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Supplementary information

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Low-voltage-area ablation for persistent atrial fibrillation: a randomized controlled trial

In the format provided by the authors and unedited

This supplementary information contains the following items:

1. **Original protocol, final protocol, summary of changes.**

2. **Original statistical analysis plan, final statistical analysis plan, summary of change**

Original protocol

Trial Title: Efficacy and Safety of Low-voltage Guided Ablation for Recurrence Prevention Compared to Pulmonary Vein Isolation Alone in Patients with Persistent Atrial Fibrillation -SUPPRESS AF-

Date and Version No: Jan 15. 2019, version 1.0

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Sponsor:

Funder:

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1. SYNOPSIS

Objectives	To investigate the effectiveness and safety of catheter ablation (hereinafter referred to as ablation) to the left intraventricular low potential area (LVA) added to pulmonary venous isolation (PVI) in patients with persistent atrial fibrillation (AF)
Trial participants	Patients who undergo ablation for persistent AF for the first time
Trial Design	Multicenter prospective randomized controlled study
Planned Sample Size	170 in one group and 340 in both groups
Planned Trial Period	From April 2019 to March 2023
Primary endpoint	AF recurrence rate for 1 year after the protocol treatment
Secondary endpoints	AF recurrence rate after the last ablation
	Death (cause of death) Symptomatic cerebral infarction Ablation-related complications Hemorrhagic complications

2. BACKGROUND AND RATIONALE

Atrial fibrillation (AF) is an arrhythmia frequently seen in older adults. AF causes cardiac dysfunction due to lack of atrial contraction, or irregular ventricular beats, and increases the risk of thromboembolism leading to severe paralysis impairing quality of life. The increasing population of AF patients requires the appropriate measures for these problems.

Pulmonary vein isolation (PVI) is an effective treatment to suppress onsets of AF. The electrical ablation between the pulmonary vein and left atrium by percutaneously inserted catheters disturbs the conduction of ectopic excitation in the pulmonary vein into the left atrium. [1-2]. The recent improvements in operators' skill and therapeutic devices enable PVI to be a standard treatment for AF [3]. However, PVI does not achieve sufficient results in maintenance of sinus rhythm particularly among the patients with persistent AF [4]. The patients with persistent AF are likely to have arrhythmogenic substrates causing AF not only in pulmonary veins but also the atrium in cases of AF recurrence after PVI. Additional procedures such as linear ablation or CFAE ablation conventionally follow PVI [5], but the efficacy of these additional uniform ablations has not been established by multicenter, randomized controlled trials [6]. We consider that the various distribution of arrhythmogenic substances among patients causes inadequate procedures.

One study comparing with historical controls suggests a promising effect of the ablation targeting LVA on recurrent AF [7]. Left atrial LVA causes electronic conduction delay of excitation propagation and acts as an arrhythmogenic substrate [8-9]. LVA ablation can suppress the onset and persistence of AF by changing the left atrial LVA to scar tissue which has no electrical activity. Two randomized controlled trials were recently conducted to evaluate the efficacy of additional LVA ablation compared with conventional substrate modification strategies [10-11]. However, these studies could not conclude the efficacy of LVA ablation because of the small sample size and outdated ablation strategies in the control group, which are not adopted by the current international guideline [12].

This study aimed to clarify the efficacy of LVA ablation in addition to PVI compared with the conventional standard ablation strategy of PVI alone in a sufficient number of persistent AF patients with LVA.

3. TRIAL DESIGN

Multicenter, prospective, randomized, open-labeled study to compare the effectiveness and safety of LVA ablation to observation in persistent AF patients with left atrial LVA after PVI

4. PARTICIPANT IDENTIFICATION

4.1. Trial Participants

In this clinical study, two-stage registration is performed, and the following selection criteria should be used at each stage. The primary registration is made after informed consent is obtained, and the secondary registration after screening.

4.2. Inclusion Criteria at the primary registration

- Patients who undergo ablation for persistent AF for the first time

4.3. Exclusion Criteria at the primary registration

The participants may not enter the trial if ANY of the following apply:

- Age < 20 years
- Diameter of left atrium > 55 mm
- History of any cardiac surgery
- Valvular AF
- Renal failure requiring dialysis
- Contraindication to ablation
- Contraindication to anti-coagulant therapy
- History of stroke, TIA, or systemic embolism within the last 6 months
- AF secondary to a reversible cause
- Pregnancy
- Any case that a physician determines inappropriate

4.4. Inclusion Criteria at the secondary registration

- Patients who have left atrial LVA

4.5. Exclusion Criteria at the primary registration

The participants may not enter the trial if ANY of the following apply:

- Patients who withdraw the consent after the primary registration
- Any case that a physician determines inappropriate

5. TRIAL PROCEDURES

5.1. Recruitment (Primary Registration)

After acquiring consent, candidates are registered. Registration is started by automatically assigning the research ID after entering personal information, clinical information including eligibility in the application for electronic registration of the computer connected to the Internet at each institution. The anonymization of personal information is automatically carried out in the computer, and only the anonymized data is sent to department of medical information, Osaka University Hospital.

5.2. Informed Consent

The participants must personally sign and date the latest approved version of the informed consent form before any trial specific procedures are performed.

Written informed consent form will be presented to the participants detailing no less than: the exact nature of the trial; what it will involve for the participants; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participants are free to withdraw from the trial at any time for any reasons without prejudice to future care, without affecting their legal rights and with no obligation to give the reasons for withdrawal.

The participants will be allowed as much time as wished to consider the information, and the opportunity to question the Investigator to decide whether they will participate in the trial. Written informed consent will then be obtained by means of dated signature of the participants and dated signature of the persons who presented and obtained the informed consent. The persons who will obtain the consent must be suitably qualified and experienced, and have been authorised to do so by the Chief Investigator. Informed consent should be done the day before the catheter ablation. A copy of the signed informed consent will be given to the participants, and another copy will be retained by principal investigator of each institute. The original signed form will be retained at the trial site.

If there is difficulty in obtaining informed consent from the patient himself, he must not be enrolled in this trial. If a patient does not wish to participate this trial, he will be treated with the same catheter ablation procedure as that in this trial without enrolment.

5.3. Screening and Eligibility Assessment

After the primary registration of candidates and the allocation of research IDs are completed, screening is carried out. Left atrial voltage mapping is enforced as the screening and the existence of LVA is confirmed. The candidates not satisfying the eligibility criteria in the screening test are treated as “screening-dropouts”. For “screening-dropouts”, no further data collection and rescreening will be conducted. "Not satisfying the eligibility criteria" includes the cases where PVI is unsuccessful, left atrial voltage mapping cannot be enforced to fail to maintain sinus rhythm, or left atrial LVA is not detected.

5.4. Randomization (secondary registration)

Subjects who satisfy eligibility criteria in screening are assigned (secondary registration). Assignment at random is 1: 1 ratio to either "Group A: LVA ablation (-)" or "Group B: LVA ablation (+)" by the DATATRAKONE system at the time of entering the research ID to the computer connected to the Internet at each institution. Minimization method using participating institutes as an adjustment factor is chosen so as not to cause large deviation within each facility. The detailed algorithm of the random assignment is not notified to investigators.

5.5. Baseline Assessments and Subsequent Visits

① Baseline information
② Information about AF
③ Comorbidities
④ Medications
⑤ Laboratory tests
⑥ Transthoracic echocardiography (TTE)
⑦ Transesophageal echocardiography (TEE)

⑧ Details of ablation

⑨ 12-lead ECG

⑩ 24-hour ECG

⑪ Portable ECG recorder

⑫ Outcome measures

⑬ Severe adverse events (SAE)

The above items are evaluated before procedure, during hospitalization (treatment day and before discharge) after discharge from the hospital. The observation schedule is shown below.

	Before procedure*1	Treatment Day	Before discharge	3 months	6 months	1 year
Days from target treatment	-	0	-	90	180	360
Allowance*2	-90 days		-7 days	±30 days	±30 days	±30 days
①Basic information	●		●*5	●*5	●*5	●*5
②Information about AF ③Comorbidities	●					
④Medications	●		●	●	●	●
⑤Laboratory tests	●				●	●
⑥TTE*3	●					●
⑦TEE	●					
⑧Details of ablation		●				
⑨12-lead ECG	●		●	●	●	●
⑩24-hour ECG					●	●
⑪Portable ECG recorder					●	●
⑫Outcome measures*4			←	—	—	→
⑬SAE		←	—	—	—	→

* 1 Data before procedure can be acquired before consent acquisition.

* 2 When multiple observations are made within an allowable range, the data on the day closest to the date calculated from treatment day is chosen.

* 3 - It is possible up to 180 days before treatment day.

* 4 The presence or absence of recurrence of atrial fibrillation is not acquired for 3 months from the treatment.

* 5 Only blood pressure and pulse rate are collected.

6. PROTOCOL TREATMENT

Protocol treatment in each group is as follows:

Group A: left atrial LVA ablation (-)

Group B: left atrial LVA ablation (+)

- The reference date of protocol treatment should be the date on which ablation is performed.
- The protocol treatment in this study means left atrial LVA ablation. Antiarrhythmic therapy, anticoagulant therapy and ablation procedures other than left atrial LVA ablation are defined as treatment before and after protocol treatment. Protocol treatment is performed during the hospitalization.

6.1. Period from consent acquisition to protocol treatment implementation

The period from consent acquisition to treatment implementation should be within 90 days. If 90 days have elapsed, the physician obtains the verbal consent, which should be recorded in the source material. In the case, research ID is not reissued.

6.2. Left Atrial LVA Ablation

In group B, ablation for the left atrial LVA is performed. The entire left atrial LVA should be ablated principally with planar ablation so that tags with 6 mm in diameter would overlap each other. The VISITAG module is used and adjusted to (1) catheter stability range of motion ≤ 2.0 mm, (2) catheter stability duration > 5 s, and (3) CF

≥ 5g (time ≥ 25%). In principle, the energization end point of each point is evaluated by the ablation index (AI), and the target is 350. Since ablation at the posterior wall is concerned about esophageal disorder, left atrial LVA may be electrically isolated by linear ablation. LVA ablation in the vicinity of Bachmann 's bundle near the ceiling of the anterior wall and the septal His bundle should be avoided by operators' judgments in order not to harm those tissues. In group A, ablation for the left atrial LVA is not performed.

6.3. End-of-Treatment Criteria and Cancellation Criteria

Protocol treatment is completed, in principle, in the case where it is judged that the end point described above is achieved or that benefits cannot be obtained even if further ablation is performed after the end point is achieved.

The protocol treatment can be postponed or canceled according to the participant's condition, at the discretion of the physician. In addition, when continuation of further treatment is judged to be dangerous for participant's safety during the protocol treatment, when it is difficult to continue the protocol treatment due to occurrence of serious adverse events, when participants wish to withdraw from the study, or when the protocol treatment is judged to increase a risk of exacerbation or recurrence of the original diseases, comorbidities or complications, protocol treatment can be discontinued promptly.

6.4. Treatment before and after ablation

Preoperative anticoagulant therapy follows the guidelines of the Japanese Cardiovascular Society Guidelines (It continues in principle).

Preoperative antiarrhythmic drugs other than Amiodarone should stop during more than 5 times the half-life of each drug before the protocol treatment, while Amiodarone stop from 60 days or more before.

Other ablation procedures except for left atrial LVA ablation are defined in separately prepared manuals so that they are unified between institutions.

Post-ablation treatments such as antiarrhythmic therapy, anticoagulant therapy, or cardioversion should be performed according to the judgment of each physician. However, antiarrhythmic drugs should be discontinued at the time of 3 months after treatment.

6.5. Re-ablation

Re-ablation should be done at the discretion of the physicians. In re-ablation, confirmation of PV re-conduction and re-PVI in this case are essential. After re-PVI, the voltage map should be performed in the manner described in the separate manual. Ablation to the left atrial LVA is not performed in group A, while performed in group B. In any group, ablations (such as linear ablation or CFAE ablation) other than left atrial LVA ablation can be performed by the judgment of the operators.

