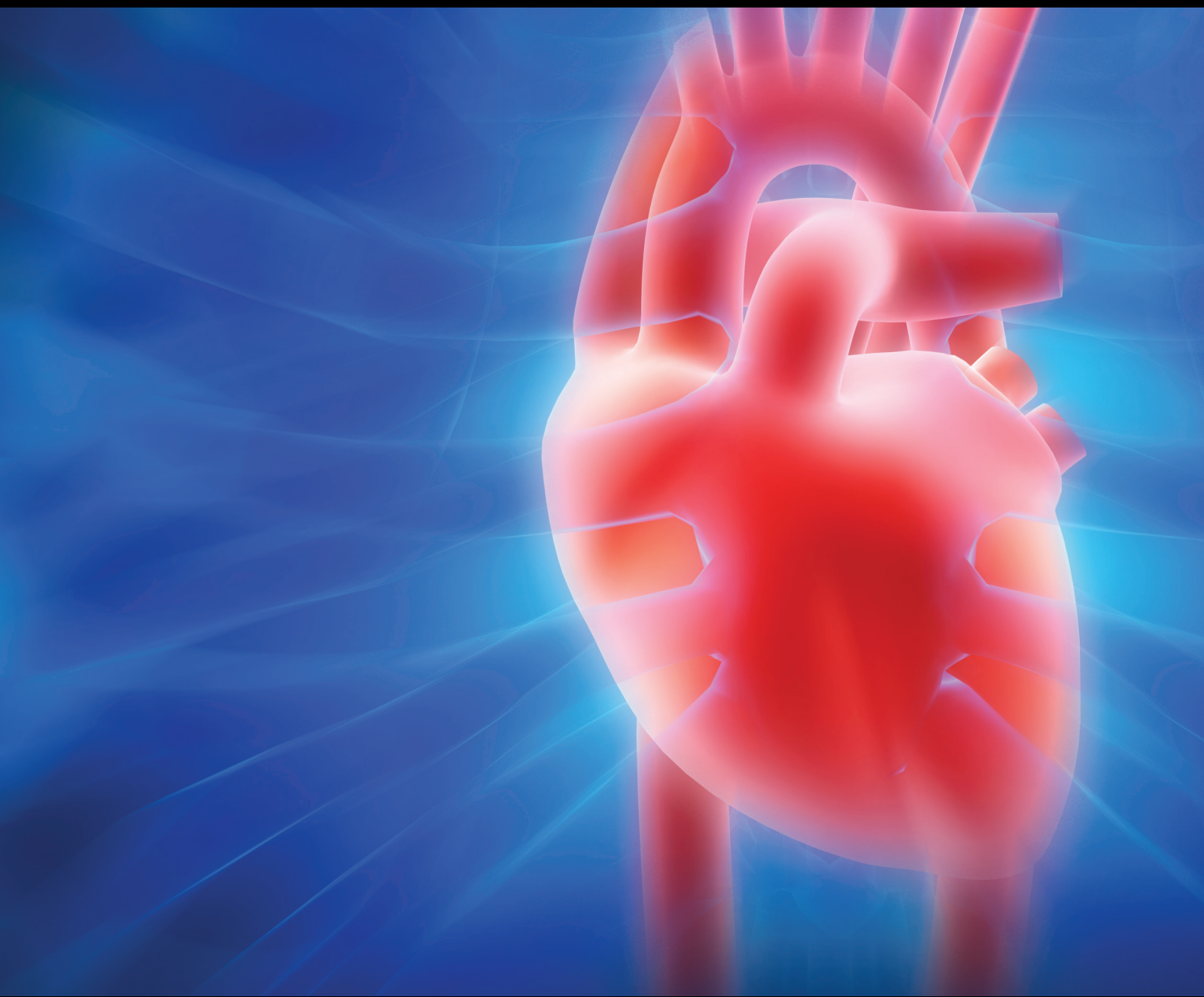
Cardiology Research and Practice

Atrial Fibrillation: Mechanisms and Management

Lead Guest Editor: Tong Liu
Guest Editors: Gary Tse, LiLei Yu, Panagiotis Korantzopoulos, and Konstantinos P. Letsas



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Editorial Board

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Editorial

Atrial Fibrillation: Mechanisms and Management

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Atrial fibrillation (AF) is the most common cardiac arrhythmia encountered in clinical practice, contributing to significantly mortality, and morbidity through the development of stroke, heart failure, and dementia. It has a prevalence of around 2% in the general population globally, but this is expected to increase exponentially in part owing to an ageing population. The current treatment modalities include pharmacological therapy and interventional procedures such as catheter ablation. However, both modalities have their shortfalls, which may be due to the fact that their pathophysiological mechanisms are incompletely elucidated. In this special issue of *Cardiology Research and Practice*, we have selected the latest original and review articles on the mechanisms and management of AF for our readers.

Regarding the risk factors and epidemiology of AF, this has been the topic of intense investigations. In addition to traditional risk factors such as diabetes mellitus, the relationship of malignancy and AF is complex. AF development is dependent on not only the cancer type, burden, and metabolic links but also different cancer therapies. The Xia group has conducted a detailed systematic review and meta-analysis, demonstrating that the risk of AF to be highest within 3 months of a cancer diagnosis. This has important implications for the area of cardio-oncology practice, where cancer patients may benefit from early intensive monitoring for the presence of AF and treatment for stroke prevention. The Fedele group provided a review on the possible pathophysiological mechanisms through which anxiety disorders can promote the onset, progression, and maintenance of AF. This highlights the importance of emotional stress as the source of triggers for this arrhythmia.

For novel biomarkers, the Zhang group conducted a prospective cohort study of patients with persistent AF and identified anti-M2-muscarinic acetylcholine receptor antibodies to be a biomarker for atrial fibrosis severity in the left atrial appendage, and this was associated with upregulation of transforming growth factor-β1 and connective tissue growth factor, which may represent future pharmacological targets. Novel biomarkers may not be readily available in all centers, and thus there has been investigation into the roles of routine markers from routine blood tests for risk stratification. In this issue, the Su group examined one of these markers, red cell distribution width, and found it to be an independent risk factor of AF and affected by the type of AF and altitude.

In terms of advances in pharmacological therapy for AF, the Liu group conducted a randomized controlled trial to investigate whether the traditional Chinese medicine, Wenxin Keli, provided additional benefits when

added to usual amiodarone treatment. They found that the use of Wenxin Keli was safe and led to reduction in conversion time to sinus rhythm in patients with recent onset AF.

Catheter ablation is an invasive strategy used for the treatment of AF. Although it can be effective, recurrence can occur in a subset of patients, which can contribute to the costs and morbidity through the need of repeated procedures and the development of procedural complications. Therefore, there is a need to improve long-term success by identifying technical and mechanistic factors that can be used by clinicians to reduce arrhythmia recurrence. The Zhou and Ding group has conducted a pilot study to investigate the clinical utility of an automated lesion tagging module based on catheter stability information, VisiTag, with the CARTO system. They found the optimal predefined criteria (OPC) to be 3 mm distance limit for at least 20 seconds and Force Over Time of 5g in pulmonary vein isolation procedures. The Wang group has identified concealed bigeminy arising from the pulmonary veins during sinus rhythm as a marker of pulmonary vein firing. This can guide interventionalists to induce a greater number of radiofrequency ablations to improve isolation. Previously, pharmacological challenge tests such as adenosine testing to unmask dormant conduction failed to demonstrate a significant benefit on long-term AF recurrence rates. Concealed pulmonary vein potentials may be unmasked electrically by left atrial stimulation. Nevertheless, the Liu group explored the use of low-dose ibutilide, a Class III anti-arrhythmic agent, during catheter ablation in persistent AF patients and found that it can be used for substrate localization and possibly reduction of ablation of unnecessary sites. To prevent stroke, left atrial appendage occlusion is a well-recognized treatment and is typically guided by a combination of transoesophageal echocardiographic imaging and fluoroscopy. The Zhang group investigated a new method of left atrial appendage occlusion using transoesophageal echocardiographic imaging alone without fluoroscopic guidance. In their article, they reported the feasibility and safety of this novel approach, with implications that the procedure can be simplified and radiation risk can be reduced to healthcare workers and patients underlying this procedure. Finally, the Mei group investigated the use of catheter-based renal denervation procedures to reduce cardiac sympathetic nerve activity, hypothesizing that this can inhibit AF in a canine model. They found that renal artery adventitial cryoablation could create transmural effects by applying lesions from outside of the renal artery, leading to neural modelling, as well as decreases in AF incidence and duration. Using a rabbit model, the Huang group investigated the pathophysiology of exercise training-induced AF and identified downregulation of two repolarizing currents, I_{K1} and I_{KACh}, as the underlying atrial remodelling mechanisms.

In conclusion, this special issue published clinical and experimental studies on recent advances regarding the epidemiology, mechanisms and pathophysiology, biomarkers, and pharmacological and nonpharmacological management of AF.

Conflicts of Interest

The editors declare that they have no conflicts of interest.

Authors' Contributions

Tong Liu and Gary Tse contributed equally to this work.

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Lilei Yu
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Review Article

Association of Cancer and the Risk of Developing Atrial Fibrillation: A Systematic Review and Meta-Analysis

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Aims. Previous studies have demonstrated epidemiological evidence for an association between cancer and the development of new-onset atrial fibrillation (AF). However, these results have been conflicting. This systematic review and meta-analysis was conducted to examine the relationship between cancer and the risk of developing atrial fibrillation. *Methods*. PubMed and Web of Science were searched for publications examining the association between cancer and atrial fibrillation risk published until June 2017. Adjusted odds ratios (ORs) or hazard ratios (HRs) and 95% CI were extracted and pooled. *Results*. A total of five studies involving 5,889,234 subjects were included in this meta-analysis. Solid cancer patients are at higher risk developing atrial fibrillation compared to noncancer patients (OR 1.47, 95% CI 1.31 to 1.66, $p < 0.00001$; $I^2 = 67\%$, $p = 0.02$). The risk of atrial fibrillation was highest within 90 days of cancer diagnosis (OR 7.62, 95% CI 3.08 to 18.88, $p < 0.00001$) and this risk diminished with time. *Conclusions*. The risk of AF was highest within 90 days of cancer diagnosis. We should take into account the increased risk of atrial fibrillation development and, after this, study the embolic risk and potential indication of oral anticoagulation.

1. Introduction

Atrial fibrillation (AF) is the commonest cardiac arrhythmia observed in clinical practice and is associated with significant morbidity and mortality globally. Its prevalence is increasing in part due to an aging population [1], from ~0.5% in those aged 40 years to 6–12% in those aged 85 years [2]. Apart from advancing age, independent predictors of AF include hypertension, obesity, diabetes, and smoking [1, 2]. Moreover, cancer remains the second most common cause of death in the United States. Several risk factors are known to contribute to both AF and cancer [3]. Thus, mechanistic studies have demonstrated a critical role of proinflammatory states in cancer [4]. A proinflammatory environment has also been linked to AF, as reflected by increases in serum inflammatory biomarkers such as C-reactive protein (CRP),

interleukin- (IL-) 2, IL-6, and IL-8 [5]. However, evidence from epidemiological studies has been controversial [6–10]. Therefore, this systematic review and meta-analysis was conducted to examine the relationship between cancer and the risk of developing AF.

2. Methods

This meta-analysis of observational studies was in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement [11].

2.1. Search Strategies. Two researchers (M. Y. and X. F.) systematically and independently searched the relevant studies from the following databases: PubMed (until June 2017) and Web of Science (from 1986 through June 2017). We used the following key words: "cancer," "carcinoma," "tumor," and "atrial fibrillation." The reference lists of the articles, conferences, and editorials were used to identify further relevant studies.

2.2. Inclusion Criteria. All observational studies reported on the epidemiological evidence for an association between solid cancer and AF were included in this meta-analysis. Because the aim of this meta-analysis is to investigate whether cancer patients are at an increased risk of developing AF in general population, study population that involved the patients who underwent surgery and chemotherapy were excluded. The inclusion criteria were articles (1) published in English language, (2) on human subjects, (3) that were case control, prospective or retrospective cohort study, (4) reporting the odds ratios (ORs) or hazard ratios (HRs) and the corresponding 95% confidence intervals (CIs) or data required for their calculations were provided, (5) assessing the association between AF and cancer, not survival of cancer or complication of AF. Where two articles with overlapping data were included, the articles with higher subjects were included.

2.3. Study Selection. Two researchers (M. Y. and X. F.) independently screened the titles and abstracts of the studies. Potential eligible studies were retrieved using the relevant inclusion criteria mentioned previously. Any disagreements or indeterminations between the two researchers were resolved through discussion or consultation with a third researcher (T. L.).

2.4. Data Extraction and Quality Assessment. Hazard ratio (HRs), odds ratio (ORs), and their 95% confidence intervals (CIs) for the association between cancer and AF were extracted from individual articles. If both unadjusted and adjusted ORs/HRs were reported, the adjusted ORs/HRs were preferentially used. And we priority to extracted multivariate adjusted ORs/HRs, not age- or gender-adjusted, to evaluate the risk of AF occurrence. The extracted data of each study included first author's last name, year of publication, geographic location of study, study design, total number of subjects, participants' age and sex, types of cancer, criterion for AF confirmation, follow-up duration, maximum adjusted covariates, HR or OR with 95% CI, and patients with heart failure, hypertension, and diabetic mellitus.

The Newcastle-Ottawa Scale (NOS) items, with total score of nine stars, were used to evaluate the quality of cohort or case control studies [12]. We defined the cohort or case control studies with NOS score ≥ 7 stars as high quality and NOS score <7 stars as low quality.

2.5. Statistical Analysis. The ORs with 95% CIs were used as the common risk estimates and then were pooled. The HR value using multivariate Cox proportional hazards model in the original research was directly considered as OR. Percent variability across studies attributable to heterogeneity beyond sampling error was evaluated using the I^2 statistic, and I^2 value of >50% represented moderate to high heterogeneity. The random effects model was used as it is better to explain heterogeneity between studies over the fixed-effects model. Subgroup analyses and sensitivity analysis were performed to identify the sources of heterogeneity. Subgroup analyses regarding study design (case control studies or cohort studies), study location (Europe and USA), and the methods for AF diagnosis (electrocardiogram and International Classification of Disease code) and time interval between cancer diagnosis and AF were performed. Sensitivity analysis was performed by omitting one study at a time to evaluate the influence of individual studies on the pooled results. The funnel plot was constructed to identify possible publication bias. P values of < 0.05 (two-tailed) were considered statistically significant. Statistical analyses were performed using the Review Manager (RevMan) software (Nordic Cochrane Center; http://ims.cochrane.org/revman, version 5.3).

3. Results

A flow diagram of the data search and study selection is shown in Figure 1. Initially, 6311 records were identified from the PubMed and Web of Science databases. Of these, 2796 were duplicate studies and were excluded. The remaining articles were screened, and 3487 were subsequently excluded because they were review articles, animal studies, or irrelevant to this analysis. The 28 remaining studies were then reviewed in detail, and 23 of the 28 were excluded: study published in Italian, Russian, or Spanish language ($n = 5$) [13–16]; study reported AF leading to cancer ($n = 4$) [17–20]; individual case reports ($n = 3$) [21–23]; letters [24, 25] or editorials [26] ($n = 3$); different article from the same center [27, 28], and a more recent series was available [8], patients in this study [29] may be repeated in the study by Conen [10] ($n = 3$); study evaluated the relation of AF and survival in cancer patients ($n = 2$) [30, 31]; OR was not provided ($n = 1$) [32]; cross-sectional study ($n = 1$) [33]; and study investigated AF as a complication in cancer patients ($n = 1$) [34]. Therefore, a total of

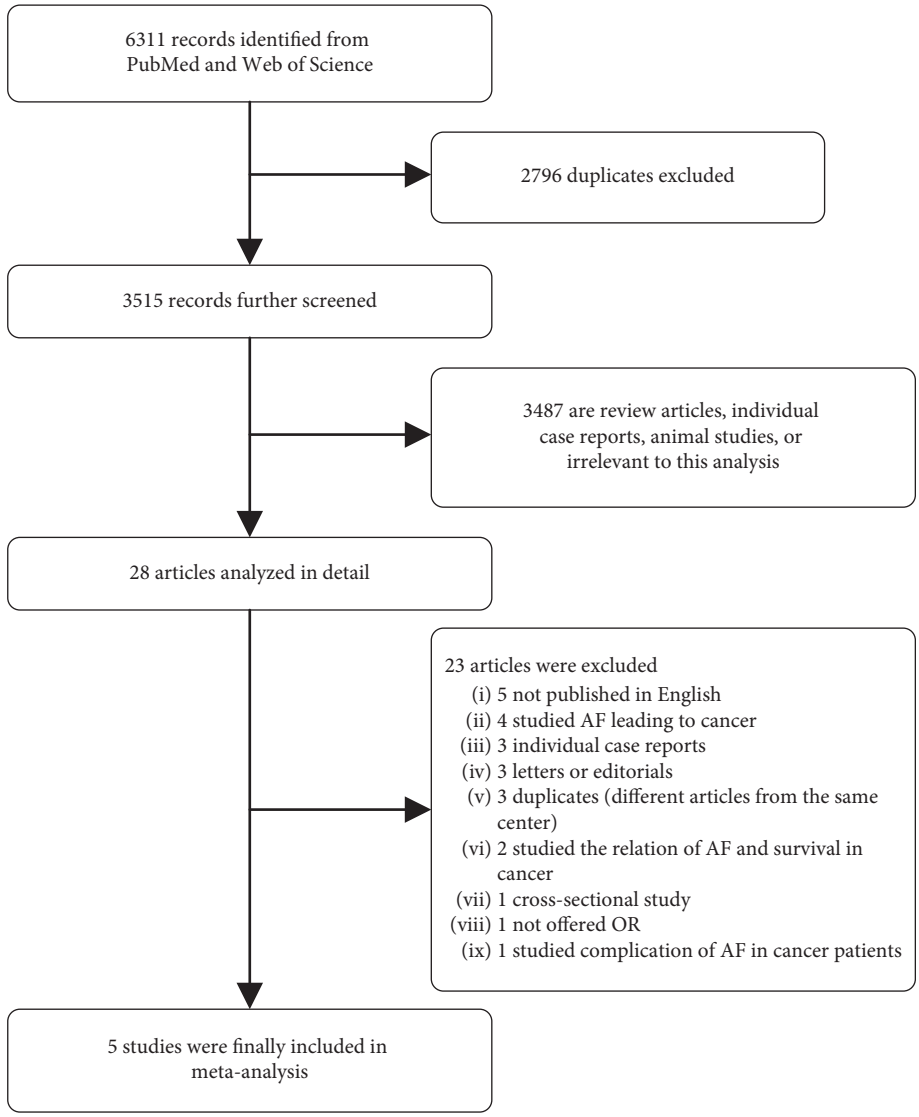

Figure 1: Flow diagram of the study selection process. AF = atrial fibrillation; OR = odds ratio.

five studies comprising 5,889,234 participants were included in our meta-analysis.

The characteristics of the included studies and their quality scores are shown in Table 1. Two were case control studies [7, 8], and three were cohort studies [6, 9, 10]. Two studies [6, 7] investigated only the association between AF and colorectal cancer, whereas three studies [8–10] examined colorectal cancer and also cancer of the breast, lung, and prostate. For the included cohort, the mean age ranged from 53 to 75 years and the proportion of male patients accounted for all patients ranged from 0% to 60%. Characteristics of the patients are presented in Table 2. NOS analysis showed that all included studies were of high quality.

Apart from one study [6] reporting no significant association between cancer and AF, the remaining four studies [7–10] consistently demonstrated a significant association between cancer and new AF risk. Overall, the summary estimate from the five separate estimates from the cohort or case-control studies united indicated that patients with cancer had an approximately 47% higher risk of AF compared to noncancer patients (OR 1.47, 95% CI 1.31 to 1.66, $p < 0.00001$; Figure 2). There was a significant heterogeneity across the studies ($I^2 = 67\%$, $p = 0.02$).

Four of five studies reported OR of incidence of AF in colorectal cancer; the pooled effect sizes of these studies [6–9] (OR 1.54, 95% CI 1.40 to 1.71, $p < 0.00001$; heterogeneity: $I^2 = 48\%$, $p = 0.12$; Figure 3(a)) showed that patients with colorectal cancer at a 54% higher risk of developing AF than those without colorectal cancer. Two of five studies offered OR of incidence of AF in breast cancer; in the pooled analysis of these studies [8, 9] (OR 2.07, 95% CI 0.96 to 4.45, $p = 0.06$; heterogeneity: $I^2 = 81\%$, $p = 0.02$; Figure 3(b)), we observed a prevalence of AF close to two times higher in breast cancer patients compared to those without breast cancer.

Subgroup analyses were subsequently performed to identify potential sources of heterogeneity. The details are

Table 1: Characteristics of the five studies included in this meta-analysis.

First author and year	Location	Period of enrollment	Study design	Total patients, *N*	Cancer type	Incident cases of AF, *N* (%)	AF type	Criterion for AF diagnosis	Covariates in adjusted model	Follow-up (year)	Quality score
Guzzetti 2008 [8]	Italy	1987–2004	Case-control	1868	Colorectal and breast cancer	49 (2.6)	New-onset AF	Routine presurgery ECG	Age, sex	NA	8
Erichsen 2012 [7]	Denmark	1999–2006	Case-control	311593	Colorectal cancer	NA	AF/ Flutter	According to the ICD	Age, sex, country	NA	8
Jakobsen 2015 [9]	Denmark	2000–2012	Prospective cohort	5539824	All types of cancer	NA	New-onset AF	NA	Age, sex	12	7
Nouraie 2015 [6]	USA	2000–2012	Retrospective cohort	1258	Colorectal cancer	93 (7.4)	New-onset AF	According to the ICD	Age, HTN, HF, DM, alcohol, tobacco	NA	8
Conen 2016 [10]	USA	1993–2013	Prospective cohort	34691	All types of cancer	824 (2.3)	New-onset AF	Electrocardiographic AF documentation or a medical report that documented a diagnosis of AF	Age, EDU, race, height, BMI, HTN, DM, smoking, HC, alcohol, physical activity, CHF, MI, stroke	19.1	9

AF = atrial fibrillation; BMI = body mass index; CHF = congestive heart failure; CRP = C-reactive protein; DM = diabetes mellitus; ECG = electrocardiography; EDU = educational lever; HC = hypercholesterolemia; HDL-c = high-density lipoprotein cholesterol; HF = heart failure; HTN = hypertension; ICD = International Classification of Diseases; LVH = left ventricular hypertrophy; MI = myocardial infarction; NA = not applicable; SBP = systolic blood pressure; TC = total cholesterol.

Table 2: Patient characteristics of the five studies.

First author and year	AF in CA/NO-CA (%)	Mean age, CA/NO-CA (year)	Male sex, CA/NO-CA (%)	Diabetes, CA/NO-CA (%)	Hypertension, CA/NO-CA (%)	Smoking, CA/NO-CA (%)	Cardiovascular disease, CA/NO-CA (%)	BMI, CA/NO-CA	Race, CA/NO-CA (%)	Education, high school, CA/NO-CA (%)
Guzzetti 2008 [8]	3.6/1.6	63.1 ± 12.6/ 63.6 ± 9.3	25.7/ 53.0	NA	NA	NA	NA	NA	NA	NA
Erichsen 2012 [7]	NA	75 (70–79)/ 74 (70–79)	54.1/ 54.1	7.1/4.5	3.3/2.3	NA	37.7/21.7	NA	NA	NA
Jakobsen 2015 [9]	NA	66.5 (66.5–66.5)/ NA	47.4/ NA	NA	NA	NA	NA	NA	NA	NA
Nouraie 2015 [6]	10.1/ 5.4	67 (58–78)/ 56 (48–69)	47/51	33/29	59/50	27/31	14/13	NA	NA	NA
Conen 2016 [10]	3.8/2.3	55 (50–61)/ 53 (49–58)	0/0	3.2/2.6	30.0/25.8	53.3/47.5	NA	25.1 (22.6–28.9)/ 24.9 (22.5–28.3)	White: 96.5/ 94.9	43.4/44.4

CA = cancer group; NA = not applicable; NO-CA = noncancer group.

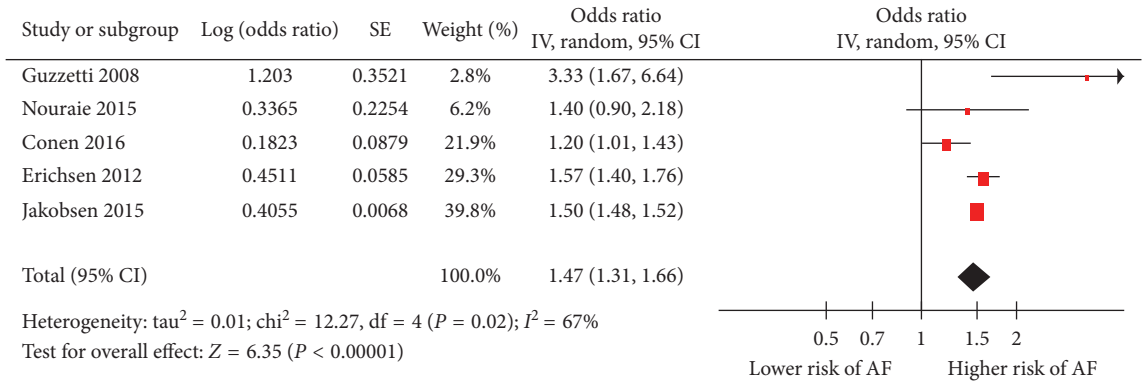

Figure 2: Forest plot for pooled odds ratio (OR) for the risk of atrial fibrillation (AF) in patients with any cancer. SE = standard error; IV = inverse variance.

shown in Table 3. Our pooled meta-analysis suggested that cancer is significantly associated with the occurrence of AF in both cohort [6, 9, 10] and case control [7, 8] studies with significant heterogeneity. As shown in Table 3, cancer was associated with an increased risk of AF in both studies originating from Europe [7–9] (OR 1.56, 95% CI 1.39 to 1.76, $p < 0.0001$, $I^2 = 65\%$) with significant heterogeneity as well as the United States [6, 10] (OR 1.22, 95% CI 1.04 to 1.44, $p = 0.01$, $I^2 = 0\%$) without heterogeneity.

Subgroup analysis was also performed for the method of AF diagnosis. Meta-analysis of two studies using electrocardiography [8, 10] reported a higher risk of AF in cancer patients (OR 1.89, 95% CI 0.70 to 5.10, $p = 21$, $I^2 = 87\%$) with significant heterogeneity. Meta-analysis of two studies that had used the International Classification of Diseases (ICD) [6, 7] also demonstrated elevated risk of AF (OR 1.56, 95% CI 1.39–1.74, $p < 0.0001$, $I^2 = 0\%$), and this was associated with minimal heterogeneity. Therefore, study location [6–10] and the method for diagnosing AF [6–8, 10] are both likely the origin of the heterogeneity in our main meta-analysis.

To examine temporal relationship between cancer and AF, ORs from two studies were further pooled [7, 10] for AF according to the time since cancer was first diagnosed, classified by time interval less than 90 days, 91 to 365 days, or more than 365 days. We found significantly increased risk of AF in cancer patients diagnosed less than 90 days (OR 7.62, 95% CI 3.08 to 18.88, $p < 0.0001$, $I^2 = 91\%$). Otherwise, pooled OR of incidence of AF was not significantly increased for longer time-points of 91 to 365 days (OR 1.06, 95% CI 0.90 to 1.25, $p = 0.46$, $I^2 = 0\%$) or beyond 365 days (OR 0.97, 95% CI 0.71 to 1.34, $p = 0.87$, $I^2 = 84\%$) (Table 3). Finally, sensitivity analysis by excluding one study at a time did not significantly alter the pooled OR. The results of the funnel plot for the association between cancer and AF was asymmetry, indicating that publication bias may be present, although the small number of studies made this somewhat difficult to interpret (Figure 4).

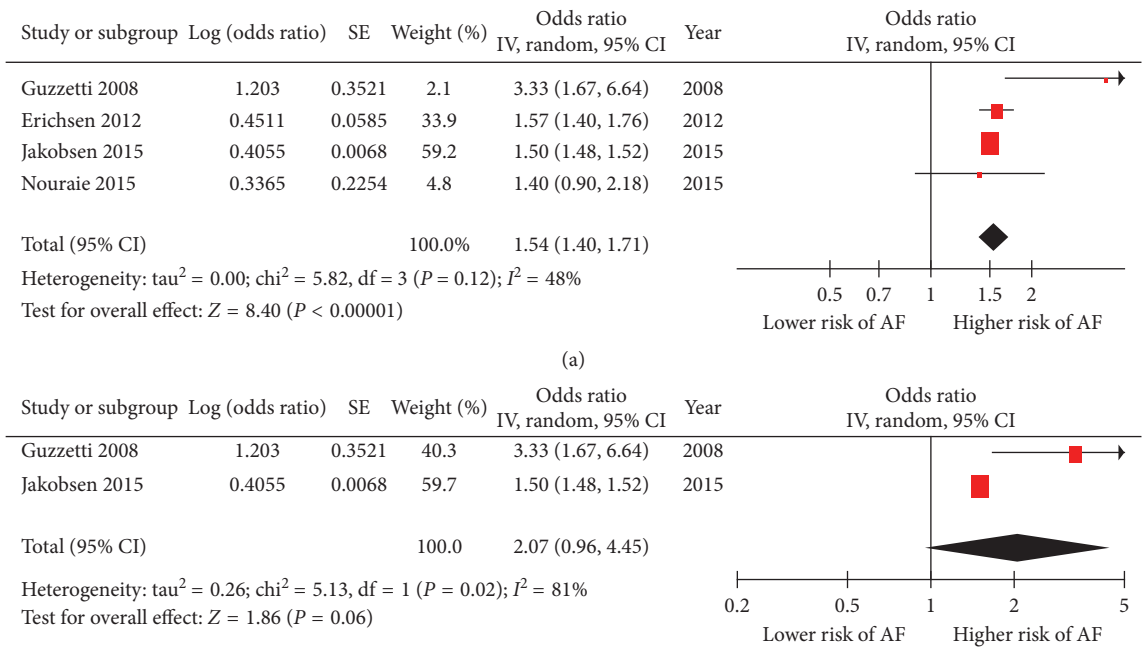

Figure 3: Outcomes for the risk of atrial fibrillation (AF) in patients with different cancer. (a) Forest plot of the odds ratio (OR) for pooled risk of atrial fibrillation (AF) in patients with colorectal cancer; (b) forest plot of the odds ratio (OR) for pooled risk of atrial fibrillation in patients with breast cancer. SE = standard error; IV = inverse variance.

Table 3: Subgroup analysis of the association between cancer and atrial fibrillation.

	Subgroup	Number of studies	Meta-analysis			Heterogeneity	
			OR	95% CI	p value	I^2 (%)	p value
Study design	Case control	2	2.11	1.03–4.34	0.04	77	0.04
	Cohort	3	1.38	1.15–1.64	0.0004	69	0.04
Study location	Europe	3	1.56	1.39–1.76	<0.0001	65	0.06
	USA	2	1.22	1.04–1.44	0.01	0	0.52
Criterion for AF diagnosis	ECG	2	1.89	0.70–5.10	0.21	87	0.005
	ICD	2	1.56	1.39–1.74	<0.0001	0	0.62
Time interval between cancer diagnosis and AF	≤90 days	2	7.62	3.08–18.88	<0.0001	91	0.0009
	91–365 days	2	1.06	0.90–1.25	0.46	0	0.48
	>365 days	2	0.97	0.71–1.34	0.87	84	0.01

AF = atrial fibrillation; CI = confidence interval; ECG = electrocardiogram; ICD = International Classification of Disease; OR = odds ratio.

4. Discussion

Our systematic review and meta-analysis of five published observational studies suggests that subjects with newly diagnosed cancer had a significantly increased risk of AF during subsequent follow-up. There was significant heterogeneity observed between the included studies which were likely due to different methods of AF diagnosis. Interestingly, the increased risk of AF was only observed within the first 90 days after cancer diagnosis, and the risk was not significant after 1 year. Our study found substantial statistical heterogeneity in the pooled effect estimates. This is partly explicable by the use of different methods to detect AF in the individual studies. There are some data to support an increased risk of stroke after a cancer diagnosis, and one could hypothesize that this relationship between cancer and AF could account for part of the elevated risk of stroke if the AF went undetected.

