it is designated as the study intervention in order to further explore its effectiveness in preventing recurrence after catheter ablation for AF; therefore, blinding will not be implemented. All treatment procedures will be conducted by qualified physicians, whereas study-related data collection and endpoint adjudication will be completed by blinded assessors to control information bias. Treatment administration and concomitant medications will be documented in detail in the CRF, and safety monitoring will be conducted throughout the study.

### 3.4 Study Flowchart

Figure 1. Study design and flow.

## IV. Study Population

### 4.1 Inclusion Criteria

- Age 20–75 years;

- Persistent atrial fibrillation;
- First catheter ablation for atrial fibrillation and restoration of sinus rhythm after the procedure;
- Left atrial diameter <50 mm;
- Ability to understand the study, voluntary participation, and signed informed consent.

#### 4.2 Exclusion Criteria

- Cardiovascular events (including stroke, transient ischemic attack, myocardial infarction, unstable angina, etc.) or cardiac surgery within the previous 3 months;
- Two consecutive in-hospital measurements (5 minutes apart) showing systolic blood pressure <100 mmHg accompanied by symptoms of hypotension;
- Severe valvular heart disease (severe structural heart disease such as moderate-to-severe aortic or mitral valve disease);
- Women who are breastfeeding, pregnant, or planning pregnancy during the follow-up period;
- Status after pacemaker implantation;
- Second- or third-degree atrioventricular block;
- Bifascicular or trifascicular block;
- Acupuncture treatment for cardiovascular disease within the previous 3 months;
- Any other serious disease or condition, including: malignancy; history of chronic infectious disease; renal impairment defined as estimated glomerular filtration rate (according to the CKD-EPI equation) <30 mL/min/1.73 m<sup>2</sup> or the need for hemodialysis at screening; moderate or severe hepatic impairment (Child–Pugh class B or C); anemia defined as hemoglobin <90 g/L; systemic disease requiring hormone therapy; severe cognitive impairment or dementia; alcohol abuse or drug abuse; expected survival <12 months; poor compliance or inability to provide informed consent;
- Current participation in another interventional study.

#### 4.3 Withdrawal Criteria

- The subject requests withdrawal of informed consent for any reason;
- In the investigator's medical judgment, continued participation is not in the patient's best interest and the study should be discontinued for the patient.

#### 4.4 Exclusion After Enrollment

- Subjects who violate the study inclusion criteria;
- Subjects who meet protocol exclusion criteria;
- Subjects who become pregnant or enter the breastfeeding period after signing informed consent.

## V. Endpoints

### 5.1 Primary Endpoint

At the end of Month 12, recurrence of atrial fibrillation/atrial flutter/atrial tachycardia in the treatment group and the control group.

Recurrence of atrial fibrillation/atrial flutter/atrial tachycardia is defined as any symptomatic episode of atrial fibrillation, atrial flutter, or other re-entrant atrial tachycardia lasting at least 30 seconds as recorded by surface electrocardiogram or 24-hour Holter monitoring after the 3-month blanking period following catheter ablation. All episodes will be reviewed by two independent electrophysiologists who are blinded to patient identity and randomization group.

### 5.2 Secondary Endpoints

- Change in left atrial diameter (measured by transthoracic echocardiography) at Month 12 compared with baseline.
- Change in left ventricular end-diastolic diameter (measured by transthoracic echocardiography) at Month 12 compared with baseline.
- Change in left ventricular end-systolic diameter (measured by transthoracic echocardiography) at Month 12 compared with baseline.
- Change in left ventricular ejection fraction (measured by transthoracic echocardiography) at Month 12 compared with baseline.
- Change in atrial fibrillation burden at Month 12 compared with baseline in the treatment and control groups. Atrial fibrillation burden is defined as the percentage of time in atrial fibrillation on 24-hour Holter monitoring.
- Change in QoL score at Month 12 compared with baseline in the treatment and control groups. The atrial fibrillation-specific AFEQT questionnaire will be used to assess changes in QoL. AFEQT contains 21 items covering 4 domains: symptoms, daily activities, treatment concerns, and treatment satisfaction; 18 items are used to calculate the overall score, ranging from 100 (no AF-related disability) to 0 (complete AF-related disability).

### 5.3 Safety Endpoints

Because acupuncture treatment is considered safe and has no obvious adverse reactions, no formal safety endpoint is specified in this study. However, safety evaluation will include:

- The nature, incidence, severity, and seriousness of adverse events;
- Changes in vital signs, symptoms, and physical examination findings relative to randomization baseline;
- Changes in clinical laboratory test results relative to randomization baseline.