6.6. Other treatments

Treatments for complications or co-morbidities other than the anticoagulant, antiarrhythmic drugs and re-ablation mentioned above can be made at the discretion of the physicians.

7. OUTCOME MEASURES

7.1. Primary Outcome

AF recurrence rate for 1 year from first ablation

AF recurrence is defined as any atrial fibrillation, atrial flutter or atrial tachycardia that persists for more than 30 seconds in the electrocardiogram or a test finding similar thereto. However, AF recurrence during blanking period, which is defined as three months from the treatment day should not be regarded as recurrence. Re-ablation during the blanking period should be analysed as AF recurrence immediately after the blanking period.

7.2. Secondary Outcomes

Efficacy assessment

- AF recurrence rate after the last ablation

Safety assessment

- Death (causes of death)

Death is defined as any causes of death that occurred during the protocol treatment until the end of the observation period. It does not matter whether it relates to protocol treatment or not. The causes of death are also investigated.

- Symptomatic cerebral infarction

Cerebral infarction with symptoms that occurred during the protocol treatment until the end of the observation period is evaluated. Symptoms caused by cerebral infarction can be transient or persistent consciousness disturbances, sensorimotor disorder of face, trunk, upper or lower extremities, etc. However, it is left to the judgment of physicians at each institution.

- Ablation-related complications

Ablation-related complications are, in principle, defined as the following events that occurred within one week of protocol treatment; hemorrhagic events including gastrointestinal bleeding and intracranial hemorrhage, systemic thromboembolism including cerebral infarction, pulmonary infarction, pneumothorax, arteriovenous fistula, pericarditis, cardiac tamponade, phrenic nerve palsy, atrioventricular block, sick sinus syndrome that prolongs discharge, pulmonary hypertension associated with pulmonary vein stenosis, atrial esophageal fistula, acute gastric distension, infection, heart failure, dermatitis, and allergy against other agents such as contrast agents.

- Hemorrhagic complications

Hemorrhagic complications are defined as the following events that occurred during the study period according to ISTH bleeding criteria; lethal bleeding, symptomatic bleeding in critical sites or organs (intracranial, intraocular, retroperitoneal, intraarticular, pericardial, or intramuscular bleeding with fascial compartment syndrome), bleeding which causes a drop

of 2g / dL or more of the hemoglobin level, bleeding requiring more than 2 units of transfusion (whole blood or erythrocyte), hemorrhage requiring hospitalization.

7.3. Evaluation Methods

12-lead ECG

12-lead ECG is performed at 3, 6, and 12 months after treatment, or at any time if necessary. If AF recurrence is confirmed by the 12-lead ECG, it should be recorded for 30 seconds or longer.

24-hour ECG

24-hour ECG is performed at 6 and 12 months after treatment, or at any time if necessary.

Portable ECG recorder

The portable ECG recorder is performed at 6 and 12 months after treatment, or at any time if necessary. HCG-901 manufactured by Omron is used. The physicians lend the portable ECG recorder to the participants, and they record electrocardiogram for 2 minutes once every morning and evening. In principle, the lease period shall be one week or more.

7.4. Outcome Evaluation Committee

The above primary endpoint is evaluated by the outcome evaluation committee. It is held after the observation period of the last case has completed. It evaluates the cases where protocol treatment is completed and the data is collected. The primary outcome is judged by three committee members appointed by the research representative on the basis of ECG findings (copy).

The outcome evaluation committee is composed independently of the research representative, the protocol creation committee, the independent data monitoring committee, the clinical research fund contributor and the research director, contributor or collaborator of the participating medical institution.

8. STATISTICAL CONSIDERATION

8.1. Sample Size Determination

Assuming that the rate of AF recurrence for 1 year from the first ablation is 40% for group A (LVA ABL (-)) and 25% for group B (LVA ABL (+)), 155 cases in each group (310 cases in both groups) are required to detect the difference according to log-rank test with a significance level of 5% and a power of 80%. In consideration of dropout of about 10%, the number of assigned cases (group A and group B) is set as 170 in one group and 340 in both groups.

8.2. Populations for Analyses

A group consisting of all registered subjects is regarded as all registered cases, and the group of efficacy analysis subjects and the group of safety analysis subjects are defined as follows.

1) Efficacy analysis group

FAS (Full Analysis Set):

Among cases registered in this study and carrying out protocol treatment, a group consisting of cases excluding the ones in which data on efficacy is missing

PPS (Per Protocol Set):

Group consisting of cases excluding serious protocol violations from FAS

2) Safety analysis group

SAS (Safety Analysis Set):

A group consisting of cases in which the protocol treatment is performed and data on safety after the protocol treatment is obtained.

8.3. Statistical Analysis

The main aggregation and analysis methods are shown below, but detailed analysis contents are separately specified in the statistical analysis plan document.

Baseline descriptive statistics

The number of registered cases, ineligible cases, deviant cases, cases to be analyzed for each group, and stopped cases are counted. Descriptive statistics are calculated for subject background information.

Primary endpoint

The rate of AF recurrence in each group is estimated by the Kaplan-Meier method for FAS. In addition, the 1-year recurrence rate and its 95% confidence interval after the first ablation are calculated by the Greenwood formula, and the difference between groups is tested by log-rank test. Cox proportional hazards model will be used to correct latent confounders, if necessary. Analysis targeting PPS is conducted to confirm the stability of the analysis.

Secondary endpoints

Efficacy evaluation

The analysis for FAS is conducted, but the analysis for PPS is also performed as a sensitivity analysis. Kaplan-Meier estimation, log-rank test, Cox proportional hazard model are applied.

Safety assessment

For SAS, 95% confidence intervals are calculated by collecting prevalence and incidence of death, symptomatic cerebral infarction, ablation-related complications for each group. Fisher's exact test is used for comparison between groups. The same analysis is also performed for cause of death and type of complication.

8.4. Interim Analysis

Interim analysis is not carried out in this study.

9. DATA COLLECTION AND MANAGEMENT

9.1. Medical Record

For each institution participating in the study, a person authorized to view the medical record collects the data by electronic data capture (EDC). The collected data is anonymized and sent to the research secretariat via the EDC system to department of medical information, Osaka University Hospital. Data communication is performed on a secure network using Virtual Private Network (VPN). The data is stored in the server at department of medical information, Osaka University Hospital, and it is managed responsibly by data center. Only the authorized person can enter the place where the server is installed, and security is adequately protected. Since personal information is stored in PC at each institution, the PC should be kept safely.

Medical records of each participant should be kept at each institution in accordance with the regulations of the institution.

9.2. Electrocardiogram and Voltage Map Data

Electrocardiograms and voltage map data acquired in the course of clinical practice are stored in paper or electronic media (USB or DVD) at each institution, and mailed or handed over directly to the research secretariat. Personal information (name and medical number) is masked and research ID is given. Data collected at the secretariat is kept on a locked shelf within department of cardiovascular medicine, Osaka University Graduate School of Medicine, and analyzed by the analysts when necessary (central analysis).

10. SAFETY REPORTING

10.1. Definitions

Adverse events are defined as any undesirable or unexpected signs including abnormal variations in clinical laboratory values, symptoms, or diseases that occur after the subject receives the protocol treatment, and it does not depend on whether or not there is a causality with the protocol treatment.

10.2. Evaluation and Report of Adverse Events

Adverse events that occur during protocol treatment or after completion of protocol treatment (or discontinuation of protocol treatment) are observed up to 6 months after the protocol treatment and are listed in the case report. Deterioration of the original disease (recurrence of atrial fibrillation) and hospitalization for the treatment (re-ablation) are not considered as adverse events.

10.3. Serious Adverse Events and Subsequent Response

Serious adverse events are defined as follows:

1) events which lead to die,

2) events which threaten life,

3) events which require hospitalization for treatment or which extend hospitalization period, except for hospitalization or extension of hospitalization period that satisfies the following requirements should not be considered as a serious adverse event;

· Hospital stay less than 6 hours (not considered as hospitalization)

· Hospitalization scheduled in advance (for example, surgery scheduled before the start of examination)

· Hospitalization or extension of hospitalization period for medical examination

4) events which cause persistent or prominent disability or malfunction, or

5) events which cause congenital anomalies in descendants.

If a "serious adverse event" occurs, the best treatment is taken and the course is carefully observed. It should be reported to the research representative (research secretariat) according to the appropriate manner shown below.

10.4. Serious Adverse Event Reporting

Primary report

When a serious adverse event occurs, the Investigators at each institution promptly reports to the research representative (research secretariat) by entering the items grasped at the time to the EDC. The Research Secretariat exports matters relating to serious adverse events entered into the EDC to the appropriate form and reports them to institutional review board at Osaka University Hospital.

Secondary (Final) Report

The Investigators at each institution should enter necessary information to the EDC when the symptoms are fixed and report it to the research representative. The Research Secretariat exports matters relating to serious adverse events entered into the EDC to the appropriate form and reports them to institutional review board at Osaka University Hospital.

10.5. Response of Chief Investigator

The Chief Investigator responds to the matter after receiving the report and informs the Investigator at each institution. The Chief Investigator, if necessary, asks for the independent data monitoring committee to evaluate and decide the response (interrupt, termination, continuation of study) based on the recommendation. The Investigators at each institution is informed immediately after the decision is made according to the recommendation.

10.6. Roles of Independent Data Monitoring Committee

The Independent data monitoring Committee evaluates the content of the report and recommends to the Chief Investigator (the research secretariat) in writing about the future action such as whether to continue, whether to revise the protocol or discontinue the entire study.

11. QUALITY ASSURANCE PROCEDURES

11.1. Progress Management

The Investigators reports progress (number of registrations) and the details of serious adverse events to the head of each institution once every year.

The protocol planning committee prepares the implementation plan, modifies and revises it, and the research secretariat maintains the quality of the study and submits the final report to the institutional review board. The members of the protocol planning committee are specified separately. The protocol planning committee holds regular meetings, but the frequency and the date of the meeting are adjusted as necessary.

11.2. Monitoring and Auditing

Monitoring is carried out based on "Standard operating procedure on implementation of monitoring". On the other hand, audits are not conducted in this clinical study.

11.3. Independent Data Monitoring Committee

The Independent data monitoring committee is composed independently of the Investigator, protocol planning committee, Outcome Evaluation Committee, and Clinical Research Funding Contributor. The Committee may review data on safety of this clinical study, monitor occurrence of adverse events and make recommendations on safety concerns, risks to subjects and scientific validity to continue the study. If recommendations for revision of the protocol or discontinuation of the study due to concerns about the safety of subjects are necessary, it should be made in writing. The final decision is left to the Chief Investigator according to the recommendations. Details are specified in the standard operating procedure.

12. ETHICAL AND REGULATORY CONSIDERATIONS

12.1. Subject's human rights protection

The Investigators conduct this study according to the ethical norm based on the Declaration of Helsinki (revised in 2013) or the ethical guidelines on medical research for people (December 22, 2014, revised on February 28, 2017).

12.2. Explanation and consent to subjects

Prior to registration to this study, the Investigators should hand the "explanatory document to the patient" which is approved in advance by the institutional review board to the subject and give a sufficient explanation about the contents. In addition, the Investigators should give time and opportunity enough to ask questions about this study, and explain that they can withdraw from this study at any time, they never receive disadvantage by withdrawal, and they are never asked for reasons for withdrawal.

12.3. Creation and revision of explanatory documents and consent form

The Investigators make two copies of signed and dated consent form based on the participant's free willing consent. The original is kept by the Investigators at each institution, and one copy is stored in the medical record, and the other is provided to the subject. When obtaining information such as efficacy and safety considered to affect the consent of the participant or when obtaining the revision of the protocol considered to affect the consent of the participant, the Investigators promptly provide the information to the participant, confirms the intention of the participant regarding the continuation of participation in this study, and obtains the consent of the participant again. The protocol planning committee revises the explanatory documents as necessary and obtains the approval of the institutional review board at Osaka university hospital.

12.4. Acquisition of permission for implementation at each institution

The protocol and its revision should be reviewed together by the institutional review board at the Osaka University Hospital, approved and subsequently reported to the head of each institution and obtained the approval of the head of each institution.