Oncocardiology is a new field of clinical medicine that addresses the close link between cancer and cardiovascular diseases [35, 36]. Recent evidence showed that cancer is closely related to the development of AF. A number of pathophysiological mechanisms, such as inflammation and autonomic dysfunction, have been proposed to explain this link [5, 37]. Firstly, clinical studies have demonstrated elevations in proinflammatory markers in both AF and cancer. A case-control study showed that patients with atrial arrhythmia compared to those without atrial arrhythmia had higher levels of the inflammatory marker, C-reactive protein (CRP). Indeed, CRP levels were higher in persistent than paroxysmal AF patients [38]. Another large population-based cohort study reported that CRP was independently

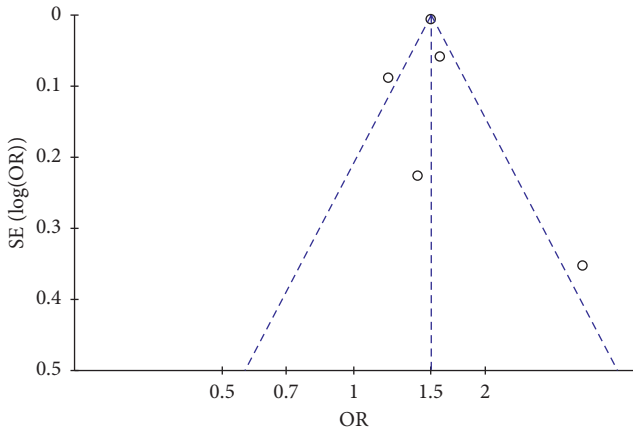

Figure 4: Funnel plot for the association between cancer and atrial fibrillation occurrence. SE = standard error; OR = odds ratio.

associated with the presence of AF and also predicted patients who were at increased risk of subsequently developing AF [39]. Marcus et al. reported that CRP levels in patients with atrial flutter that were initially elevated fell after successful ablation [40]. These findings are consistent with notion that inflammation plays a key role in structural and electrophysiological modeling that underlies arrhythmic substrates [41]. Similarly, cancer is associated with a proinflammatory state [42]. Thus, CRP levels were significantly elevated in colorectal [43] and breast [44] cancer patients compared to controls. However, in the included studies, data on cancer staging were not available, which could alter the degree of inflammation and the risk of AF.

The second factor is dysfunction of the autonomic nervous system. Altered balance of sympathetic versus parasympathetic activity has been associated with AF [45], and patients with cancer also show some evidence of altered autonomic activity or dysfunction [46]. Pain and emotional or physical stress in cancer may increase sympathetic nervous activity and predispose to atrial fibrillation [47]. Therefore, the patients who diagnosed with cancer underwent mental stress and sympathetic challenge during the first 90 days of receiving such anxiety provoking news which may have predisposed the patients to triggers of AF. Besides, Faber et al. reported that subclinical thyroid diseases seemed to change the structure and function of heart with subsequent alters in morbidity and mortality [48]. And the outcome of another analysis demonstrates that patients with clinical or subclinical hyperthyroidism are at increased risk of AF [49]. A hypothesis concerns that tumors may release thyroid hormones such as thyroid-stimulating hormone (TSH) and triiodothyronine (T3) [8]. Thus, it is possible that cancer enhances the incidence of AF through the abnormal production of thyroid hormones-like peptides [50]. These findings suggest altered autonomic activity in occult cancer, suggesting that undiagnosed cancer may precede AF. Finally, the epidemiological link between AF and cancer may be due to shared risk factors, diagnostic bias, undiagnosed occult cancer leading to AF, and anticoagulation unmasking cancer.

There are several potential limitations of our meta-analysis. Firstly, the data on the epidemiological evidence for the relationship between new-onset cancer and the risk of AF are sparse, and only a few studies have addressed this issue. And given that the total number of studies was small, we included a letter [28] and an abstract of conference [9] to maximize the use of available data. Secondly, some studies only presented the sex- or age-adjusted ORs/HRs, so not all ORs/HRs used in this analysis were extracted from multivariate analysis. This may have introduced a degree of random error in our pooled analysis. Finally, the epidemiological evidence for an association between cancer and risk of AF is mainly investigated in this study, and we will perform further study to uncover the potential relationship between surgery or chemotherapy and development of atrial fibrillation.

In summary, this meta-analysis demonstrates that cancer is associated with atrial fibrillation that was significant within the first three-month period. These findings would prompt us to suggest that AF patients should be screened for occult cancer. Future studies are needed to examine the potential mechanisms linking cancer to AF, and further analyses that can examine the association between AF and subsequent cancer-related mortality may exclude to possibility of diagnostic bias.

Disclosure

An earlier version of the manuscript has been presented as conference abstract in Global Cardio-Oncology Summit 2017, available in the following link: http://cardiaconcology.ca/wp-content/uploads/GCOS2017_029_YXia_Abstract.pdf.

Conflicts of Interest

The authors declare no conflicts of interest.

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Research Article

Red Cell Distribution Width as a Novel Marker for Different Types of Atrial Fibrillation in Low and High Altitude

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Background. Increased red cell distribution width (RDW) can predict the incidence and mortality of cardiovascular diseases. However, there are limited data on the relationship between RDW and altitude and the subtype of atrial fibrillation (AF). We investigated the effects of altitude on RDW in patients with different types of AF. *Methods*. A total of 303 patients with nonvalvular AF were included. Of these, 156 lived in low altitude (77 paroxysmal AF, PAF; 79 persistent AF, PeAF) and 147 in high altitude (77 paroxysmal AF, PAF; 70 persistent AF, PeAF). In these groups, baseline characteristics, complete blood counts, serum biochemistry, and echocardiography were evaluated. Multivariate logistic regression analysis was conducted to determine the independent predictors of AF at the different altitudes. *Results*. In both low and high altitudes, RDW and left atrial diameter (LAD) were higher in AF than control subjects ($P < 0.05$) and higher in persistent AF than paroxysmal AF ($P < 0.05$). Compared with any groups (PAF group, PeAF group, or control group) of low-altitude, RDW and LAD were found higher in high-altitude corresponding groups. Multivariate logistic regression analysis demonstrated that RDW, mean corpuscular volume (MCV), and LAD levels independently associated with AF patients in low altitude (RDW, OR 1.687, 95% CI 1.021–2.789; $P < 0.05$), while in high altitude, RDW, MCV, creatinine (Cr), and LAD were independent predictors for AF patients (RDW, OR 1.755, 95% CI 1.179–2.613; $P < 0.05$). *Conclusion*. Elevated RDW levels may be an independent risk marker for nonvalvular AF, affected by type of AF and altitude.

1. Introduction

Red blood cell distribution width (RDW) is a parameter of anisocytosis or heterogeneity in the volume of circulating erythrocytes and is traditionally used in laboratory hematology for differential diagnosis of anemias, which is easily available from a standard complete blood cell count (CBC) [1, 2]. Some recent studies have shown that higher RDW level can predict morbidity and mortality of cardiovascular disease, for example, myocardial infarction, heart failure, and atrial fibrillation [3–6]. Atrial fibrillation (AF) is one of the most common arrhythmias worldwide, seriously threatens people's quality of life, and increases the risk of stroke, heart failure, and death. The incidence and prevalence of AF is significantly increased in China [7, 8]. The specific mechanism between the elevated RDW and AF is unclear. Recent studies indicate that inflammatory reaction and oxidative stress play an important role in the connection of RDW and AF [9–11].

High-altitude exposure is known for its strong ultraviolet light and low oxygen pressure. Among them, hypoxia is closely related to the occurrence and development of cardiovascular diseases, mainly manifested by changes in the structure and function of the cardiovascular system, thereby aggravating the occurrence of cardiovascular diseases [12–14]. Some studies shown that the mechanisms of cardiovascular disease associated with altitude include mainly sympathetic activation, inflammatory reaction, and oxidative stress [15–17].

However, there were no data on the association of RDW with different types of nonvalvular AF at different altitude areas. We aimed to investigate the role of RDW as

Table 1: Baseline characteristics in all subjects at low altitude (3.5 m).

	AF (n = 156)	Control (n = 72)	χ^2/T	P
Male (n %)	77.00 (49.40)	31.00 (43.10)	0.785	0.376
Age (years)	67.92 ± 10.80	66.26 ± 10.54	0.025	0.280
Body mass index (kg/m^2)	25.42 ± 3.93	25.26 ± 2.83	6.75	0.717
Smoking (n %)	22.00 (14.10)	5.00 (6.90)	2.418	0.120
Diabetes mellitus (n %)	31.00 (19.90)	10.00 (13.90)	1.196	0.274
Hypertension (n %)	97.00 (62.20)	48.00 (66.70)	0.428	0.513
Previous stroke (n %)	26.00 (16.70)	11.00 (15.30)	0.070	0.791
WBC ($\times 10^9$/L)	6.86 ± 1.85	6.68 ± 1.74	1.422	0.484
RBC ($\times 10^{12}$/L)	4.45 ± 0.55	4.52 ± 0.44	2.976	0.400
Hb (g/L)	139.37 ± 18.59	140.67 ± 15.98	1.008	0.610
RDW (%CV)	12.85 ± 0.78	12.59 ± 0.58	2.844	0.013
HCT (%)	41.26 ± 5.25	41.22 ± 4.48	2.26	0.954
MCV (fL)	92.81 ± 5.57	90.24 ± 9.69	0.019	0.012
MCHC (g/L)	337.67 ± 10.87	341.13 ± 8.90	2.972	0.019
Plt ($\times 10^9$/L)	219.06 ± 58.22	222.13 ± 46.30	1.581	0.695
MPV (fL)	9.48 ± 1.00	9.33 ± 0.99	0.116	0.283
Cr (μmol/L)	70.29 ± 27.62	66.29 ± 17.65	1.606	0.261
UA (mmol/L)	325.35 ± 95.49	336.63 ± 87.22	0.670	0.395
TBIL (μmol/L)	14.64 ± 6.16	12.30 ± 4.18	4.203	0.001
LAD (mm)	40.45 ± 7.54	33.78 ± 4.63	14.865	0.001
LVEDD (mm)	48.34 ± 6.33	47.13 ± 5.37	1.693	0.163
IVST (mm)	9.52 ± 2.32	9.06 ± 1.87	0.461	0.148
LVPWT (mm)	9.21 ± 1.90	9.06 ± 3.09	1.503	0.636
LVEF (%)	56.70 ± 9.64	60.76 ± 5.54	7.489	0.001
Aspirin (n %)	105.00 (67.30)	57.00 (79.20)	3.368	0.066
ACEIs or ARBs (n %)	49.00 (31.40)	19.00 (26.40)	0.593	0.441
β-blocker (n %)	67.00 (42.90)	28.00 (38.90)	0.334	0.563
CCBs (n %)	51.00 (32.70)	22.00 (30.60)	0.103	0.748
Statins (n %)	71.00 (45.50)	38.00 (52.80)	1.042	0.307

AF, atrial fibrillation; BMI, body mass index; WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; RDW, red cell distribution width; HCT, hematocrit; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; Plt, platelet count; MPV, mean platelet volume; Cr, creatinine; UA, uric acid; TBIL, total bilirubin; LAD, left atrial diameter; LVEDD, left ventricular end-diastolic diameter; IVST, interventricular septal thickness; LVPWT, left ventricular posterior wall thickness; LVEF, left ventricular ejection fraction.

a novel marker for different types of AF at different altitude in China.

2. Methods

2.1. Study Population. Our study included 303 patients with nonvalvular AF in total, 156 in low altitude (77 paroxysmal AF, PAF; 79 persistent AF, PeAF), 147 in high altitude (77 PAF; 70 PeAF), respectively, and 167 patients without AF (72 in low altitude, 95 in high altitude) matched for sex, age, atherosclerotic risk factors, and history of medicine who were admitted in cardiology department from March 2016 to March 2018 in Second Hospital of Tianjin Medical University and Qinghai Provincial People's Hospital from two cities (Tianjin and Xining), respectively. Tianjin is located at 3.5 m. Xining is located at 2260 m above the sea level. AF was defined as absence of P waves and irregular R-R interval in a 12-lead electrocardiogram (ECG) or 24 h Holter recording. The different types of AF were defined according to the ESC guidelines for the management of atrial fibrillation [18]. Each subject had at least one ECG showing AF. Exclusion criteria were congenital heart disease, coronary artery disease, cardiomyopathy, concomitant valvular heart disease, previous cardiac surgery, renal insufficiency, thyroid dysfunction, acute or chronic inflammatory disease, hematological diseases, or unavailable medical records. Moreover, patients who had a recent 3-month history of blood transfusion were also excluded.

2.2. Study Protocol. All baseline demographic, clinical characteristics and laboratory examinations including CBC and transthoracic echocardiography were carefully recorded. CBC testing utilized clinical laboratory methods (Coulter BC-5380/6800 Hematology Analyzer, Mindray, Shenzhen, China) for white blood cell (WBC) count, red blood cell (RBC), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelet count (Plt), mean platelet volume (MPV), hemoglobin (Hb) levels, and RDW. Serum creatinine (Cr), uric acid (UA), and total bilirubin levels (TBIL) were measured by using an automatic blood biochemical analyzer (TBA-120 FR analyzer Toshiba, Japan). Both laboratory and biochemical tests were obtained after the second sky-abdomen examination after admission. Left atrial diameter (LAD), interventricular septal thickness (IVST), left ventricular posterior wall thickness (LVPWT), left ventricular end-diastolic diameter (LVEDD), and left

Table 2: Baseline characteristics in all subjects at high altitude (2260 m).

	AF (n = 147)	Control (n = 95)	χ^2/T	P
Male (n %)	85.00 (57.80)	46.00 (48.40)	2.055	0.152
Age (years)	68.16 ± 9.58	69.28 ± 8.99	1.359	0.363
Body mass index (kg/m$^{2)}$	25.55 ± 3.43	25.97 ± 2.64	4.467	0.290
Smoking (n %)	25.00 (17.00)	11.00 (11.60)	1.343	0.247
Diabetes mellitus (n %)	30.00 (20.40)	14.00 (14.70)	1.248	0.264
Hypertension (n %)	104.00 (70.70)	64.00 (67.40)	0.311	0.577
Previous stroke (n %)	32.00 (21.80)	15.00 (15.80)	1.318	0.251
WBC ($\times 10^9$/L)	5.63 ± 1.56	5.72 ± 1.63	0.004	0.668
RBC ($\times 10^{12}$/L)	4.91 ± 0.68	4.92 ± 0.57	2.798	0.824
Hb (g/L)	152.66 ± 19.07	150.98 ± 17.28	1.245	0.488
RDW (% CV)	13.83 ± 1.12	13.10 ± 0.85	5.765	0.001
HCT (%)	46.26 ± 5.64	45.82 ± 5.01	1.726	0.538
MCV (fL)	94.56 ± 4.93	92.52 ± 9.75	0.110	0.032
MCHC (g/L)	330.24 ± 9.84	330.21 ± 9.44	0.038	0.983
Plt ($\times 10^9$/L)	154.60 ± 52.49	180.98 ± 47.62	0.342	0.001
MPV (fL)	12.16 ± 1.52	11.96 ± 1.26	0.041	0.302
Cr (μmol/L)	80.15 ± 20.68	63.47 ± 14.12	10.889	0.001
UA (mmol/L)	374.16 ± 114.71	331.38 ± 83.48	7.007	0.001
TBIL (μmol/L)	18.00 ± 8.00	16.93 ± 8.09	0.115	0.316
LAD (mm)	41.58 ± 6.56	34.21 ± 4.14	15.665	0.001
LVEDD (mm)	46.23 ± 4.73	46.01 ± 3.78	4.54	0.689
IVST (mm)	10.44 ± 1.49	9.88 ± 1.30	3.288	0.004
LVPWT (mm)	10.18 ± 1.30	9.66 ± 1.17	0.306	0.002
LVEF (%)	64.70 ± 6.89	64.57 ± 5.70	3.524	0.876
Aspirin (n %)	107.00 (72.80)	59.00 (62.10)	3.058	0.080
ACEIs or ARBs (n %)	40.00 (27.20)	16.00 (16.80)	3.488	0.062
β-blocker (n %)	55.00 (37.40)	25.00 (26.30)	3.212	0.073
CCBs (n %)	49.00 (33.30)	30.00 (31.60)	0.081	0.776
Statins (n %)	65.00 (44.20)	39.00 (41.10)	0.236	0.627

AF, atrial fibrillation; BMI, body mass index; WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; RDW, red cell distribution width; HCT, hematocrit; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; Plt, platelet count; MPV, mean platelet volume; Cr, creatinine; UA, uric acid; TBIL, total bilirubin; LAD, left atrial diameter; LVEDD, left ventricular end-diastolic diameter; IVST, interventricular septal thickness; LVPWT, left ventricular posterior wall thickness; LVEF, left ventricular ejection fraction.

ventricular ejection fraction (LVEF) were evaluated by a transthoracic echocardiographic examination (the Vivid-7 system USA).

2.3. Statistical Analysis. Categorical variables were reported as counts (percentage) and continuous variables as means ± standard deviation. Comparison between groups were used the Student's t-test or ANOVA with Tukey's post hoc test for continuous variables (as appropriate) while categorical variables were tested by Chi square. Multivariate logistic regression analysis was performed to identify the independent predictors of AF. The regression coefficients of the confounders included in the multivariable analysis were used to generate a nomogram for calculating the patient-specific probabilities of the occurrence of AF. Only P values < 0.05 were regarded as statistically significant. The statistical studies were carried out using Statistical Package for Social Sciences software (SPSS 24.0 for Windows, SPSS Inc., Chicago, IL, USA) and R 3.4.3 (The R Foundation, Vienna, Austria).

3. Results

The mean age of the included patients was 68 ± 10 years old, and 49.1% were male. There was no significant difference in age, gender, the presence of diabetes mellitus, hypertension, stroke, and smoking between the groups. Both low and high altitude, the levels of RDW and LAD in patients with AF group were higher than those in the control group ($P < 0.05$) in Tables 1 and 2, and the PeAF group was higher than PAF-group ($P < 0.05$) in Table 3. RDW, RBC, HCT, MPV, LAD, and LVEF levels were higher in the PAF group, PeAF group, or control group of high-altitude than those of corresponding groups in low altitude (Table 3).

Multivariate logistic regression analysis demonstrated that RDW, MCV, and LAD levels independently associated with AF patients in low altitude (RDW, OR 1.687, 95% CI 1.021–2.789; $P < 0.05$) in Table 4, while in high altitude, RDW, MCV, Cr, and LAD were independent predictors for AF patients (RDW, OR 1.755, 95% CI 1.179–2.613; $P < 0.05$) (Table 5).

Therefore, we integrated RDW, MCV, Cr, and LAD into two nomograms for prediction of probability of occurrence of AF in low altitude (Figure 1) and high altitude (Figure 2), respectively. For the nomogram, each predictor was assigned a point in the graphic interface of the nomogram, and the total points were assigned as a linear combination of the points of each predictor on a scale from 0 to 100% to find out the corresponding risk of AF.

Table 3: Baseline characteristics in patients with AF at two altitudes.

	Low altitude (3.5 m)			High altitude (2260 m)			P
	PAF (n = 77)	PeAF (n = 79)	Control (n = 72)	PAF (n = 77)	PeAF (n = 70)	Control (n = 95)	
Male (n %)	32.00 (41.60)	45.00 (57.00)	31.00 (43.10)	48.00 (62.30)	37.00 (52.90)	46.00 (48.40)	0.075
Age (years)	66.92 ± 12.61	68.89 ± 8.66	66.26 ± 10.54	67.23 ± 10.67	69.19 ± 8.17	69.28 ± 8.99	0.250
BMI (kg/m$^{2)}$	25.10 ± 4.02	25.74 ± 3.84	25.26 ± 2.83	25.18 ± 3.63	25.96 ± 3.18	25.97 ± 2.64	0.351
Smoking (n %)	11.00 (14.30)	11.00 (13.90)	5.00 (6.90)	12.00 (15.60)	13.00 (18.60)	11.00 (11.60)	0.434
Diabetes mellitus (n %)	11.00 (14.30)	20.00 (25.30)	10.00 (13.90)	16.00 (20.80)	14.00 (20.00)	14.00 (14.70)	0.340
Hypertension (n %)	46.00 (59.70)	51.00 (64.60)	48.00 (66.70)	55.00 (71.40)	49.00 (70.00)	64.00 (67.40)	0.704
Previous stroke (n %)	10.00 (13.00)	16.00 (20.30)	11.00 (15.30)	13.00 (16.90)	19.00 (27.10)	15.00 (15.80)	0.276
WBC ($\times 10^9$/L)	6.89 ± 1.92	6.82 ± 1.79	6.68 ± 1.74	5.56 ± 1.78^a	5.70 ± 1.28^a	5.72 ± 1.63^a	0.001
RBC ($\times 10^{12}$/L)	4.40 ± 0.53	4.51 ± 0.58	4.52 ± 0.44	4.90 ± 0.75^a	4.92 ± 0.59^a	4.92 ± 0.57^a	0.001
Hb (g/L)	138.48 ± 17.28	140.24 ± 19.85	140.67 ± 15.98	152.55 ± 19.45^a	152.79 ± 18.78^a	150.98 ± 17.28^a	0.001
RDW (%CV)	12.71 ± 0.74	12.98 ± 0.80^c	12.59 ± 0.58	13.52 ± 0.99^a	14.17 ± 1.16^{abc}	13.10 ± 0.85^a	0.001
HCT (%)	40.55 ± 4.75	41.97 ± 5.64	41.22 ± 4.47	46.13 ± 5.68^a	46.40 ± 5.64^a	45.82 ± 5.01^a	0.001
MCV (fL)	92.37 ± 5.12	93.24 ± 5.98^c	90.24 ± 9.69	94.69 ± 5.26^{ac}	94.42 ± 4.57	92.52 ± 9.75^a	0.002
MCHC (g/L)	341.39 ± 9.97	334.04 ± 10.52	341.13 ± 8.90	330.94 ± 10.05^a	329.47 ± 9.62^a	330.21 ± 9.44^a	0.001
Plt ($\times 10^9$/L)	227.84 ± 62.69	210.51 ± 52.50^b	222.13 ± 46.30	156.94 ± 49.28^{ac}	152.03 ± 56.06^{ac}	180.98 ± 47.62^a	0.001
MPV (fL)	9.37 ± 0.97	9.58 ± 1.02	9.33 ± 0.99	12.04 ± 1.44^a	12.29 ± 1.60^a	11.96 ± 1.26^a	0.001
Cr (μmol/L)	69.73 ± 32.44	70.83 ± 22.15	66.29 ± 17.65	81.05 ± 22.14^{ac}	79.16 ± 19.05^{ac}	63.47 ± 14.12	0.001
UA (mmol/L)	310.62 ± 94.97	339.70 ± 94.39	336.63 ± 87.22	362.09 ± 100.77^a	387.44 ± 127.74^c	331.38 ± 83.48	0.001
TBIL (μmol/L)	13.47 ± 6.07	15.79 ± 0.06^c	12.30 ± 4.18	16.43 ± 5.87^a	19.73 ± 9.57	16.93 ± 8.09	0.001
LAD (mm)	37.00 ± 6.48^c	43.82 ± 6.99^{bc}	33.78 ± 4.63	38.08 ± 4.95^c	45.43 ± 5.95^{bc}	34.21 ± 4.14	0.001
LVEDD (mm)	46.83 ± 5.34	49.81 ± 6.88^b	47.13 ± 5.38	45.97 ± 4.11	46.51 ± 5.34^a	46.01 ± 3.80	0.001
IVST (mm)	9.45 ± 2.25	9.58 ± 2.41	9.06 ± 1.87	10.48 ± 1.60^a	10.39 ± 1.37	9.88 ± 1.30^a	0.001
LVPWT (mm)	9.26 ± 2.18	9.17 ± 1.60	9.06 ± 3.09	10.21 ± 1.40^a	10.14 ± 1.20^a	9.66 ± 1.17	0.001
LVEF (%)	59.47 ± 6.63	54.00 ± 11.27	60.76 ± 5.54	65.68 ± 7.10^a	63.63 ± 6.52^a	64.57 ± 5.70^a	0.001
Aspirin (n %)	51.00 (62.20)	54.00 (68.40)	57.00 (79.20)	55.00 (71.40)	52.00 (74.30)	59.00 (62.10)	0.223
ACEIs or ARBs (n %)	22.00 (28.60)	27.00 (34.20)	19.00 (26.40)	19.00 (24.70)	21.00 (30.00)	16.00 (16.80)	0.174
β-blocker (n %)	35.00 (45.50)	32.00 (40.50)	28.00 (38.90)	28.00 (36.40)	27.00 (38.60)	25.00 (26.30)	0.180
CCBs (n %)	24.00 (31.20)	27.00 (34.20)	22.00 (30.60)	26.00 (33.80)	23.00 (32.90)	30.00 (31.60)	0.996
Statins (n %)	41.00 (53.20)	30.00 (38.00)	38.00 (52.80)	30.00 (39.00)	35.00 (50.00)	30.00 (31.60)	0.017

AF, atrial fibrillation; PAF, paroxysmal AF; PeAF, persistent AF; BMI, body mass index; WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; RDW, red cell distribution width; HCT, hematocrit; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; Plt, platelet count; MPV, mean platelet volume; Cr, creatinine; UA, uric acid; TBIL, total bilirubin; LAD, left atrial diameter; LVEDD, left ventricular end-diastolic diameter; IVST, interventricular septal thickness; LVPWT, left ventricular posterior wall thickness; LVEF, left ventricular ejection fraction; a: compared to the different altitude correspondence groups; b: compared to the same altitude PAF-group; c: compared to the same altitude control group.

4. Discussion

The main findings of our study are that AF patients have higher RDW and LAD levels than patients without AF at both high and low altitudes. Moreover, RDW and LAD levels were in high altitude compared to low altitude. Multivariate logistic regression analysis showed that RDW was a risk factor for AF.

RDW represents the variability in the size of circulating red blood cells obtained by an automatic blood count instrument, which measures 100,000 red blood cell volume over ten seconds. A number of studies have reported that RDW is a novel marker for proinflammatory reaction and oxidative stress in the body. The latter may inhibit the maturation of red blood cells, leading to the entry of immature red blood cells into the general circulation, resulting in an increase in the heterogeneity of peripheral red cell morphology [1, 9, 19]. RDW has been associated with, and used as a prognostic marker for outcomes, in many cardiovascular diseases [3–5, 20]. Liu et al. showed that not only the RDW level in patients with paroxysmal AF was significantly higher than that in the control group, but also related to the CHADS2 and CHA2DS2-VASc scores and thromboembolic risk in patients with AF [6, 21, 22]. Aksu et al. found that high RDW levels can be used to predict AF recurrence after cryoballoon ablation [23]. Moreover, previous comprehensive systematic reviews and meta-analyses confirmed that increased RDW can predict new-onset and recurrent AF generally, and after isolated coronary artery bypass grafting, valvular surgery, or combined procedures [24, 25]. This study found in two altitudes that not only RDW in patients with AF were higher than non AF patients,

Table 4: Multiple logistic regression analysis to detect the independent predictors of the occurrence of AF at low altitude (3.5 m).

Variables	β	Wald χ^2	P	OR	95% CI
RDW	0.523	4.160	0.041	1.687	1.021~2.789
MCV	0.074	3.956	0.047	1.077	1.001~1.159
TBIL	0.031	0.774	0.379	1.031	0.963~1.104
LAD	0.142	21.823	0.001	1.152	1.086~1.223
LVEF	−0.032	1.718	0.190	0.968	0.923~1.016

RDW, red cell distribution width; MCV, mean corpuscular volume; TBIL, total bilirubin; LAD, left atrial diameter; LVEF, left ventricular ejection fraction.

Table 5: Multiple logistic regression analysis to detect the independent predictors of the occurrence of AF at high altitude (2260 m).

Variables	β	Wald χ^2	P	OR	95% CI
RDW	0.562	7.669	0.006	1.755	1.179~2.613
MCV	0.084	4.251	0.039	1.088	1.004~1.178
Hb	0.003	0.091	0.762	1.003	0.975~1.019
Cr	0.063	20.331	0.001	1.065	1.036~1.094
UA	0.001	0.070	0.791	1.001	0.996~1.003
LAD	0.233	30.785	0.001	1.262	1.162~1.370
IVST	0.133	0.163	0.687	1.142	0.599~2.180
LVPWT	0.218	0.330	0.565	1.244	0.382~1.692

RDW, red cell distribution width; MCV, mean corpuscular volume; Hb, hemoglobin; Cr, creatinine; UA, uric acid; LAD, left atrial diameter; IVST, interventricular septal thickness; LVPWT, left ventricular posterior wall thickness.

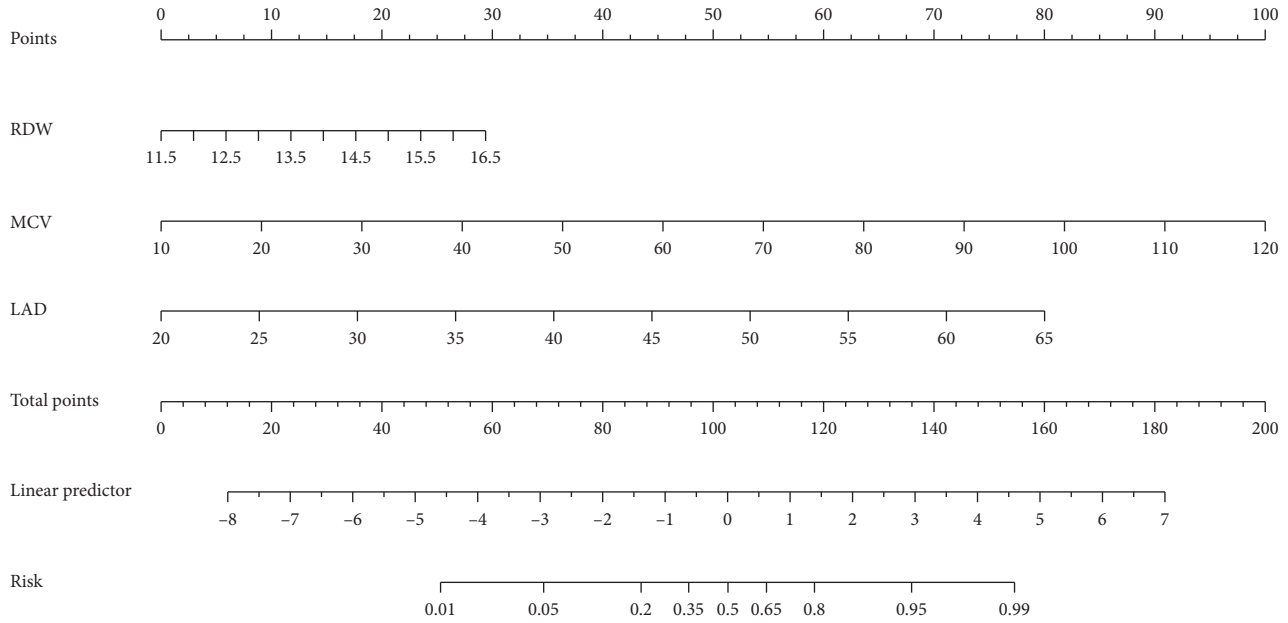

Figure 1: The nomogram for predicting the occurrence of AF at low altitude (3.5 m). To calculate the risk of AF, first identify the values of each axis, and than draw a vertical line upward to the point axis from each axis. Sum up the points of all variables and locate the value on to the total point line. Subsequently, draw a vertical line down to the risk of AF. AF, atrial fibrillation; RDW: red cell distribution width; MCV: mean corpuscular volume; LAD: left atrial diameter.

but also patients with persistent AF had higher levels than patients with paroxysmal AF, speculating that RDW levels were associated with AF subtypes.