### 5.4 Exploratory Endpoints (if applicable)

Not applicable.

## VI. Study Procedures

### 6.1 Study Steps and Related Assessments

#### 6.1.1 Screening Phase

##### 6.1.1.1 Preliminary screening of potential subjects:

Patients aged 20–75 years with persistent atrial fibrillation who are hospitalized in the cardiology ward of the study site and are scheduled to undergo first-time catheter ablation for atrial fibrillation will be preliminarily screened.

##### 6.1.1.2 Screening for enrollment:

Potential subjects will be screened from the cardiology ward of the study center, and inclusion and exclusion criteria will be reviewed. Informed consent will be obtained before screening.

For those who provide informed consent, the CRF will be completed further. Regardless of randomization group, the following baseline data will be collected:

- Sociodemographic characteristics: sex, age, marital status, height, weight, etc.;
- Medical history and medication use history;
- Physical examination: blood pressure, heart rate, cardiac examination, etc.;
- QoL assessment: AFEQT;
- Instrumental examinations: 12-lead ECG, 24-hour Holter monitoring, transthoracic echocardiography, transesophageal echocardiography, pulmonary vein CTV;
- Laboratory tests: complete blood count, blood glucose, blood lipids, liver and renal function, electrolytes, coagulation, cardiac troponin, NT-proBNP, etc.

#### 6.1.2 Enrollment Phase

Randomization and allocation concealment: Subjects who sign informed consent will be randomized. Central randomization will be used in this study. According to random codes generated by SAS computer software, subjects will be randomized at a ratio of 1:1 to one of the two treatment groups. Randomization will use simple block randomization with variable block lengths. The randomization sequence will be generated and retained by an independent biostatistician. Investigators will obtain the randomization number and group assignment through the “randomization platform.” The randomization center computer system will automatically generate a randomization result list containing the patient number, randomization number, treatment assignment information, and randomization date for monitoring purposes.

Blinding procedures: This is an open-label study.

Dose and administration: Starting on the second postoperative day, patients in the treatment group will receive electroacupuncture (Neiguan and Jianshi; low frequency, 2 Hz; twice weekly for 5 weeks). Whether other antiarrhythmic drugs should be discontinued or added will be decided by the treating clinician. Patients in the control group will receive standard drug treatment (blank control) according to the physician’s judgment.

Concomitant medication: All medications used during the study period (prescription or OTC) must be recorded in the corresponding section of the follow-up observation form.

Compliance: Study personnel will maintain detailed records and coordination of medications for each subject during the study.

### 6.1.3 Follow-up Phase

Each enrolled subject will undergo the study visits shown in Table 1, with a total follow-up duration of 12 months. Follow-up will be conducted by study personnel and research nurses who have received specialized education in the care of patients with atrial fibrillation and training on the study protocol. All patients will attend in-person visits at Months 1, 3, 6, and 12 after AF catheter ablation. Each visit will include: (1) whether any adverse events have occurred; (2) changes in concomitant medication (drug name and dosage); (3) physical examination, including blood pressure, heart rate, and body weight; (4) QoL assessment (AFEQT); (5) any AF-related symptoms and documented recurrence of arrhythmia; and (6) instrumental examinations (12-lead ECG, transthoracic echocardiography, and 24-hour Holter monitoring). Transthoracic echocardiography will be performed in the echocardiography laboratory of Zhongshan Hospital according to a standardized protocol and reviewed in a blinded manner. No additional biological sample collection or outsourced testing will be performed in this study.

During follow-up, if the physician judges that AF is inadequately controlled, other antiarrhythmic drugs may be added at any time according to relevant guidelines.

Table 1. Schedule of Assessments

Assessment	Screening	1 Month	3 Months	6 Months	12 Months
Informed consent	X				
Inclusion/exclusion criteria	X				
Sociodemographic characteristics	X				
Disease and procedural history	X				
Concomitant medication	X	X	X	X	X
Vital signs	X	X	X	X	X
Cardiac physical examination	X	X	X	X	X
QoL assessment	X	X	X	X	X
12-lead ECG	X	X	X	X	X
24-hour Holter monitoring	X		X	X	X
Transthoracic echocardiography	X		X	X	X
Transesophageal echocardiography	X				
Pulmonary vein CTV	X				
Complete blood count	X				X
Fasting blood	X				X

glucose					
Liver and renal function	X				X
Electrolytes	X				X
Blood lipids	X				X
High-sensitivity troponin T	X				X
NT-proBNP	X				X
C-reactive protein	X				X
Drug accountability		X	X	X	X
Adverse events		X	X	X	X

## 6.2 Investigational Drug (if applicable)

Not applicable.