12.5. Protection of personal information

The subject's personal information is protected throughout the course of this study. For this purpose, all data of each subject is identified by using identification (ID) code which is not a medical record number. However, the identification of the subjects is performed by a method in which the original source can be retroactively identified by the ID code.

When providing or disclosing data about this study to a third party (core laboratory, DMC, audit, or outcome evaluation committee), personal information is masked.

The institutions participating in this study should protect personal information of subjects according to guidelines such as "ethical guidelines on research on medical science for human beings" and requirements of the institutional review board.

13. FINANCE AND INSURANCE

13.1. Funding source and financial relationship

This clinical study will be conducted with funds provided by Biosense Webster, Inc. Biosense Webster, Inc. is responsible for the planning of this study and interpretation and publication of the results, but does not involve the duty including data collection, data management, statistical analysis, creation of progress reports, or monitoring.

13.2. Costs related to the study

This study is carried out within the range that is done in routine practice. Therefore, medical expenses (including inspection and treatment expenses) of this study are generally handled within the scope of health insurance.

13.3. Compensation for health damage

If health damage occurs during the study, the Investigators promptly take appropriate measures. However, the costs for health damage due to treatment provided are applied to the health insurance and should not be compensated in money.

14. PUBLICATION POLICY

14.1. Publication of outcomes

The outcomes of this study should be determined by the Chief Investigator after consultation with the Osaka Cardiovascular Committee Arrhythmia Team and published to academic societies or academic journals.

14.2. Attribution of outcomes

The main outcomes obtained from this study belong to the Osaka Cardiovascular Committee Arrhythmia Team. When publishing these results in academic meetings and articles, authors, co-authors and procedures are individually determined by discussion within the team. The intellectual property right such as patent right created by the outcomes of this study belongs to Osaka University.

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16. ABBREVIATIONS

ACEI	Angiotensin Converting Enzyme Inhibitor
AF	Atrial Fibrillation
ARB	Angiotensin Receptor Blocker
ATP	Adenosine Triphosphate
CFAE	Complex Fractionated Atrial Electrogram
COPD	Chronic Obstructive Pulmonary Disease
CTI	Cavo-Tricuspid Isthmus
Dd	Left ventricular end-diastolic dimension

DOAC	Direct Oral Anticoagulant
Ds	Left ventricular end-systolic dimension
EDC	Electronic Data Capture
FAS	Full Analysis Set
ISTH	International Society of Thrombosis and Hemostasis
IVS	Intra-Ventricular Septum thickness
LA	Left Atrium
LVA	Low Voltage Area
LVEF	Left Ventricular Ejection Fraction
MR	Mitral Regurgitation
NYHA	New York Heart Association
PPS	Per Protocol Set
PV	Pulmonary Vein
PVI	Pulmonary Vein Isolation
PW	Posterior Wall Thickness
QOL	Quality of Life
RF	Radiofrequency
TR	Tricuspid Regurgitation
VPN	Virtual Private Network

Final protocol

Trial Title: Efficacy and Safety of Low-voltage Guided Ablation for Recurrence Prevention Compared to Pulmonary Vein Isolation Alone in Patients with Persistent Atrial Fibrillation -SUPPRESS AF-

Date and Version No: Apr 19. 2022, version 8.0

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17. SYNOPSIS

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Planned Trial Period	From the approval of the head of the research institution until December 2024.
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Secondary endpoints	AF recurrence rate after the last ablation
	Death (cause of death) Symptomatic cerebral infarction Ablation-related complications Hemorrhagic complications

18. BACKGROUND AND RATIONALE

Atrial fibrillation (AF) is an arrhythmia frequently seen in older adults. AF causes cardiac dysfunction due to lack of atrial contraction, or irregular ventricular beats, and increases the risk of thromboembolism leading to severe paralysis impairing quality of life. The increasing population of AF patients requires the appropriate measures for these problems.

Pulmonary vein isolation (PVI) is an effective treatment to suppress onsets of AF. The electrical ablation between the pulmonary vein and left atrium by percutaneously inserted catheters disturbs the conduction of ectopic excitation in the pulmonary vein into the left atrium. [1-2]. The recent improvements in operators' skill and therapeutic devices enable PVI to be a standard treatment for AF [3]. However, PVI does not achieve sufficient results in maintenance of sinus rhythm particularly among the patients with persistent AF [4]. The patients with persistent AF are likely to have arrhythmogenic substrates causing AF not only in pulmonary veins but also the atrium in cases of AF recurrence after PVI. Additional procedures such as linear ablation or CFAE ablation conventionally follow PVI [5], but the efficacy of these additional uniform ablations has not been established by multicenter, randomized controlled trials [6]. We consider that the various distribution of arrhythmogenic substances among patients causes inadequate procedures.

One study comparing with historical controls suggests a promising effect of the ablation targeting LVA on recurrent AF [7]. Left atrial LVA causes electronic conduction delay of excitation propagation and acts as an arrhythmogenic substrate [8-9]. LVA ablation can suppress the onset and persistence of AF by changing the left atrial LVA to scar tissue which has no electrical activity. Two randomized controlled trials were recently conducted to evaluate the efficacy of additional LVA ablation compared with conventional substrate modification strategies [10-11]. However, these studies could not conclude the efficacy of LVA ablation because of the small sample size and outdated ablation strategies in the control group, which are not adopted by the current international guideline [12].

This study aimed to clarify the efficacy of LVA ablation in addition to PVI compared with the conventional standard ablation strategy of PVI alone in a sufficient number of persistent AF patients with LVA.

19. TRIAL DESIGN

Multicenter, prospective, randomized, open-labeled study to compare the effectiveness and safety of LVA ablation to observation in persistent AF patients with left atrial LVA after PVI

20. PARTICIPANT IDENTIFICATION

20.1. Trial Participants

In this clinical study, two-stage registration is performed, and the following selection criteria should be used at each stage. The primary registration is made after informed consent is obtained, and the secondary registration after screening.

20.2. Inclusion Criteria at the primary registration

- Patients who undergo ablation for persistent AF for the first time

20.3. Exclusion Criteria at the primary registration

The participants may not enter the trial if ANY of the following apply:

- Age < 20 years
- Diameter of left atrium > 55 mm
- History of any cardiac surgery
- Valvular AF
- Renal failure requiring dialysis
- Contraindication to ablation
- Contraindication to anti-coagulant therapy
- History of stroke, TIA, or systemic embolism within the last 6 months
- AF secondary to a reversible cause
- Pregnancy
- Any case that a physician determines inappropriate

20.4. Inclusion Criteria at the secondary registration

- Patients who have left atrial LVA

20.5. Exclusion Criteria at the primary registration

The participants may not enter the trial if ANY of the following apply:

- Patients who withdraw the consent after the primary registration
- Any case that a physician determines inappropriate

21. TRIAL PROCEDURES

21.1. Recruitment (Primary Registration)

After acquiring consent, candidates are registered. Registration is started by automatically assigning the research ID after entering personal information, clinical information including eligibility in the application for electronic registration of the computer connected to the Internet at each institution. The anonymization of personal information is automatically carried out in the computer, and only the anonymized data is sent to department of medical information, Osaka University Hospital.

21.2. Informed Consent

The participants must personally sign and date the latest approved version of the informed consent form before any trial specific procedures are performed.

Written informed consent form will be presented to the participants detailing no less than: the exact nature of the trial; what it will involve for the participants; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participants are free to withdraw from the trial at any time for any reasons without prejudice to future care, without affecting their legal rights and with no obligation to give the reasons for withdrawal.

The participants will be allowed as much time as wished to consider the information, and the opportunity to question the Investigator to decide whether they will participate in the trial. Written informed consent will then be obtained by means of dated signature of the participants and dated signature of the persons who presented and obtained the informed consent. The persons who will obtain the consent must be suitably qualified and experienced, and have been authorised to do so by the Chief Investigator. Informed consent should be done the day before the catheter ablation. A copy of the signed informed consent will be given to the participants. , and another copy will be retained by principal investigator of each institute. The original signed form will be retained at the trial site.

If there is difficulty in obtaining informed consent from the patient himself, he must not be enrolled in this trial. If a patient does not wish to participate this trial, he will be treated with the same catheter ablation procedure as that in this trial without enrolment.

21.3. Screening and Eligibility Assessment

After the primary registration of candidates and the allocation of research IDs are completed, screening is carried out. Left atrial voltage mapping is enforced as the screening and the existence of LVA is confirmed. The candidates not satisfying the eligibility criteria in the screening test are treated as “screening-dropouts”. For “screening-dropouts”, no further data collection and rescreening will be conducted. "Not satisfying the eligibility criteria" includes the cases where PVI is unsuccessful, left atrial voltage mapping cannot be enforced to fail to maintain sinus rhythm, or left atrial LVA is not detected.

21.4. Randomization (secondary registration)

Subjects who satisfy eligibility criteria in screening are assigned (secondary registration). Assignment at random is 1: 1 ratio to either "Group A: LVA ablation (-)" or "Group B: LVA ablation (+)" by the DATATRAKONE system at the time of entering the research ID to the computer connected to the Internet at each institution. Minimization method using participating institutes as an adjustment factor is chosen so as not to cause large deviation within each facilities. The detailed algorithm of the random assignment is not notified to investigators.

21.5. Baseline Assessments and Subsequent Visits

⑭ Baseline information

⑮ Information about AF

⑯ Comorbidities

⑰ Medications

⑱ Laboratory tests

⑲ Transthoracic echocardiography (TTE)

⑳ Transesophageal echocardiography (TEE)

21 Details of ablation

22 12-lead ECG

23 24-hour ECG

24 Portable ECG recorder

25 Outcome measures

26 Severe adverse events (SAE)

The above items are evaluated before procedure, during hospitalization (treatment day and before discharge) after discharge from the hospital. The observation schedule is shown below.

	Before procedure*1	Treatment Day	Before discharge	3 months	6 months	1 year
Days from target treatment	-	0	-	90	180	360
Allowance*2	-90 days		-7 days	＋30 days	±30 days	±30 days
①Basic information	●		●*6	●*6	●*6	●*6
②Information about AF ③Comorbidities	●					
④Medications	●		●	●	●	●
⑤Laboratory tests	●				●	●
⑥TTE*3	●					●
⑦TEE	●					
⑧Details of ablation		●				
⑨12-lead ECG	●		●	●	●	●
⑩24-hour ECG					●	●
⑪Portable ECG recorder*4					←	→
⑫Outcome measures*5			←	—	—	→
⑬SAE		←	—	—	—	→

* 1 Data before procedure can be acquired before consent acquisition.

* 2 When multiple observations are made within an allowable range, the data on the day closest to the date calculated from treatment day is chosen.

* 3 - It is possible up to 180 days before treatment day.

* 4 Record twice a day in the morning and evening for six months from the 6 months after the treatment, and additionally record when symptoms appear.

* 5 The presence or absence of recurrence of atrial fibrillation is not acquired for 3 months from the treatment.

* 6 Only blood pressure and pulse rate are collected.

22. PROTOCOL TREATMENT

Protocol treatment in each group is as follows:

Group A: left atrial LVA ablation (-)

Group B: left atrial LVA ablation (+)

- The reference date of protocol treatment should be the date on which ablation is performed.
- The protocol treatment in this study means left atrial LVA ablation. Antiarrhythmic therapy, anticoagulant therapy and ablation procedures other than left atrial LVA ablation are defined as treatment before and after protocol treatment. Protocol treatment is performed during the hospitalization.

22.1. Period from consent acquisition to protocol treatment implementation

The period from consent acquisition to treatment implementation should be within 90 days. If 90 days have elapsed, the physician obtains the verbal consent, which should be recorded in the source material. In the case, research ID is not reissued.