The high-altitude environment results in chronic hypoxia. Several studies have shown that hypoxia causes transcription translational changes of multiple genes mediated by transcription mediators such as hypoxia-inducible factors, which in turn leads to imbalance of energy metabolism, neuroendocrine alterations, body fluid imbalance, increased oxidative stress, vascular dysfunction, and consequent pathophysiological changes [17, 26]. Indeed, exposure to acute and chronic hypoxic conditions in high altitude, increased sympathetic activation, plasma adrenaline levels, cardiac output, heart rate, and elevated blood pressure ultimately lead to cardiac structure and function change [27, 28]. In healthy people, high sympathetic activity that arises from sporting activity can induce atrial or ventricular arrhythmias through mechanisms such as increased automaticity, triggered activity, or reentry [29–31]. The commonest hematological adaptations are increased adaptive red blood cells and hemoglobin levels and polycythemia, leading to hyperviscosity, impairing the oxygen supply to multiple organs. Hypoxia can stimulate erythropoietin (EPO) synthesis and release that is the main mechanism of the occurrence of high-altitude polycythemia [26]. RDW can weaken the response of bone marrow to EPO and hinder the maturation of red blood cells, eventually leading to an increase in red blood cell volume dispersion. In our study, this may be the explanation as to why the RDW levels of each group in the high-altitude area were higher than the low altitude. The possible mechanism by which hypoxia leads to oxidative stress is increasing the generation of reactive oxygen species (ROS). Due to the limited oxygen supply, less oxygen receives electrons from oxidative phosphorylation, which may lead to accumulate reduction equivalents, and this reducing environment is beneficial to the mass production of superoxide, peroxide, and hydroxyl radicals. However, the

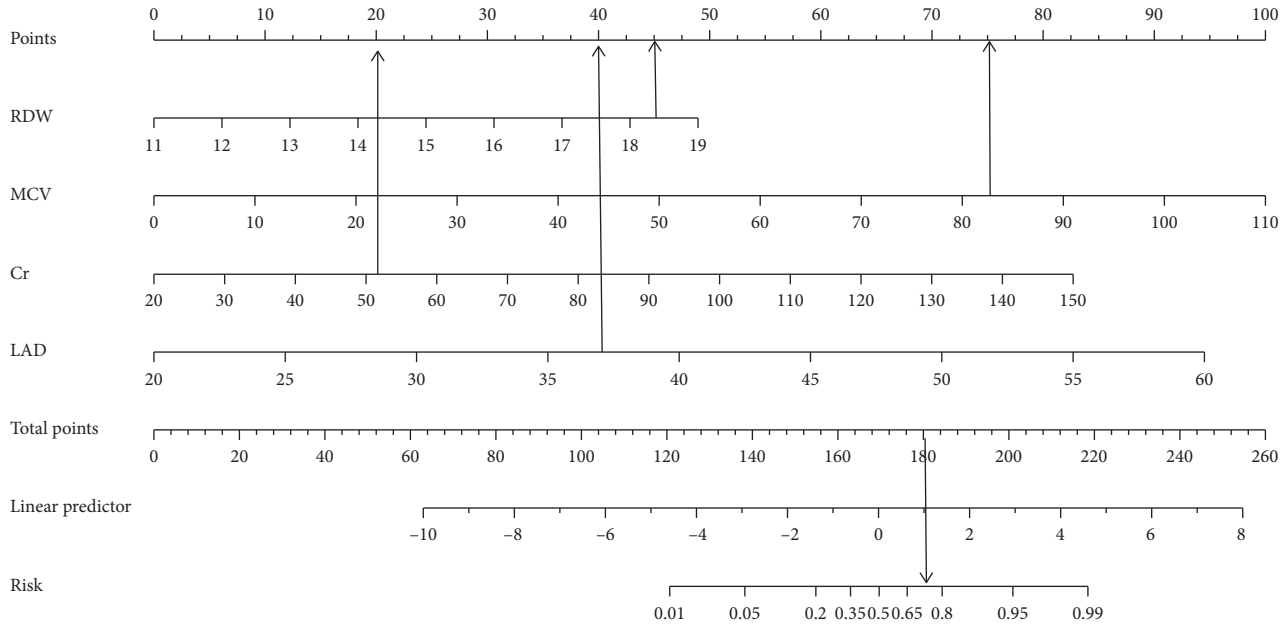

Figure 2: The nomogram for predicting the occurrence of AF at low altitude (2260 m). For example, the RDW (45 points), MCV (75 points), Cr (20 points), and LAD (40 points) arrive at a total of 180 points, which gives an estimated probability of 70% for occurrence of AF. AF, atrial fibrillation; RDW: red cell distribution width, MCV: mean corpuscular volume; Cr, creatinine; LAD: left atrial diameter.

potential source of ROS is due to the large-scale activation of xanthine oxidase and phospholipase A_2, which not only increases the release of NO, but also increases the availability of free iron and increases the release of oxygen free radicals from red blood cells. The balance between the oxidation and antioxidant systems is broken and oxidative stress occurs when too much ROS is produced or antioxidant is depleted [17, 32–35]. Al-Hashem et al. [16] found that compared to low-altitude natural rats, serum inflammatory cytokine levels and urinary norepinephrine levels were significantly increased in high-altitude rats, and while using beta and alpha adrenergic receptor blockers, inflammatory mediator levels is lower. Hartmann et al. [36] found that at 3458 m and 4559 m, interleukin (IL) -6, IL-1, and C-reactive protein (CRP) levels were higher than baseline in healthy people in the short-term. In this study, although the WBC level in high-altitude areas is lower than that in low altitude, the level of RDW is significantly increased which is considered to be related to the sensitivity of WBC to the inflammatory state of the body is not as sensitive as high-sensitive C-reactive protein (hs-CRP) and ILs [37]. Inflammatory reaction and oxidative stress play an important role in the process of high-altitude hypoxia injury.

5. Limitations

Several limitations of our study should be noted. Firstly, this took a cross-sectional design without follow-up, so the impact of RDW on the progression of AF and the occurrence of AF complications has not been explored. Second, a number of inflammatory markers, such as hs-CRP, IL, and tumor necrosis factor, were not evaluated. Thirdly, the high-altitude subjects have a higher RDW level than those at low altitude, excepting hypoxia, the living habits of residents are different from those in domestic plains, which may also have an impact on RDW levels.

6. Conclusion

Elevated RDW levels may be an independent risk marker for nonvalvular AF, affected by type of AF and altitude. RDW is a simple and economical marker that is routinely taken during complete blood counts and could be assessed in high-altitude residents for AF risk stratification.

Data Availability

The data of this study can be obtained from the corresponding author.

Conflicts of Interest

There are no conflicts of interest to declare between the authors.

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Review Article

Triggers for Atrial Fibrillation: The Role of Anxiety

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Atrial fibrillation (AF) is the most widely recognized arrhythmia. Systemic arterial hypertension, diabetes, obesity, heart failure, and valvular heart diseases are major risk factors for the onset and progression of AF. Various studies have emphasized the augmented anxiety rate among AF patients due to the poor quality of life; however, little information is known about the possibility of triggering atrial fibrillation by anxiety. The present review sought to underline the possible pathophysiological association between AF and anxiety disorders and suggests that anxiety can be an independent risk factor for AF, acting as a trigger, creating an arrhythmogenic substrate, and modulating the autonomic nervous system. The awareness of the role of anxiety disorders as a risk factor for AF may lead to the development of new clinical strategies for the management of AF.

1. Introduction

Atrial fibrillation (AF) is the most common arrhythmia in clinical practice, with an overall prevalence of 1-2% in the general population [1] and an incidence that increases with age up to 20% in octogenarians. In the next 50 years, its prevalence is expected to double, as a consequence of the prolongation of life expectancy [2]. Five types of AF are classified: first diagnosed, paroxysmal, persistent, long-standing persistent, and permanent AF (KirchhoffAF and psychological factors). AF is associated with high relative risk of all-cause mortality, stroke, cardiovascular mortality, cardiac events, heart failure, and chronic cognitive impairment and represents the most common arrhythmia that requires hospitalization and one of the most frequent causes of hospitalization for heart diseases. Several risk factors and heart diseases are known to be involved in the genesis and/or perpetuation of AF, acting by different pathophysiological pathways on the presence of a susceptible atrial electro-anatomic substrate. Among risk factors [3], both unmodifiable, as genetic susceptibility, age, gender, race, and modifiable, as systemic arterial hypertension, diabetes mellitus, smoking, obstructive sleep apnea, and obesity, have adverse effects on cardiovascular hemodynamic as well as on cardiac structure and function, increasing the prevalence of AF. Moreover, heart failure (HF) and AF frequently coexist: HF predisposes AF and vice versa. Left ventricular dysfunction, independently from ejection fraction, is linked to AF by a hemodynamic overload [4]; on the other hand, as well known, AF can decrease overall cardiac output from loss of atrial kick [5].

Over the last decades, epidemiological studies associated various risk factors with AF. Moreover, many reports have advanced the hypothesis of a mutual relationship between AF and anxiety disorders, in which the latter can pave a background that is favourable for the initiation and progression of the former. Anxiety is generally defined as a psychobiological emotional state or reaction that consists of unpleasant feelings of tension, apprehension, nervousness, worry, and activation of the autonomic nervous system [6].

The diagnosis of anxiety includes specific phobia, social phobia, panic disorder, agoraphobia, and generalized anxiety disorder [7]. Traditionally, anxiety has been considered a consequence of AF due to the impairment in quality of life associated with this arrhythmia. While it is well known that anxiety is an independent risk factor for cardiovascular disease, associated with a 26% increased risk of incident coronary heart disease (CHD) and a 48% increased risk of cardiac death [8–10], less is known about the role of anxiety disorders in AF onset, severity, and clinical outcomes. The recognition of the involvement of such psychological factors in the development of AF may help the identification of new clinical strategies for the management of AF.

2. Pathophysiological Insights for the Link between AF and Anxiety Disorders

The onset and progression of AF is the result of the interaction between three elements that form Coumel's triangle of arrhythmogenesis: the arrhythmogenic substrate, the trigger factors, and the modulation factors, of which the most common is the autonomic nervous system.

Several studies show a possible association of anxiety disorders and AF. Nevertheless, a clear relationship has never been demonstrated, this association should be based on the pathophysiological consequences of the anxious state on the neuroendocrine, coagulative, microcirculatory, and immune systems.

It is known that inflammation and oxidative stress are key players for the development of AF through atrial fibrosis, myocyte apoptosis and/or necrosis, and irregular myocellular hypertrophy with disarrangement of lines of cells and recruitment of macrophages to the endothelial surface [11]. All these factors constitute the anatomical arrhythmogenic substrate that results in shortening and dispersion of refractory period, conduction velocity slowing, and formation of reentry circuits. The arrhythmogenic substrate resulting from anatomical and electrophysiological atrial remodelling predisposes to onset and maintenance of AF.

The relationship between inflammatory cytokines and AF risk has been previously described in multiple setting, and both C-reactive protein and interleukin-6 are independently associated with AF [12–15]. Many studies showed that anxiety and depressive disorders are linked to low-grade systemic inflammation [12, 16–18]. For example, the ATTICA study evaluated various inflammation and coagulation markers among healthy adults in relation to the anxious state (assessed by Spielberger's State-Trait Anxiety Inventory, STAI). STAI score was positively correlated with C-reactive protein, interleukin-6, homocysteine, and fibrinogen levels. The ATTICA study provided strong evidence that anxiety is associated with systemic inflammation and abnormal coagulation process, possibly leading to increased cardiovascular events [6].

Moreover, stress response has been described as resulting from a "fight or flight" reaction that can be the result of endocrine, nervous, and immune systems [18].

The inflammatory state found in anxious and depressive disorders is presumably related to a hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis which is commonly seen in patients with chronic stress. Although the HPA axis in normal situations should temper inflammatory reactions, prolonged hyperactivity of the HPA axis might result in blunted anti-inflammatory responses to glucocorticoids resulting in increased inflammation [16, 19, 20] Furthermore, hyperactivity of the HPA axis with cortisol hyperproduction and corticotropin-releasing hormone (CRH) overdrive produces an imbalance of monoamines; in particular, chronic stress leads to a reduced activity of dopaminergic, serotoninergic, and noradrenergic neurons [18]. In fact, persisting hypercortisolemia decreases serotonin production. For this reason, antidepressants act on monoamines replacement as well as on modulation of cerebral glucocorticoid receptors [21, 22].

Additionally, patients suffering from anxious and depressive disorders are more likely to have increased activity of sympathetic nervous system [23] and subsequently catecholamine overload [24, 25]. It is known that elevated serum catecholamine levels can trigger Takotsubo (or stress) cardiomyopathy through microvascular endothelial damage and catecholamine cardiotoxic effects [26, 27]. On the other hand, acute emotional stress and chronic anxiety disorders are considered predisposing risk factors for stress cardiomyopathy because of the higher prevalence of these psychosocial factors than that in the acute coronary syndrome patients as well as in general population [28, 29]. In anxiety disorders, serum catecholamine levels are elevated; thus, the susceptibility to AF may be in part related to anxiety disorders through the same catecholamine-mediated myocardial injury seen in stress cardiomyopathy. Catecholamine overload in anxiety disorders could lead to the formation of the arrhythmogenic substrate and could be a trigger for the onset of paroxysmal AF. The morphological alterations caused by catecholamine overload include: extracellular matrix overproduction, contraction band necrosis, and mononuclear cell infiltration [26, 27]. Catecholamine overload leads to an extracellular accumulation of collagen alfa-1 (I) chain and to an increased ratio of collagen alfa-1 (I) chain to collagen alfa-1 (III) chain that results in a large and rapid increase in atrial fibrosis [27]. The increased release of catecholamines results in an enhanced catecholamine degradation, which in turn leads to production of reactive oxygen species [30, 31], and in increased level of profibrotic mediator like angiotensin II, TGF beta, and osteopontin [32]. On the other hand, matrix metalloproteinase are not correspondingly activated with the result of augmentation of extracellular matrix proteins, myocardial disarray, and negative atrial remodelling. Lastly, the overstimulation of beta-adrenoreceptors by supraphysiologic levels of catecholamines alters the expression of calcium-regulatory protein genes [33, 34]. The impairment of the calcium-handling system causes ultrastructural atrial remodelling and predisposes to the onset and progression of AF. Anxiety disorders can trigger AF through the increased activity of sympathetic nervous system that is known to be the most important modulation factor of Coumel's triangle of arrhythmogenesis. Patients with anxiety have reduced heart rate variability and vagal tone [35], which suggests an

abnormal autonomic system regulation and represents an independent risk factor for AF [36].

Lastly, individuals who suffer from anxiety have a stimulated renin-angiotensin-aldosterone system (RAAS) [37]. Elevated levels of angiotensin II stimulate mitogen-activated protein kinases and reduce collagenase activity, which results in cardiac fibrosis and left ventricular hypertrophy. Binding of angiotensin II to angiotensin II type I receptors induces transforming growth factor-1 (TGF-1) production which promotes atrial fibrosis [27]. Thus, the hyperactivity of RAAS results in detrimental cardiac remodelling with abnormal ventricle relaxation, diastolic impairment, and increased atrial pressure and stretch. All these mechanisms can promote AF by slowing atrial conduction velocity and providing a greater atrial surface for reentry.

3. Discussion

As mentioned above, chronic stress and anxious state can promote AF through several mechanisms acting at different levels as trigger, modulating the autonomic nervous system and modifying the atrial substrate. In sum, anxiety disorders can interact with all the three elements of the triangle of Coumel resulting in the arrhythmogenesis of AF.

We are underlying the possible pathophysiological consequences of the chronic stress and anxious state on the neuroendocrine, coagulative, microcirculatory, and immune systems; however, a strict relationship between AF and anxiety has never been demonstrated so far. Nevertheless, several studies clearly show the strong association of anxiety symptoms and onset or recurrence of AF strengthening the hypothesis of the existence of causal link between these two disorders.

For example, Eaker et al. showed that anxiety is a risk factor for incident AF in males and females over a 10-year time period [38, 39]. Moreover, it has been reported that anxiety symptoms increased the incidence of AF after cardiac surgery and the use of beta-blockers may reduce this correlation [40, 41].

Increased sympathetic tone, lessened vagal tone, and the cardinal symptoms of anxiety could be major provocative factors of postoperative AF acting as trigger and modulating the autonomic nervous system [24, 40].

Additionally, Lange and Herrmann-Lingen [42] found that after successful electrical cardioversion, risk of recurrence of AF remains existent due to anxiety. For those AF patients who scored more than 7 on Hospital Anxiety and Depression Scale (HADS), 85% have the possibility of recurrence. In another study, Yu et al. reported an increased risk of AF recurrence due to anxiety after taking circumferential pulmonary vein ablation [24, 43]. These studies show that anxiety disorders have an impact on AF treatment success, suggesting that these psychological disturbances can have a major role on the development and progression of AF.

There is a small study which found that paroxetine reduces drug-resistant paroxysmal AF, presumably modulating vagal tone at the level of the midbrain and inhibiting the vasovagal reflex [9, 44]. In the last decades, a bulk of data demonstrated the efficacy of selective serotonin reuptake inhibitors (SSRIs) and serotonin and norepinephrine reuptake inhibitors (SNRIs) in anxiety disorders [45]. These studies may suggest the usefulness of SSRIs and SNRIs in patients with AF and anxiety disorders. Pragmatic randomized controlled trials with antidepressants are needed to explore a possible effect on the course of AF in patients with comorbid psychosocial disorders.

All these evidences suggest that anxious disorders may create an environment that is favourable of the initiation and perpetuation of AF [9]. Nevertheless, the above-mentioned studies have several limitations as such as the small sample size, the short follow-up period, and they are single-center experiences using different questionnaires with heterogeneous validity and reliability. Additionally, the rating scales used in epidemiological studies, as Eaker's one, are not necessarily the same scales used to diagnose anxiety disorders in daily clinical practice. These findings suggest that future research should involve large multicentre prospective trials, with long follow-up period using thorough, exhaustive, and homogeneous interviews and scales for the diagnoses of anxiety disorders.

The identification of anxiety disorders as independent risk factor for AF opens new scenarios in the management of this arrhythmia.

Firstly, in patients with multiple risk factors for AF, anxiety assessment should be routinely performed through standardized questionnaire, as the Hamilton Anxiety Rating Scale (HAM-A) [46], Spielberger's State Anxiety Inventory (STAI) [47], and the Zung Self-Rating Anxiety Scale (SAS) [48], to identify this psychosocial risk factor and possibly prevent AF onset and progression. Moreover, in this setting, it is important to underline the need of a clinical diagnosis of anxiety disorders in order to make a differential diagnosis with depressive disorders that might also be present as comorbidity.

Secondly, it might be interesting to evaluate whatever identification and treatment of anxious states could be useful to improve outcomes and optimize the management of this arrhythmia in patients with recurrent AF, or treatment-resistance AF.

Lastly, future prospective well-designed studies should clarify the possible causal role of anxiety disorders in the onset of AF in patients after surgery. Considering the possible causal link between AF and anxiety in patients after cardiac surgery, the usefulness of beta-blockers, benzodiazepines, and SSRI should be evaluated in reducing the incidence and in the management of postoperative AF.

4. Conclusions

The present review underlines the possible pathophysiological mechanisms through which anxiety disorders can promote the onset, progression, and maintenance of AF. A relationship may exist between the most common clinical arrhythmia and anxious states. The recognition of the involvement of such psychological factors in the development of AF may help the identification of new clinical strategies

for the management of AF. Nevertheless, a few studies pointed out the possible role of psychological factors on the development of AF, and a clear association has not been demonstrated yet. Considering the limitations of the present studies addressing a role of anxiety disorders as risk factor for AF and the high health burden of AF, large prospective studies are necessary to elucidate this multifaceted relationship and to assess the benefits of routine anxiety assessment in AF patients and the usefulness of anxiolytics and antidepressants in the prevention and treatment of AF.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Research Article

Association of Autoantibodies against M2-Muscarinic Acetylcholine Receptor with Atrial Fibrosis in Atrial Fibrillation Patients

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Objectives. To investigate the association of serum autoantibodies against M2-muscarinic acetylcholine receptor (anti-M2-R) with atrial fibrosis in long-standing persistent atrial fibrillation (AF) patients. *Methods*. Twenty-four long-standing persistent AF patients, scheduled to undergo hybrid ablation surgery, were enrolled in the study. Twenty-six patients with sinus rhythm, scheduled to undergo coronary artery bypass grafting surgery, were enrolled into the non-AF group. We detected serum anti-M2-R levels. Left atrial appendages were subjected to histological and molecular biological assays. Patients in the AF group received follow-up for two years. *Results*. The AF group showed significantly higher serum anti-M2-R levels compared to the non-AF group (496.2 ± 232.5 vs. 86.3 ± 25.7 pmol/L, $p < 0.001$). The AF group exhibited severe fibrosis in the left atrial appendages, as indicated by increased collagen volume fraction ($45.2 \pm 4.7\%$ vs. $27.6 \pm 8.3\%$, $p < 0.001$), and higher levels of collagen I (0.52 ± 0.04 vs. 0.24 ± 0.06, $p < 0.001$) and collagen III (0.51 ± 0.07 vs. 0.36 ± 0.09, $p < 0.001$). TGF-β1 and CTGF were also upregulated in the AF group. A positive correlation between serum anti-M2-R levels and fibrosis of the left atrial appendage and fibrogenic indexes was observed. *Conclusions*. Serum anti-M2-R levels are higher in AF patients and are associated with the severity of atrial fibrosis. In addition, serum anti-M2-R levels are positively correlated to TGF-β1 and CTGF expression in the left atrial appendage.

1. Introduction

Atrial fibrillation (AF) is the most common arrhythmia in the clinical setting. The estimated number of AF patients worldwide in 2010 was 33.5 million, which included 20.9 million males and 12.6 million females [1]. The incidence and prevalence rates of AF are generally higher in developed countries. Approximately 25% of middle-aged adults in Europe and the USA are predicted to develop AF, with higher prevalence in older people and in patients with hypertension, diabetes mellitus, coronary artery disease, or heart failure [2–4]. The underlying pathogenesis of AF has long been disputed, but atrial remodeling, including electrical, structural, and autonomic remodeling, has always been recognized to contribute to the maintenance of AF in animal models and humans [5]. There is growing evidence confirming that autoimmunity may play an important role in the initiation and perpetuation of AF [6].

M2-muscarinic acetylcholine receptor belongs to the family of cardiac G-protein-coupled receptors. Circulating autoantibodies against M2-muscarinic acetylcholine receptors (anti-M2-R) have been found in several cardiac arrhythmias [7]. Our previous study showed that serum anti-M2-R levels are higher in AF patients and positive for anti-M2-R as an independent predictor for the recurrence of AF one year after radiofrequency catheter ablation [8]. A recent report has shown that serum anti-M2-R has a predictive value for moderate-extensive left atrial fibrosis defined by delayed-enhancement magnetic resonance imaging (DE-MRI) in patients following cryoablation [9]. Atrial fibrosis is one of the most fundamental mechanisms involved in the physiopathology of AF [10]. TGF-β1 and connective

tissue growth factor (CTGF) are strong fibrogenic indexes. Studies have shown that plasma TGF-β1 and CTGF levels are correlated with the degree of left atrial fibrosis and are associated with the development and maintenance of AF [11–13]. However, the relationship between anti-M2-R and atrial fibrosis and the association between anti-M2-R and fibrogenic indexes, such as TGF-β1 and CTGF, in AF patients are not clear.

Therefore, the aim of this study was to test the hypothesis that anti-M2-R is associated with atrial fibrosis. We tested serum anti-M2-R levels of AF patients, non-AF patients, and healthy controls by ELISA. We assessed atrial fibrosis and examined the expression of M2 receptor, TGF-β1, CTGF, collagen I, and collagen III in the left atrial appendage (LAA) of long-standing persistent AF patients receiving hybrid ablation surgery and patients receiving coronary artery bypass grafting (CABG) surgery with sinus rhythm. The relationship between anti-M2-R and fibrogenic indexes was also investigated.

2. Materials and Methods

2.1. Study Population. During the period between January 2015 and June 2016, 24 patients diagnosed with long-standing persistent AF who were scheduled to undergo hybrid ablation surgery were enrolled into the study. Twenty-six patients with sinus rhythm and scheduled for CABG surgery were also enrolled as the non-AF group. We also recruited 25 healthy controls. Baseline demographic and clinical characteristics were collected for all of the patients. Data relating to the duration of AF, history of stroke, and other comorbidities were also gathered. Prior to the surgery, all of the patients underwent transthoracic echocardiographic examination and pulmonary function testing to assess cardiopulmonary function. Enhanced computed tomography of left atrial and pulmonary vein and transesophageal echocardiography were routinely examined to clarify local anatomy and rule out thrombus in the LAA. Autoimmune or infectious diseases were excluded. This study complied with the Declaration of Helsinki and was approved by the Ethics Committee of Beijing Chao-yang Hospital, Beijing, China (13-S-84). All of the subjects were provided a written informed consent before study.

2.2. ELISA. Peripheral venous blood samples were acquired from the median cubital vein of the AF patients before hybrid ablation surgery and centrifuged at 1,000g for 10 min. Levels of serum anti-M2-R were measured using the "human muscarinic acetylcholine receptor M2 (mAChRM2) autoantibody enzyme-linked immunosorbent assay (ELISA) kit" (JIANGLAI, Shanghai, People's Republic of China, Catalogue No. JL45683) following the manufacturer's instructions. The variation coefficient between two wells was <5%, and the R^2 values were always >0.98.

2.3. Histological Assessment. During surgery, about 200 mg of the LAA were collected. The sample was divided into two parts; one part was rapidly placed into liquid nitrogen and then transferred to a −80°C freezer, and the other was immediately fixed with 10% neutral buffered formalin and then embedded in paraffin. Hematoxylin and eosin (H&E) staining were performed to assess the morphology of myocardial tissue. Evidence of collagen deposition in the extracellular matrix was detected by Masson's trichrome staining. Light microscopy was used to capture high-magnification light micrographs. The image quantitative digital analysis system (Image-Pro Plus 6.0, Media Cybernetics, Inc., Rockville, MD, USA) was used to measure collagen volume fraction (CVF).

2.4. Immunohistochemical Analysis. Immunohistochemical staining was performed with paraffin section using the EnVision™ two-step method. M2 receptor antibody (ab2805, Abcam, Cambridge, USA), TGF-β1 antibody (ab92486, Abcam), and CTGF antibody (ab6992, Abcam) were added at dilutions of 1 : 100, 1 : 1,000, and 1 : 400, respectively, followed by incubation for 60 min at room temperature and washing three times with phosphate-buffered saline (PBS) solution. Then, 50 μL of EnVision™ reagent was added, and the sections were incubated for 60 min at room temperature and then washed with PBS solution. Finally, the sections were stained with 3,3′-diaminobenzidine and counterstained with hematoxylin. The appearance of brownish yellow granules in the cytomembrane or extracellular matrix demonstrates a positive result.

2.5. Immunofluorescence Staining. Indirect immunofluorescence staining was performed to detect the expression of collagen I and collagen III in the paraffin sections of LAA tissues. Tissue sections were blocked with normal bovine serum albumin (Zhongshan Goldenbridge Biotechnology, China) for 30 min, and then, the sections were incubated with polyclonal rabbit anti-human collagen I antibody (1 : 50, ab34710, Abcam) or monoclonal mouse anti-human collagen III antibody (1 : 200, ab6310, Abcam) overnight at 4°C. After washing with PBS solution with 0.1% polysorbate, the sections were incubated with the corresponding secondary antibodies at 37°C for 1 h. Fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (1 : 100, Zhongshan Goldenbridge Biotechnology) was used as the secondary antibody for collagen I, and fluorescein isothiocyanate-conjugated goat anti-mouse IgG (1 : 100, Zhongshan Goldenbridge Biotechnology) was used as the secondary antibody for collagen III. Finally, the sections were washed with PBS solution thrice and counterstained with 4′,6-diamidino-2-phenylindole (1 : 100) for 1 h at room temperature. Negative controls were conducted without incubation with primary antibodies.

2.6. Western Blotting. About 50 μg of protein was extracted from the LAA tissues that were stored in the −80°C freezer by lysing in a radio-immunoprecipitation assay lysis buffer. Protein concentration was detected using the bicinchoninic acid assay. An equivalent amount of protein was added to 10% sodium dodecyl sulfate-polyacrylamide gel

electrophoresis, and the proteins were transferred onto nitrocellulose membrane. Membranes were blocked with 5% skimmed milk in TBST (10 mM Tris-HCl (pH 8), 150 mM NaCl, and 0.1% Tween 20) for 60 min at room temperature. The primary antibodies were added following the manufacturer's instructions. The nitrocellulose membranes were incubated overnight at 4°C with monoclonal mouse anti-human M2 receptor antibody (1 : 1000, ab2805, Abcam), polyclonal rabbit anti-human TGF-β1 antibody (1 : 1,000, ab92486, Abcam), polyclonal rabbit anti-human CTGF antibody (1 : 1,000, ab6992, Abcam), polyclonal rabbit anti-human collagen I antibody (1 : 500, ab34710, Abcam), or monoclonal mouse anti-human collagen III antibody (1 : 500, ab6310, Abcam). Horseradish peroxidase-labeled goat anti-rabbit antibody and goat anti-mouse antibody (1 : 10,000, Zhongshan Goldenbridge Biotechnology) were used as secondary antibodies. After incubation with the secondary antibody diluted in 5% skimmed milk in TBST at room temperature for 40 min, the membranes were washed thrice with TBST, and the film was placed in 10 mL of Ponceau solution for 3 min. The images were placed into an automatic image analyzer, and the density of each band was quantified using the Gel Image System ver.4.00 (Tanon, China). Monoclonal anti-GAPDH antibody was used as the loading control.

2.7. Postprocedural Follow-Up. Hybrid ablation surgery was performed according to Pison et al. [14]. All of the patients needed endocardial touch-up ablation to reach bidirectional block. The LAA was removed using a stapling device. All of the patients were transferred to the intensive care unit after hybrid ablation surgery. Amiodarone and warfarin/dabigatran etexilate capsules (Pradaxa®, Boehringer Ingelheim Pharma GmbH and Co., KG) were restarted 6 h after the surgery for three months. If with warfarin, enoxaparin 1 mg/kg was used until the target international normalized ratio of 2.0-3.0 was achieved. Outcomes of hybrid ablation surgery were assessed by off antiarrhythmic drug. All 24 patients were scheduled for visits at 1, 3, 6, and 12 months after hospital discharge and every 6 months thereafter in the outpatient clinics. At each visit, all of the patients received cardiological physical examination, questionnaire for AF-related symptoms (palpitations, fatigue, dizziness, and chest discomfort) and a 12-lead electrocardiogram. Long-attached ambulatory electrocardiographic recorder and software (NSSP-A-01, Ensense Biomedical Technologies (Shanghai) Co., Ltd.) for 14 days after routine 24 h Holter recording was conducted every 6 months after the hybrid ablation surgery for all of the patients. Any episode of AF, atrial flutter, or atrial tachycardia lasting at least 30 sec and occurring after the 90-day blanking period was classified as recurrence [15].

2.8. Statistical Analysis. Quantitative data with normal distribution were presented as the mean ± standard deviation. Qualitative data were presented as frequencies and percentages. Quantitative data were compared by one-way ANOVA and unpaired Student's t-test among the three groups or between the two groups. Qualitative data were compared using the chi-square test. Pearson correlation analysis was used to test the correlation between serum anti-M2-R levels and fibrosis of LAA-related parameters. Statistical analysis was performed using SPSS statistical software (version 20.0; SPSS Inc.). A two-tailed $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Patients Characteristics and Clinical Findings. The patients' basic information and echocardiographic data are shown in Table 1. Demographic characteristics were similar among the three groups. Comorbidities were similar between the AF and the non-AF groups. There were no significant differences in pulmonary function and echocardiographic data, including left ventricular end-diastolic dimension and left ventricular ejection fraction among the three groups. The mean left atrium diameter of the AF group estimated by echocardiography was significantly larger than that in the non-AF group (58.0 ± 5.3 mm vs. 36.3 ± 1.1 mm; $p < 0.001$).

In the AF group, the duration of AF was 103.3 ± 61.8 months. The CHA2DS2-VASc score was 2.6 ± 1.8, and HAS-BLED score was 1.3 ± 1.0. The pulmonary function test and the anatomy of left atrium and pulmonary vein were normal. All 24 of the patients underwent hybrid ablation surgery and LAA excision successfully. The mean anesthesia duration was 375.6 ± 118.6 min, and the mean duration of operation was 280.9 ± 117.3 min. The hospital stay was 20.1 ± 8.4 days. At the two-year follow-up, the success rate of hybrid ablation was 87.5% (21/24).

3.2. Elevated Serum Anti-M2-R and M2 Receptor in AF Patients. Serum anti-M2-R levels were significantly higher in the AF group compared to the non-AF group (496.2 ± 232.5 vs. 86.3 ± 25.7 pmol/L, $p < 0.001$). There were no differences between the non-AF and the control groups (86.3 ± 25.7 vs. 82.4 ± 34.9 pmol/L, $p = 0.654$) (Figure 1). The expression of M2 receptor was determined by immnunohistochemical and Western blotting analyses (Figures 2(a) and 3). M2 receptor expression was higher in the AF group than the non-AF group (0.35 ± 0.04 vs. 0.18 ± 0.02, $p < 0.001$). Pearson correlation analysis showed a close relationship between serum anti-M2-R and M2 receptor levels ($r = 0.90$, $p < 0.001$), Figure 4.

3.3. Occurrence of Atrial Fibrosis in AF Patients. Representative sections of LAA tissues from the two groups subjected to H&E and Masson's trichrome staining are shown in Figure 5. H&E staining of sections from the AF group indicated hypertrophy, vacuolar degeneration, and necrosis of cardiomyocytes. The cell surface area increased in the AF group compared to the non-AF group (76.2 ± 7.7% vs. 64.4 ± 3.9%, $p < 0.001$; Figure 5(a)). There were abundant collagen fibers distributed in the extracellular matrix in the AF group. CVF increased in the AF group than in the non-AF group (45.2 ± 4.7% vs. 27.6 ± 8.3%, $p < 0.001$; Figure 5(b)).