## 6.3 Concomitant Medications and Treatment (if applicable)

Not applicable.

## 6.4 Dose Adjustment (if applicable)

Not applicable.

## 6.5 End of Study

The end of study is defined as the time when all enrolled patients have completed the 12-month follow-up.

## 6.6 Early Termination or Suspension of the Study

The study may be terminated early or suspended under the following circumstances: unexpected, significant, or unacceptable risks to participants are identified; major protocol errors are found during study conduct; continuation of the study is deemed meaningless; etc.

## 6.7 Clinical Observation, Follow-up, and Measures to Ensure Subject Compliance

- A complete visit schedule (baseline and Months 1, 3, 6, and 12) will be provided to ensure that patients are informed;
- The treatment group will receive 5 weeks of acupuncture treatment free of charge;
- Safety follow-up will be completed for subjects who withdraw or are lost to follow-up.

# VII. Collection and Reporting of Adverse Events

## 7.1 Definition of Adverse Events

Adverse event: any unfavorable medical event occurring after a subject receives acupuncture treatment, which does not necessarily have a causal relationship with the treatment.

Serious adverse event: any event occurring during participation in this study that requires hospitalization, prolongs hospitalization, causes disability, affects the ability to work, is life-threatening, or results in death.

- Mild: tolerable to the subject, does not affect treatment, requires no special management, and has no effect on recovery.
- Moderate: difficult for the subject to tolerate, requires special management, and has a direct impact on recovery.
- Severe: life-threatening, fatal, or disabling, requiring immediate emergency treatment.

## 7.2 Recording and Reporting of Adverse Events

Clinical adverse events may occur during subject treatment. Once an adverse event occurs (including important adverse events), the time of onset, clinical manifestations, management process and duration, outcome, and relationship to acupuncture treatment will be recorded in detail in the case report form. If an abnormal laboratory finding occurs, the patient must be followed until the test result returns to normal, returns to the pre-acupuncture level, or is determined to be unrelated to the study intervention (acupuncture treatment). If a serious adverse event occurs, a serious adverse event form must be completed and reported within 24 hours after the event to the sponsor, the Ethics Committee, the National Medical Products Administration (NMPA), and the local health administrative authority.

## 7.3 Risk Prevention and Management

- Bleeding: local bleeding after needle withdrawal during follow-up generally can be stopped by compression; if persistent bleeding or similar symptoms occur, timely treatment will be provided.
- Hematoma: local hematoma after needle withdrawal during follow-up; if hematoma occurs, prompt compression or other treatment will be provided.
- Pain: pain during needle retention and after needle withdrawal during follow-up; if the pain is intolerable, treatment will be stopped if necessary and appropriate management will be provided.
- Vasovagal reaction: factors that may cause vasovagal reactions will be evaluated before treatment. After treatment begins, heart rate and blood pressure will be monitored, along with signs and symptoms of vasovagal reaction. If a vasovagal reaction occurs, acupuncture will be stopped immediately and treatment will be given. Mild vasovagal reactions may be relieved by lying supine or appropriate fluid supplementation; severe cases may require intravenous atropine.
- Infection: infection-related symptoms will be evaluated before treatment and monitored after treatment. If symptoms such as local redness, swelling, suppuration, or fever occur, the patient will seek medical attention promptly and receive appropriate treatment.

# VIII. Data Management

## 8.1 Case Report Form Design and Data Collection (Case Report Form, CRF)

This study will use paper case report forms (CRFs) for data collection. The CRFs will be designed strictly in accordance with the study endpoints and key data elements in the protocol to ensure complete and accurate recording of subject information, clinical assessment results, laboratory test results, and safety-related information required for the study.

The main content modules of the CRF include:

- Basic subject information: general demographic data such as age, sex, height, and weight;
- Disease-related information: clinical data including AF history, previous diagnoses, treatments, and concomitant diseases;
- Treatment and intervention records: detailed records of the acupuncture regimen, frequency, treatment course, and standard treatment;
- Laboratory and imaging examination results: complete blood count, liver and renal function, cardiac biomarkers (such as cardiac troponin and NT-proBNP), ECG, echocardiography, etc.;
- Adverse event records: detailed records of adverse events and serious adverse events occurring during the study, including time of occurrence, severity, management measures, and outcomes.