22.2. Left Atrial LVA Ablation

In group B, ablation for the left atrial LVA is performed. The entire left atrial LVA should be ablated principally with planar ablation so that tags with 6 mm in diameter would overlap each other. The VISITAG module is used and adjusted to (1) catheter stability range of motion ≤ 2.0 mm, (2) catheter stability duration > 5 s, and (3) CF ≥ 5g (time ≥ 25%). In principle, the energization end point of each point is evaluated by the ablation index (AI), and the target is 350. Since ablation at the posterior wall is concerned about esophageal disorder, left atrial LVA may be electrically isolated by linear ablation. LVA ablation in the vicinity of Bachmann 's bundle near the ceiling of the anterior wall and the septal His bundle should be avoided by operators' judgments in order not to harm those tissues. In group A, ablation for the left atrial LVA is not performed.

22.3. End-of-Treatment Criteria and Cancellation Criteria

Protocol treatment is completed, in principle, in the case where it is judged that the end point described above is achieved or that benefits cannot be obtained even if further ablation is performed after the end point is achieved.

The protocol treatment can be postponed or canceled according to the participant's condition, at the discretion of the physician. In addition, when continuation of further treatment is judged to be dangerous for participant's safety during the protocol treatment, when it is difficult to continue the protocol treatment due to occurrence of serious adverse events, when participants wish to withdraw from the study, or when the protocol treatment is judged to increase a risk of exacerbation or recurrence of the original diseases, comorbidities or complications, protocol treatment can be discontinued promptly.

22.4. Treatment before and after ablation

Preoperative anticoagulant therapy follows the guidelines of the Japanese Cardiovascular Society Guidelines (It continues in principle).

Preoperative antiarrhythmic drugs other than Amiodarone should stop during more than 5 times the half-life of each drug before the protocol treatment, while Amiodarone stop from 60 days or more before.

Other ablation procedures except for left atrial LVA ablation are defined in separately prepared manuals so that they are unified between institutions.

Post-ablation treatments such as antiarrhythmic therapy, anticoagulant therapy, or cardioversion should be performed according to the judgment of each physician. However, antiarrhythmic drugs should be discontinued at the time of 3 months after treatment.

22.5. Re-ablation

Re-ablation should be done at the discretion of the physicians. In re-ablation, confirmation of PV re-conduction and re-PVI in this case are essential. After re-PVI, the voltage map should be performed in the manner described in the separate manual. Ablation to the left atrial LVA is not performed in group A, while performed in group B. In any group, ablations (such as linear ablation or CFAE ablation) other than left atrial LVA ablation can be performed by the judgment of the operators.

22.6. Other treatments

Treatments for complications or co-morbidities other than the anticoagulant, antiarrhythmic drugs and re-ablation mentioned above can be made at the discretion of the physicians.

23. OUTCOME MEASURES

23.1. Primary Outcome

AF recurrence rate for 1 year from first ablation

AF recurrence is defined as any atrial fibrillation, atrial flutter or atrial tachycardia that persists for more than 30 seconds in the electrocardiogram or a test finding similar thereto. However, AF recurrence during blanking period, which is defined as three months from the treatment day should not be regarded as recurrence. Re-ablation during the blanking period should be analysed as AF recurrence immediately after the blanking period.

23.2. Secondary Outcomes

Efficacy assessment

- AF recurrence rate after the last ablation

Safety assessment

- Death (causes of death)

Death is defined as any causes of death that occurred during the protocol treatment until the end of the observation period. It does not matter whether it relates to protocol treatment or not. The causes of death are also investigated.

- Symptomatic cerebral infarction

Cerebral infarction with symptoms that occurred during the protocol treatment until the end of the observation period is evaluated. Symptoms caused by cerebral infarction can be transient or persistent consciousness disturbances, sensorimotor disorder of face, trunk, upper or lower extremities, etc. However, it is left to the judgment of physicians at each institution.

- Ablation-related complications

Ablation-related complications are, in principle, defined as the following events that occurred within one week of protocol treatment; hemorrhagic events including gastrointestinal bleeding and intracranial hemorrhage, systemic thromboembolism including cerebral infarction, pulmonary infarction, pneumothorax, arteriovenous fistula, pericarditis, cardiac tamponade, phrenic nerve palsy, atrioventricular block, sick sinus syndrome that prolongs discharge, pulmonary hypertension associated with pulmonary vein stenosis, atrial esophageal fistula, acute gastric distension, infection, coronary artery spasm, heart failure, dermatitis, and allergy against other agents such as contrast agents.

- Hemorrhagic complications

Hemorrhagic complications are defined as the following events that occurred during the study period according to ISTH bleeding criteria; lethal bleeding, symptomatic bleeding in critical sites or organs (intracranial, intraocular, retroperitoneal, intraarticular, pericardial, or intramuscular bleeding with fascial compartment syndrome), bleeding which causes a drop of 2g / dL or more of the hemoglobin level, bleeding requiring more than 2 units of transfusion (whole blood or erythrocyte), hemorrhage requiring hospitalization.

23.3. Evaluation Methods

12-lead ECG

12-lead ECG is performed at 3, 6, and 12 months after treatment, or at any time if necessary. If AF recurrence is confirmed by the 12-lead ECG, it should be recorded for 30 seconds or longer.

24-hour ECG

24-hour ECG is performed at 6 and 12 months after treatment, or at any time if necessary.

Portable ECG recorder

The portable ECG recorder is performed for six months from the 6 months after the treatment, or at any time if necessary. HCG-901 or HCG-801 manufactured by Omron is used. The physicians lend the portable ECG recorder to the participants, and they record electrocardiogram for 30 seconds once every morning and evening and additionally at the time symptoms appear. If for any reason the portable ECG monitor was not used at all, the case will be considered a dropout due to the potential for significant changes in outcomes. This should be documented in the medical records.

23.4. Outcome Evaluation Committee

The above primary endpoint is evaluated by the outcome evaluation committee. It is held after the observation period of the last case has completed. It evaluates the cases where protocol treatment is completed and the data is collected. The primary outcome is judged by

three committee members appointed by the research representative on the basis of ECG findings (copy).

The outcome evaluation committee is composed independently of the research representative, the protocol creation committee, the independent data monitoring committee, the clinical research fund contributor and the research director, contributor or collaborator of the participating medical institution.

24. STATISTICAL CONSIDERATION

24.1. Sample Size Determination

Assuming that the rate of AF recurrence for 1 year from the first ablation is 40% for group A (LVA ABL (-)) and 25% for group B (LVA ABL (+)), 155 cases in each group (310 cases in both groups) are required to detect the difference according to log-rank test with a significance level of 5% and a power of 80%. In consideration of dropout of about 10%, the number of assigned cases (group A and group B) is set as 170 in one group and 340 in both groups.

24.2. Populations for Analyses

A group consisting of all registered subjects is regarded as all registered cases, and the group of efficacy analysis subjects and the group of safety analysis subjects are defined as follows.

1) Efficacy analysis group

FAS (Full Analysis Set):

Among cases registered in this study and carrying out protocol treatment, a group consisting of cases excluding the ones in which data on efficacy is missing

PPS (Per Protocol Set):

Group consisting of cases excluding serious protocol violations from FAS

2) Safety analysis group

SAS (Safety Analysis Set):

A group consisting of cases in which the protocol treatment is performed and data on safety after the protocol treatment is obtained.

24.3. Statistical Analysis

The main aggregation and analysis methods are shown below, but detailed analysis contents are separately specified in the statistical analysis plan document.

Baseline descriptive statistics

The number of registered cases, ineligible cases, deviant cases, cases to be analyzed for each group, and stopped cases are counted. Descriptive statistics are calculated for subject background information.

Primary endpoint

The rate of AF recurrence in each group is estimated by the Kaplan-Meier method for FAS. In addition, the 1-year recurrence rate and its 95% confidence interval after the first ablation are calculated by the Greenwood formula, and the difference between groups is tested by log-rank test. Cox proportional hazards model will be used to correct latent confounders and logistic regression analysis will be performed, if necessary. Analysis targeting PPS is conducted to confirm the stability of the analysis.

Secondary endpoints

Efficacy evaluation

The analysis for FAS is conducted, but the analysis for PPS is also performed as a sensitivity analysis. Kaplan-Meier estimation, log-rank test, Cox proportional hazard model are applied.

Safety assessment

For SAS, 95% confidence intervals are calculated by collecting prevalence and incidence of death, symptomatic cerebral infarction, ablation-related complications, hemorrhagic complications for each group. Fisher's exact test is used for comparison between groups. The same analysis is also performed for cause of death and type of complication.

24.4. Interim Analysis

Interim analysis is not carried out in this study.

25. DATA COLLECTION AND MANAGEMENT

25.1. Medical Record

For each institution participating in the study, a person authorized to view the medical record collects the data by electronic data capture (EDC). The collected data is anonymized and sent to the research secretariat via the EDC system to department of medical information, Osaka University Hospital. Data communication is performed on a secure network using Virtual Private Network (VPN). The data is stored in the server at department of medical information, Osaka University Hospital, and it is managed responsibly by data center. Only the authorized person can enter the place where the server is installed, and security is adequately protected. Since personal information is stored in PC at each institution, the PC should be kept safely.

Medical records of each participant should be kept at each institution in accordance with the regulations of the institution.

25.2. Electrocardiogram and Voltage Map Data

Electrocardiograms and voltage map data acquired in the course of clinical practice are stored in paper or electronic media (USB or DVD) at each institution, and mailed or handed over directly to the research secretariat. Personal information (name and medical number) is masked and research ID is given. Data collected at the secretariat is kept on a locked shelf within department of cardiovascular medicine, Osaka University Graduate School of Medicine, and analyzed by the analysts when necessary (central analysis).

26. SAFETY REPORTING

26.1. Definitions

Adverse events are defined as any undesirable or unexpected signs including abnormal variations in clinical laboratory values, symptoms, or diseases that occur after the subject receives the protocol treatment, and it does not depend on whether or not there is a causality with the protocol treatment.

26.2. Evaluation and Report of Adverse Events

Adverse events that occur during protocol treatment or after completion of protocol treatment (or discontinuation of protocol treatment) are observed up to 6 months after the protocol treatment and are listed in the case report. Deterioration of the original disease (recurrence of atrial fibrillation) and hospitalization for the treatment (re-ablation) are not considered as adverse events.

26.3. Serious Adverse Events and Subsequent Response

Serious adverse events are defined as follows:

1) events which lead to die,

2) events which threaten life,

3) events which require hospitalization for treatment or which extend hospitalization period, except for hospitalization or extension of hospitalization period that satisfies the following requirements should not be considered as a serious adverse event;

· Hospital stay less than 6 hours (not considered as hospitalization)

· Hospitalization scheduled in advance (for example, surgery scheduled before the start of examination)

· Hospitalization or extension of hospitalization period for medical examination

4) events which cause persistent or prominent disability or malfunction, or

5) events which cause congenital anomalies in descendants.

If a "serious adverse event" occurs, the best treatment is taken and the course is carefully observed. It should be reported to the research representative (research secretariat) according to the appropriate manner shown below.

26.4. Serious Adverse Event Reporting

Primary report

When a serious adverse event occurs, the Investigators at each institution promptly report detail of the event without delay tothe research representative (research secretariat). The Research Secretariat reports them to institutional review board at Osaka University Hospital.

Secondary (Final) Report

When the symptoms are fixed, the Investigators at each institution should report it to the research representative. The Research Secretariat reports them to institutional review board at Osaka University Hospital.

26.5. Response of Chief Investigator

The Chief Investigator responds to the matter after receiving the report and informs the Investigator at each institution. The Chief Investigator, if necessary, asks for the independent data monitoring committee to evaluate and decide the response (interrupt, termination, continuation of study) based on the recommendation. The Investigators at each institution is informed immediately after the decision is made according to the recommendation.

26.6. Roles of Independent Data Monitoring Committee

The Independent data monitoring Committee evaluates the content of the report and recommends to the Chief Investigator (the research secretariat) in writing about the future

action such as whether to continue, whether to revise the protocol or discontinue the entire study.

27. QUALITY ASSURANCE PROCEDURES

27.1. Progress Management

The Investigators reports progress (number of registrations) and the details of serious adverse events to the head of each institution once every year.

The protocol planning committee prepares the implementation plan, modifies and revises it, and the research secretariat maintains the quality of the study and submits the final report to the institutional review board. The members of the protocol planning committee are specified separately. The protocol planning committee holds regular meetings, but the frequency and the date of the meeting are adjusted as necessary.