Table 1: Baseline characteristics of the control group and study population.

	Control group ($n = 25$)	AF group ($n = 24$)	Non-AF group ($n = 26$)
Clinical parameters			
Age (years)	65.6 ± 7.8	62.5 ± 9.9	65.8 ± 7.3
Gender, male (%)	13 (52.0)	16 (66.7)	14 (53.8)
Smoking (%)	9 (36.0)	9 (37.5)	10 (38.5)
Alcohol consumption (%)	7 (28.0)	8 (33.3)	8 (30.8)
BMI (kg/m^2)	25.4 ± 2.9	26.6 ± 3.8	25.7 ± 3.1
Comorbidity			
Hypertension (%)	0	12 (50.0)	14 (53.8)
Diabetes mellitus (%)	0	9 (37.5)	10 (38.5)
History of stroke (%)	0	10 (41.7)	11 (42.3)
Echocardiographic parameters			
Left atrium diameter (mm)	36.0 ± 1.2	58.0 ± 5.3*	36.3 ± 1.1
LVEDD (mm)	48.9 ± 1.8	50.0 ± 3.3	49.2 ± 2.0
LVEF (%)	62.4 ± 4.3	60.3 ± 8.6	62.1 ± 5.0
Pulmonary function			
FEV1 (L)	2.8 ± 0.5	2.6 ± 0.4	2.6 ± 0.2
FEV1/FVC (%)	77.6 ± 2.8	76.6 ± 4.1	76.8 ± 3.6

BMI, body mass index; LVEDD, left ventricular end diastolic diameter; LVEF, left ventricular ejection fraction; Anti-M2-R, autoantibodies against M2-muscarinic acetylcholine receptors. One-way ANOVA and the unpaired Student's two-tailed t-test were performed among the three groups or between two groups. * $p < 0.001$.

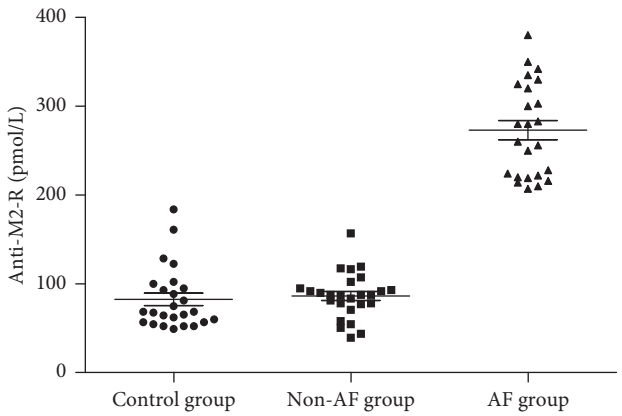

Figure 1: Comparison in serum anti-M2-R levels among three groups. The serum anti-M2-R level in the AF group was 496.2 ± 232.5 pmol/L, which was significantly higher than 86.3 ± 25.7 pmol/L in the non-AF group, $p < 0.001$. No difference in serum anti-M2-R levels between the non-AF and control group was observed (86.3 ± 25.7 vs. 82.4 ± 34.9 pmol/L, $p = 0.654$).

Furthermore, we determined the expression of collagen I and collagen III by immunofluorescence staining and Western blotting (Figures 3 and 6). Although collagen I and collagen III were expressed in both groups, the levels were considerably higher in the LAA of the AF group than in the non-AF group (collagen I: 0.52 ± 0.04 vs. 0.24 ± 0.06, $p < 0.001$; collagen III: 0.51 ± 0.07 vs. 0.36 ± 0.09, $p < 0.001$).

3.4. Increased Fibrogenic Indexes in AF Patients. The expression of TGF-β1 and CTGF were determined by immnunohistochemistry and Western blotting (Figures 2(b)-2(c) and 3). These indicators all increased in the AF group compared to the non-AF group (TGF-β1: 0.38 ± 0.06 vs. 0.09 ± 0.04, $p < 0.001$; CTGF: 0.55 ± 0.04 vs. 0.37 ± 0.05, $p < 0.001$).

3.5. Correlation between Fibrosis and Serum Anti-M2-R Levels. CVF and collagen I and collagen III were all correlated with serum anti-M2-R levels (CVF: $r = 0.84$, $p < 0.001$; collagen I: $r = 0.87$, $p < 0.001$; collagen III: $r = 0.78$, $p < 0.001$). Fibrogenic indexes were also correlated with serum anti-M2-R levels (TGF-β1: $r = 0.92$, $p < 0.001$; CTGF: $r = 0.89$, $p < 0.001$), Figure 4.

4. Discussion

4.1. Major Findings. Our findings indicate that serum anti-M2-R levels are significantly higher in AF patients and are correlated with the severity of atrial fibrosis as shown by histology and molecular biology assays. Atrial fibrosis is probably one of the important factors between anti-M2-R and the occurrence and maintenance of AF. Our results further revealed a positive correlation between serum anti-M2-R levels and TGF-β1 and CTGF expression in LAA tissues. These findings suggest that serum anti-M2-R plays an important role in fibrosis-associated AF and its potential mechanism.

4.2. Elevated Serum Anti-M2-R Levels in AF Patients. Anti-M2-R was first detected in idiopathic dilated cardiomyopathy patients in 1993 by Fu et al. [16]. Later, anti-M2-R was reported as the strongest independent predictor for the presence of AF in patients with idiopathic dilated cardiomyopathy and Graves' hyperthyroidism [17]. We further found that the frequency of anti-M2-R in AF patients with normal heart function was 40.8%. Yalcin et al. found that the serum anti-M2-R level was 142.3 ng/mL in lone paroxysmal AF patients, markedly higher than 69.0 ng/mL in healthy

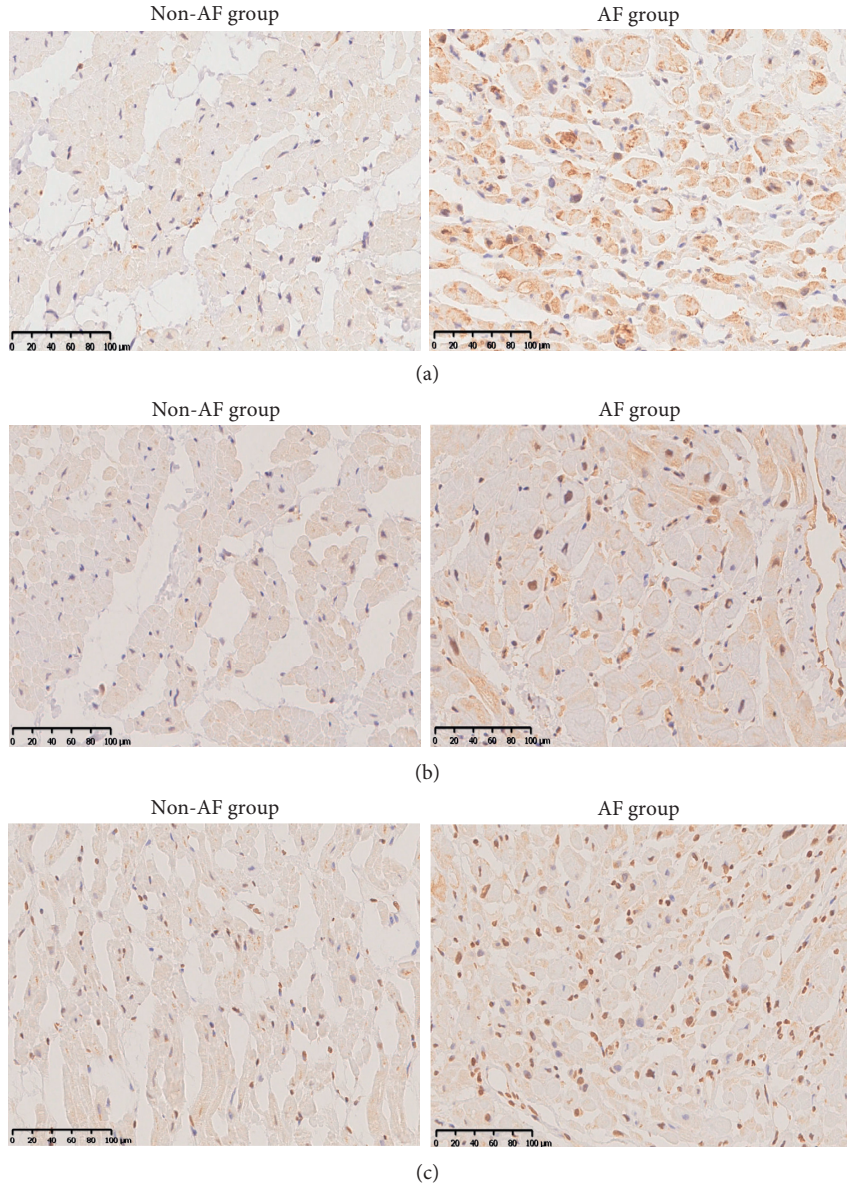

Figure 2: Immunohistochemical analysis of M2 receptor, TGF-β1, and CTGF expression. Representative sections of the immunohistochemistry- (IHC-) stained left atrial appendage tissue of patients in the non-AF and AF group. (a) M2 receptor; (b) TGF-β1; (c) CTGF.

controls. Using a cutoff value of 101.83 ng/mL for predicting the presence of lone paroxysmal AF, the sensitivity was 94.68% and the specificity was 81.33% [18]. In this study, we found that the serum anti-M2-R level in long-term persistent AF patients was 496.2 pmol/L, significantly higher than 86.3 pmol/L in non-AF controls. Some investigators have suggested that anti-M2-R probably is a novel mediator or upstream target in AF [6, 19, 20].

4.3. Role of Serum Anti-M2-R in the Pathogenesis of AF. The atrial electrical, structural, and autonomic remodeling has been recognized to contribute to the maintenance of AF. Circulating anti-M2-R can recognize and bind to 169–193 amino acids of the second extracellular loop of the M2 receptor specifically. It is not only able to bind to the target receptor in the myocardium but also to induce biological responses as partial agonist [16, 21]. Anti-M2-R binds to the M2 receptor, resulting in the activation of the muscarinic-gated potassium channel $I_{K,Ach}$, and the primary cellular electrophysiological effects are hyperpolarization and shortening of action potential duration [22]. In knockout mice lacking this channel, M-receptor stimulation did not induce AF [23]. Thereafter, Hong et al. [22] reported that atrial tissues clearly exhibit atrial fibrosis in rabbits immunized with a synthetic peptide corresponding to the M2 receptor. DE-MRI has recently been used for visualizing left atrial fibrosis for AF ablation procedures to estimate the degree of atrial fibrosis. Gurses et al. [9] showed that serum anti-M2-R levels may be associated with the severity of left atrial fibrosis as quantified by DE-MRI. However, the histological changes and underlying molecular mechanism

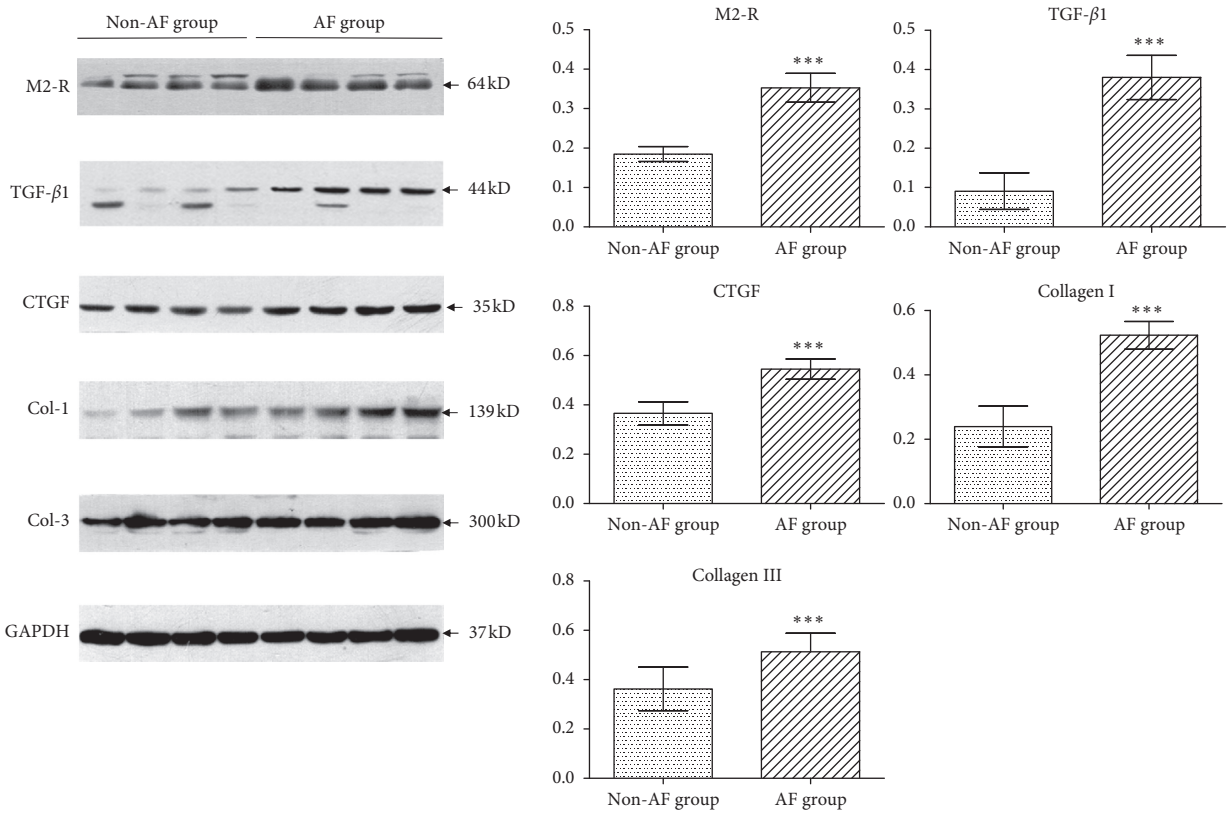

Figure 3: Western blotting analysis. Western blotting examined the left atrial appendage of patients in the non-AF group and the AF group. Left, representative western blot analysis the expression in the non-AF group and the AF group; right, quantitative results of the protein levels. *** $p < 0.001$. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

have not been extensively studied. In this study, we quantified serum anti-M2-R levels and we found that they are associated with CVF and fibrogenic indexes related to fibrosis. These results agree with the findings of previous studies on the ventricles [24, 25]. Earlier investigations have demonstrated that the mRNA level of the inhibitory G protein α subunit is higher in heart failure patients and pace-induced heart failure dogs [26, 27]. Redfern et al. [28] reported that fibronectin, laminin, and collagen were all upregulated in transgenic mice that conditionally expressed receptors that constitutively activated the inhibitory G protein signaling pathway. We hypothesize that the cause of atrial fibrosis in these AF patients may be via the activation of the inhibitory G protein pathway.

4.4. Increased M2 Receptor Expression in AF Patients. Previous studies have shown that AF dogs with vagal stimulation can significantly increase the expression of M2 receptor in the atrium, particularly in the atrial appendage, which indicated that the atrial appendage perhaps play an important role in initiation of cholinergic AF [29]. The density of M2 receptor in the left atrium increases with aging in rabbits, which contributed to the increased age-related AF vulnerability [30]. Hong et al. [22] found the expression of the M2 receptor in the atrium of anti-M2-R positive rabbits was significantly upregulated. Our study found that the expression of M2 receptor is higher in AF patients compared to the non-AF group, which agrees with the results of previous reports on rabbits [22, 30]. Zhao et al. [31] proposed that the remodeling of the M2 receptor might not be associated with AF, but with the dilated left atrium. Cisatracurium, a neuromuscular blocker, with allosteric binding to the M2 receptor in the atrium, demonstrates a dose-dependent suppression of AF and shortening of the atrial action potential accompanied by vagus nerve stimulation without facilitating sinus or atrioventricular nodal function [32]. Selective M2 receptor antagonist may thus be potentially utilized as a novel therapeutic target for the treatment of AF patients.

4.5. TGF-β1 and CTGF in Atrial Fibrosis. Data from biopsy specimens of patients with AF have uncovered the presence of atrial fibrosis [33]. Collagen I and collagen III, the major matrix proteins of cardiomyocytes, constitute 85% of the matrix. TGF-β1 is a key regulator of fibrosis that enhances collagen synthesis [11, 34]. CTGF is upregulated via the TGF-β1/Smad pathway in the atrial myocardium of AF patients [12]. In this study, we found that the expression of TGF-β1 and CTGF in LAA tissues is correlated with serum anti-M2-R levels. It is reported that muscarinic receptors exert stimulatory effects on collagen synthesis in human lung fibroblasts [35], and crosstalk between TGF-β1 and M2 receptor augments airway smooth muscle proliferation [36]. Earlier studies have shown that TGF-β1 stimulation of chick

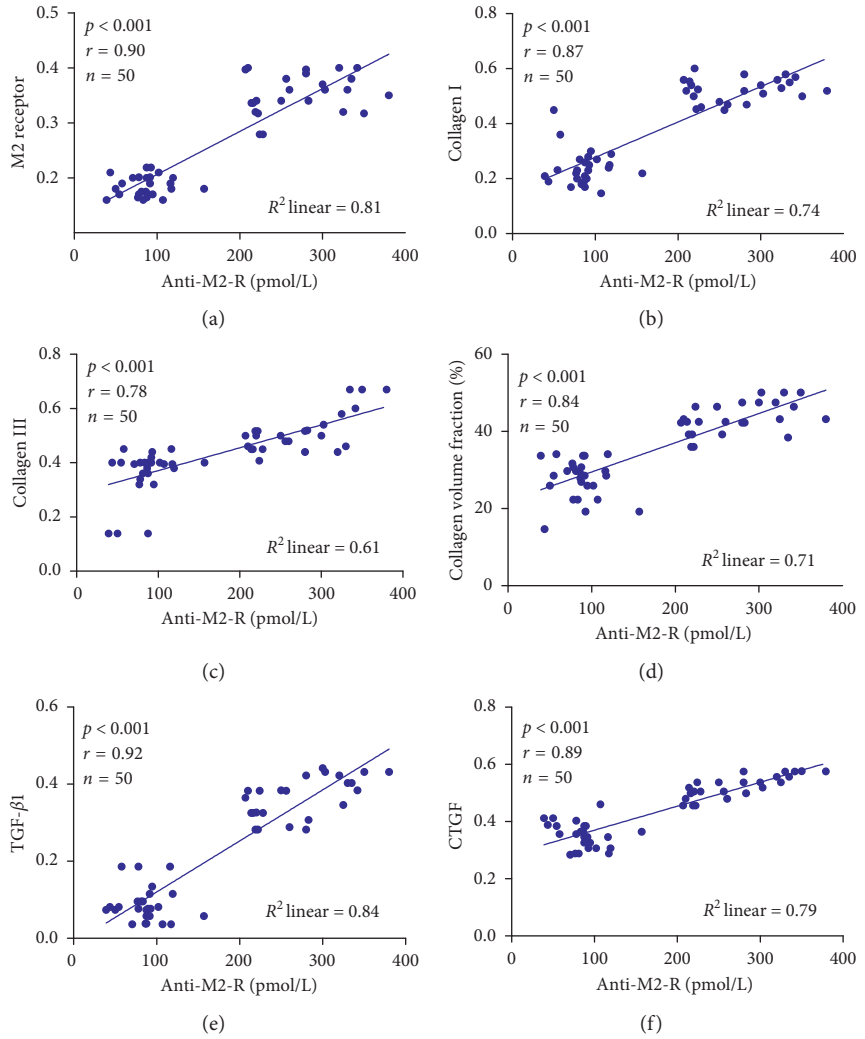

Figure 4: Correlation between serum anti-M2-R levels and fibrosis-related indexes. Pearson correlation analysis showed that M2 receptor, CVF, and collagen I and collagen III were all correlated with serum anti-M2-R levels (M2 receptor: $r = 0.90$, $p < 0.001$; CVF: $r = 0.84$, $p < 0.001$; collagen I: $r = 0.87$, $p < 0.001$; collagen III: $r = 0.78$, $p < 0.001$). Fibrogenic indexes were also correlated with serum anti-M2-R levels (TGF-β1: $r = 0.92$, $p < 0.001$; CTGF: $r = 0.89$, $p < 0.001$).

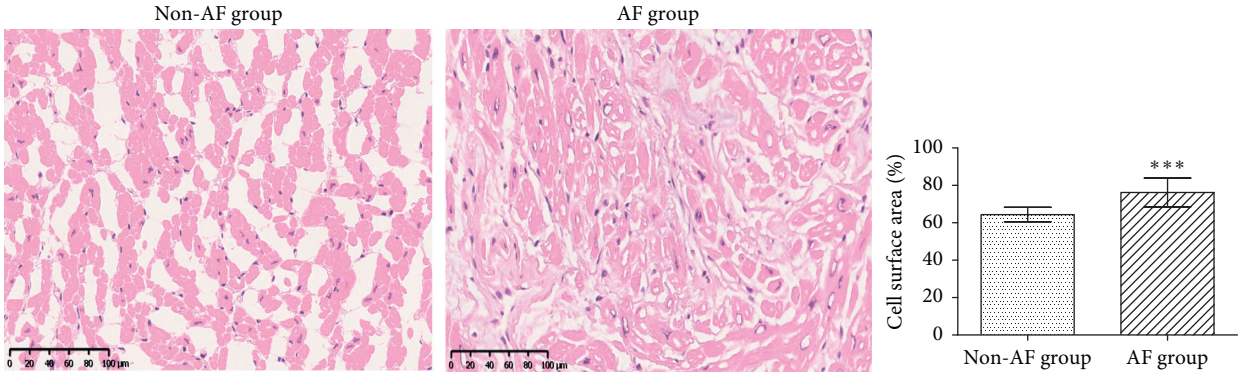

Figure 5: Continued.

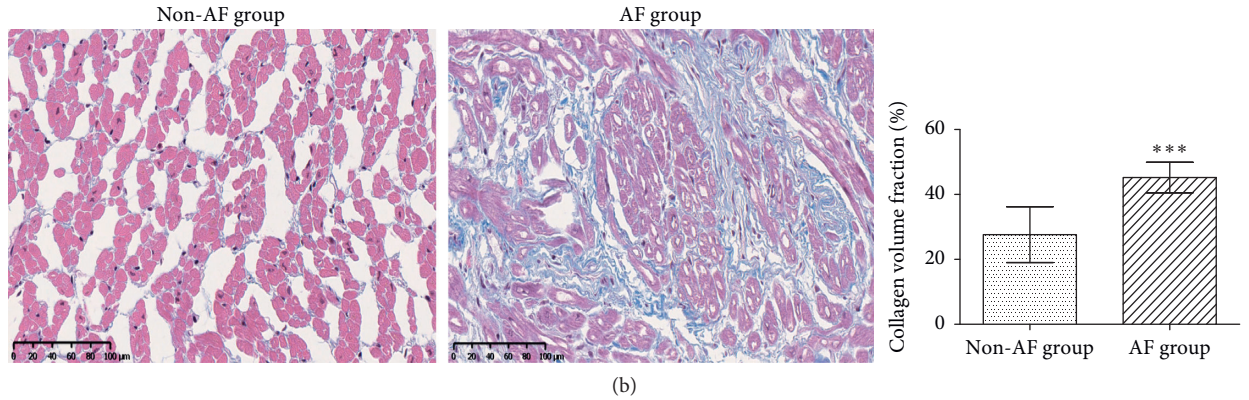

Figure 5: Histological analysis of the left atrial appendage. (a) Hematoxylin and eosin (H&E) staining of the left atrium appendage in the non-AF and the AF groups. Left, representative image; right, statistical results for the cell surface area. (b) Masson's trichrome staining of the left atrium appendage in the non-AF and AF groups. Left, representative image; right, quantification of the collagen volume fraction. $^{**}p < 0.01$; $^{***}p < 0.001$.

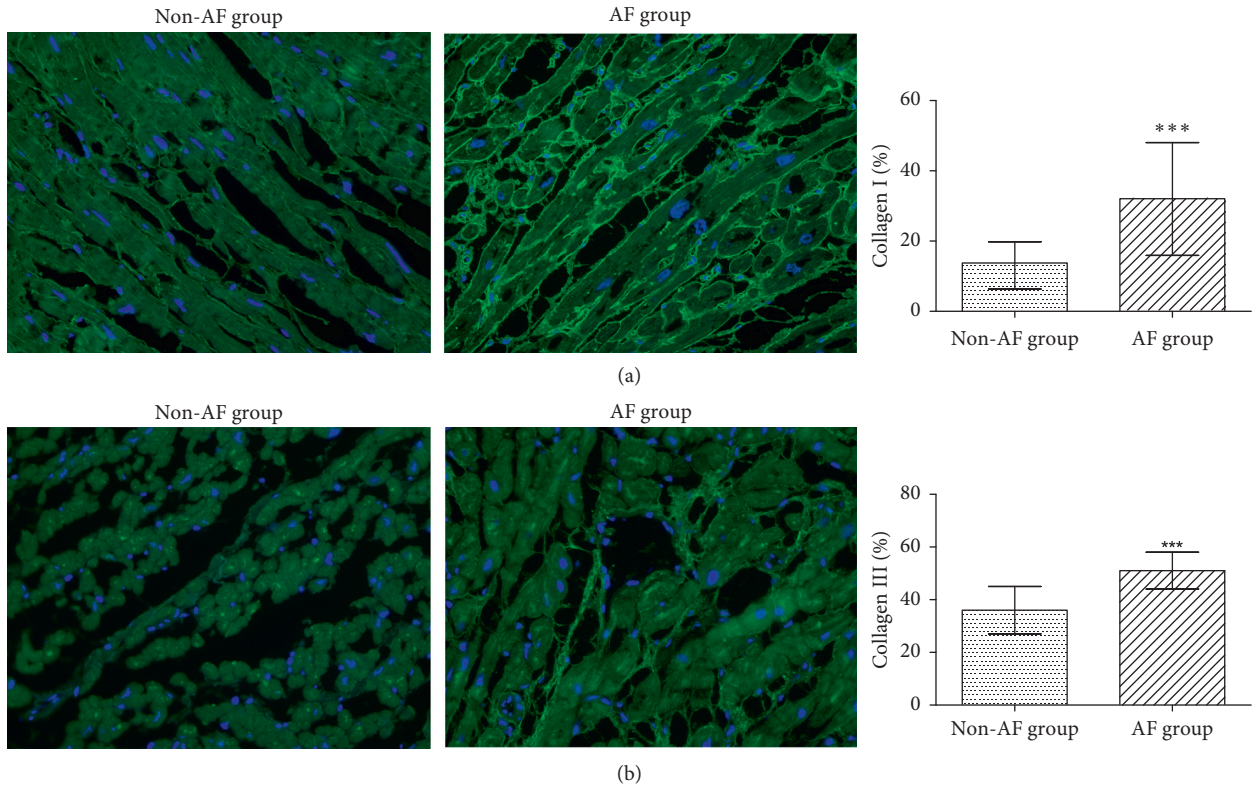

Figure 6: Immunofluorescence analysis the expression of collagen I and collagen III. Immunofluorescence staining of the left atrial appendage of patients in the two groups. Left, representative image; right, statistical results for the collagen (a) I and (b) III. $^{***}p < 0.001$; magnification, 400×.

heart cells results in the downregulation of the M2 receptor and muscarinic responsiveness [37], while we observed the upregulation of M2 receptor and TGF-β1 expression in LAA in AF patients. As the small number of patients enrolled in this study, further study is needed to verify the association between M2 receptor and TGF-β1.

4.6. Limitations. This study has a number of limitations. First, this is a single-center, small-sample population study. The results may thus be exaggerated for selection bias, and they cannot be directly expanded to all AF patients. A multicenter, large-sample population study is thus needed to verify our results. Second, as none of the patients had an implanted internal loop recorder, AF recurrence might be underestimated. Finally, this is a clinical study on the association between serum anti-M2-R levels and fibrosis of LAA. These results do not reveal a causal relationship but only demonstrate a correlation. The molecular mechanism

of anti-M2-R in atrial fibrosis in AF patients require further investigation.

5. Conclusions

Serum anti-M2-R levels are significantly higher in AF patients and are associated with the severity of atrial fibrosis. In addition, a positive correlation between serum anti-M2-R levels and the expressions of TGF-β1 and CTGF in LAA tissues in AF patients was observed.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Clinical Study

Left Atrial Appendage Occlusion Guided Only by Transesophageal Echocardiography

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Aims. To investigate a new method of left atrial appendage occlusion without fluoroscopy. *Methods and Results*. We performed left atrial appendage occlusion for 14 patients with atrial fibrillation in our hospital. All of the surgeries were completed in a general surgery setting, avoiding fluoroscopy, and in each case, the entire procedure was guided by transesophageal echocardiography (TEE). All of the surgeries were performed through the femoral vein pathway. All operations went smoothly with no serious complications. Postoperative TEE indicated that each device was in a good position, and there was no residual shunt around any of the devices. *Conclusions*. TEE-guided left atrial appendage occlusion is safe and reliable, simplifies the procedure, protects doctors and patients from radiation, and is gradually becoming the mainstream operation for left atrial appendage occlusion. This trial is registered with ChiCTR1800018387.

1. Introduction

Atrial fibrillation is the most common type of persistent arrhythmia. In addition to causing palpitations and discomfort, the onset of atrial fibrillation increases the risk of thromboembolism [1]. Evidence reported in the literature both at home and abroad indicates that thrombosis from the left atrial appendage is the main cause of stroke in atrial fibrillation [2]. Percutaneous left atrial appendage occlusion (LAAO), a new technique for preventing thromboembolism, offers more options for patients with atrial fibrillation who are at increased risk of blood clotting and cannot take anticoagulants. Currently, the clinical application of LAAO technology is guided by fluoroscopy under general anesthesia. The technology has a long learning curve, increases radiation risk, and is expensive. Moreover, the implantation procedure can cause serious complications [3–6]. Therefore, we chose to perform LAAO under the guidance of transesophageal echocardiography (TEE) in a general surgery operating theater and truly achieved zero exposure to radiation. The operating times were short, and there were fewer complications compared with the fluoroscopy-guided procedure. This technology should be available in the clinic as it is safe, radiation-free, and effective.

2. Methods

Written informed consent was obtained from each patient. Procedures were performed in accordance with ethical standards. From March 2018 to November 2018, percutaneous LAAO was performed with the guidance of TEE in a total of 14 patients. All patients underwent LAA closure under general anesthesia.

2.1. Inclusion/Exclusion Criteria. The CHA2DS2-VASC scores of the patients included in this group were greater than or equal to 2. Furthermore, these patients were not suitable for long-term oral anticoagulation, and stroke or

embolism events could still occur on the basis of the INR standard after taking warfarin.

Exclusion criteria were inner diameter of LA >65 mm, spontaneous development of intracardiac thrombus, severe mitral valve lesions and pericardial effusion >3 mm, life expectancy <1 year, low risk of stroke or low risk of bleeding, taking warfarin for other reasons, and complex atherosclerotic plaques in the ascending aorta/aortic arch. Patients undergoing elective cardiac surgery whose patent foramen ovale (PFO) was not closed with an atrial septal aneurysm and right-left shunt and who were in cardiac dysfunction were also excluded.

2.2. Occluder Device. We choose the LAmbre™ occluder device (Lifetech) for occlusions by the femoral vein pathway because it can be applied to a variety of left appendage structures and has a unique hook anchoring design that creates a more stable block.

2.3. Device Implantation. The procedure for percutaneous occlusion of the left appendage through the femoral vein pathway was as follows (Figure 1). After successfully puncturing the right femoral vein, the head end of the soft guidewire was implanted into the superior vena cava under the guidance of TEE. The transseptal guiding introducer pushed the atrial septum to the left side of the atrial septum under the guidance of TEE, creating a structure similar to a "tent." After the transseptal needle successfully punctured the atrial septum, the puncture sheath was transported to the left atrium. Then, the Amplatzer super stiff guidewire was inserted, and its head end was placed in the upper left pulmonary vein under the guidance of TEE. The transseptal guiding introducer was replaced by the delivery sheath, and the head end of the sheath was placed on the left atrium, and then, the Amplatzer super stiff guidewire was replaced with a pigtail catheter. Under the guidance of TEE and the pigtail catheter, the head end of the delivery sheath was transported to the left appendage. The left appendage occlusion device, which was measured in advance and well vented, was transported to the LAA through the sheaths with close TEE monitoring, and the push-and-pull test showed that there was almost no residual blood in the left appendage. When the occluder was in a good position, the cable and sheath were removed.