The CRF will not collect directly identifiable personal information such as subjects' real names, inpatient numbers, identity card numbers, or mobile phone numbers. Each subject will be identified only by a unique study number.

## 8.2 Data Entry and Verification

During the study, research personnel who have received uniform training will promptly enter relevant subject information into the paper CRF after each visit. To improve data accuracy and facilitate subsequent statistical analysis, research personnel will use password-protected electronic spreadsheets during the study to perform double entry of CRF data or to collate and summarize the data. The electronic data must be consistent with the paper CRF.

The study team will regularly cross-check the paper CRFs against the electronic data. If inconsistencies or missing information are identified, they will be verified and corrected in a timely manner, and modification records will be retained in the CRF to ensure data integrity and accuracy.

## 8.3 Data Review and Quality Control

The principal investigator will organize the study team to conduct regular data review and quality control, focusing on completeness, consistency, and logical plausibility of the data. All modifications must be clearly documented, with the date of modification and the person making the modification specified. Important data will be verified by two-person review to reduce human error and ensure the reliability of the study data.

## 8.4 Data Lock and Statistical Analysis

After all subjects have completed follow-up and data review has been completed, the study team will perform final confirmation of the study data and lock the database. In principle, no modifications will be made after data lock. If modification is absolutely necessary, approval from the principal investigator must be obtained and a complete modification record must be retained. The locked dataset will be used for statistical analysis and preparation of the study report.

## 8.5 Data Retention and Transfer

After the study is completed, all paper CRFs will be uniformly organized, numbered, and stored centrally in a secure location designated by Fudan University Zhongshan Hospital. Electronic data will be stored in encrypted form on access-restricted computers or storage media and backed up regularly.

For statistical analysis, the study data will be provided to statistical analysts in de-identified form, containing only study numbers and no information that can identify subjects.

## 8.6 Data Security and Privacy Protection

This study will strictly comply with relevant laws, regulations, and GCP requirements, and will adopt necessary administrative and technical measures to protect subject privacy. All study data will be managed using coded identifiers. Study team members may access study data only within the scope of their study responsibilities. Without approval from the Ethics Committee, study data must not be disclosed to irrelevant personnel or institutions.

## IX. Statistical Analysis

### 9.1 Sample Size Estimation

The sample size estimation is based on the primary study endpoint—the recurrence rate of atrial fibrillation/atrial flutter/atrial tachycardia within 12 months after catheter ablation. According to previous studies, the 12-month recurrence rate after catheter ablation in patients with persistent AF is approximately 39.8% [9,10], corresponding to a sinus rhythm maintenance rate of approximately 60.2%. Data from a systematic review indicate that acupuncture treatment can significantly improve the restoration of sinus rhythm [8], with a pooled relative risk (RR) of approximately 1.40. Based on this, the sinus rhythm maintenance rate in the acupuncture combination group is estimated to reach approximately 84.3%, corresponding to an AF/atrial flutter/atrial tachycardia recurrence rate of approximately 15.7%. For the purpose of sample size estimation in this study, the recurrence rate in the acupuncture group is conservatively assumed to be 16%.

With  $\alpha=0.05$ ,  $\beta=0.20$ , a two-sided test, and a 1:1 allocation ratio, using the sample size calculation method for comparing the difference between two proportions, the required sample size is 52 subjects per group. Considering an approximately 15% dropout rate, the final planned total sample size is 120 patients (60 per group).

The planned enrollment is 120 subjects. Based on the clinical practice experience at the study center, among AF patients who undergo first-time catheter ablation and successfully restore sinus rhythm, approximately 89% are aged 20–75 years, approximately 68% have persistent AF, and approximately 81% have a left atrial diameter <50 mm. Taking inclusion and exclusion criteria together into account, approximately 30% of patients undergoing catheter ablation for AF are expected to be eligible for enrollment. Therefore, it is estimated that at least 400 eligible AF patients will need to be screened during the screening phase to identify potential study subjects and ensure enrollment of 120 subjects.

Regarding the feasibility of study conduct, based on data from 2024, more than 450 patients at the study center met the eligibility criteria, which is sufficient to satisfy recruitment requirements.