27.2. Monitoring and Auditing

Monitoring is carried out based on "Standard operating procedure on implementation of monitoring". On the other hand, audits are not conducted in this clinical study.

27.3. Independent Data Monitoring Committee

The Independent data monitoring committee is composed independently of the Investigator, protocol planning committee, Outcome Evaluation Committee, and Clinical Research Funding Contributor. The Committee may review data on safety of this clinical study, monitor occurrence of adverse events and make recommendations on safety concerns, risks to subjects and scientific validity to continue the study. If recommendations for revision of the protocol or discontinuation of the study due to concerns about the safety of subjects are necessary, it should be made in writing. The final decision is left to the Chief Investigator according to the recommendations. Details are specified in the standard operating procedure.

28. ETHICAL AND REGULATORY CONSIDERATIONS

28.1. Subject's human rights protection

The Investigators conduct this study according to the ethical norm based on the Declaration of Helsinki (revised in 2013) or the ethical guidelines on medical research for people (December 22, 2014, revised on February 28, 2017).

28.2. Explanation and consent to subjects

Prior to registration to this study, the Investigators should hand the "explanatory document to the patient" which is approved in advance by the institutional review board to the subject and give a sufficient explanation about the contents. In addition, the Investigators should give time and opportunity enough to ask questions about this study, and explain that they can withdraw from this study at any time, they never receive disadvantage by withdrawal, and they are never asked for reasons for withdrawal.

28.3. Creation and revision of explanatory documents and consent form

The Investigators make two copies of signed and dated consent form based on the participant's free willing consent. The original is kept by the Investigators at each institution, and one copy is stored in the medical record, and the other is provided to the subject. When obtaining information such as efficacy and safety considered to affect the consent of the participant or when obtaining the revision of the protocol considered to affect the consent of the participant, the Investigators promptly provide the information to the participant, confirms the intention of the participant regarding the continuation of participation in this study, and obtains the consent of the participant again. The protocol planning committee revises the explanatory documents as necessary and obtains the approval of the institutional review board at Osaka university hospital.

28.4. Acquisition of permission for implementation at each institution

The protocol and its revision should be reviewed together by the institutional review board at the Osaka University Hospital, approved and subsequently reported to the head of each institution and obtained the approval of the head of each institution.

28.5. Protection of personal information

The subject's personal information is protected throughout the course of this study. For this purpose, all data of each subject is identified by using identification (ID) code which is not a medical record number. However, the identification of the subjects is performed by a method in which the original source can be retroactively identified by the ID code.

When providing or disclosing data about this study to a third party (core laboratory, DMC, audit, or outcome evaluation committee), personal information is masked.

The institutions participating in this study should protect personal information of subjects according to guidelines such as "ethical guidelines on research on medical science for human beings" and requirements of the institutional review board.

29. FINANCE AND INSURANCE

29.1. Funding source and financial relationship

This clinical study will be conducted with funds provided by Biosense Webster, Inc. Biosense Webster, Inc. does not involve the duty including the planning of this study, interpretation and publication of the results, data collection, data management, statistical analysis, creation of progress reports, or monitoring.

29.2. Costs related to the study

This study is carried out within the range that is done in routine practice. Therefore, medical expenses (including inspection and treatment expenses) of this study are generally handled within the scope of health insurance.

29.3. Compensation for health damage

If health damage occurs during the study, the Investigators promptly take appropriate measures. However, the costs for health damage due to treatment provided are applied to the health insurance and should not be compensated in money.

30. PUBLICATION POLICY

30.1. Publication of outcomes

The outcomes of this study should be determined by the Chief Investigator after consultation with the Osaka Cardiovascular Committee Arrhythmia Team and published to academic societies or academic journals.

30.2. Attribution of outcomes

The main outcomes obtained from this study belong to the Osaka Cardiovascular Committee Arrhythmia Team. When publishing these results in academic meetings and articles, authors, co-authors and procedures are individually determined by discussion within the team. The intellectual property right such as patent right created by the outcomes of this study belongs to Osaka University.

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32. ABBREVIATIONS

ACEI	Angiotensin Converting Enzyme Inhibitor
AF	Atrial Fibrillation
ARB	Angiotensin Receptor Blocker
ATP	Adenosine Triphosphate
CFAE	Complex Fractionated Atrial Electrogram
COPD	Chronic Obstructive Pulmonary Disease

CTI	Cavo-Tricuspid Isthmus
Dd	Left ventricular end-diastolic dimension
DOAC	Direct Oral Anticoagulant
Ds	Left ventricular end-systolic dimension
EDC	Electronic Data Capture
FAS	Full Analysis Set
ISTH	International Society of Thrombosis and Hemostasis
IVS	Intra-Ventricular Septum thickness
LA	Left Atrium
LVA	Low Voltage Area
LVEF	Left Ventricular Ejection Fraction
MR	Mitral Regurgitation
NYHA	New York Heart Association
PPS	Per Protocol Set
PV	Pulmonary Vein
PVI	Pulmonary Vein Isolation
PW	Posterior Wall Thickness
QOL	Quality of Life
RF	Radiofrequency
TR	Tricuspid Regurgitation

VPN Virtual Private Network

Summary of changes

Version	Creation date	Author	Major changes
1.0	1/15/2019	Akihiro Sunaga	New Creation
2.0	11/26/2019	Akihiro Sunaga	The period for conducting the portable ECG has been changed.
3.0	11/9/2020	Akihiro Sunaga	Coronary artery spasm has been added as an ablation-related complication.
4.0	4/19/2022	Akihiro Sunaga	The trial period has been extended.

Original statistical analysis plan

Efficacy and Safety of Low-voltage Guided Ablation for Recurrence Prevention Compared to Pulmonary Vein Isolation Alone in Patients with Persistent Atrial Fibrillation

-SUPPRESS-AF trial-

Statistical analysis plan

Version 1.0

Table of Contents

1. 1. Purpose

The purpose of this Statistical Analysis Plan is to specify the detailed statistical methods to be applied in the analysis of the study " Efficacy and Safety of Low-voltage Guided Ablation for Recurrence Prevention Compared to Pulmonary Vein Isolation Alone in Patients with Persistent Atrial Fibrillation" before data lock.

2. 2. Overview of the Study

Purpose	To clarify the efficacy and safety of catheter ablation for left atrial low-voltage areas (LVA) added to pulmonary vein isolation (PVI) in cases of persistent atrial fibrillation (AF).
Key Inclusion Criteria	Cases with persistent AF undergoing initial ablation where LVA is detected.
Target Enrollment Number	The target enrollment number is 170 cases per group, totaling 340 cases for both groups.
Study Period	The study period is from the approval of the head of the research institution until December 2024.
Study Design	A multicenter prospective randomized controlled study.
Primary Endpoint	Atrial fibrillation recurrence rate within 12 months from the initial ablation.
Secondary Endpoint	Efficacy endpoint: • Recurrence rate after multiple ablation procedures. Safety endpoints: • Mortality (cause of death). • Incidence of symptomatic cerebral infarction. • Ablation-related complications. • Hemorrhagic complications.

	Before Ablation*1	Ablation Day/ Screening	Day before discharge	3 Months	6 Months	12 Months
Date Calculated from Treatment	-	0	-	90	180	360
Allowed Range*2	-90 days		-7 days	+30 days	±30 days	±30 days
1. Basic Information	●		●*6	●*6	●*6	●*6
2. Information on Atrial Fibrillation 3. Medical History	●					
4. Medication Information	●		●	●	●	●
5. Blood Tests	●				●	●
6. Transthoracic Echocardiography	●*3					●
7. Transesophageal Echocardiography	●					
8. Details of Ablation		●				
9. 12-Lead Electrocardiogram	●		●	●	●	●
10. Holter Electrocardiogram					●	●
11. Portable Electrocardiogram*4					◄———	———►
12. Evaluation of Trial Outcomes*5		◄———	———	———	———	———►
13. Adverse Events		◄———	———	———	———►	

*1 Preoperative data may be obtained before the date of obtaining consent.

*2 If multiple observations are made within the allowable range, the information closest to the date calculated from the treatment will be used.

*3 Data can be obtained up to -180 days.

*4 Record twice daily (morning and evening), and additionally record during the appearance of symptoms.
*5 Data can be obtained at any time from after treatment to 12 months. The presence or absence of atrial fibrillation recurrence will not be obtained within 3 months post-treatment.
*6 Only blood pressure and pulse rate will be obtained.

3. 3. Analysis Population

The population consisting of all enrolled subjects will be referred to as the total enrolled set, and the populations for efficacy analysis and safety analysis will be defined as follows:

-Efficacy Analysis Population

Full Analysis Set (FAS):

The population consisting of subjects enrolled in the study and who received protocol treatment, excluding those with missing efficacy data.

Per Protocol Set (PPS):

The population consisting of subjects from the FAS, excluding those with major protocol violations.

-Safety Analysis Population

Safety Analysis Set (SAS):

The population consisting of subjects who received the trial treatment and for whom post-treatment safety information is available.

4. 4. Definition of Endpoints

The results determined by the Clinical Events Committee will be used for the analysis of both primary and secondary endpoints.

5. 4.1. Primary Endpoint

Atrial fibrillation recurrence rate within 12 months from the initial ablation

Atrial fibrillation recurrence is defined as the presence or suspicion of atrial fibrillation, atrial flutter,

or atrial tachycardia lasting for more than 30 seconds on an electrocardiogram or equivalent examination findings. However, the first 3 months from the day of protocol treatment will be considered a blanking period, and any recurrence of atrial fibrillation during this period will not be considered a recurrence. For any re-ablation performed during the blanking period, the recurrence will be analyzed as atrial fibrillation recurrence immediately after the blanking period. The start date of the evaluation period will be the date of protocol treatment. If atrial fibrillation recurrence is confirmed, the recurrence date will be the earliest date among the examinations confirming recurrence after 3 months from the initial ablation. If there is no recurrence of atrial fibrillation, the final date of confirmed non-recurrence will be the latest date among the following: the date of the last 12-lead ECG or Holter ECG test, or the date of the end of the portable ECG monitoring. At this point, the observation will be considered as censored. However, for cases where the recurrence date or the last non-recurrence confirmation date exceeds the observation period of 360 days (+ a permissible range of 30 days), censoring will be done at 390 days.

6. 4.2. Secondary Endpoints

4.2.1. Efficacy Endpoints

-Recurrence Rate after Multiple Ablations

The starting point of the evaluation period will be the day each case undergoes the last ablation treatment. If atrial fibrillation recurrence is confirmed, the recurrence date will be the earliest date among the examinations confirming recurrence after the final ablation date. However, any re-ablation performed during the blanking period of 3 months from the day of protocol treatment will be considered as re-ablation immediately after the blanking period. If there is no recurrence of atrial fibrillation, the last non-recurrence confirmation date will be the latest date under the physician's management, such as the last visit date, and this point will be considered as censored. However, for cases where the recurrence date or the last non-recurrence confirmation date exceeds the observation period of 360 days (+ a permissible range of 30 days), censoring will be done at 390 days.

-Mortality Rate

The period up to death occurring from the start of protocol treatment to the end of the observation period will be evaluated. The relationship with protocol treatment is not questioned. The starting point of the evaluation period will be the date of protocol treatment initiation. If death is not confirmed, the last date recorded in the case report form will be considered as the last survival confirmation date, and this point will be considered as censored. However, for cases where the death

date or the last survival confirmation date exceeds the observation period of 360 days (+ a permissible range of 30 days), censoring will be done at 390 days.

-Incidence Rate of Symptomatic Cerebral Infarction

The period up to the onset of symptomatic cerebral infarction occurring from the start of protocol treatment to the end of the observation period will be evaluated. Symptoms of cerebral infarction may include transient or persistent consciousness disturbances, motor or sensory abnormalities of the face, trunk, or limbs, among others, but the judgment is left to the researchers at each study institution. The starting point of the evaluation period will be the date of protocol treatment initiation. If the onset is not confirmed, the last date recorded in the case report form will be considered as the last non-onset confirmation date, and this point will be considered as censored. However, for cases where the onset date or the last non-onset confirmation date exceeds the observation period of 360 days (+ a permissible range of 30 days), censoring will be done at 390 days.