2.4. End Points. The end points of this study refer to the standardized end points/criteria included in the Munich consensus paper on LAAO by Tzikas et al. [7]. The primary end point of the study included successful implantation of the LAA occlusion device. Successful closure of the LAA was determined by TEE as the absence of flow or minimal flow (jet of <5 mm width; we set it to be < 3 mm width) around the device according to the echocardiographic sealing criteria was described previously [8]. The second end points were the occurrence of adverse events, which included composite end points such as all-cause mortality, ischemic stroke or systemic embolism, and periprocedural complications

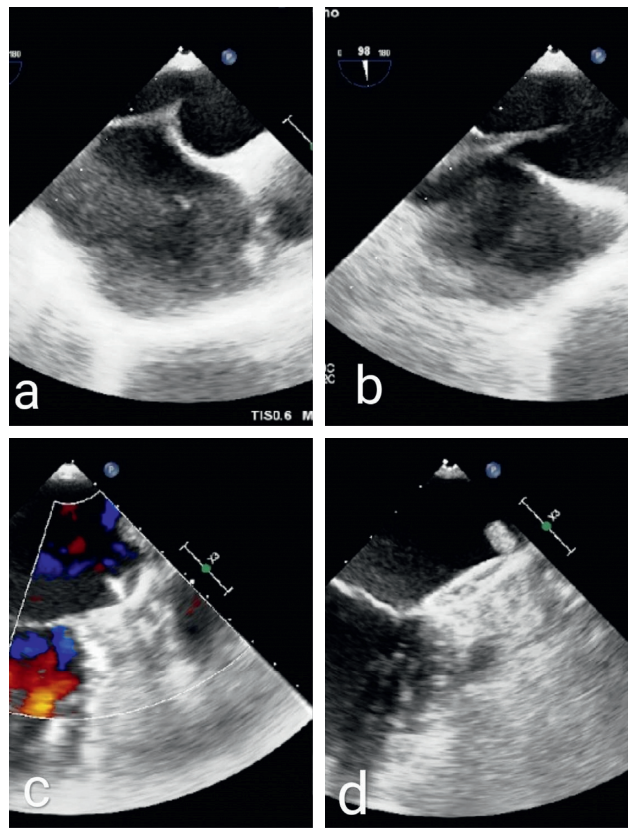

Figure 1: Operation procedure of percutaneous occlusion of the left atrial appendage through the femoral vein pathway. (a) The tent structure. (b). Successful atrial septum puncture. (c) Push-and-pull test. (d) Closure with successful release.

including pericardial effusion/tamponade, bleeding, pericarditis, myocardial infarction, access-related complications, renal and hepatic injuries, and device-related complications.

2.5. Statistical Analysis. SPSS 22.0 software was used for the statistical analysis. Estimated frequencies of event occurrences are expressed as percentages or rates. Continuous variables are summarized as the mean and SD.

3. Results

3.1. Baseline Characteristics. The average age of patients in this group, including 9 males and 5 females, was 67.1 ± 9.5 years, and all of them had atrial fibrillation. Nine patients had hypertension before the operation, 4 patients had a history of cerebral infarction, one of the patients had diabetes, and none of the patients had valve disease. The average CHA2DS2VASc score was 3.5 ± 2.1, and the average HASBLED score was 3.6 ± 0.9 (Table 1).

3.2. Primary End Points. TEE-guided LAAO was successfully completed for all patients in the group. The mean LAA width was 23.9 ± 1.9 mm (Table 2), and the mean LAA work length was 22.8 ± 7.2 mm. The mean diameter of the seal

Table 1: Baseline patient characteristics.

	$n = 14$
Age (y)	67.1 ± 9.5
Sex (male/female)	9 (64.3%)/5 (35.7%)
Congestive heart failure/LV dysfunction	5 (35.7%)
Hypertension	9 (64.3%)
Diabetes mellitus	1 (7.1%)
Stroke/TIA/TE	4 (28.6%)
Vascular disease	5 (35.7%)
CHA2DS2VASc score, *n*	
0 to 1	0
2	7
3	2
4	1
5	0
6	1
7	1
8	1
Mean CHA2DS2VASc score	3.5 ± 2.1
Mean HASBLED score	3.6 ± 0.9

LV, left ventricular; TIA, transient cerebral ischemic attacks; TE, thromboembolism.

Table 2: Procedure findings.

	$n = 14$
TEE assessment	
LAA ostium width (mm)	23.9 ± 1.9
LAA work length (mm)	22.8 ± 7.2
Diameter of seal plate of the devices (mm)	27.7 ± 3.3
Number of devices, *n*	1

LAA, left atrial appendage; TEE, transesophageal echocardiography.

plate of the devices was 27.7 ± 3.3 mm. All of the devices were implanted successfully using the first choice with no changes to other types of occluders. Because some of the procedures were combined with radiofrequency ablation of atrial fibrillation, the plugging time alone could not be calculated accurately. Simple percutaneous closure of the LAA without radiofrequency ablation of atrial fibrillation could be completed within 30 minutes.

3.3. Secondary End Point. No complications that seriously affected a patient's life occurred during the perioperative period. During the operation, there was one patient who developed a reaction to the anesthesia (nausea and vomiting after surgery) (Table 3). The endotracheal tube was removed immediately after the completion of surgery. Postoperative TEE reexamination showed successful closure of the LAA in all patients with no flow or minimal residual flow of 2 mm in one patient. The mean postoperative hospitalization time was 4.5 ± 1.3 days.

4. Discussion

Patients with atrial fibrillation (AF), which is the most common arrhythmia, have an increased risk for stroke [1] ranging from 2% to >10% per year, depending on additional risk factors [9]. As a result, AF is responsible for 15% to 20% of all ischemic strokes [10].

Table 3: Perioperative complications.

	$n = 14$
Major complications	0 (0%)
Cardiac tamponade	0
Stroke	0
Myocardial infarction	0
Device dislocation	0
Malignant arrhythmia	0
Death	0
Minor complications	2 (14.3%)
Pericardial effusion	0
Residual shunt	0
Thrombus	0
Access-related complications/hematoma	1
Anesthesia reaction	1
Pericarditis	0
Renal and hepatic injuries	0
Device-related complications	0
All complications	2 (14.3%)

The mortality and disability rates of atrial fibrillation are high, which seriously threaten the life and quality of life of patients. Warfarin anticoagulation is the first choice of therapy, but due to the existence of anticoagulant contraindications and other factors, some patients refuse drug treatment or are not allowed for drug treatment. According to the statistics, more than 90% of thrombi of patients with nonvalvular atrial fibrillation originate in the left atrial appendage. In recent years, many clinical studies in home and abroad have shown that LAAO can reduce the risk of stroke in patients with atrial fibrillation. A multicenter clinical study of 110 patients showed that treatment with LAAO can reduce the risk of stroke, major bleeding, and death compared with other therapeutic strategies [11].

The European Society of Cardiology Guidelines for managing atrial fibrillation stated in 2016 that LAA occlusion may be considered for stroke prevention in patients with AF and contraindications for long-term anticoagulant treatment (e.g., those with a previous life-threatening bleed without a reversible cause). Class IIb, level B surgical occlusion or exclusion of the LAA may be considered for stroke prevention in patients undergoing thoracoscopic AF surgery. Class IIb, level B surgical LAA occlusion or exclusion concomitant to cardiac surgery has been performed for many decades and with various techniques. Multiple observational studies have indicated the feasibility and safety of surgical LAA occlusion/exclusion, but only limited controlled trial data are available [12–15]. Residual LAA flow or incomplete LAA exclusion can increase stroke risk [16].

Currently, the most commonly used technique in clinical practice is LAAO via percutaneous transcutaneous catheter under the guidance of angiography and TEE. Such an operation process is complex, the operating time is long, and the incidence of complications is relatively high; rates for major complications, including significant pericardial effusion, tamponade, and periprocedural stroke, are reported to be between 3.7% and 7.7% [2, 17]. More importantly,

it increases the exposure time of doctors and patients to X-rays. According to the relevant literature and our accumulated experience in the occlusion of congenital heart disease induced by pure esophageal ultrasound, we believe that the above procedures can be completely replaced by the 2D and 3D functions of TEE, which can demonstrate the shape of the LAA, the diameter of the LAA inlet and waist, and the location of the occlusion device. Radinovic et al. [18] reported the technique of atrial septal puncture guided by pure esophageal ultrasound but did not carry out further left heart operations under pure esophageal ultrasound guidance. Since the beginning of this year, our center has successively developed percutaneous catheter LAAO technology that is completely guided by TEE, avoiding exposure to radiation, shortening the operating time greatly, and reducing perioperative complications.

The regular 2D transesophageal echocardiogram can show each side of the LAA, enabling observation of the presence of a thrombus and measurement of its largest and least diameters and the depth of the LAA. However, there is no imaging advantage for an LAA with a complex structure or different opening forms.

Three-dimensional TEE can be used to quickly obtain a perpendicular LAA section, and the multisection surface can display the diameter of the LAA opening in real time, reduce the steps required during surgery, and shorten the measurement time. It can directly image the complex anatomical structure of the LAA and display its shape, internal structure, and thrombus [19] in 3D images. Therefore, it plays an important role in screening patients, selecting a suitable plugging device, and ensuring the sealing effect.

After the successful release of the plugging device, TEE can evaluate its position and residual shunt at multiple angles and on multiple planes. More importantly, TEE can dynamically display the changes in the above observation indexes during the pushing and pulling experiment in real time.

To summarize, the successful completion of percutaneous transcatheter LAAO, purely TEE-guided atrial septal puncture, and the release of the occluder is the key to the success of this procedure. Since many patients in atrial fibrillation have a right atrium enlargement, and the inferior vena cava and atrial septum are not completely in the same plane, and the normal atrial septum puncture catheter may not succeed in moving the atrial septum to the left atrial surface after it is ultrasonically guided to the atrial septum position, so that the puncture may cause the heart to rupture. We used a modified atrial septal puncture catheter, which has a larger angle and a longer length from the angle to the tip of the catheter. Under the protection of a guidewire and the guidance of TEE, it is easy to create a "tent" structure at the atrial septum. We still choose to puncture the lower part of the atrial septum, so that the guidewire and catheter can enter the LAA more easily. 2D and 3D TEE can monitor the puncture process in real time to ensure the safety of the procedure. Secondly, before releasing the occluder, the conveying sheath should be placed in the upper left pulmonary vein, and it is afterwards guided by a pigtail catheter to the left atrial appendage; this prevents penetration of bleeding from the left atrial appendage. In one patient, the postoperative X-ray suggested a high density shadow in the upper left lungs, considered to be upper left pulmonary vein branch bleeding, but if it were LAA bleeding, it could lead to serious cardiac tamponade.

Berti et al. reported intracardiac echocardiography-(ICE-) guided LAAO [20]. According to their experience, ICE represents a safe and useful ultrasound option for guiding the LAA transcatheter occlusion procedure and for preventing short- and midterm complications. It has the advantage over TEE of not requiring the support of general anesthesia and anesthesiology. But crucially, it still requires fluoroscopy. With the wide applications of ICE, we have reasons to believe that LAAO by the femoral vein pathway can be carried out under local anesthesia with the guidance of pure ICE, with minimal injury to patients in a minimally invasive and green procedure.

4.1. Study Limitations. There were several limitations in our present study. This single-center study was small and nonrandomized. There was no control group. Further study will analyze LAAO guided by pure TEE and fluoroscopy and compare the two methods.

5. Conclusions

LAAO is feasible and safe under the guidance of TEE.

5.1. Impact on Daily Practice. TEE-guided LAAO is safe and reliable, and the role of TEE is most likely to be further explored.

Data Availability

The data used to support the findings of this study are included within the tables of the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Low-Dose Ibutilide Combined with Catheter Ablation of Persistent Atrial Fibrillation: Procedural Impact and Clinical Outcome

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Background. In patients with persistent atrial fibrillation (AF), the procedural and clinical outcomes of ablation combined with infusion of antiarrhythmic drug are unknown. *Objectives*. To determine the impact of low-dose ibutilide after circumferential pulmonary vein isolation (CPVI) and/or left atrial (LA) substrate modification on acute procedural and clinical outcome of persistent AF. *Methods*. In a prospective cohort of 135 consecutive patients with persistent AF, intravenous 0.25 mg ibutilide was administered 3 days before the procedure and intraprocedurally, if required, after CPVI and/or additional LA substrate modification of sites with continuous, rapid or fractionated, and low-voltage (0.05–0.3 mv) atrial activity. *Results*. Persistent AF was terminated by CPVI alone ($n = 15$) or CPVI + ibutilide ($n = 32$) in 47 (34.8%) patients (CPVI responders). Additional LA substrate modification without ($n = 33$) or with subsequent administration of 0.25 mg ibutilide ($n = 19$) terminated AF in another 52 (38.5%) patients (substrate modification responders). Sinus rhythm was restored by electrical cardioversion in the remaining 36 (26.7%) patients (nonresponders). The mean LA substrate ablation time was 14 ± 6 minutes. At follow-up of 24 ± 10 months, the rates of freedom from atrial tachyarrhythmias among the responders in CPVI and substrate modification groups were mutually comparable (66.0% and 69.2%) and higher than among the nonresponders (36.1%; $P < 0.01$). Among the responders, there was no difference in clinical outcome between patients whose persistent AF was terminated without or with low-dose ibutilide. *Conclusion*. Administration of low-dose ibutilide during ablation of persistent AF may allow select patients wherein substrate ablation is not or minimally required to optimize procedural and clinical outcomes.

1. Introduction

Persistent atrial fibrillation (AF) represents a major challenge in catheter ablation of arrhythmias [1]. Although various strategies have been proposed [2–6], the long-term efficacy of ablation remains disappointingly low, particularly in long-standing persistent AF [7–9]. Thus, there is an important unmet clinical need for an individualized and a truly substrate-based approach for ablation of persistent AF.

Recent studies using novel technologies highlighted the diversity of the left atrial (LA) substrate and the importance of specific atrial areas, harboring rotor and focal impulses, in maintaining persistent AF [10, 11]. It is worth noting that these technologies, at their current stage of development, are not widely diffused. Using conventional mapping techniques, on the other hand, it is difficult to identify the key atrial substrate of persistent AF because of the chaotic pattern of atrial activity in most patients. However, this raises an important question: can these key atrial substrates be revealed by conventional mapping potentiated by drugs which have little effect on AF drivers but significantly reduce bystander atrial activities? In a canine model of pacing-

induced sustained AF, Chou et al. found that ibutilide had significant effects on reentrant wave fronts in the pulmonary vein (PV) and PV-LA junction but did not suppress the fast, repetitive, and rapid activities responsible for the maintenance of AF [12]. In addition, a study that included 11 patients with persistent AF suggested that intraprocedural administration of low-dose ibutilide after circumferential PV isolation (CPVI) was effective in organizing AF activity [13]. Yet, the long-term outcomes in persistent AF patients terminated by ablation with or without infusion of low-dose ibutilide remain unknown.

We conducted a prospective study aiming to test three hypotheses: (1) low-dose ibutilide could facilitate acute termination of persistent AF; (2) ibutilide could help to discriminate AF drivers from passive bystander activities and thereby limit ablation of the atrial substrate after CPVI; and (3) acute AF termination by this combined approach could be associated with favorable long-term outcome.

2. Methods

2.1. Study Cohort. The study cohort consisted of 139 consecutive patients who were referred for catheter ablation of symptomatic persistent AF refractory to antiarrhythmic drugs. The inclusion criteria were age between 18 and 80 years, nonvalvular AF, no previous catheter ablation of AF, and availability of informed consent. Patients with any of the following characteristics were excluded from this study: AF with a reversible cause (such as hyperthyroidism); LA diameter ≥55 mm on echocardiography; New York Heart Association class IV heart failure, left ventricular ejection fraction less than 35%, and severe hypertrophic cardiomyopathy (ventricular septal thickness >20 mm); and a baseline-corrected QT interval ≥480 ms. Persistent and long-lasting persistent AF were defined as continuous AF that persisted for >1 week and >1 year, respectively. The study protocol was approved by the institutional review board.

2.2. Preablation Preparation. All antiarrhythmic drugs were discontinued for at least 5 half-lives, and amiodarone was stopped for at least 2 months prior to ablation. Warfarin was discontinued 3–5 days before ablation and was replaced with subcutaneous injections of low-molecular-weight heparin. Transesophageal echocardiography and cardiac-computed tomography were performed 1 day before the procedure to exclude LA thrombi and reconstruct 3-dimensional anatomy of the PVs and LA. Three days before the procedure, 0.25 mg ibutilide was injected intravenously over 3 minutes, and rhythm was monitored for 24 hours in all patients. If persistent AF was terminated by this low dose of ibutilide, the patient was excluded from the study.

2.3. Electrophysiological Study. The procedure was performed under conscious sedation using fentanyl and midazolam, as required. A steerable decapolar catheter (XT™, Bard Electrophysiology, Lowell, MA, USA) was positioned in the coronary sinus via the left femoral vein. After transseptal catheterization, two long sheaths (SL1, St. Jude Medical, MN, USA) were advanced into the LA and flushed with continuous injection of saline (20 mL/h) to avoid thrombus formation or air embolism. During the whole procedure, the activated clotting time was maintained between 250 and 300 seconds by intravenous administration of heparin. The surface electrocardiogram (ECG) and bipolar endocardial electrograms were continuously monitored and recorded with a computer-based digital amplifier and recording system (Bard Electrophysiology, Lowell, MA, USA).

After PV angiography, the AF cycle length (AFCL) was recorded at baseline within each PV and left atrial appendage (LAA) using a decapolar circular catheter (Lasso™, Biosense Webster, Diamond Bar, CA, USA). Three-dimensional electroanatomical LA reconstruction was performed by using the CARTO 3 system (Biosense Webster, Diamond Bar, CA, USA), and ablation was performed with a 3.5 mm-tip irrigated catheter (TheromoCool NaviStar, Biosense Webster, Diamond Bar, CA, USA).

2.4. Ablation Protocol. The ablation protocol is shown in Figure 1. Briefly, the first step was CPVI in all patients. If persistent AF converted to sinus rhythm (SR) during CPVI, no further ablation was performed. If AF continued, 0.25 mg ibutilide was administered over 3 minutes, and AFCL within the LAA was recorded every 5 minutes, thereafter. If AF converted to SR within a 30-minute period, no further ablation was performed. In patients with SR, a voltage map of the LA was created and the percentage of areas with low-amplitude (<0.5 mV) atrial signals was calculated. When AF continued, the LA substrate was ablated (see descriptions below) until it terminated or until the total ablation time reached 30 minutes, whichever was earlier. In the latter situation, a second dose of 0.25 mg ibutilide was injected over 3 minutes, and the patient was observed for another 30 minutes. Unless AF was terminated within 30 minutes, SR was restored by electrical cardioversion. If AF converted to AT anytime during the procedure, it was mapped and ablated until SR was restored. Finally, the completeness of the 2 circular lines each surrounding the ipsilateral PVs was checked by bolus injection of 20 mg adenosine triphosphate in all patients. If a patient received any atrial linear lesions for macro-reentrant ATs, bidirectional conduction block across the lesions was confirmed by using the differential pacing maneuver.

Radiofrequency energy was delivered from the Stockert generator at a temperature setting of 43°C, with power limited to 30 to 35 W at an irrigation speed of 17 mL/min in the LA and to 20 to 25 W at an irrigation speed of 30 mL/min within the coronary sinus.

2.5. Target Sites for LA Substrate Modification. The sites with the following characteristics were tagged and ablated: (a) continuous (>5 s), low-amplitude (0.05–0.3 mV), and fractionated atrial electrograms [3] and (b) local atrial deflections more rapid than those from the adjacent sites (Figure 2).

2.6. Repeat Ablation Procedure. If an atrial tachyarrhythmia recurred, ablation was repeated at least 3 months after the

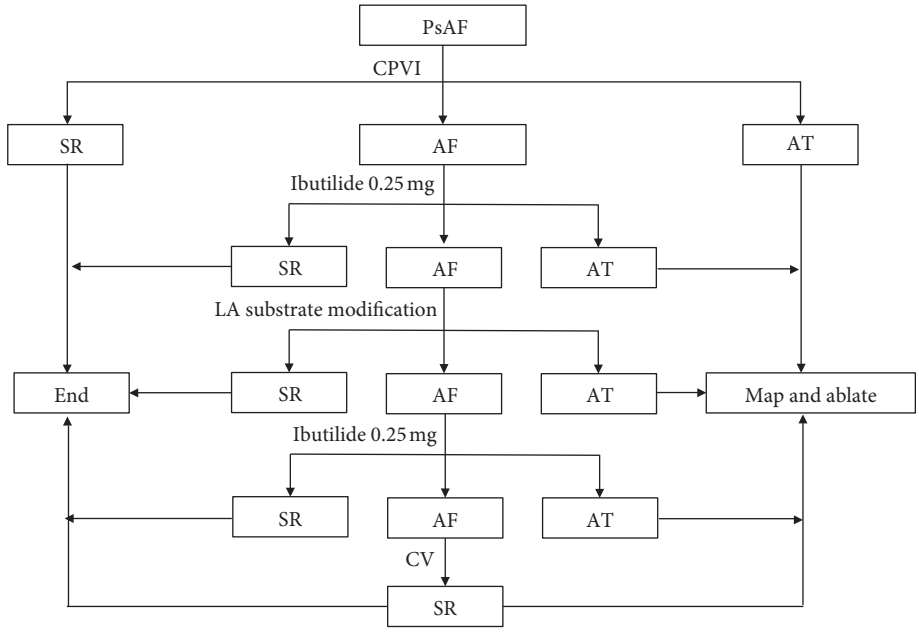

Figure 1: The ablation protocol used in this study. PsAF = persistent atrial fibrillation; SR = sinus rhythm; AT = atrial tachycardia; LA = left atrial; CV = cardioversion.

index procedure. During the repeat procedure, the PVs were checked for recovery of conduction and reisolated if they were reconnected. Isoproterenol and/or adenosine triphosphate were used to trigger non-PV foci in patients with recurrent paroxysmal AF. If the recurrent AF was unrelated to PV, the LA substrate was ablated during the repeat procedure. All recurrent ATs were also mapped and ablated.

2.7. Postprocedural Care and Follow-Up. Antiarrhythmic medications were resumed postablation, and patients were monitored inhospital for 2 to 3 days prior to discharge. Arrhythmias recurring within a 3-month blanking period after ablation were cardioverted, if required. Follow-up at the outpatient clinic was scheduled for 2 weeks and 1, 3, 6, 9, and 12 months after the procedure and 6 monthly thereafter. Twelve-lead ECG was obtained at each visit, and serial 72-hour Holter was undertaken at 6, 12, 18, and 24 months. Additional 24-hour Holter or ECG was undertaken in patients reporting with symptoms. Recurrence was defined as documented AF/AT lasting >30 s after the 3-month blanking period.

Postprocedural anticoagulation was continued for 6 months in the absence of arrhythmia recurrence or for a longer period based on the CHA_2DS_2-VASc score. Repeat ablation was encouraged in patients with recurrent AF/AT beyond the blanking period.

2.8. Statistics. All data are reported as a mean ± SD for continuous variables and number of subjects (%) for categorical variables unless otherwise indicated. For baseline demographics and procedure parameters, continuous variables were compared using ANOVA with the modality by which SR was achieved during the procedure as the factor and categorical variables were compared using Fisher's exact test. Changes in AFCL were compared using paired *T*-test. Freedom from atrial tachyarrhythmias was determined and compared using the Kaplan–Meier analysis and the log-rank test. To identify independent predictors of termination of AF during ablation and of clinical success, multivariate logistic regression was conducted, with only covariates. All hypotheses were 2-sided tests with a type I error level set to 0.05.

3. Results

3.1. Patient Characteristics. Among consecutive 139 eligible patients, administration of ibutilide 3 days before the ablation procedure terminated persistent AF in 4 (2.8%), who were, therefore, excluded from the study. The baseline characteristics of the remaining 135 patients are presented in Table 1.

3.2. Acute Outcome of CPVI. The baseline AFCL within the LAA, left superior PV, left inferior PV, right superior PV, and right inferior PV were 148 ± 21 ms, 147 ± 22 ms, 145 ± 19 ms, 151 ± 24 ms, and 147 ± 21 ms, respectively. All PVs were isolated during AF. During CPVI, persistent AF converted directly to SR in 7 patients (5.2%) and organized to typical atrial flutter in 8 patients (5.9%). AF persisted in the remaining 120 (88.9%) patients. Pre- and post-CPVI and AFCLs within the LAA were similar (148 ± 21 ms vs. 149 ± 20 ms, $P = 0.689$).

3.3. Effect of Intraprocedural Ibutilide after CPVI. After CPVI, persistent AF was converted into SR in 27/120 patients and organized to AT in 5/120 patients within 30 minutes of ibutilide infusion. In the remaining 88/120

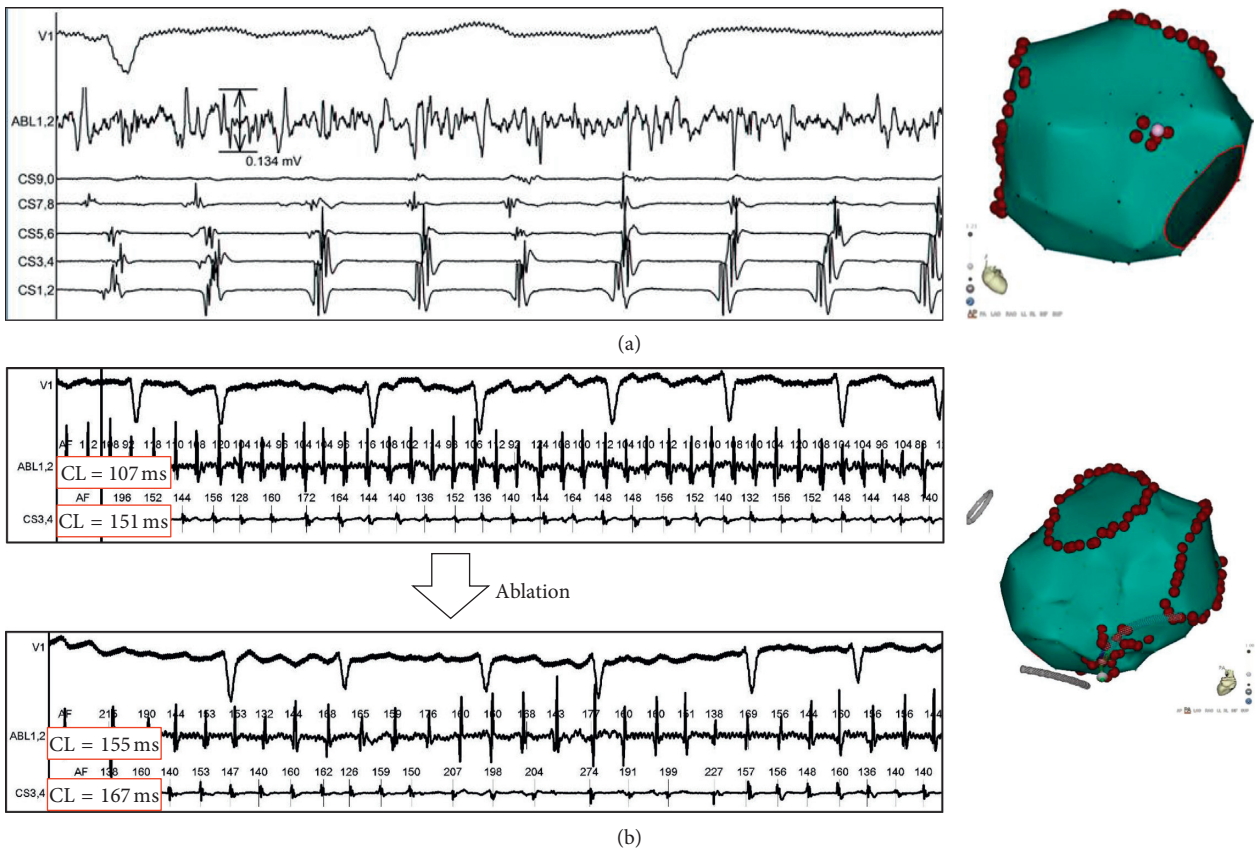

Figure 2: Examples of ablation targets. (a) Surface ECG lead V1, intracardiac recordings from an ablation catheter (abl), and a mapping catheter within the coronary sinus (CS). Note that, after circumferential pulmonary vein isolation (CPVI) and ibutilide, the atrial activity with CS had become very organized; however, an area on the anterior wall of the left atrium (LA) still presented with continuous, low-voltage (0.134 mV), and fractionated atrial electrograms. (b) Surface ECG lead V1 and intracardiac recording from an ablation catheter and CS 3-4 electrodes. After CPVI and ibutilide, the local atrial fibrillation cycle length (AFCL) was 107 ms on the lower part of the posterior wall of the LA, whereas the AFCL recorded by the adjacent CS catheter was more rapid (151 ms) (top). After ablation, the local AFCLs at the ablation site and CS were prolonged to 155 and 167 ms, respectively (bottom).

Table 1: Patient characteristics.

	Total (n = 135)	CPVI responders (n = 47)	LA substrate responders (n = 52)	Nonresponders (n = 36)	P value
Age, years	63 ± 10	62 ± 12	63 ± 9	63 ± 10	0.913
Male, n (%)	85 (63.0)	26 (55.3)	31 (59.6)	28 (77.8)	0.090
BMI	26 ± 5	26 ± 5	26 ± 5	27 ± 3	0.873
Hypertension, n (%)	95 (70.4)	29 (61.7)	38 (73.1)	25 (69.4)	0.470
Underlying heart disease, n (%)	25 (18.5)	9 (19.1)	12 (23.1)	4 (11.1)	0.361
Diabetes mellitus, n (%)	31 (23.0)	6 (12.8)	18 (34.6)	7 (19.4)	0.030
History of stroke, n (%)	13 (9.6)	3 (6.4)	8 (15.4)	2 (5.6)	0.198
Atrial fibrillation duration					
Duration (month)	14 ± 18	8 ± 14	13 ± 14	23 ± 24	0.001
Short lasting/long lasting/ uncertain (n)	76/40/19	32/7/8	26/19/7	18/14/4	0.1064
CHA_2DS_2-VASc score	2.4 ± 1.5	2.3 ± 1.5	2.6 ± 1.5	2.3 ± 1.5	0.402
HAS-BLED score	1.3 ± 0.8	1.2 ± 0.8	1.4 ± 0.8	1.3 ± 0.6	0.352
LA volume (ml)	123 ± 29	111 ± 24	124 ± 28	138 ± 29	0.000
Left ventricular diastolic diameter (mm)	48 ± 5	47 ± 5	48 ± 5	50 ± 5	0.036
Left ventricular ejection fraction (%)	62 ± 10	62 ± 10	62 ± 10	62 ± 8	0.963

Values are presented as mean ± SD or as n (%). CPVI: circumferential pulmonary vein isolation; LA: left atrium; BMI: body mass index.

(73.3%) patients, LAA-AFCL was significantly prolonged. The AFCL prolongation started at the end of the infusion (0 min, 161 ± 23 ms, $P < 0.05$) and peaked at 5 or 10 minutes later (173 ± 23 and 173 ± 24 ms, respectively; paired T-test $P < 0.05$ for both). It shortened insignificantly thereafter within 30 minutes.

3.4. LA Voltage Map in SR. An LA voltage map in SR was obtained for 39/47 patients in whom persistent AF was terminated by CPVI alone or combined with ibutilide. Low-voltage (<0.5 mV) areas were found in 6 patients, covering mean 15 ± 6% of the LA surface. In others, the LA voltage map did not show any area of low voltage.

3.5. Acute Outcome of LA Substrate Ablation without and with Ibutilide. Ablation terminated persistent AF in 33/88 (37.5%) patients, restoring SR directly in 12/88 (13.6%) patients and via intermediate AT in 21/88 (23.9%) patients. The AF termination sites were posteroinferior LA ($n = 10$), LA septum ($n = 6$), LAA ($n = 4$), anterior LA ($n = 4$), posterior LA ($n = 3$), lateral LA ($n = 3$), and LA roof ($n = 3$). Figures 3 and 4 show 2 different examples of LA substrate ablation leading to termination of AF. In 55 (62.5%) patients, whose AF persisted after 30 minutes of LA substrate ablation, a second bolus of 0.25 mg ibutilide restored SR in 11 (12.5%) patients and converted AF to AT in 8 (9.1%) patients. AF was electrically cardioverted in the remaining 36 (40.9%) patients.

In total, intraprocedural AF termination by ablation ± ibutilide was achieved in 99/135 (73.3%) patients. The ablation times for CPVI and LA substrate modification were 28 ± 10 min and 14 ± 6 min, respectively. Other procedural parameters are summarized in Table 2.

3.6. Mapping and Ablation of Intermediate ATs. Totally, 49 ATs were encountered in 46 (34.1%) patients. The most common AT was cavotricuspid isthmus-dependent flutter ($n = 34$, 57.6%), followed by LA roof-dependent flutter ($n = 10$, 16.9%), perimitral flutter ($n = 10$, 16.9%), and focal AT ($n = 5$, 5.7%). All but 4 ATs were terminated by ablation. Cardioversion was performed in 4 patients with perimitral flutter, and further ablation of mitral isthmus was performed during pacing from the distal coronary sinus. The bidirectional block across the linear lesions was achieved in all patients who underwent linear ablation.