### 9.2 Definition and Selection of Analysis Sets

The statistical analysis of this study will follow the ICH E9 guideline and the relevant requirements of the Biostatistical Guideline for Drug Clinical Trials issued by the National Medical Products Administration. Study data will be centrally managed and analyzed by the statistical team of Fudan University Zhongshan Hospital and the Shanghai Institute of Cardiovascular Diseases using SAS 9.4 statistical software. The

primary analysis populations will include the Full Analysis Set (FAS) and the Per Protocol Set (PPS). The FAS will be constructed according to the Intention-to-Treat (ITT) principle and will include all randomized subjects who have received at least one intervention. The PPS will consist of the subset of subjects with good treatment compliance and no major protocol violations and will be used mainly for sensitivity analyses. Safety analyses will also be conducted in the FAS population.

### 9.3 Statistical Analysis of Study Data

#### 1. Descriptive Statistics and Baseline Comparisons

All baseline demographic and clinical variables will be presented using descriptive statistics. Continuous variables with a normal distribution will be expressed as mean  $\pm$  standard deviation (SD), while continuous variables with a non-normal distribution will be expressed as median and interquartile range (IQR). Categorical variables will be presented as counts and percentages. Comparisons of baseline characteristics between groups will be performed using the independent-samples t-test, Wilcoxon rank-sum test, chi-square test, or Fisher's exact test, as appropriate according to variable type.

#### 2. Primary Endpoint: recurrence rate of atrial fibrillation/atrial flutter/atrial tachycardia at Month 12

A recurrence event is defined as an episode of atrial fibrillation, atrial flutter, or atrial tachycardia occurring after the post-ablation blanking period (3 months), lasting  $\geq 30$  seconds, diagnosed by ECG or 24-hour Holter monitoring, and independently confirmed by two blinded electrophysiology experts. Kaplan–Meier survival analysis will be used to plot event-free survival curves for recurrence, and the log-rank test will be used for significance testing. Comparisons of recurrence rates will be further analyzed using the Cox proportional hazards regression model to estimate the hazard ratio (HR) and its 95% confidence interval between the treatment group and the control group. The FAS will be the primary analysis set, and the PPS will be used for sensitivity validation.

#### 3. Secondary Endpoint Analysis

All secondary endpoints are continuous variables, including changes in echocardiographic parameters (left atrial diameter, LVEDD, LVESD, LVEF), atrial fibrillation burden, and the QoL questionnaire AFEQT score, all compared between Month 12 and baseline. Analyses will be performed using an analysis of covariance (ANCOVA) model adjusted for pre-treatment baseline values and other important covariates. Results will be expressed as the difference in least-squares mean (LS-mean) changes between groups and the corresponding 95% confidence intervals.

#### 4. Safety Analysis

Safety analyses will include the frequency and proportion of adverse events (AEs) and serious adverse events (SAEs). Between-group comparisons will be performed using the chi-square test or Cochran–Mantel–Haenszel test. The results will be summarized by treatment group and will describe the incidence of each type of AE and its possible association with the intervention.

All statistical tests will be two-sided, and a P value  $< 0.05$  will be considered statistically significant. Unless otherwise specified, missing data will not be imputed and analyses will be performed on complete-case data.

## X. Study Ethics

### 10.1 Ethics Committee Review

This protocol, the written informed consent form, and materials directly related to subjects must be submitted to the Ethics Committee, and the study may formally begin only after written approval has been obtained from the Ethics Committee. The investigator must submit a continuing review report one month before expiration of the ethics approval letter to apply for renewal. Upon study suspension and/or completion, the investigator must notify the Ethics Committee in writing. The investigator must promptly report all changes occurring during the study (such as revisions to the protocol and/or informed consent form) to the Ethics Committee, and such changes must not be implemented before Ethics Committee approval is obtained, unless the change is necessary to eliminate an obvious and immediate risk to subjects. In such cases, the Ethics Committee will be notified.

### 10.2 Informed Consent

The investigator must provide the subject or the subject's legal representative with an easily understandable informed consent form approved by the Ethics Committee, and must give the subject or legal representative sufficient time to consider participation in the study. Subjects must not be enrolled before written informed consent signed by the subject is obtained. During the subject's participation, all updated versions of the informed consent form and written information will be provided to the subject. The informed consent form shall be retained as an important clinical trial document for inspection.

## XI. Confidentiality Measures

The results of this study may be published in medical journals; however, patients' personal information will be kept confidential in accordance with legal and regulatory requirements. Unless required by applicable law, patients' personal information will not be disclosed. When necessary, government regulatory authorities, the hospital Ethics Committee, and related personnel may review patient data in accordance with regulations.

## XII. Expected Study Timeline and Completion Date

The planned enrollment period is 1 year (January 1, 2026 to December 31, 2026), with a follow-up period of 12 months. The latest study completion date is April 30, 2028. The total study duration is 28 months.

## XIII. References

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