4.2.2. Safety Endpoints

- Ablation-Related Complications

Ablation-related complications are defined, in principle, as the following events occurring within one week of protocol treatment: bleeding or hematoma formation at the puncture site requiring intervention, hemorrhagic events including gastrointestinal bleeding or cerebral hemorrhage, systemic thromboembolism including cerebral infarction or pulmonary embolism, pneumothorax, arteriovenous fistula, pericarditis, cardiac tamponade, phrenic nerve paralysis, atrioventricular block, persistent sinus node dysfunction at discharge, pulmonary hypertension due to pulmonary vein stenosis, atrial-esophageal fistula, acute gastric dilation, infections, heart failure, dermatitis, allergic reactions to therapeutic drugs, contrast agents, or test agents, and others.

- Incidence Rate of Hemorrhagic Complications

The period up to the onset of hemorrhagic complications occurring during the trial period will be evaluated. Hemorrhagic complications are defined as the following events according to the ISTH bleeding criteria for major bleeding:

- Fatal bleeding
- Symptomatic bleeding in a critical area or organ (e.g., intracranial, intraocular, retroperitoneal, intra-articular, pericardial, intramuscular with compartment syndrome)
- Bleeding causing a decrease in hemoglobin level of 2 g/dL or more

- Bleeding requiring transfusion of 2 or more units of whole blood or red cells
- Bleeding necessitating hospitalization

If the onset is not confirmed, the last date recorded in the case report form will be considered as the last non-onset confirmation date, and this point will be considered as censored. However, for cases where the onset date or the last non-onset confirmation date exceeds the observation period of 360 days (+ a permissible range of 30 days), censoring will be done at 390 days.

7. 5. Analysis Methods

8. 5.1. Breakdown of Subjects

Using the primary registration cases as the target, illustrate the transformation process of the target population in a diagram. In this diagram, aggregate the numbers of primary and secondary registration cases, the number of ineligible cases, the number of discontinued and dropped-out cases, and the number of cases belonging to each analysis population, separately for Group A: observation and Group B: left atrial LVA ablation. For discontinued and dropped-out cases, as well as cases not included in each analysis population, aggregate them according to the reasons.

9. 5.2. Subject Background

For the FAS and PPS populations, summarize the key background characteristics of the subjects using descriptive statistics, separately for Group A: observation and Group B: left atrial LVA ablation. For continuous variables, calculate the number of subjects, mean, standard deviation, minimum, median, interquartile range, and maximum. For categorical variables, calculate frequencies and percentages. In subsequent analyses, use the same descriptive statistics for summarization. The items to be summarized are as follows. Additionally, for the study institutions, which are allocation adjustment factors, aggregate the number of subjects by group.

1. Basic Information

   Age at consent acquisition, Sex, Height (cm), Weight (kg), BMI (body weight (kg)/height (m)^2), Systolic and diastolic blood pressure (mmHg), Pulse rate (bpm)

2. Information on Atrial Fibrillation

   Type of atrial fibrillation (persistent or long-standing persistent), Duration of atrial fibrillation, Duration of atrial fibrillation persistence

3. Medical History

Hypertension, Diabetes mellitus, Dyslipidemia, Congestive heart failure, NYHA class Cardiomyopathy, History of myocardial infarction, Peripheral artery disease, Valvular disease (excluding post-surgical repair), COPD, Thyroid disease, Tachyarrhythmias other than atrial fibrillation (ventricular tachycardia, paroxysmal supraventricular tachycardia, atrial tachycardia, atrial flutter), Sinus node dysfunction, Implanted devices, History of stroke, History of transient ischemic attack, History of thromboembolism, CHA_2DS_2VASc score (congestive heart failure: 1 point, hypertension: 1 point, age 75 or older: 2 points or age 65 or older: 1 point, diabetes mellitus: 1 point, stroke or transient ischemic attack or thromboembolism: 2 points, myocardial infarction or peripheral artery disease: 1 point, female: 1 point)

4. Medication Information

Presence and type of anticoagulants, Presence of antiplatelet drugs, Presence of antiarrhythmic drugs

5. Blood Tests

Hemoglobin (g/dL), Creatinine (mg/dL), CRP (mg/dL), eGFR (mL/min/1.73m²): Male eGFR = 194 × Cr (mg/dL) ^ (-1.094) × Age (years) ^ (-0.287), Female eGFR = Male eGFR × 0.739

6. Transthoracic Echocardiography

LVDd (mm), LVDs (mm), LA diameter (mm), LVEF (Teichholz): LVEF = (LVEDV - LVESV) / LVEDV × 100, LVEDV = (7 × $LVDd^3$) / (2.4 + LVDd), LVESV = (7 × $LVDs^3$) / (2.4 + LVDs)

7. Transesophageal Echocardiography

Presence of spontaneous echo contrast, Left atrial appendage inflow velocity (cm/sec), Left atrial appendage outflow velocity (cm/sec)

8. Details of Ablation

Use of deflectable sheath, Success of pulmonary vein isolation, Number of gaps in first pass isolation (left and right separately), Presence of time-dependent reconduction, Presence, ablation sites, and success of non-pulmonary vein origin AF trigger ablation, Presence and reasons of CTI ablation, and success of block formation, Presence, type, ablation sites, success, and reasons of ablation for other atrial tachycardia or flutter, Type of mapping catheter, Number of mapping points, Mapping time, LVA area, Left atrial surface area, LVA ablation energy application time,

LVA ablation energy, Success of total LVA ablation, Residual LVA area, Reasons for incomplete ablation of LVA ablation, Total energy application time, Total energy, Treatment time

10. 5.3. Medication Information

For the FAS and PPS populations, aggregate the medication details of antiarrhythmic drugs at 3 months, 6 months, and 12 months, separately by group.

11. 5.4. Status of Portable ECG Implementation

For the FAS and PPS populations, calculate the implementation rate and implementation period of the portable ECG, and the mean, standard deviation, minimum, median, interquartile range, and maximum values for each group. The implementation rate and implementation period will be calculated using the following two definitions:

Implementation Rate 1 = Number of Implementations / ((Portable ECG Return Date - Portable ECG Lending Date) × 2)
Implementation Rate 2 = Number of Implementations / ((Portable ECG End Date - Portable ECG Start Date) × 2)

Implementation Period 1 = Portable ECG Return Date - Portable ECG Lending Date
Implementation Period 2 = Portable ECG End Date - Portable ECG Start Date

12. 5.5. Primary Endpoint

For the FAS population, If death is confirmed during the observation period, deaths suspected to be related to atrial fibrillation will be treated as events, while deaths considered unrelated to atrial fibrillation will be treated as censored. The event onset date or the censoring date will be calculated with the start date set as day 1.

The atrial fibrillation recurrence rate will be estimated using the Kaplan-Meier method, separately for Group A: observation and Group B: left atrial LVA ablation. The recurrence rate at 12 months after the initial ablation and its 95% confidence interval will be calculated using Greenwood's formula, and the log-rank test will be used for group comparison.

Additionally, using a Cox proportional hazards model that includes only the treatment variable, the hazard ratio of Group B to Group A and its 95% confidence interval will be estimated. Furthermore, using a Cox proportional hazards model to adjust for potential confounding factors, the hazard ratio

of Group B to Group A and its 95% confidence interval will be estimated. The candidate confounding factors to be adjusted for are as follows: Left atrial diameter (LA diameter), duration of atrial fibrillation, age, sex, and CHA2DS2VASc score.
If an imbalance of these candidate confounding factors is confirmed between groups, adjustment will be performed in order of priority among the imbalanced variables. The priority for adjustment will be in the order listed. If multiple confounding factors require adjustment, they will be added to the model in order of priority until the model fails to converge. The stability of the estimated results will be confirmed, and the final model will be selected.

As a sensitivity analysis, the definition of atrial fibrillation recurrence will be “the presence or suspicion of atrial fibrillation, atrial flutter, or atrial tachycardia lasting for more than 30 seconds on an electrocardiogram or equivalent examination findings. However, the first 3 months from the day of protocol treatment will be considered a blanking period, and any recurrence of atrial fibrillation during this period will not be considered a recurrence. For any re-ablation performed during the blanking period, the recurrence will be analyzed as atrial fibrillation recurrence immediately after the blanking period.” This analysis will be performed in the same manner as described above.

The same analyses described above will be performed for the PPS population.

13. 5.6. Secondary Endpoints

5.6.1. Efficacy Endpoints

- Recurrence Rate after Multiple Ablations

  Perform the same analysis as for the primary endpoint. Additionally, aggregate the number of cases that underwent multiple ablations by the number of ablations and by group.

- Mortality, Cause of Death

  For the SAS and FAS populations, to compare between groups, calculate the difference in rates and its 95% confidence interval, and perform Fisher's exact test. Aggregate the details of mortality. Additionally, estimate the mortality rate using the Kaplan-Meier method for Group A: observation and Group B: left atrial LVA ablation. Calculate the mortality rate at 12 months after the initial ablation and its 95% confidence interval using Greenwood’s formula, and use the log-rank test for group comparison. For the cause of death, aggregate the number of cases and the incidence rates by group. Also, using a Cox proportional hazards model that includes only the

treatment variable, estimate the hazard ratio of Group B to Group A and its 95% confidence interval. Adjust for confounding factors in the same way as in the primary endpoint analysis. Additionally, in calculating the event onset date or the censoring date, the start date will be considered as day 1.

- Incidence of Symptomatic Cerebral Infarction

For the SAS and FAS populations, to compare between groups, calculate the difference in rates and its 95% confidence interval, and perform Fisher's exact test. Additionally, estimate the incidence rate of symptomatic cerebral infarction using the Kaplan-Meier method for Group A: observation and Group B: left atrial LVA ablation. Calculate the incidence rate at 12 months after the initial ablation and its 95% confidence interval using Greenwood's formula, and use the log-rank test for group comparison. Also, using a Cox proportional hazards model that includes only the treatment variable, estimate the hazard ratio of Group B to Group A and its 95% confidence interval. Adjust for confounding factors in the same way as in the primary endpoint analysis. Handle mortality in the same way as in the primary endpoint analysis. Additionally, in calculating the event onset date or the censoring date, the start date will be considered as day 1.

5.6.2. Safety Endpoints

- Ablation-Related Complications

For the SAS population, aggregate the number of cases and incidence rates of ablation-related complications, and the number of events separately for Group A: observation and Group B: left atrial LVA ablation. Calculate the 95% confidence intervals of the incidence rates using the Clopper-Pearson method. Additionally, compare the incidence rates between groups using the difference in rates and its 95% confidence interval, and perform Fisher's exact test. Furthermore, provide detailed aggregation of the number of cases, incidence rates, and number of events of ablation-related complications by group.

- Hemorrhagic Complications

For the SAS population, to compare between groups, calculate the difference in rates and its 95% confidence interval, and perform Fisher's exact test. Aggregate the details of hemorrhagic complications. Additionally, estimate the incidence rate of hemorrhagic complications using the Kaplan-Meier method separately for Group A: observation and Group B: left atrial LVA ablation. Calculate the incidence rate at 12 months after the initial ablation and its 95% confidence interval using Greenwood's formula, and use the log-rank test for group comparison. Furthermore,

provide detailed aggregation of the number of cases, incidence rates, and number of events of hemorrhagic complications by group. Also, using a Cox proportional hazards model that includes only the treatment variable, estimate the hazard ratio of Group B to Group A and its 95% confidence interval. Adjust for confounding factors in the same way as in the primary endpoint analysis. Handle mortality in the same way as in the primary endpoint analysis. Additionally, in calculating the event onset date or the censoring date, the start date will be considered as day 1.