3.7. Long-Term Clinical Outcome. At 12 months and 24 ± 10 months after the index procedure, freedom from atrial tachyarrhythmias off antiarrhythmic drugs was achieved in 98 (72.6%) and 80 (59.3%) patients, respectively. Recurrent arrhythmias included persistent AF, paroxysmal AF, persistent AT, and persistent AF alternating with AT in 18, 15, 7, and 15 patients, respectively.

The study population was divided into 3 groups as follows: CPVI responders (SR/AT following CPVI without or with ibutilide, $n = 47$), LA substrate responders (SR/AT following LA substrate ablation without or with ibutilide, $n = 52$), and nonresponders (electrical cardioversion of AF, $n = 36$). The clinical characteristics of each of these groups are shown in Table 1. The duration of persistent AF and the LA volume were significantly greater in the nonresponders than in the responders, suggesting a more severe arrhythmogenic substrate in nonresponders.

The 24-month success rates were comparable between the CPVI and LA substrate responders (66% versus 69.2%, $P = 0.830$), but significantly higher than that of the nonresponders (36.1%, $P < 0.001$, Figure 5). Among the CPVI responders, the 2-year success rates did not differ between the patients who received and who did not receive intraprocedural ibutilide (62.5% versus 73.3%, $P = 0.528$). Similarly, in the LA substrate responders' group, the 2-year success rates did not differ between the patients who received and who did not receive ibutilide (73.7% versus 66.7%, $P = 0.758$).

3.8. Redo Procedures and Outcome. A total of 23/135 (17%) patients underwent a second ablation procedure, and 1 of them had 3 ablation procedures. After the last procedure, 14/23 (60.9%) patients were in stable SR at mean 21 ± 15 months of follow-up.

3.9. Periprocedural Complications. A severe left hemothorax occurred in 1 patient just after ablation. Thoracocentesis relieved the symptoms without any sequelae. Hematoma in the right groin was observed in 4 (3%) patients. Ibutilide induced premature ventricular complexes with right bundle branch block morphology during the procedure in 20 (14.8%) patients but no ventricular tachycardia up to 24 hours after procedure. No cardiac tamponade, stroke, or atrial-esophageal fistula were observed.

3.10. Predictors of Long-Term Outcome after the Index Ablation Procedure. Multivariate logistic regression analysis identified small left atrial volume (OR 0.976, 95% CI 0.956–0.996, $P = 0.020$) and intraprocedural AF termination (OR 7.675, 95% CI 2.362–24.943, $P = 0.001$) as the independent predictors of long-term outcome after the index ablation procedure.

4. Discussion

In this study, we systematically investigate the role of intraprocedural administration of low-dose ibutilide during catheter ablation for persistent AF. It confirms that (1) intraprocedural termination of persistent AF is associated with a favorable long-term outcome and demonstrates that low dose of ibutilide may extend important benefit and (2) the long-term outcome was similar between patients in whom AF was terminated by ablation alone or in combination with intraprocedural ibutilide.

4.1. Use of Low-Dose Ibutilide in Persistent AF Ablation. The clinical indication for administering ibutilide is recent-onset AF or atrial flutter with a routine dose of 1 to 2 mg [14].

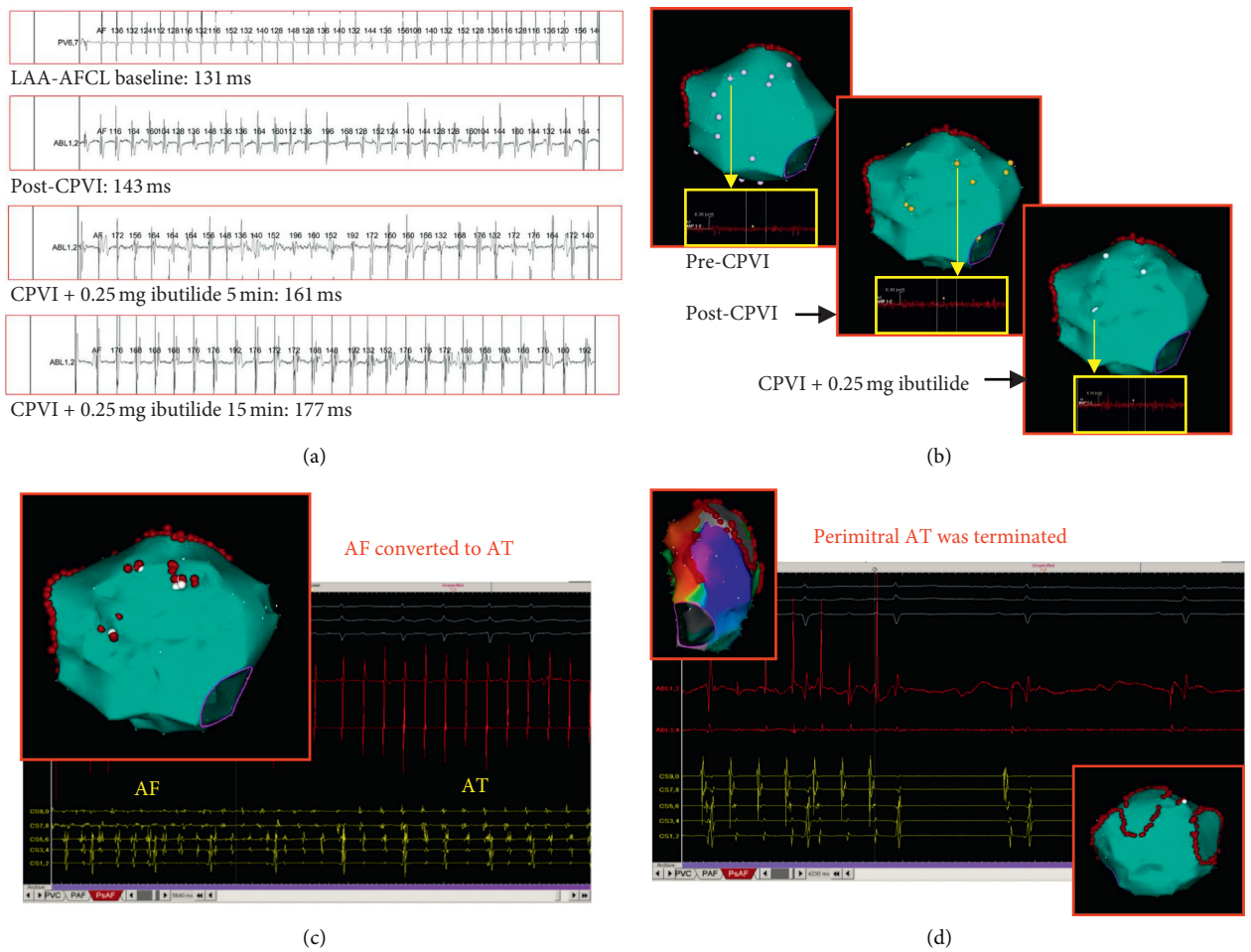

Figure 3: An example of persistent atrial fibrillation (AF) ablation with this low-dose ibutilide-guided approach. (a) The AF cycle length (AFCL) within the left atrial appendage (LAA) at baseline, after circumferential pulmonary vein isolation (CPVI), and 5 and 15 minutes after administration of 0.25 mg ibutilide. (b) Three electroanatomic maps showing the sites where low-amplitude complex fractionated atrial electrograms (CFAEs) were recorded on the anterior wall of the left atrium (LA). The pink, yellow, and white points represent CFAEs recorded before CPVI, after CPVI, and after administration of 0.25 mg ibutilide, respectively. The CFAE areas were minimized after CPVI plus ibutilide. (c) Ablation targeting these areas converted AF to atrial tachycardia (AT). (d) The 3D electroanatomic mapping of LA suggested that the mechanism of this AT was perimitral reentrant tachycardia (left upper), and ablation (alone) at the mitral isthmus terminated this AT.

In a dose-response study, Ellenbogen et al. found that AF termination could only be achieved in 12% of such patients if a very low dose (0.005 mg/kg) of ibutilide was used [15]. In the current study, the AF termination rate using 0.25 mg (≈0.004 mg/kg for a body weight of 60 kg) of ibutilide, 3 days before the procedures, was only 2.9% ($n = 4$). Of note, the AF was not recent-onset in this study. Interestingly, when the same dose of ibutilide was used intraprocedurally again after 3 more days (beyond >5 half-lives (2–12, mean 6 hours)), the AF termination rate increased to 23.7% ($n = 32$) after CPVI and additionally by 14.1% ($n = 19$) after both CPVI and LA substrate modification. These results suggest that ablation can modify the atrial substrate facilitating termination of persistent AF with low-dose ibutilide.

4.2. Acute Intraprocedural AF Termination and Long-Term Outcome. In line with most studies of persistent AF ablation, this study showed a clear difference in long-term outcome between patients with and without intraprocedural AF termination [7]. Failure to terminate persistent AF during ablation could indicate that the critical substrate was not being targeted by ablation most likely because it was not identified [16, 17]. On the other hand, ablation targeting those sites presenting with low-dose ibutilide refractory atrial activities which can acutely terminate persistent AF in 59.1% (52/88) of patients suggests that these sites are potentially the areas harboring drivers of persistent AF. Also, for patients treated with either CPVI or CPVI plus LA substrate ablation, an important finding of this study is that the long-term outcome was similar between patients in whom AF was terminated by ablation or by ablation combined with ibutilide. This means that the atrial substrate "erased" by low-dose ibutilide was not critical to the maintenance of persistent AF. This role of ibutilide in discriminating AF drivers from passive bystander activities

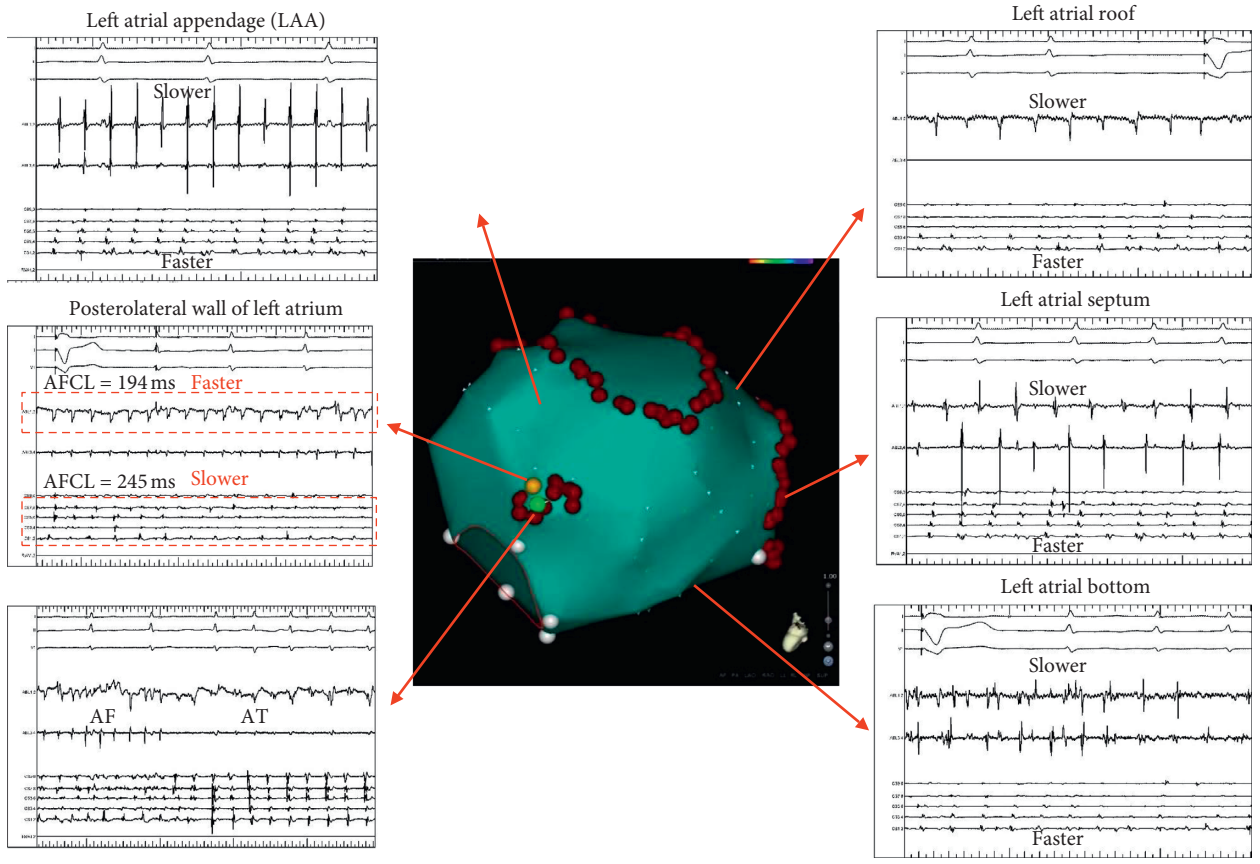

Figure 4: Ablation of the site with the most rapid atrial fibrillation cycle length (AFCL) uncovered by low-dose ibutilide administration terminated persistent AF. The middle figure is the 3D electroanatomic map of the left atrium (LA) in this patient. The red points represent the lesions surrounding the PV antrum. After administration of 0.25 mg ibutilide, the LA activity became organized, and the AFCL could be measured at most of the sites in the LA. From top to bottom on the right panel are the intracardiac recordings (abl) from the LA roof, septum, and bottom. The activity at these sites and in the LA appendage (left upper) was longer than that in the coronary sinus. However, the AFCL at the posterolateral aspect of the LA (left middle, yellow point) was significantly shorter than that in the CS (194 vs 245 ms), suggesting that this area was harboring an AF driver. During regional ablation of this small region, AF converted to atrial tachycardia (AT, green point).

Table 2: Procedural parameters.

	Total (n = 135)	CPVI responders (n = 47)	LA substrate responders (n = 52)	Nonresponders (n = 36)	P value
Ablation time (sec)					
Total ablation time (sec)	2542 ± 783	1981 ± 566	2860 ± 736	2813 ± 702	<0.001
RPV ablation time (sec)	808 ± 249	813 ± 255	805 ± 260	809 ± 228	0.984
LPV ablation time (sec)	897 ± 272	955 ± 281	851 ± 249	911 ± 294	0.259
LA ablation time (sec)	812 ± 384		777 ± 403	862 ± 355	0.319
Fluoroscopy time (min)	23 ± 7	22 ± 7	22 ± 7	25 ± 7	0.084
Fluoroscopy dose (mGy)	303 ± 152	287 ± 132	305 ± 179	323 ± 133	0.561
Saline (ml)	1012 ± 277	804 ± 224	1162 ± 257	1065 ± 195	<0.001
LAA baseline AFCL (ms)	148 ± 21	159 ± 23	145 ± 16	138 ± 18	<0.001
LAA maximal AFCL (ms)	186 ± 25	213 ± 25	185 ± 20	172 ± 17	<0.001

Values are presented as mean ± SD. RPV: right pulmonary vein; LPV: left pulmonary vein; LA: left atrium; LAA: left atrial appendage; AFCL: atrial fibrillation cycle length.

raises an intriguing hypothesis, where ibutilide may be considered to affect preferentially a functional and reversible part of the substrate, such as electrical remodeling. In this study, the mean ablation time taken for LA substrate modification was only 14 minutes, suggesting that the combined approach limited the atrial damage. It underscores the desirable influence of ibutilide in limiting the ablation of the atrial substrate after CPVI.

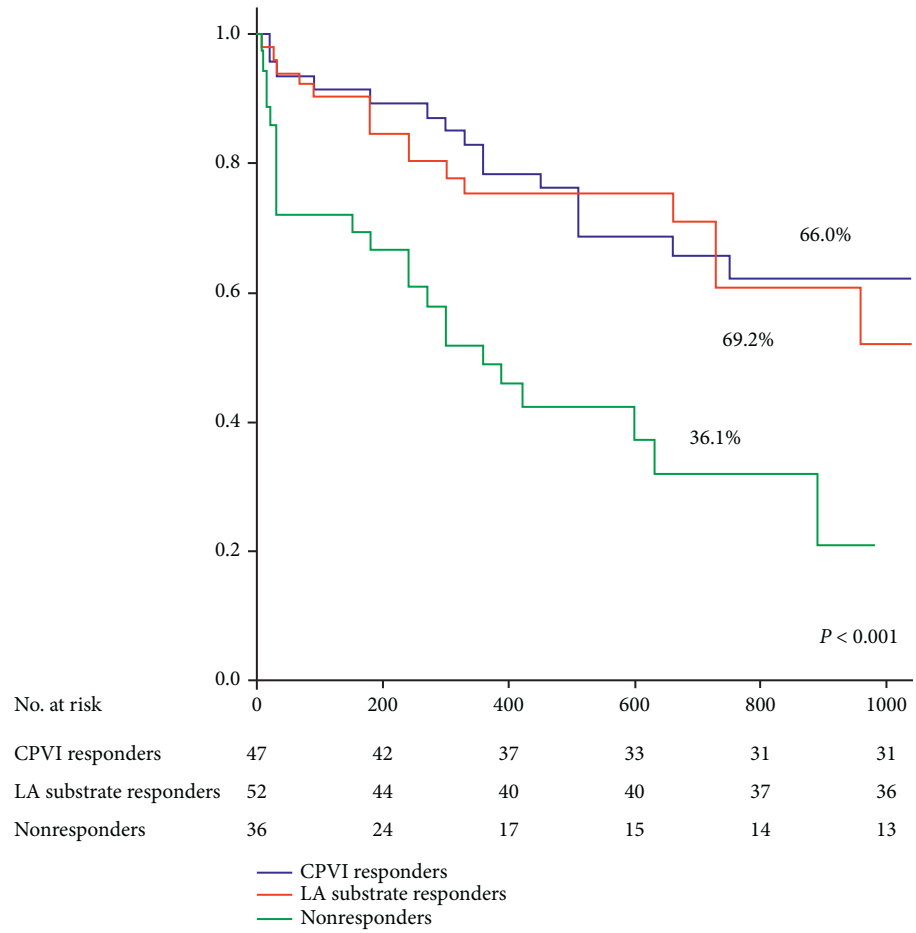

Figure 5: Kaplan–Meier plots depicting atrial tachyarrhythmia-free survival at 2 years after the first procedure. Persistent atrial fibrillation (AF) was terminated by circumferential pulmonary vein isolation (CPVI) alone (blue line), CPVI combined with left atrial modification guided by low-dose ibutilide (red line), and cardioversion (CV) (green line).

4.3. Identification of Patients in Whom CPVI May Be Enough. Recently, the STAR AF II trial reported that a CPVI alone approach is effective in 41% of patients with persistent AF [18]. The question, however, is how to identify these patients intraprocedurally. In the present study, CPVI without and with ibutilide terminated persistent AF in 1/3 of study population, and more importantly, the follow-up data, showed that the rate of freedom from atrial tachyarrhythmias among these patients was 83% at 12 months and 66% after 2-year-long follow-up, which supports the hypothesis that further LA substrate ablation is not be required in this subset of patients. Notably, in the CPVI responders' group, there was no difference in the 2-year success rate between patients whose PsAF was terminated without or with ibutilide, suggesting that low-dose ibutilide avoided LA substrate ablation in 32 more patients actually.

4.4. Localization of the Key LA Substrate by Using Low-Dose Ibutilide. Considering that there is substantial variability in the characteristics of atrial substrate and that the majority of complex fractionated atrial electrogram are actually functional [19], an individualized approach targeting the key atrial drivers, such as rotors and foci, has gained interest recently [10, 11]. Although the ability of conventional bipolar electrograms to diagnose such key driver locations remains subpar, some of the characteristics have been regarded as indicators of driver activity [20]. In the current study, we found that 0.25 mg ibutilide significantly prolonged the AFCL by organizing atrial electrograms in several areas, possibly the bystander sites. The sites presenting continuous, rapid or low-voltage, and fractionated activity were regarded as key locations of AF drivers. Identification of limited atrial sites with characteristic signals as ablation targets favourably reduced the extent and duration of substrate ablation to achieve AF termination.

4.5. Limitations. There are three main limitations of this study. First, the right atrium was not mapped after administration of ibutilide. Recent studies have shown that the right atrium harbors about 30% of drivers of persistent AF [12, 13]. Second, this is a single-center study and its findings need to be reproduced in a multicenter trial. However, its sample size is quite large, and the duration of follow-up is aptly long. Currently, such a multicenter study utilizing biatrial mapping is underway. Finally, we only monitored the AFCL in the LAA after CPVI. If we also checked the

AFCL in other atrial sites simultaneously, such as right atrial appendage, the following atrial substrate mapping process may be facilitated.

5. Conclusion

In patients undergoing ablation of persistent AF, intraprocedural use of low-dose ibutilide may allow select patients in whom additional substrate ablation after CPVI can be avoided or reduced without compromising the long-term clinical success.

6. Clinical Perspectives

During catheter ablation of persistent atrial fibrillation (AF), it is difficult to identify the key atrial substrate of AF because of the chaotic pattern of atrial activity in most patients. In this prospective study enrolling 135 patients with persistent AF, we have systematically tested the hypotheses that intraprocedural low-dose (0.25 mg) ibutilide would distinguish between the patients who will do well just with circumferential pulmonary vein isolation (CPVI) versus those who would require additional atrial substrate ablation. In the latter group, low-dose ibutilide could unravel the key AF driver locations from passive bystander sites. Persistent AF was terminated in 99/135 (73.3%) patients using the combination of "ablation + ibutilide" approach. Notably, left atrial ablation was not required in 47 (34.8%) patients to terminate AF and in other 52 (38.5%) patients, where it was required, and its duration was reduced to mean 14 minutes. After 2-year-long follow-up, freedom from atrial tachyarrhythmias was similar between 52 patients who required it and 47 patients, who did not (69.2% and 66%). In patients wherein the combination approach did not result in AF termination ($n = 36$), the long-term arrhythmia freedom was significantly worse (36.1%; $P < 0.01$). Thus, combining the two may allow selection of a large subset of patients in whom CPVI is good enough and atrial ablation is unnecessary. Also, in patients who remain to be the candidates for atrial ablation, the potentiating effect of ibutilide on ablation facilitates AF termination with minimal duration of atrial ablation. All acute effects of ibutilide-facilitated ablation translate similarly into long-term clinical outcome.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Drs. Sun and Tian contributed equally to this work.

Acknowledgments

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Research Article

Influence of Continuous Training on Atrial Myocytes I_{K1} and I_{KAch} and on Induction of Atrial Fibrillation in a Rabbit Model

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Background. Elucidation of mechanisms underlying continuous training-related atrial fibrillation (AF) may inform formulation of novel therapeutic approaches and training method selection. This study was aimed at assessing mechanisms underlying continuous training-induced AF in an animal model. *Methods*. Healthy New Zealand rabbits were divided into three groups (n=8 each), namely, control (C), and moderate intensity (M), and high intensity (H) continuous training according to treadmill speed. Atrial size andintrinsic and resting heart rates were measured by transthoracic echocardiography before, and 8 and 12 weeks after training. Using a Langendorff perfusion system, AF was induced by S1S2 stimulation and the induction rate was recorded. Atrial I_{K1} and I_{KAch} ion current densities were recorded using whole-cell patch-clamp technique in isolated atrial myocytes. Changes in atrial Kir2.1, Kir2.2, Kir3.1, and Kir3.4 mRNA expression were assessed by reverse transcriptase-coupled polymerase chain reaction. *Results*. After 8 and 12 weeks, Groups M and H vs. Group C had greater (all $P < 0.05$) atrial anteroposterior diameter; greater incidence of AF (60% and 90% vs. 45%, respectively; $P < 0.05$, also between Groups H and M); and greater atrial IK_{Ach} current density. In Group H, Kir2.1 and Kir2.2 mRNA expression in the left and right atria was increased ($P < 0.05$, vs. Groups C and M) as was left atrial Kir3.1 and Kir3.4 mRNA expression ($P < 0.05$, vs. Group C). *Conclusion*. In a rabbit model, continuous training enlarges atrial diameter leading to atrial structural and electrical remodeling and increased AF incidence.

1. Background

Atrial fibrillation (AF), a common cardiac arrhythmia, is associated with increased risk of stroke, heart failure, and death [1]. Among athletes, AF has an incidence of about 0.2%–0.3%, which is greater than that in the general population [2]. Continuous (vs. interval) training remains common and can induce AF via the autonomic nervous system and atrial structural changes (dilatation and fibrosis) secondary to chronic volume and pressure overload [3]. In the human heart, atrial stretching might result in excessive I_{K1} currents, likely decreasing the effective refractory period, thereby initiating and/or maintaining AF [4]. I_{KACh} is crucial in AF generation as shown in a murine I_{KACh}-deficient knockout model [5].

Improved understanding of the mechanisms of continuous training-related AF may help in the development of novel therapeutic approaches [6] and in training method selection. In current study, we designed a rabbit model to test if continuous training can increase AF vulnerability and the potential mechanisms including changes in ion channel currents and gene expression.

2. Methods

2.1. Ethics Committee Approval. Animal welfare and the relevant experiment were carried out in compliance with the guide for the care and use of laboratory animals. And the

experiment was approved by the Ethics Committee of the PLA Army General Hopsital.

2.2. Animal Model and Experimental Protocol. This study was approved by the institutional animal research ethics committee and conformed to the Guide for the Care and Use of Laboratory Animals. Forty-two pathogen-free New Zealand rabbits weighing 2.5 to 3.0 kg were fed pellet rabbit diet adlibitum and had free access to water. The rabbits were randomly divided into three groups (initially $n = 14$ each, finally $n = 8$ each after eliminating rabbits unwilling to exercise): control(C); and moderate- (M-) and high- (H-) intensity continuous training. Exercise training was performed on a low-speed, levelled, motorized treadmill; the treadmill was fabricated in the Integrated runway and divided into four tracks by using a dummy plate; it was a total volume of 405 L with a total length of 150 cm, height 30 cm, and width 90 cm to keep running the four in sync; it was automatic and adjustable with the angle of 0~35° and speed of 0~67 cm/s (the rabbit running platform, which had not been reported so far, was improved based on Gaustad treadmill using a high-powered motor drive and a concurrent four runway operation by Beijing Zhi Bao Biotechnology Co. Ltd. production of rats, Figure 1). The training program was preceded by a 1-week period of adaptation to the treadmill exercise, with 30 min running time and 25 cm/s treadmill speed. Exercise training consisted [7–9] of a 12-week period of running at a speed of 25 cm/s (Group M) and 50 cm/s (Group H) for 60 min (or one-time exhaustive exercise less than 1 h) 5 days per week; all rabbits were tested at inclination ranging 0°; Group C did not do any exercise. Investigators observed the treadmill sessions daily to ensure effective running.

2.3. Echocardiography. Transthoracic echocardiographic studies were performed with a phased-array probe 10S (4.5–11.5 Megahertz) in an IE 33 system and under 0.5% sodium pentobarbital anesthesia. Measurements were made at weeks 0, 8, and 12. The atrial anteroposterior diameters were recorded.

2.4. Electrophysiological Study. At the end of the study period, all rabbits were anaesthetized and sacrificed with 3% pentobarbital at 30 mg/kg. After midsternal thoracotomy, the heart was rapidly removed and placed in cold perfusion fluid (0°C–10°C). The aorta was cannulated and connected to a Langendorff perfusion system filled with warmed (37°C) Krebs-Henseleit's solution. The composition of the perfusion fluid was (in mM) as follows: NaCl 118, $NaHCO_3$ 25, KCl 2.8, $CaCl_2$ 2.5, $MgSO_4$ 0.5, KH_2PO_4 1.2, Na_2EDTA 0.57, pyruvic acid 2.0, and glucose 5.5; it was gassed with a mixture of 95% O_2/5% CO_2, and pH was kept at 7.35–7.45. The perfusion pressure was maintained at 65 mmHg, with an initial coronary flow of 20 ml/min. To test the inducibility of AF and measure atrial effective refractory periods (AERPs), electrical stimulation was performed with a bipolar electrode attached to the right atrial appendage. The noncontact 12-lead electrocardiogram (ECG) recording system with Wilson terminal was used to record the ECG signals. These signals then were processed using the Biopac ECG amplification system and stored in the computer. ADP90 (the action potential duration of cardiac repolarization of 90%) was automatic analysis with CED Spike II analysis software.

AERPs were measured during sinus rhythm at a S1S2 programmed ectopic stimulation [10, 11]. After an eight-beat train (S1-S1), a single premature stimulus (S2) was delivered. The S1-S2 coupling interval was decreased in 2 ms steps, starting with 120 ms. The shortest S1-S2 interval resulting in a propagated response was defined as an AERP.

Then, AF was induced by S1S2 incremental stimulation (Figure 2), with the cycle length starting from AERPs. Stimuli of 3.3 Hz, 3 ms pulse duration, and threefold diastolic pacing threshold were delivered. The measurement was repeated five times, and episodes of AF were recorded. AF was defined as >1 second of irregular atrial electrograms with irregular ventricular response. Sustained AF (lasting >1 minute) was terminated by bursts.

Following these baseline measurements, one group of pharmacologic agents was added to the perfusate incontinuous infusion for 15–20 minutes and the experiment was repeated. The groups were as follows: acetylcholine (Ach 1 μM/L), Ach+atropine (0.0001, 0.0005, 0.001, and 0.002 mg/ml), and Ach + barium chloride ($BaCL_2$ 100 and 500 nM/L and 1, 3, and 6 μM/L).

2.5. Patch Clamp Technique. After the electrophysiological study (EPS), the hearts were perfused on a Langendorff apparatus at 37°C by pumping with Tyrode's solution (calcium, 1.8 mmol/L). Following a 5 min perfusion with calcium-free Tyrode's solution, the enzyme solution (50 ml Tyrode's solution with 20 mg type II collagenase and 2 mg trypsin) was perfused for 20–25 min.

When the atrium was loose and translucent, the left atrium and right atrium were sliced into small pieces and placed in Krebs buffer solution containing (mmol/L) KCL 39.97, KH_2PO_4 25, $MgSO_4$ 7, H_2O 3, glucose 10.09, KOH 80, HEPES 10.7, EGTA 0.53, taurine 19.98, and L-glutamic acid 50.3. The cell suspension was filtered through nylon gauze (150 μm mesh), and the cells were stored in the same solution at room temperature for at least 1 h before use. The isolated cells were perfused with extracellular fluid containing *N*-methyl-D-glucamine 149, $MgCl_2$ 5, HEPES5, and $CaCL_2$ 0.65 (pH 7.4) for 10 minutes.

Only quiescent, rod-shaped cells with clear cross striations were studied. Ionic currents were recorded with whole-cell clamp methods, using an Axopatch 700B amplifier (Axon Instruments). Glass microelectrodes with resistances of 2–3 MΩ were used to record I_{K1}. Data were sampled with an A/D converter (Digidata 1322, Axon Instruments) and stored for subsequent analysis. A 3D manipulator (Mp-225, Sutter Instruments) was adjusted to form a stable connection of the electrode tip to the cell surface.

Tip potentials were zeroed before formation of the membrane-pipette seal in tyrode solution. The capacitance and series resistance (Rs) were electrically compensated to

Figure 1: Four rabbits running on the treadmill at the same time.

Figure 2: AF was induced by S1S2 incremental stimulation.

minimize the duration of the capacitive surge on the current recording and the voltage drop across the clamped cell membrane. Dof 5 nm/L, 100 μmol/L $CdCl_2$, 100 μmol/LTTX, 50 μmmol/L4-aminopyridine (4-AP), and glibenclamide 10 μmol/L were added to the extracellular solution to inhibit current contamination by I_{Kr}, L-type Ca^{2+}-current, I_{Na}, transient outward K^+-current, and adenosine triphosphate- (ATP-) dependent K^+-current, respectively. The sampling frequency depended on step stimulation of 10 mV, from −1000 mV to 60 mV, holding potential −40 mV, and clamping time 300 ms, with a frequency of 0.2 kHz used for slowly changing currents.

2.6. RNA Isolation and cDNA Synthesis. The atria were snap-frozen in liquid nitrogen and stored at −80°C. Total RNA was isolated with the TRIzol reagent (Invitrogen, USA). RNA was DNase-treated (Fermentas, USA). The quality of isolated RNA was assessed by electrophoresis on polyacrylamide gels (Biowest, Spain).