14. 5.7. Exploratory Analysis

For the FAS and PPS populations, conduct exploratory subgroup analyses based on the following items: Age (75 years and older vs. under 75 years), Gender, BMI (25 and above vs. under 25), CHA2DS2VASc score (4 and above vs. under 4), LVEF (three groups: less than 40%, 40% to less than 50%, 50% and above), Left atrial diameter (45 mm and above vs. under 45 mm), AF type (duration of 1 year and longer vs. under 1 year), NYHA class (class II and above vs. class I), Hypertension, Diabetes mellitus, LVA area (20 and above vs. under 20)

For each of these items, estimate the atrial fibrillation recurrence rate using the Kaplan-Meier method for each subgroup, separately for Group A: observation and Group B: left atrial LVA ablation. Calculate the recurrence rate at 12 months after the initial ablation and its 95% confidence interval using Greenwood's formula, and use the log-rank test for group comparisons. Additionally, estimate the hazard ratio using a Cox proportional hazards model that includes the treatment variable, the variable used for subgroup analysis, and their interaction term.

15. 5.8. Units and Digits

In the summary tables, display units and digits according to the following rules. Percentages should be displayed to one decimal place. However, the standard digits refers to the number of digits specified in the database definition document.

- Minimum and maximum values: standard digits
- Mean, standard deviation, first quartile, median, third quartile, and confidence intervals: standard digits + 1 decimal digit

For derived variables (items requiring calculation), do not round during the calculation process; round to the specified number of digits just before displaying in the summary tables or lists.

16. 5.9. Missing Values

Missing values will not be imputed.

17. 5.10. Significance Levels and Confidence Intervals

Unless otherwise specified, the significance level for statistical tests will be set at a two-sided 5%, and the confidence coefficient will be set at a two-sided 95%.

18. 6. Software to be Used

SAS Version 9.4 (SAS Institute Inc., Cary, NC, USA) will be used for the analysis.

19. 7. Creation and Revision of the Statistical Analysis Plan

The procedures for the creation, approval, and revision of this Statistical Analysis Plan will follow the Standard Operating Procedures (SOPs) established separately.

20. 8. Storage and Management of Documents

The storage and management of documents generated from the statistical analysis tasks of this study will follow the Standard Operating Procedures (SOPs) established separately.

Final statistical analysis plan

Efficacy and Safety of Low-voltage Guided Ablation for Recurrence Prevention Compared to Pulmonary Vein Isolation Alone in Patients with Persistent Atrial Fibrillation

-SUPPRESS-AF trial-

Statistical analysis plan

Version 2.1

Table of Contents

21. 1. Purpose

The purpose of this Statistical Analysis Plan is to specify the detailed statistical methods to be applied in the analysis of the study " Efficacy and Safety of Low-voltage Guided Ablation for Recurrence Prevention Compared to Pulmonary Vein Isolation Alone in Patients with Persistent Atrial Fibrillation" before data lock.

22. 2. Overview of the Study

Purpose	To clarify the efficacy and safety of catheter ablation for left atrial low-voltage areas (LVA) added to pulmonary vein isolation (PVI) in cases of persistent atrial fibrillation (AF).
Key Inclusion Criteria	Cases with persistent AF undergoing initial ablation where LVA is detected.
Target Enrollment Number	The target enrollment number is 170 cases per group, totaling 340 cases for both groups.
Study Period	The study period is from the approval of the head of the research institution until December 2024.
Study Design	A multicenter prospective randomized controlled study.
Primary Endpoint	Atrial fibrillation recurrence rate within 12 months from the initial ablation.
Secondary Endpoint	Efficacy endpoint: • Recurrence rate after multiple ablation procedures. Safety endpoints: • Mortality (cause of death). • Incidence of symptomatic cerebral infarction. • Ablation-related complications. • Hemorrhagic complications.

	Before Ablation*1	Ablation Day/ Screening	Day before discharge	3 Months	6 Months	12 Months
Date Calculated from Treatment	-	0	-	90	180	360
Allowed Range*2	-90 days		-7 days	+30 days	±30 days	±30 days
1. Basic Information	●		●*6	●*6	●*6	●*6
2. Information on Atrial Fibrillation 3. Medical History	●					
4. Medication Information	●		●	●	●	●
5. Blood Tests	●				●	●
6. Transthoracic Echocardiography	●*3					●
7. Transesophageal Echocardiography	●					
8. Details of Ablation		●				
9. 12-Lead Electrocardiogram	●		●	●	●	●
10. Holter Electrocardiogram					●	●
11. Portable Electrocardiogram*4					◄———	———►
12. Evaluation of Trial Outcomes*5		◄———	———	———	———	———►
13. Adverse Events		◄———	———	———	———►	

*1 Preoperative data may be obtained before the date of obtaining consent.

*2 If multiple observations are made within the allowable range, the information closest to the date calculated from the treatment will be used.

*3 Data can be obtained up to -180 days.

*4 Record twice daily (morning and evening), and additionally record during the appearance of symptoms.
*5 Data can be obtained at any time from after treatment to 12 months. The presence or absence of atrial fibrillation recurrence will not be obtained within 3 months post-treatment.
*6 Only blood pressure and pulse rate will be obtained.

23. 3. Analysis Population

The population consisting of all enrolled subjects will be referred to as the total enrolled set, and the populations for efficacy analysis and safety analysis will be defined as follows:

-Efficacy Analysis Population

Full Analysis Set (FAS):

The population consisting of subjects enrolled in the study and who received protocol treatment, excluding those with missing efficacy data.

Per Protocol Set (PPS):

The population consisting of subjects from the FAS, excluding those with major protocol violations.

-Safety Analysis Population

Safety Analysis Set (SAS):

The population consisting of subjects who received the trial treatment and for whom post-treatment safety information is available.

24. 4. Definition of Endpoints

The results determined by the Clinical Events Committee will be used for the analysis of both primary and secondary endpoints.

25. 4.1. Primary Endpoint

Atrial fibrillation recurrence rate within 12 months from the initial ablation

Atrial fibrillation recurrence is defined as the presence or suspicion of atrial fibrillation, atrial flutter,

or atrial tachycardia lasting for more than 30 seconds on an electrocardiogram or equivalent examination findings, or the use of antiarrhythmic drugs. However, the first 3 months from the day of protocol treatment will be considered a blanking period, and any recurrence of atrial fibrillation during this period will not be considered a recurrence. For any re-ablation performed during the blanking period, the recurrence will be analyzed as atrial fibrillation recurrence immediately after the blanking period. The start date of the evaluation period will be the date of protocol treatment. If atrial fibrillation recurrence is confirmed, the recurrence date will be the earliest date among the examinations confirming recurrence after 3 months from the initial ablation. If there is no recurrence of atrial fibrillation, the last non-recurrence confirmation date will be the latest date under the physician's management, such as the last visit date, and this point will be considered as censored. However, for cases where the recurrence date or the last non-recurrence confirmation date exceeds the observation period of 360 days (+ a permissible range of 30 days), censoring will be done at 390 days.

26. 4.2. Secondary Endpoints

4.2.1. Efficacy Endpoints

-Recurrence Rate after Multiple Ablations

The starting point of the evaluation period will be the day each case undergoes the last ablation treatment. If atrial fibrillation recurrence is confirmed, the recurrence date will be the earliest date among the examinations confirming recurrence after the final ablation date. However, any re-ablation performed during the blanking period of 3 months from the day of protocol treatment will be considered as re-ablation immediately after the blanking period. If there is no recurrence of atrial fibrillation, the last non-recurrence confirmation date will be the latest date under the physician's management, such as the last visit date, and this point will be considered as censored. However, for cases where the recurrence date or the last non-recurrence confirmation date exceeds the observation period of 360 days (+ a permissible range of 30 days), censoring will be done at 390 days.

-Mortality Rate

The period up to death occurring from the start of protocol treatment to the end of the observation period will be evaluated. The relationship with protocol treatment is not questioned. The starting point of the evaluation period will be the date of protocol treatment initiation. If death is not confirmed, the last date recorded in the case report form will be considered as the last survival confirmation date, and this point will be considered as censored. However, for cases where the death

date or the last survival confirmation date exceeds the observation period of 360 days (+ a permissible range of 30 days), censoring will be done at 390 days.

-Incidence Rate of Symptomatic Cerebral Infarction

The period up to the onset of symptomatic cerebral infarction occurring from the start of protocol treatment to the end of the observation period will be evaluated. Symptoms of cerebral infarction may include transient or persistent consciousness disturbances, motor or sensory abnormalities of the face, trunk, or limbs, among others, but the judgment is left to the researchers at each study institution. The starting point of the evaluation period will be the date of protocol treatment initiation. If the onset is not confirmed, the last date recorded in the case report form will be considered as the last non-onset confirmation date, and this point will be considered as censored. However, for cases where the onset date or the last non-onset confirmation date exceeds the observation period of 360 days (+ a permissible range of 30 days), censoring will be done at 390 days.

4.2.2. Safety Endpoints

- Ablation-Related Complications

Ablation-related complications are defined, in principle, as the following events occurring within one week of protocol treatment: bleeding or hematoma formation at the puncture site requiring intervention, hemorrhagic events including gastrointestinal bleeding or cerebral hemorrhage, systemic thromboembolism including cerebral infarction or pulmonary embolism, pneumothorax, arteriovenous fistula, pericarditis, cardiac tamponade, phrenic nerve paralysis, atrioventricular block, persistent sinus node dysfunction at discharge, pulmonary hypertension due to pulmonary vein stenosis, atrial-esophageal fistula, acute gastric dilation, infections, heart failure, dermatitis, allergic reactions to therapeutic drugs, contrast agents, or test agents, and others.

- Incidence Rate of Hemorrhagic Complications

The period up to the onset of hemorrhagic complications occurring during the trial period will be evaluated. Hemorrhagic complications are defined as the following events according to the ISTH bleeding criteria for major bleeding:

- Fatal bleeding
- Symptomatic bleeding in a critical area or organ (e.g., intracranial, intraocular, retroperitoneal, intra-articular, pericardial, intramuscular with compartment syndrome)
- Bleeding causing a decrease in hemoglobin level of 2 g/dL or more

- Bleeding requiring transfusion of 2 or more units of whole blood or red cells
- Bleeding necessitating hospitalization

If the onset is not confirmed, the last date recorded in the case report form will be considered as the last non-onset confirmation date, and this point will be considered as censored. However, for cases where the onset date or the last non-onset confirmation date exceeds the observation period of 360 days (+ a permissible range of 30 days), censoring will be done at 390 days.

27. 5. Analysis Methods

28. 5.1. Breakdown of Subjects

Using the primary registration cases as the target, illustrate the transformation process of the target population in a diagram. In this diagram, aggregate the numbers of primary and secondary registration cases, the number of ineligible cases, the number of discontinued and dropped-out cases, and the number of cases belonging to each analysis population, separately for Group A: observation and Group B: left atrial LVA ablation. For discontinued and dropped-out cases, as well as cases not included in each analysis population, aggregate them according to the reasons.

29. 5.2. Subject Background

For the FAS and PPS populations, summarize the key background characteristics of the subjects using descriptive statistics, separately for Group A: observation and Group B: left atrial LVA ablation. For continuous variables, calculate the number of subjects, mean, standard deviation, minimum, median, interquartile range, and maximum. For categorical variables, calculate frequencies and percentages. In subsequent analyses, use the same descriptive statistics for summarization. The items to be summarized are as follows. Additionally, for the study institutions, which are allocation adjustment factors, aggregate the number of subjects by group.