Briefly, first strand of cDNA (deoxyribose nucleic acid) was synthesized by incubation of 4 μg of RNA specimen in reverse transcription 5 buffer, with 4 μl M-MLVreverse transcriptase (Tresure Biological Engineering (Dalian) Co., Ltd.), 4 μl of T18 primers (China Beijing Taihe Biological Technology Co. Ltd.), 2 μl of dNTP (Tresure Biological Engineering (Dalian) Co., Ltd.), and 1 μl of RNase inhibitor. Synthesis reaction was performed for 5 min at 70°C, 60 min at 42°C, 5 min at 90°C, and 5 min at 4°C.

2.7. Semiquantitative Polymerase Chain Reaction (PCR). The cDNA of interest and the cDNA of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were co-amplified in a single PCR. Primers (China Beijing Taihe Biological Technology Co. Ltd.) were designed for Kir2.1, Kir2.2, Kir3.1, Kir3.4, and GAPDH (Table 1).

The PCR products were separated on agarose gel by electrophoresis and stained with 2 × Ex TaqMix. The density of the PCR products was quantified by densitometry. Linearity for the PCR was established by making a correlation between the number of cycles and the density of gene of interest and GAPDH.

The synthesized cDNA (1 μl) was then used as an amplification template in a 25 μl reaction mixture. After an initial denaturing step at 95°C for 5 minutes, PCR mixes were amplified during 40 cycles with denaturing at 95°C for 30 seconds, annealing at 65°C for 25 seconds, and extension at 72°C for 40 seconds (final extension, 72°C for 7 minutes).

2.8. Data Analysis. Group data are expressed as mean ± SE. Statistical analysis was performed with ANOVA. All PCR procedures were performed in duplicate series. One-way or two-way analysis of variance was used for all group comparisons. A P value <0.05 was considered statistically significant. SPSS version 19.0 was used for all statistical analyses.

3. Results

3.1. Structural Remodeling and AF Induction. Compared with Group C, the left and right atrial anteroposterior diameters increased in Group M and H after 8 w and 12 w (all $P < 0.05$). Compared with Group M, the left and right atrial anteroposterior diameters increased in Group H after 8 w

Table 1: Primer information.

Primer name	Sequence (5′ to 3′)	Base number	Product length
GAPDH	F: CAAGTTCCACGGCACGGTCA	20	118 bp
	R: CTCGGCACCAGCATCACCC	19	
Kir2.1	F: TGGTGGTGTTCCAGTCAATC	20	102 bp
	R: CCAGGGTCTCGTTTCTCTTC	20	
Kir2.2	F: GCCAACATGGACGAGAAGTC	20	100 bp
	R: AGGCCAGCGAGAAGATAAGC	20	
Kir3.1	F: CTCTCGGACCTCTTCACCAC	20	114 bp
	R: GATCACCCACCACATGGAC	19	
Kir3.4	F: CTCAGGTCCATCCAAGTCCT	20	104 bp
	R: AGGTGGCAGAGACAACCAAG	20	

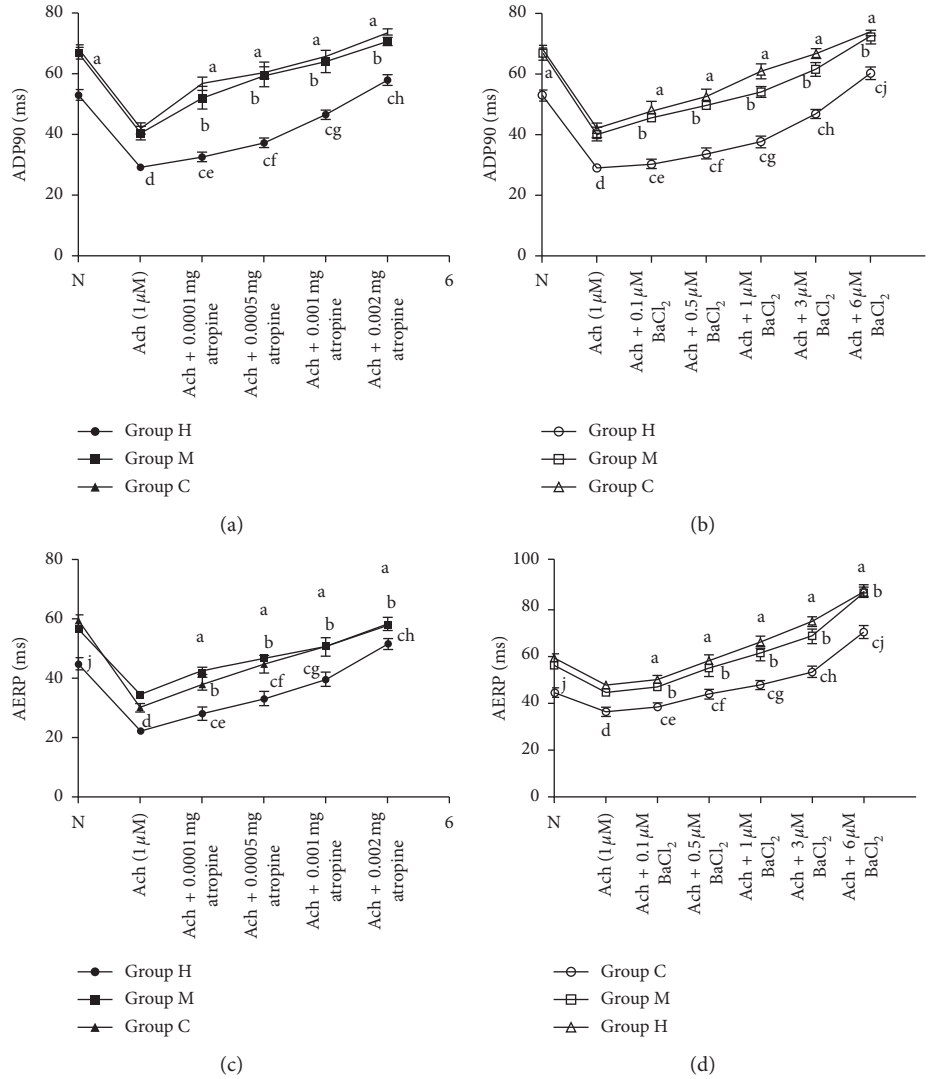

Figure 3: Continued.

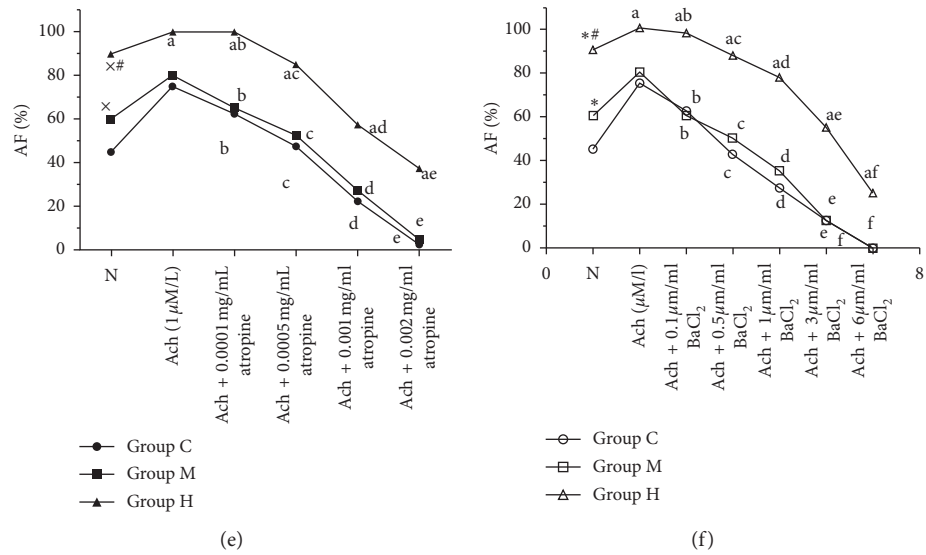

Figure 3: (a) Comparison of atrial APD90 in rabbits with different-intensity continuous training under different Ach + atropine concentrations; (b) comparison of atrial APD90 in rabbits with different-intensity continuous training under different Ach + barium chloride concentrations; (c) comparison of atrial AERP(ms) in rabbits with different-intensity continuous training under different Ach + atropine concentrations; (d) comparison of atrial AERP(ms) in rabbits with different-intensity continuous training under different Ach + barium chloride concentrations. Compared with Groups C and M, $^{d}P < 0.05$, $^{e}P < 0.05$, $^{f}P < 0.05$, $^{h}P < 0.05$, and $^{j}P < 0.05$; Compared with Ach alone, $^{a}P < 0.05$, $^{b}P < 0.05$, $^{c}P < 0.05$. (e, f) Comparison of the inductivity of AF in rabbits with different-intensity continuous training under different drug concentrations. (e) Ach + different atropine concentrations; (f) Ach + different barium chloride concentrations. Compared with Group C, $^{a}P < 0.05$; compared with Ach alone, $^{b}P < 0.05$, $^{c}P < 0.05$, $^{d}P < 0.05$, $^{e}P < 0.05$, and $^{f}P < 0.05$.

and 12 w (all $P < 0.05$) (Figure 3). Compared with Group C, the incidence of AF increased in Group M and H (45% vs. 60%, 45% vs. 90%, all $P < 0.01$).

The rabbit atrial APD90 and AERP trend to shorten with training-related is developed increasingly (Groups H vs. Groups C and M, *all* $P < 0.05$). The ADP90 and AERP prolonged with increasing atropine concentration (0.0001 mg/ml~0.002 mg/ml) and $BaCl_2$ (100 nm~6M). The ADP90 and AERP were significantly reduced in Group H and were intensity dependent at the same drug concentration ($P < 0.001$). APD90 and AERP were not significantly different between Group M and C ($P > 0.05$). Ach reduced the atrial APD90 and AERP and increased the inducibility of AF. AF could be 100% induced in Group H by Ach, in contrast with 75% and 80% in Groups C and M ($P < 0.05$). The incidence of AF with Ach + atropine (0.002 mg/ml) was 2.5%, 5%, and 37.5% in Groups C, M, and H, respectively (all $P < 0.05$). The incidence of AF with Ach + $BaCl_2$ (6 μM) was 0%, 0%, and 25% in Groups C, M, and H, respectively ($P < 0.05$) (Figures 3(a)–3(f)).

3.2. Patch Clamping. The I_{KAch} current density of left atrial myocytes in Group H was increased (−100 mV: 14.18 ± 1.74 pA/pF; +50 mV: 10.75 ± 1.68 pA/pF), while I_{KAch} current density was 9.92 ± 1.20 pA/pF (−100 mV), 5.57 ± 0.59 pA/pF (+50 mV) in Group C, and 11.07 ± 1.95 pA/pF (−100 mV), 8.25 ± 0.85 pA/pF (+50 mV) in Group M, respectively (Figure 4(a)–4(d), Figure 5(a), 5(b) and Figure 6(a)–6(c)).

The voltage-dependent inward currents could be recorded in both atrial myocytes by I_{KAch} stimulation protocol. I_{KAch} inward current decreased with increasing stimulus pulse. The current density increased when the voltage was more negative in Group H, which was absent in Group M. Reversal potential was about −50mv, above which the current became outward flowing with an obviously outward rectification. The current density of I_{KAch} increased with increasing training intensity, and the current-voltage curve moved up. However, the reversal potential and rectifying properties were not influenced by training intensity ($P > 0.05$).

3.3. RT-PCR. Kir2.1, Kir2.2, Kir3.1, and Kir3.4 mRNA expression of the left and right atria as assessed by reverse-transcription polymerase chain reaction (RT-PCR) was higher in Group H relative to Groups C and M ($P < 0.05$). Compared with Group C, Kir2.1 and Kir3.1 mRNA expression in the left atrium was higher in Group M ($P < 0.05$). Compared with Group M, Kir3.4 mRNA expression in the left atrium was higher in Group H ($P < 0.05$) (Figure 7(a)–7(d)).

4. Discussion

It is commonly believed that maximal oxygen uptake (VO2max) has been the best measure of heart-lung function in humans [12], which is being used more and more as animal studies [13, 14] to estimate medicinal effect

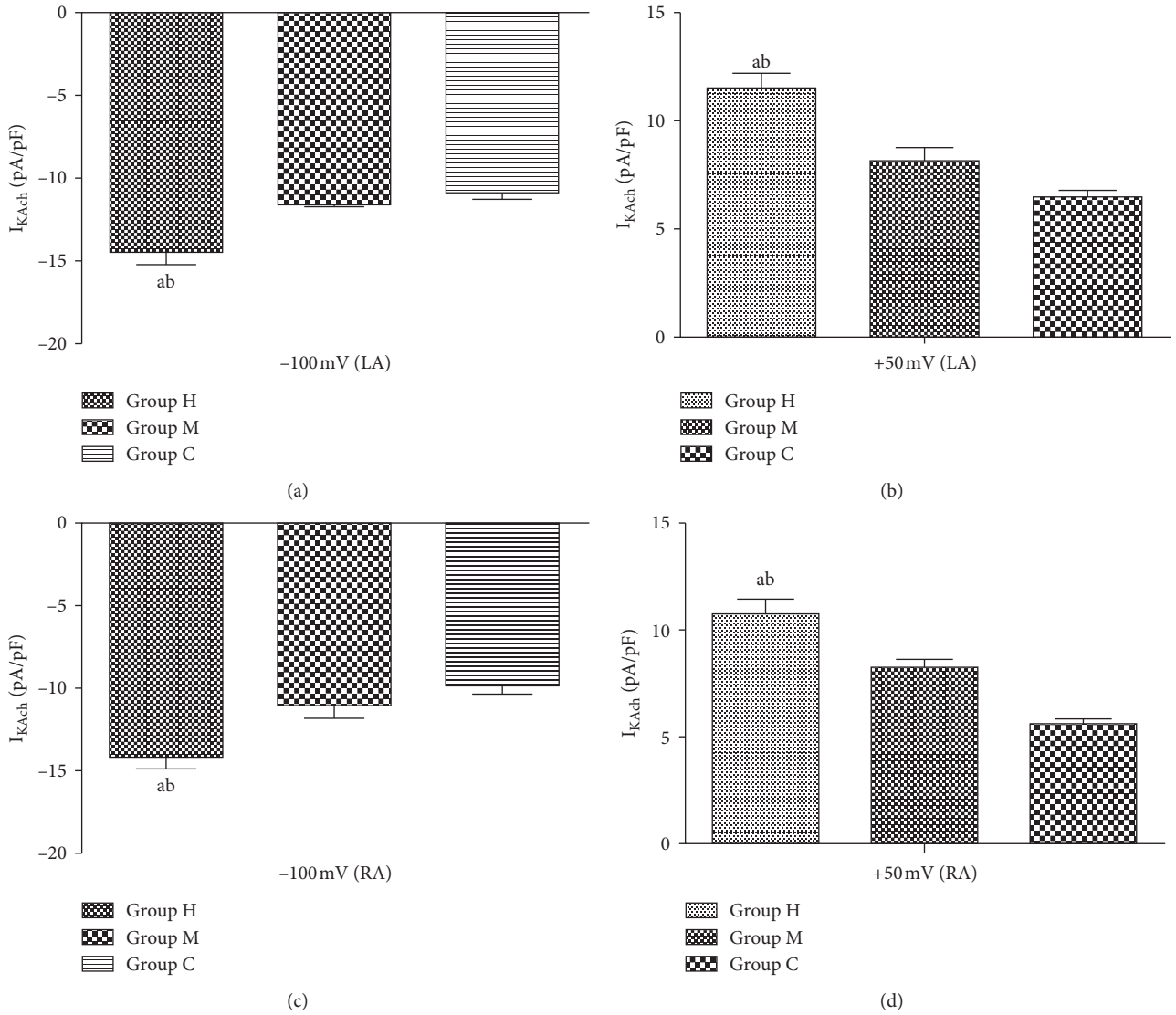

Figure 4: Effects of different-intensity continuous training on atrial tissue IKAch current density in rabbits. (a, c) Clamping voltage: 100 mV; (b, d): clamping voltage +50 mV. Compared with Group C, $^{a}P < 0.05$; compared with Group M, $^{b}P < 0.05$.

[15]. Some studies [13, 16, 17] have shown that VO2max is connected with inclination of the treadmill at aerobic exercise. Bedford [18] studies have shown intensity training according to oxygen uptake at maximal aerobic exercise: high-intensity at 90% of VO2max and moderate-intensity at 60%~70% of VO2max; Gaustad [9]studies have shown that all the rabbits' running speed at VO2max was 51 ± 9 cm/s and inclinations of the treadmill was 0–20°, when they looked more comfortable at 0–10° of inclinations ranging. The rabbits were tested at inclination ranging 0°, high-intensity: running speed at 50 cm/s; moderate-intensity: running speed at 25 cm/s in the experimental procedures.

Atrial enlargement is an independent risk factor for AF [19]. This study showed that high-intensity training for 12 weeks could lead to increased atrial diameter and incidence of AF in rabbits, which is consistent with the previous studies [20].

We posit that exercise-induced AF is triggered by left atrial enlargement following increased cardiac preload and ventricular pressure after long-term high-intensity endurance training. Stretch-activated channels of atrial myocytes are activated, and thus ADP90 and AERP are shortened.

K-Ach is a specific potassium channel that is mainly present in atrial myocytes and is activated by acetylcholine released from the vagal nerve. Many studies have shown increased atrial myocyte I_{KAch} current density in patients with AF. In this study, Ach was added into the perfusate, then ADP and AERP were recorded, and AF was induced. The results showed significant shortening of ADP and AERP and inducibility of AF in Group H. Different concentrations (0.0001 mg/ml~0.002 mg/ml) of the I_{KAch} inhibitor atropine prolonged ADP and AERP and reduced AF inducibility. AF inducibility in Group H was higher than in the remaining two groups at the same concentration of atropine, which

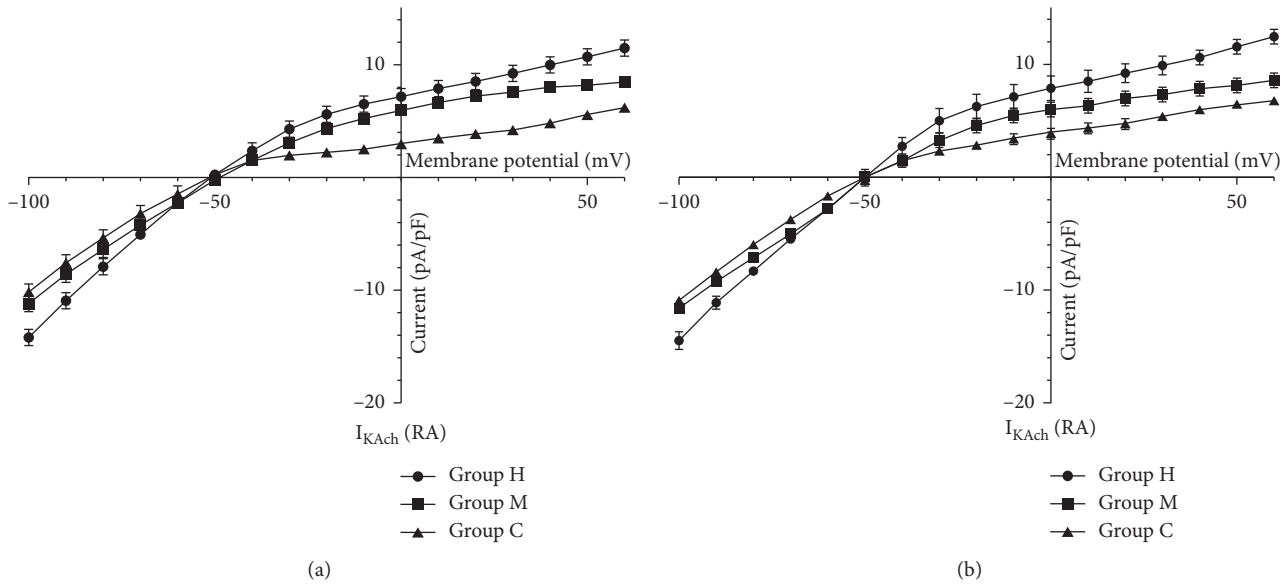

Figure 5: Effects of different-intensity training on atrial tissue IK_{Ach} current-voltage in rabbits. Clamping voltage: 100 mV: (a) RA; (b) LA.

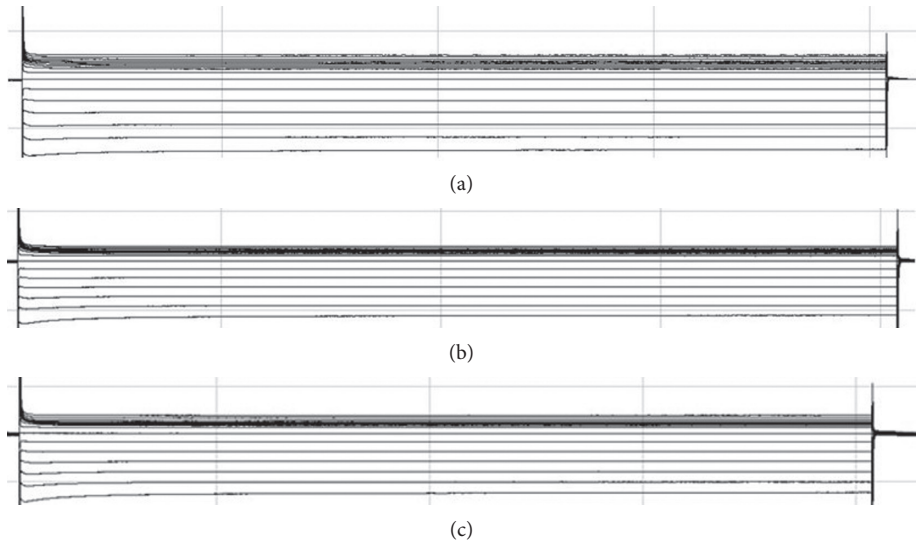

Figure 6: Effects of different-intensity continuous training on atrial tissue IKAch current density in rabbits: (a) Group C; (b) Group M; (c) Group H.

suggested that activated I_{KAch} increased the incidence of AF, and inversely, that blocking I_{KAch} reduced the incidence of AF. The latter result is consistent with that of the study by Kovoor [5] et al. Therefore, high-intensity endurance exercise enhanced I_{KAch} response. The increased vagal tone induced by exercise released Ach, which activated receptor type M through G protein, thereby increasing K^+ outflow and accelerating repolarization, resulting in shortening of APD and ERP.

Atrial myocytes resting potential mainly depends on I_{K1}.K^+ outflow gradually reduced during depolarization, closely relevant with 3rd phase repolarization and ADP [21]. AF inducibility decreased with increasing concentrations (100 nm~6 M) of the I_{K1} blocker$BaCl_2$. At the same $BaCl_2$ concentration, AF inducibility in the high-intensity group was higher than in the other two groups, and ADP and AERP were shorter. Therefore, high-intensity endurance exercise enhances I_{K1} response. Tan and her colleagues have confirmed that long-term high-intensity training causes rabbit atrial expansion and increases the inducibility of AF [22].

Subunit compositions of I_{K1} differ among species [23–25]. Human myocardium includes Kir2.1, Kir2.2, and Kir2.3, while only Kir2.1 and Kir2.2 are present in rabbit myocardium. The present study, which is the first to explore the effect of different-intensity endurance exercise on

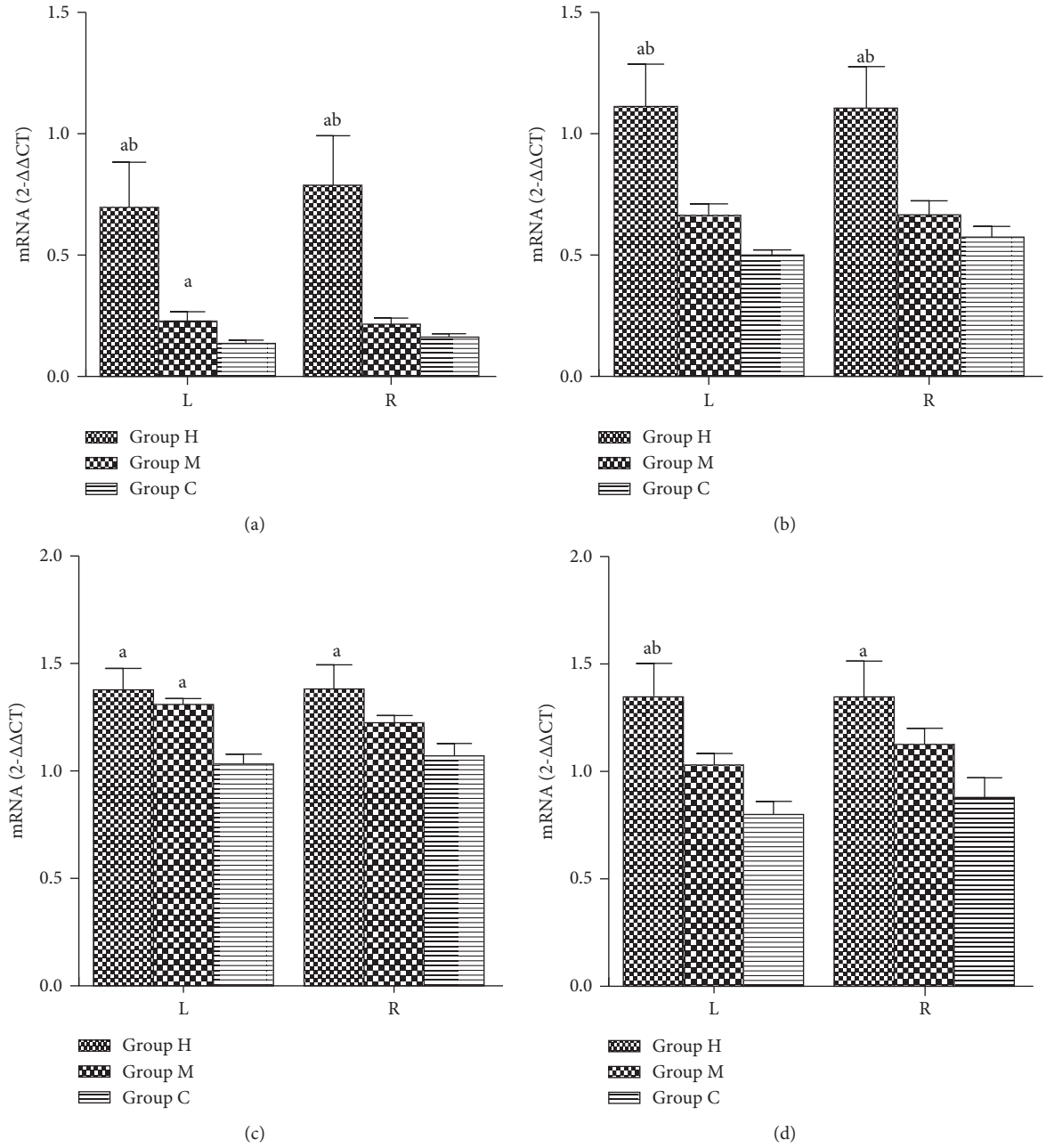

Figure 7: (a–d) Effects of different-intensity exercise training on Kir2.1, Kir2.2, Kir3.1, and Kir3.4 mRNA expression of left and right atria in rabbits. Compared with Group C, $^{a}P < 0.05$; compared with Group M, $^{b}P < 0.05$.

the expression of the K1 subunit in rabbit atrial myocytes, showed that high-intensity continuous training promoted the expression of Kir2.1 and Kir2.2 in rabbit atrial myocytes. Dobrev [26] et al found that I_{K1} increased Kir2.1 mRNA expression in patients with chronic AF. The sympathetic nerve and renin-angiotensin system are activated after exercise, and the adrenergic response acts on protein kinase A (PKA) and C (PKC) [27–29], increasing the Kir2.1 and Kir2.2 expression. K-Ach which is mediated by potassium channels encoded by Kir3.1 and Kir3.4 is mainly present in the mammalian atrium. K-Ach regulates atrial myocytes membrane potential and action potential repolarization and decreases myocardial contractility, excitability, and automaticity.

5. Conclusion

The present study showed that long-term high-intensity continuous training increased Kir3.4 and Kir3.1 mRNA expression in the rabbit atrium and I_{KAch} current density.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Supplementary Materials

Supplementary material contains revised articles on parameters of how the treadmill was made; exercise training was performed on a low-speed, levelled, motorized treadmill; the treadmill was fabricated in the Integrated runway by dividing into four tracks by a dummy plate; it was a total volume of 405L with a total length of 150 cm, height 30 cm, and width 90 cm to keep running the four rabbits in sync; it was automatic and adjustable with angle 0–35° and speed 0–67 cm/s (the rabbit running platform, which had not been reported so far, was improved based on the Gaustad treadmill using a high-powered motor drive and a concurrent four runway operation by Beijing Zhi Bao Biotechnology Co. Ltd. production of rats, Figure 1). The training program was preceded by a 1-week period of adaptation to the treadmill exercise, with 30 min running time and 25 cm/s treadmill speed. (*Supplementary Materials*)

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Research Article

Effects of Renal Denervation via Renal Artery Adventitial Cryoablation on Atrial Fibrillation and Cardiac Neural Remodeling

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Introduction. Catheter-based renal denervation (RDN) could reduce cardiac sympathetic nerve activity (SNA) and inhibit atrial fibrillation (AF). However, the reliability is uncertain, because the renal sympathetic nerves are mainly distributed in the adventitial surface of the renal artery. *Objective*. The aims of this study were to test the hypothesis that renal artery adventitial ablation (RAAA) definitely had the effects of RDN and to study the effects of RDN via renal artery adventitial cryoablation (RAAC) on AF and cardiac neural remodeling. *Methods*. Twenty beagle canines were randomly assigned to two groups: the left RDN group (LRDN, $n = 10$), which underwent left RDN via RAAC; the Sham group ($n = 10$). After 2 months of postoperative recovery, AF vulnerability, AF duration, and histological examination were performed in both groups. *Results*. Compared with the Sham group, left stellate ganglion (LSG) tissue fibrosis was increased in the LRDN group. LRDN significantly increased the percentage of TH-negative ganglionic cells and decreased the density of TH-positive nerves in the LSG ($P < 0.001$). Also, the densities of TH-positive nerves and GAP43 immunoreactivity within the left atrium (LA) were significantly decreased in the LRDN group ($P < 0.05$). After LA burst pacing, all 10 canines (100%) could be induced AF in the Sham group, but only 4 of 10 canines (40%) could be induced AF in the LRDN group ($P = 0.011$). The percentage of LA burst stimulation with induced AF was 26.7% (8/30) in the LRDN group, which was significantly decreased compared with that of the Sham group (53.3%, 16/30) ($P = 0.035$). In addition, AF duration was also significantly decreased in the LRDN group (13.3 ± 5.1 s) compared with that of the Sham group (20.3 ± 7.3 s, $P = 0.024$). *Conclusions*. RDN via RAAC could cause cardiac neural remodeling and effectively inhibit AF inducibility and shorten AF duration. It may be useful in selecting therapeutic approaches for AF patients.

1. Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia [1]. Autonomic neural system (ANS) activation played a key role in the occurrence and maintenance of AF, which could induce atrial structural remodeling and the changes of atrial electrophysiology. Reasonable autonomic nerve intervention could reduce cardiac sympathetic nerve activity (SNA) and improve the treatment of AF [2–4]. Several studies have showed that catheter-based renal denervation (RDN) could reduce cardiac SNA and inhibit AF [5–7]. Linz et al. [5] demonstrated that catheter-based RDN could reduce atrial sympathetic nerve sprouting, structural alterations, and AF complexity in goats with persistent AF. Wang et al. [6] found that catheter-based RDN could inhibit the progression of paroxysmal AF by reducing the incidences of AF and shortening the duration of AF. However, the reliability of catheter-based RDN is uncertain, because the renal sympathetic nerves are mainly distributed in the adventitial surface of the renal artery [8, 9]. We speculated that renal artery adventitial ablation (RAAA) may have better effects of RDN than catheter-based RDN. At present,

epicardial cryoablation has been widely used in the surgical AF ablation, which could achieve satisfactory integrity and transmurality of ablation lines. Therefore, we aimed to perform this study as following: (1) to test the hypothesis that RAAA definitely had the effects of RDN; (2) to study the effects of RDN via renal artery adventitial cryoablation (RAAC) on AF and cardiac neural remodeling.

2. Methods

2.1. Animals. This study was approved by the Ethics Committee of Xinhua Hospital, Shanghai Jiao Tong University, School of Medicine. All animal experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. A total of 20 beagle canines (male, 15–20 kg) were studied in the Experimental Animal Center of Xinhua Hospital. The 20 beagle canines were randomly assigned to the following two groups: (1) the left RDN group (LRDN, $n = 10$), which underwent left RDN via RAAC; (2) the Sham group ($n = 10$).