1. Basic Information

   Age at consent acquisition, Sex, Height (cm), Weight (kg), BMI (body weight (kg)/height (m)^2), Systolic and diastolic blood pressure (mmHg), Pulse rate (bpm)

2. Information on Atrial Fibrillation

   Type of atrial fibrillation (persistent or long-standing persistent), Duration of atrial fibrillation, Duration of atrial fibrillation persistence

3. Medical History

Hypertension, Diabetes mellitus, Dyslipidemia, Congestive heart failure, NYHA class Cardiomyopathy, History of myocardial infarction, Peripheral artery disease, Valvular disease (excluding post-surgical repair), COPD, Thyroid disease, Tachyarrhythmias other than atrial fibrillation (ventricular tachycardia, paroxysmal supraventricular tachycardia, atrial tachycardia, atrial flutter), Sinus node dysfunction, Implanted devices, History of stroke, History of transient ischemic attack, History of thromboembolism, CHA_2DS_2VASc score (congestive heart failure: 1 point, hypertension: 1 point, age 75 or older: 2 points or age 65 or older: 1 point, diabetes mellitus: 1 point, stroke or transient ischemic attack or thromboembolism: 2 points, myocardial infarction or peripheral artery disease: 1 point, female: 1 point)

4. Medication Information

Presence and type of anticoagulants, Presence of antiplatelet drugs, Presence of antiarrhythmic drugs

5. Blood Tests

Hemoglobin (g/dL), Creatinine (mg/dL), CRP (mg/dL), eGFR (mL/min/1.73m²): Male eGFR = 194 × Cr (mg/dL) ^ (-1.094) × Age (years) ^ (-0.287), Female eGFR = Male eGFR × 0.739

6. Transthoracic Echocardiography

LVDd (mm), LVDs (mm), LA diameter (mm), LVEF (Teichholz): LVEF = (LVEDV - LVESV) / LVEDV × 100, LVEDV = (7 × $LVDd^3$) / (2.4 + LVDd), LVESV = (7 × $LVDs^3$) / (2.4 + LVDs)

7. Transesophageal Echocardiography

Presence of spontaneous echo contrast, Left atrial appendage inflow velocity (cm/sec), Left atrial appendage outflow velocity (cm/sec)

8. Details of Ablation

Use of deflectable sheath, Success of pulmonary vein isolation, Number of gaps in first pass isolation (left and right separately), Presence of time-dependent reconduction, Presence, ablation sites, and success of non-pulmonary vein origin AF trigger ablation, Presence and reasons of CTI ablation, and success of block formation, Presence, type, ablation sites, success, and reasons of ablation for other atrial tachycardia or flutter, Type of mapping catheter, Number of mapping points, Mapping time, LVA area, Left atrial surface area, LVA ablation energy application time,

LVA ablation energy, Success of total LVA ablation, Residual LVA area, Reasons for incomplete ablation of LVA ablation, Total energy application time, Total energy, Treatment time

30. 5.3. Medication Information

For the FAS and PPS populations, aggregate the medication details of antiarrhythmic drugs at 3 months, 6 months, and 12 months, separately by group.

31. 5.4. Status of Portable ECG Implementation

For the FAS and PPS populations, calculate the implementation rate and implementation period of the portable ECG, and the mean, standard deviation, minimum, median, interquartile range, and maximum values for each group. The implementation rate and implementation period will be calculated using the following two definitions:

Implementation Rate 1 = Number of Implementations / ((Portable ECG Return Date - Portable ECG Lending Date) × 2)
Implementation Rate 2 = Number of Implementations / ((Portable ECG End Date - Portable ECG Start Date) × 2)

Implementation Period 1 = Portable ECG Return Date - Portable ECG Lending Date
Implementation Period 2 = Portable ECG End Date - Portable ECG Start Date

32. 5.5. Primary Endpoint

For the FAS population, the event onset date or the censoring date will be calculated with the start date set as day 1. The atrial fibrillation recurrence rate will be estimated using the Kaplan-Meier method, separately for Group A: observation and Group B: left atrial LVA ablation. The recurrence rate at 12 months after the initial ablation and its 95% confidence interval will be calculated using Greenwood's formula, and the log-rank test will be used for group comparison.
Additionally, using a Cox proportional hazards model that includes only the treatment variable, the hazard ratio of Group B to Group A and its 95% confidence interval will be estimated. Furthermore, using a Cox proportional hazards model to adjust for potential confounding factors, the hazard ratio of Group B to Group A and its 95% confidence interval will be estimated. The candidate confounding factors to be adjusted for are as follows: Left atrial diameter (LA diameter), duration of atrial fibrillation, age, sex, and CHA2DS2VASc score.

If an imbalance of these candidate confounding factors is confirmed between groups, adjustment will be performed in order of priority among the imbalanced variables. The priority for adjustment will be in the order listed. If multiple confounding factors require adjustment, they will be added to the model in order of priority until the model fails to converge. The stability of the estimated results will be confirmed, and the final model will be selected.

As a sensitivity analysis, the definition of atrial fibrillation recurrence will be “the presence or suspicion of atrial fibrillation, atrial flutter, or atrial tachycardia lasting for more than 30 seconds on an electrocardiogram or equivalent examination findings, or the use of antiarrhythmic drugs. However, the first 3 months from the day of protocol treatment will be considered a blanking period, and any recurrence of atrial fibrillation during this period will not be considered a recurrence. For any re-ablation performed during the blanking period, the recurrence will be analyzed as atrial fibrillation recurrence immediately after the blanking period.” This analysis will be performed in the same manner as described above.

Furthermore, regarding the pattern of atrial fibrillation recurrence, create a cross-table by classifying “atrial fibrillation” and “atrial flutter” for each recurrence count, calculate the occurrence rate and the difference in occurrence rates with their 95% confidence intervals for each group, and use Fisher’s exact test for group comparison.

The same analyses described above will be performed for the PPS population.

33. 5.6. Secondary Endpoints

5.6.1. Efficacy Endpoints

- Recurrence Rate after Multiple Ablations

  Perform the same analysis as for the primary endpoint. Additionally, aggregate the number of cases that underwent multiple ablations by the number of ablations and by group.

- Mortality, Cause of Death

  For the SAS and FAS populations, aggregate the number of cases and the incidence rates by group, and calculate the 95% confidence intervals. To compare between groups, calculate the difference in rates and its 95% confidence interval, and perform Fisher's exact test. Aggregate the details of mortality. Additionally, estimate the mortality rate using the Kaplan-Meier method for Group A: observation and Group B: left atrial LVA ablation. Calculate the mortality rate at 12

months after the initial ablation and its 95% confidence interval using Greenwood's formula, and use the log-rank test for group comparison. For the cause of death, aggregate the number of cases and the incidence rates by group. Also, using a Cox proportional hazards model that includes only the treatment variable, estimate the hazard ratio of Group B to Group A and its 95% confidence interval. Adjust for confounding factors in the same way as in the primary endpoint analysis. Additionally, in calculating the event onset date or the censoring date, the start date will be considered as day 1.

- Incidence of Symptomatic Cerebral Infarction

For the SAS and FAS populations, aggregate the number of cases, number of events, and incidence rates by group, and calculate the 95% confidence intervals. To compare between groups, calculate the difference in rates and its 95% confidence interval, and perform Fisher's exact test. Additionally, estimate the incidence rate of symptomatic cerebral infarction using the Kaplan-Meier method for Group A: observation and Group B: left atrial LVA ablation. Calculate the incidence rate at 12 months after the initial ablation and its 95% confidence interval using Greenwood's formula, and use the log-rank test for group comparison. Also, using a Cox proportional hazards model that includes only the treatment variable, estimate the hazard ratio of Group B to Group A and its 95% confidence interval. Adjust for confounding factors in the same way as in the primary endpoint analysis. Handle mortality in the same way as in the primary endpoint analysis. Additionally, in calculating the event onset date or the censoring date, the start date will be considered as day 1.

5.6.2. Safety Endpoints

- Ablation-Related Complications

For the SAS population, aggregate the number of cases and incidence rates of ablation-related complications, and the number of events separately for Group A: observation and Group B: left atrial LVA ablation. Calculate the 95% confidence intervals of the incidence rates using the Clopper-Pearson method. Additionally, compare the incidence rates between groups using the difference in rates and its 95% confidence interval, and perform Fisher's exact test. Furthermore, provide detailed aggregation of the number of cases, incidence rates, and number of events of ablation-related complications by group. For the following "serious ablation-related complications," compare the incidence rates between groups using the difference in rates and its 95% confidence interval, and perform Fisher's exact test. Also, aggregate the number of cases, incidence rates, and number of events of serious ablation-related complications by group. Serious

ablation-related complications: Systemic thromboembolism including cerebral infarction and pulmonary embolism, Cardiac tamponade, Atrial-esophageal fistula, Esophageal-pericardial fistula, Major bleeding classified as an ablation-related complication.

- Hemorrhagic Complications

For the SAS population, aggregate the number of cases and incidence rates by group, and calculate the 95% confidence intervals. To compare between groups, calculate the difference in rates and its 95% confidence interval, and perform Fisher's exact test. Aggregate the details of hemorrhagic complications. Additionally, estimate the incidence rate of hemorrhagic complications using the Kaplan-Meier method separately for Group A: observation and Group B: left atrial LVA ablation. Calculate the incidence rate at 12 months after the initial ablation and its 95% confidence interval using Greenwood’s formula, and use the log-rank test for group comparison. Furthermore, provide detailed aggregation of the number of cases, incidence rates, and number of events of hemorrhagic complications by group. Also, using a Cox proportional hazards model that includes only the treatment variable, estimate the hazard ratio of Group B to Group A and its 95% confidence interval. Adjust for confounding factors in the same way as in the primary endpoint analysis. Handle mortality in the same way as in the primary endpoint analysis. Additionally, in calculating the event onset date or the censoring date, the start date will be considered as day 1.

34. 5.7. Exploratory Analysis

For the FAS and PPS populations, conduct exploratory subgroup analyses based on the following items: Age (75 years and older vs. under 75 years), Gender, BMI (25 and above vs. under 25), CHA2DS2VASc score (4 and above vs. under 4), LVEF (three groups: less than 40%, 40% to less than 50%, 50% and above), Left atrial diameter (45 mm and above vs. under 45 mm), AF type (duration of 1 year and longer vs. under 1 year), NYHA class (class II and above vs. class I), Hypertension, Diabetes mellitus, LVA area (20 and above vs. under 20)

For each of these items, estimate the atrial fibrillation recurrence rate using the Kaplan-Meier method for each subgroup, separately for Group A: observation and Group B: left atrial LVA ablation. Calculate the recurrence rate at 12 months after the initial ablation and its 95% confidence interval using Greenwood’s formula, and use the log-rank test for group comparisons. Additionally, estimate the hazard ratio using a Cox proportional hazards model that includes the treatment variable, the variable used for subgroup analysis, and their interaction term. Furthermore, create a forest plot. In the subgroup analysis of NYHA, treat the NYHA class as I for cases without congestive heart failure.

35. 5.8. Units and Digits

In the summary tables, display units and digits according to the following rules. Percentages should be displayed to one decimal place. However, the standard digits refers to the number of digits specified in the database definition document.

- Minimum and maximum values: standard digits
- Mean, standard deviation, first quartile, median, third quartile, and confidence intervals: standard digits + 1 decimal digit

For derived variables (items requiring calculation), do not round during the calculation process; round to the specified number of digits just before displaying in the summary tables or lists.

36. 5.9. Missing Values

Missing values will not be imputed.

37. 5.10. Significance Levels and Confidence Intervals

Unless otherwise specified, the significance level for statistical tests will be set at a two-sided 5%, and the confidence coefficient will be set at a two-sided 95%.

38. 6. Software to be Used

SAS Version 9.4 (SAS Institute Inc., Cary, NC, USA) will be used for the analysis.

39. 7. Creation and Revision of the Statistical Analysis Plan

The procedures for the creation, approval, and revision of this Statistical Analysis Plan will follow the Standard Operating Procedures (SOPs) established separately.

40. 8. Storage and Management of Documents

The storage and management of documents generated from the statistical analysis tasks of this study

will follow the Standard Operating Procedures (SOPs) established separately.

Summary of changes

Revision history

Version	Creation date	Author	Major changes
1.0	4/12/2024	Kazutaka Nishio	New creation
1.1	5/1/2024	Kazutaka Nishio	-Definition of censoring in the analysis of atrial fibrillation recurrence was revised. -Handling of NYHA classification in subgroup analysis was specified.
2.0	6/7/2024	Kazutaka Nishio	-In the primary endpoint section, added analysis for comparing recurrence patterns between groups. -In the secondary safety endpoint section, added analysis methods (proportion and 95% confidence intervals, Fisher's exact test).
2.1	6/27/2024	Kazutaka Nishio	In the primary endpoint section, clarified the handling of antiarrhythmic drugs