2.2. Renal Artery Adventitial Ablation (RAAA). Routine clinical monitoring was performed during the surgical procedure. Anesthesia was induced with ketamine (5–10 mg/kg) and midazolam (0.1–0.2 mg/kg IV). After intubation and mechanical ventilation, anesthesia was maintained with 2.0% isoflurane. The canines were placed in the right lateral decubitus position. A 6 to 8 cm subcostal incision was made on the abdomen to separate and expose the left renal artery (LRA). The proximal renal artery close to the renal artery ostium was subjected to cryoablation (both ventral side and dorsal side of the renal artery, cryoablation temperature −70°C, 120 seconds for each side) using a cryoprobe (5 cm in length, with a diameter of 6 mm on the tip, CryoICE, Atricure, USA) (Figure 1(a)). The incision was then closed. Cefuroxime sodium (30 mg/kg) was administered by intravenous perfusion during the surgery and used for three days after the surgery.

RAAC was performed for all canines in the LRDN group. For the Sham group, we only separated and exposed the LRA through the same incision, but RAAC was not performed.

2.3. AF Vulnerability Studies. After 2 months of postoperative recovery, electrophysiology experiment was performed. After the canines were anesthetized, the tips of two pairs of looping electrodes were directly sutured to the surface of the left atrial appendage (LAA) through the left third intercostal incision. One pair of looping electrodes was used to record the left atrial (LA) electric signal, and the other pair of looping electrodes was used for pacing. The electrocardiograms and electric signals were recorded simultaneously using the LabChart system (ADInstruments, AUS). Continuous 6 hours' rapid atrial pacing (RAP) (600 bpm, 0.5 ms, twice threshold current) was administered at the LA site for all canines. After that, AF inducibility was assessed by the burst pacing protocol at the LA site. Burst pacing was performed for each canine, at a cycle length of 50 ms and a stimulus output of 0.5 V plus twice threshold current for 60 s. Signals were sampled at 2 kHz and stored with the LabChart system. AF was defined as irregular atrial rates >500 bpm and lasting over 5 s associated with irregular atrioventricular conduction. AF inducibility was repeated for 3 times by burst pacing.

2.4. Histologic Analysis. After the electrophysiology experiment, the canines were then euthanized, and the tissues of LRA, left stellate ganglion (LSG), and LA were harvested. Small portions of the tissues were fixed in 4% formalin for 45 min and then stored in 70% alcohol for analysis. The tissues were paraffin embedded and cut into 5 μm thick sections routinely. The LRA was stained with haematoxylin-eosin (HE). Immunohistochemical staining of the LSG was performed using an anti-tyrosine hydroxylase- (TH-) antibody (22941, Immunostar, USA). Immunohistochemical staining of the LA was performed using an anti-TH-antibody (22941, Immunostar, USA) and an anti-growth-associated protein 43- (GAP-43-) antibody (NB300-143SS, Novus, USA). LSG tissues were also stained with the Masson trichrome stain. All slides were examined manually under a DP72 microscope (Olympus, Tokyo, Japan).

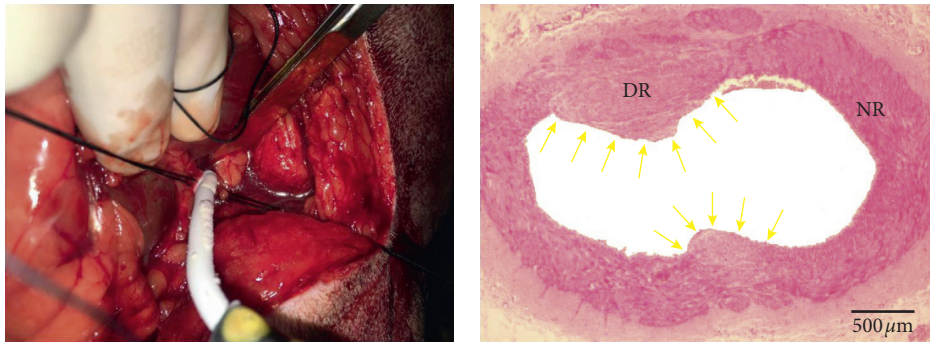
2.5. Statistical Analysis. The software SPSS 22.0 (SPSS, USA) was used for the statistical analysis. Continuous variables were expressed as mean ± standard deviation (SD) and 95% confidence interval (CI). Student's t test was used to compare continuous variables between the two groups. Categorical variables were presented as frequencies and proportions. The chi-square test or Fisher's exact test was used to compare categorical variables. A P value of <0.05 was considered to be significant for these comparisons.

3. Results

3.1. Effects of LRDN on the Morphological Changes of LRA. After 2 months of postoperative recovery, the canines were sacrificed and LRA was harvested. Figure 1(b) shows the morphological features of the LRA with H&E staining at 2 months after RAAC. The adventitia, media, and intima of renal artery wall were injured by RAAC, which displayed as the damaged region (DR). Compared with the normal region (NR), neointima formation (yellow arrow head) could be found corresponding to the DR. These features of the LRA demonstrated that RAAC could create transmural ablation from the adventitia to the intima of renal artery.

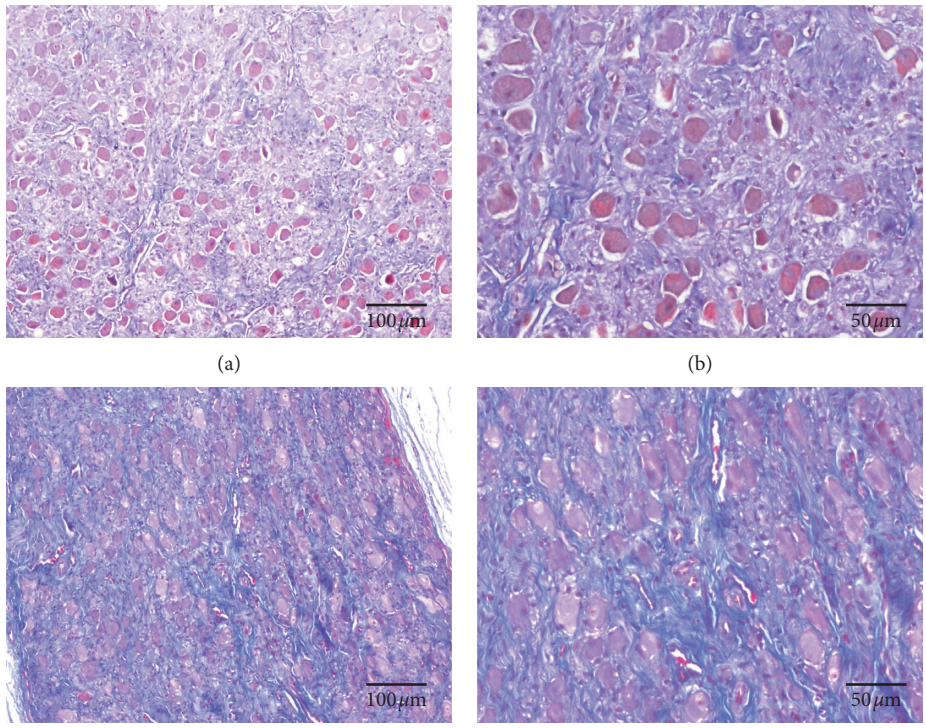
3.2. Effects of LRDN on Neural Remodeling of LSG. Figure 2 shows typical examples of LSG structural remodeling in both the Sham group and the LRDN group. Compared with the Sham group (Figures 2(a) and 2(b)), LSG tissue fibrosis was increased in the LRDN group (Figures 2(c) and 2(d)).

Figure 3 shows TH staining of the LSG in both the Sham group (Figure 3(a)) and the LRDN group (Figure 3(b)).



(a) (b)

Figure 1: Renal artery ablation. (a) Renal artery adventitial cryoablation (RAAC). (b) Morphological changes of the LRA after LRDN. DR: damage region; NR: normal region.



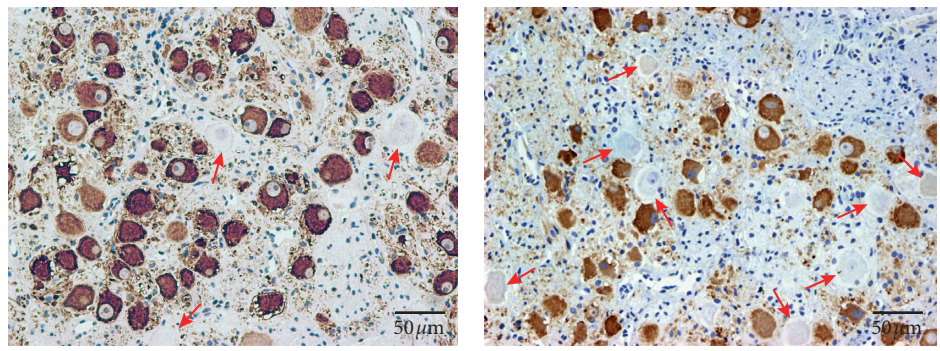
(a) (b)

(c) (d)

Figure 2: Masson trichrome staining of LSG. (a) and (b): Tissue fibrosis seen at low magnification in Sham group; (c) and (d): Tissue fibrosis seen at high magnification in LRDN group.

Compared with that of the Sham group, LRDN significantly increased the percentage of TH-negative ganglionic cells in the LSG in canines of the LRDN group. The mean percentage of TH-negative ganglionic cells in canines of the LRDN group (9.2 ± 1.7% (95% CI, 8.2% to 10.3%)) was significantly higher than that in the Sham group (4.6 ± 1.9% (95% CI, 3.4% to 5.8%)) ($P < 0.001$, Figure 4(a)). For canines with LRDN, there was a significantly decreased density of TH-positive nerves in the LSG (116835.8 ± 15794.8 $\mu m^2/mm^2$ (95% CI, 106510.7 to 125826.5)) compared to the canines of the Sham group (169784.5 ± 21284.6 $\mu m^2/mm^2$ (95% CI, 156184.9 to 182772.4)) ($P < 0.001$, Figure 4(b)).

3.3. Effects of LRDN on Neural Remodeling of LA. Figure 5 compares the results of immunostaining of TH and GAP43 in the LA between the Sham group and the LRDN group. The nerve density of LA for each group was expressed as a mean of nerve densities. Compared with the Sham group (Figure 5(a), 1660.6 ± 468.4 $\mu m^2/mm^2$ (95% CI, 1385.5 to 1958.3)), the densities of TH-positive nerves within the LA were significantly decreased in the LRDN group (Figure 5(b), 1233.4 ± 345.4 $\mu m^2/mm^2$ (95% CI, 1032.4 to 1455.7)) ($P = 0.032$, Figure 4(c)). The density of GAP43 immunoreactivity in the LA was (2313.9 ± 411.0 $\mu m^2/mm^2$ (95% CI, 2048.4 to 2560.6)) in the Sham group (Figure 5(c)), which was



(a) (b)

Figure 3: Tyrosine hydroxylase (TH) immunostaining of LSG. (a) TH staining showed TH-negative ganglion cells (red arrows) and TH-positive ganglion cells in LSG in Sham group; (b) TH staining showed TH-negative ganglion cells (red arrows) and TH-positive ganglion cells in LSG in LRDN group.

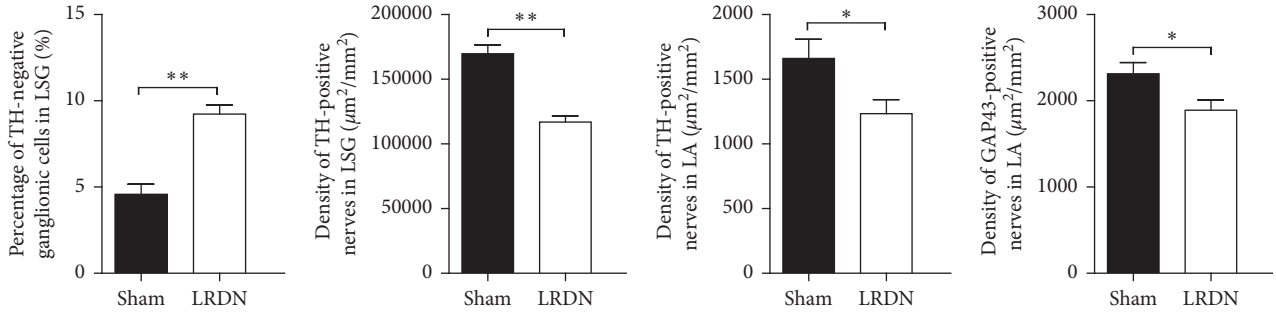


Figure 4: Immunostaining results in LSG or LA between Sham group and LRDN group. (a) The percentage of TH-negative ganglionic cells in LSG in both Sham group and LRDN group; (b) comparison between the density of TH-positive nerves in LSG between Sham group and LRDN group; (c) the density of TH-positive nerves in LA in both Sham group and LRDN group; (d) comparison between the density of GAP43-positive nerves in LA between Sham group and LRDN group ($^{*}P < 0.05$ vs. the Sham group; $^{**}P < 0.01$ vs. the Sham group).

significantly higher than that of the LRDN group (Figure 5(d), 1890.9 ± 383.8 $\mu m^2/mm^2$ (95% CI, 1648.9 to 2115.1)) ($P = 0.029$, Figure 4(d)).

3.4. Effects of LRDN on AF Inducibility and AF Duration. After LA burst pacing, all 10 canines (100%) could be induced AF in the Sham group, but only 4 of 10 canines (40%) could be induced AF in the LRDN group ($P = 0.011$, Figure 6(a)). The percentage of LA burst stimulation with induced AF was 26.7% (8/30) in the LRDN group, which was significantly decreased compared with that of the Sham group (53.3%, 16/30) ($P = 0.035$, Figure 6(b)). In addition, AF duration was also significantly decreased in the LRDN group (13.3 ± 5.1 s, 95% CI 10.2~17.2) compared with that of the Sham group (20.3 ± 7.3 s, 95% CI 16.9~24.0, $P = 0.024$, Figure 6(c)).

4. Discussion

ANS activation could induce significant and heterogeneous changes of atrial electrophysiology. Multiple evidences have demonstrated that ANS, especially sympathetic nervous system, may play a key role in the occurrence and maintenance of AF [10, 11]. In anatomical structure, the heart is innervated by the extrinsic nervous system and the intrinsic nervous system. Both the extrinsic and intrinsic cardiac nervous systems are important for arrhythmogenesis, such as AF [2, 12]. The extrinsic cardiac nervous system mainly includes stellate ganglion (SG) and vagal nerve. The intrinsic cardiac nerves are mostly found in the atrial wall. Neuromodulation methods that reduce sympathetic nerve activity may be helpful in controlling AF [13, 14]. In the previous study, Shen et al. has reported that vagal nerve stimulation (VNS) could effectively suppress SGNA and reduce the incidences of paroxysmal atrial tachyarrhythmias (PAT) [15]. Therefore, the modulation of ANS may be a promising target for intervention in AF patients. At present, ganglionated plexi (GP) ablation has been used to cure AF in patients [16–18]. However, nerve regeneration after GP ablation is still related to AF recurrence [19, 20].

In order to improve the effects of ANS modulation, several methods have been developed. RDN was considered to be one of the recommended methods to modulate ANS. Catheter-based approach has been developed for RDN, which has been proved to be able to reduce cardiac SNA and

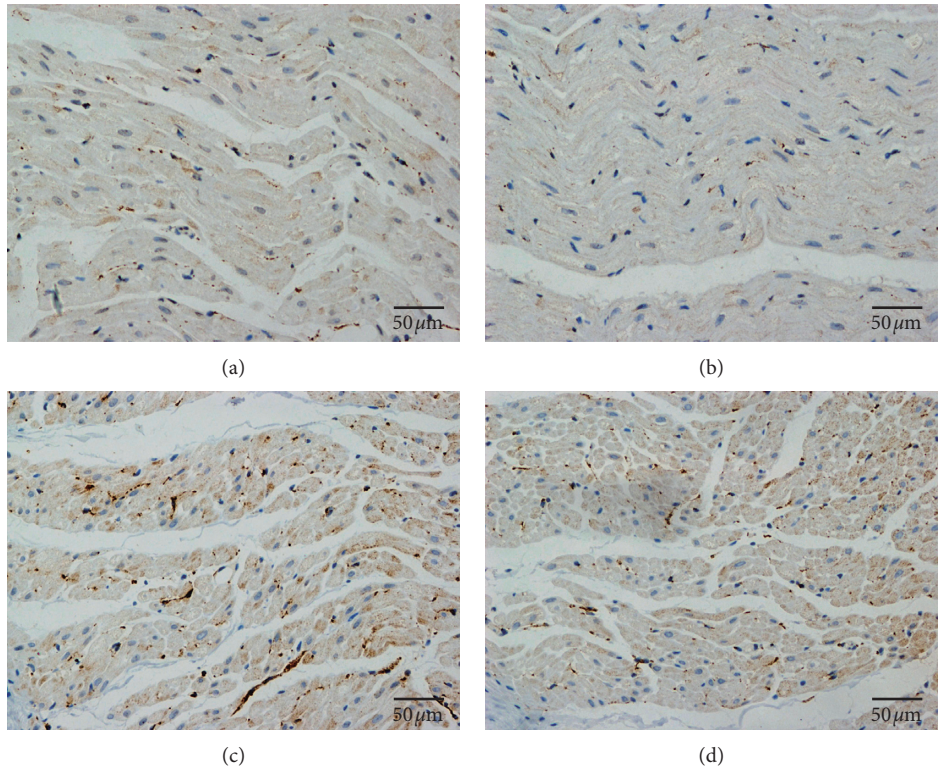


Figure 5: TH and GAP43 immunostaining of LA. (a) TH staining showed the densities of TH-positive nerves (brown) within the LA in Sham group; (b) TH staining showed the densities of TH-positive nerves within the LA in LRDN group; (c) GAP43 staining showed the density of GAP43 immunoreactivity in the LA in Sham group; (d) GAP43 staining showed the density of GAP43 immunoreactivity in the LA in LRDN group.

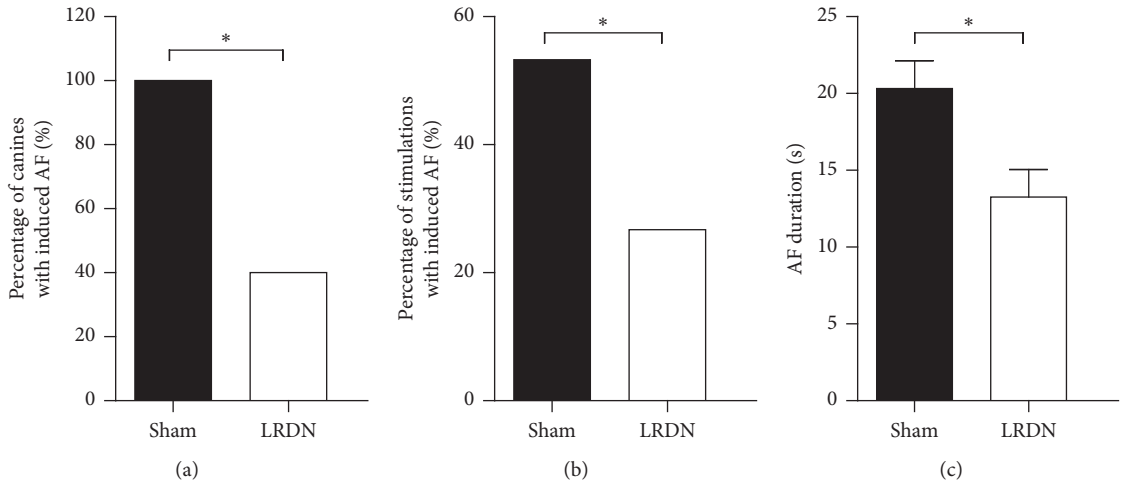


Figure 6: AF inducibility and AF duration between Sham group and LRDN group. (a) The percentage of canines with induced AF in LSG in both Sham group and LRDN group; (b) comparison between the percentage of stimulations with induced AF between Sham group and LRDN group; (c) comparison between the AF duration between Sham group and LRDN group ($^*P < 0.05$ vs. the Sham group).

inhibit AF [21]. However, the outcomes of catheter-based RDN are still uncertain, because the afferent and efferent nerves are mainly distributed in the adventitia of the renal artery [8, 9]. In this study, we performed RDN via RAAC (both ventral side and dorsal side of the renal artery, cryoablation temperature −70°C, 120 seconds for each side). LRA with H&E staining showed that the adventitia, media, and intima of renal artery wall were injured by RAAC, which demonstrated that RAAC could create transmural ablation of renal artery for RDN.

In the previous studies, Huang et al. demonstrated that 3 hours of left-sided electrical stimulation of renal sympathetic

nerve was able to increase both systemic and cardiac sympathetic nerve activities and cause neural remodeling in the LSG, which can be proarrhythmic in dogs in the presence of acute myocardial infarction. It suggested that there were direct or indirect connections between renal sympathetic nerve and LSG [22, 23]. Tsai et al. founded that RDN could reduce SG nerve activity and cause sympathetic nerve remodeling. His findings in part certificated the connections between renal sympathetic nerve and LSG [24]. According to these previous reports, increasing renal SNA could increase SG nerve activity and cause corresponding SG neural remodeling, whereas decreasing renal SNA could decrease SG nerve activity and cause corresponding SG neural remodeling. In our study, we also found that RDN via RAAC could increase LSG tissue fibrosis, increase the percentage of TH-negative ganglionic cells, and significantly decrease the density of TH-positive nerves in the LSG. That is, sympathetic components in LSG were significantly reduced by RDN via RAAC. Therefore, our findings demonstrated that RAAC definitely had the effects of RDN and inhibited SG nerve activity.

SG is the sympathetic ganglion formed by fusion of the inferior cervical ganglion and the first thoracic ganglion. SG is considered to be an important source of cardiac sympathetic innervation. It gave rise to sympathetic nerves that innervate atrium and ventricle [25, 26]. Cao et al. induced cardiac sympathetic nerve sprouting by infusing nerve growth factor (NGF) to the LSG in dogs with myocardial infarction and complete atrioventricular block [27]. This demonstrated that neural remodeling of intrinsic cardiac nerve was related to the extrinsic cardiac nerve. In our study, we harvested the LA tissues and examined with immunostaining of TH and GAP43. Both the density of TH-positive nerve and GAP43 immunoreactivity within the LA were significantly decreased in the LRDN group compared with that of the Sham group. Our findings demonstrated that LRDN not only significantly decreased the components of sympathetic nerve but also significantly inhibited novel sympathetic nerve sprouting in LA.

Overactivity of the sympathetic nervous system played an important role in the occurrence and maintenance of AF. Increased SNA or atrial sympathetic innervation is associated with increased incidence and duration of AF. In chronic AF patients, atrial sympathetic nerve densities are also significantly increased [28, 29]. Now, it was suggested that RDN could reduce SNA and decrease susceptibility to AF [5–7]. In our study, we also examined the effects of LRDN on AF inducibility and AF duration. Compared with the Sham group (100%), only 40% canines could be induced AF by LA burst pacing in the LRDN group. The percentage of LA burst stimulation with induced AF was 26.7% in the LRDN group, which was significantly lower than that of the Sham group (53.3%). Besides, LRDN also significantly decreased AF duration. Our data confirmed that RDN via RAAC could effectively inhibit AF inducibility and shorten AF duration.

5. Clinic Implications

The present study showed that RAAC could achieve the effects of RDN and inhibit cardiac SNA. Compared with the Sham group, RDN via RAAC could effectively inhibit AF inducibility and shorten AF duration. Therefore, we conclude that additional RDN via RAAC may be able to inhibit the occurrence and maintenance of AF and improve the therapeutic effects of AF.

6. Limitations

The present study has few limitations. Firstly, we did not evaluate the SG function by directly recording SG nerve activity, which may reflect SG function more objectively. Secondly, we only performed left side RDN. The reason is that previous studies [22, 23] have demonstrated that left-sided electrical stimulation of renal sympathetic nerve was able to increase both systemic and cardiac sympathetic activity and cause neural remodeling in LSG. We guess that LRDN may be able to decrease systemic and cardiac SNA. So, we would like to examine the effects of LRDN by this study. Thirdly, we got satisfactory short-term effects of RDN, but the midterm or long-term effects were not observed. The midterm or long-term effects of RDN via RAAC remain to be explored in the future. Fourthly, RDN via adventitial ablation may be potentially better than catheter-based RDN, but RDN via adventitial ablation have more trauma than that of catheter-based RDN.

7. Conclusions

Renal artery adventitial ablation (RAAA) definitely had the effects of RDN. RDN via renal artery adventitial cryoablation (RAAC) could cause cardiac neural remodeling and effectively inhibit AF inducibility and shorten AF duration. It may be useful in selecting therapeutic approaches for AF patients.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

There are no conflicts of interest to report.

Authors' Contributions

Wei Wang and Zhaolei Jiang contributed equally to this work.

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Research Article

Concealed Pulmonary Vein Bigeminy during Sinus Rhythm in Patients with Paroxysmal Atrial Fibrillation: A Useful Marker for Pulmonary Vein Firing

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Introduction. A concealed pulmonary vein (PV) bigeminy (cPVB) may be found in some patients with atrial fibrillation (AF) during sinus rhythm (SR). The aim of this study was to investigate whether the presence of cPVB during SR is associated with a higher PV firing. *Methods and Results*. Seven hundred seventy-six PVs (excluding 5 right middle PVs and 8 left common trunks) were mapped in 198 patients with paroxysmal AF (PAF) who underwent circumferential PV isolation. cPVB with a mean coupling interval of 136 ± 16 ms during SR was observed prior to ablation in 22 (11%) patients. Focal firing was provoked prior to ablation in 144 (19%) PVs. The incidence of focal firing was greater in PVs exhibiting cPVB compared with PVs without cPVB (89% vs. 16%; $P < 0.001$). Also, the number of radiofrequency applications required for isolation was greater in ipsilateral PVs, exhibiting cPVB compared with ipsilateral PVs without cPVB (21.6 ± 6.8 vs. 18.2 ± 5.6; $P = 0.024$). During a follow-up of 32 ± 20 months, the single ablation success rate was 82%. Compared with patients without cPVB, patients with cPVB were associated with higher recurrence rate of AF (27% vs. 17%; $p = 0.032$). *Conclusion*. cPVB during SR was observed prior to index ablation in 11% of PAF patients. Such a potential itself may be a PV firing in a concealed manner, which does not reactivate LA. The PV exhibiting cPVB required a greater number of radiofrequency applications for isolation. Compared to patients without cPVB, the recurrence rate of AF in patients with cPVB was greater.

1. Introduction

The triggers initiating atrial fibrillation (AF) originate most often from sleeves of left atrial myocardium extending onto the pulmonary veins (PVs) or the PV antrum [1–6]. It is for this reason that electrical isolation of the PVs forms the cornerstone for catheter ablation of AF. A single PV potential closely following or fused with an atrial potential in the ostium of the PVs is the most commonly observed pattern during sinus rhythm (SR) [7, 8]. Isolated reports also showed that a concealed PV bigeminy (cPVB) or an unusual double PV potential (PVP) at the PV ostium could be found in some patients with AF during SR, and focal ablation or isolation of related PV can cure AF [9–11]. However, whether these observations are applicable to a larger series is unknown. Such cPVB may reflect triggered activity in PV or reentry in PV which plays an important role in the PV firing, or may simply be a phenomenon without clinical implication. Therefore, the aim of the study is to investigate whether the presence of cPVB during SR is associated with a higher PV firing and clinical outcomes after the AF ablation.

2. Methods

One hundred ninety-eight consecutive drug refractory paroxysmal AF (PAF) patients (mean age 66 ± 20 years, 62% males) who underwent circumferential PV isolation were included in this study from July 2014 to June 2017. To minimize the influence of known clinical predictors, only patients with PAF were included.

2.1. Electrophysiologic Study and Catheter Ablation. After obtaining written informed consent, all patients underwent

an electrophysiologic study in the fasting state. The antiarrhythmic agents were stopped for at least five half-lives prior to the ablation procedure. Amiodarone was discontinued for at least three months. Before the procedure, cardiac spiral computerized tomography scans were performed to visually define the anatomy of the left atrium (LA) and PVs. All patients underwent transthoracic and transesophageal echocardiography to evaluate the left atrial thrombus and size.

Our technique used in this study has been described in our previous study [12]. Coronary sinus was mapped with a decapolar catheter inserted via the right internal jugular vein. Double separate transseptal punctures were performed. After transseptal puncture, anticoagulation with unfractionated heparin was begun to maintain an activated clotting time above 350 seconds, and selective multiview pulmonary venograms were obtained. To validate mapping, a circular decapolar catheter (Lasso, Biosense Webster) placed around the PV ostium was used. A 3D electroanatomic reconstruction of the LA was made guided by a 3D navigation system (CARTO, Biosense Webster) with a mapping/ablation catheter (Navistar ThermoCool, Biosense Webster) or a Lasso catheter. Before ablation, cPVB was recorded by using the circular mapping catheter in each vein during SR. If the initial rhythm was AF, the patient was cardioverted and then for following 5 minutes. After assessment of cPVB, the circular mapping catheter and mapping/ablation catheter were positioned sequentially into all PVs to identify the PVs exhibiting firing (at least three rapid consecutive ectopies with or without activating the LA). The methods used to provoke PV firing included the use of isoproterenol (3–10 μg/min) combined with cardioversion of sustained AF. During the whole procedure, the heart rate was recorded for further analysis.

Catheter ablation was performed to encircle the right- and left-sided PV in pairs until ipsilateral PVs' isolation was achieved. Septal and lateral continuous circular lesions around the ipsilateral PVs were deployed about 1 cm posterior and 5 mm anterior from their ostia as defined by PV angiography and the 3D map. The circular mapping catheter or ablation catheter was then advanced deeper into the PVs to exclude residual PVPs. After ablation, isoproterenol (3–10 μg/min) was repeated to provoke firing within the atria and isolated PVs. A waiting time >30 minutes was respected.

2.2. Definition of cPVB. A Lasso catheter placed at the PV ostium was used to record the presence and characteristics of cPVB. cPVB was defined as wide double PV potentials with an interval >120 ms following far-field LA and right atrial potentials at the PV ostium during SR for at least 15 min in a continuous form before ablation (Figure 1).

2.3. Follow-Up. All patients were followed-up at 1 and 3 months and thereafter every 3 months after discharge. Standard 12-lead electrocardiogram and 24-hour Holter recordings were examined routinely. All patients were instructed to contact us with any symptoms or documented AF recurrences. Recurrent AF was defined as any occurrence of sustained atrial tachyarrhythmia lasting at least 30 seconds after a postablation 3-month blanking period.

2.4. Redo Ablation Procedure. All patients with AF recurrences were offered redo ablation procedures after the postablation blanking period. Redo ablation procedure was started as described above. After baseline mapping of the PVs, a new CARTO-guided 3D electroanatomical map of the LA was constructed.

2.5. Statistical Analysis. Continuous data are presented as mean ± standard deviation. Categorical variables are expressed as number (%). Groups were compared using an unpaired *t*-test, Fisher's exact test, or the chi-square test, as appropriate. Statistical significance was established at a P value of <0.05.

3. Results

Mapping and ablation were performed in 789 PVs (198 patients): 198 right superior PVs (RSPVs), 198 right inferior PVs (RIPVs), 5 right middle PVs (RMPVs), 190 left superior PVs (LSPVs), 190 left inferior PVs (LIPVs), and 8 left common trunks. Complete isolation (elimination of all PVPs) was obtained in all 789 PVs. For comparison between the 4 principal PVs, the data from the 5 RMPVs and 8 left common trunks were eliminated, leaving 776 PVs for analysis. With the exception of one right femoral pseudoaneurysm and one right internal jugular hematoma, no major complication was found.

3.1. Incidence of cPVB during Sinus Rhythm. cPVB during SR was seen prior to ablation in 22 (11%) patients. cPVB was seen in 27 of 776 (3.5%) PVs targeted at the index procedure with a mean of 1.2 cPVB per patient. The characteristics of the cPVB are shown in Table 1. The mean interval between the two PV potentials, the wide double potential interval, was 136 ± 16 ms. Atrial pacing at cycle lengths <400 ms suppressed the second PV potentials in all patients. The cPVB was more common in the superior pulmonary veins compared with inferior veins (LSPV 37%, RSPV 33%, LIPV 19%, and RIPV 11%). The heart rate during sinus rhythm was higher in the patients with cPVB compared with the patients without cPVB (72 ± 8 beats/min vs. 68 ± 8 beats/min; $P = 0.0019$) (Table 2).

3.2. Relation between PV Firing and cPVB. Focal firing was provoked prior to ablation in 144 (19%) PVs. Twenty-four (89%) PVs exhibiting cPVB during SR showed focal firing spontaneously or with use of isoproterenol combined with cardioversion of sustained AF. The rate of focal firing was greater in PVs exhibiting cPVB compared with PVs without cPVB during SR (89% vs. 16%; $P < 0.001$). Besides, the number of radiofrequency applications required for isolation was greater in ipsilateral PVs exhibiting cPVB

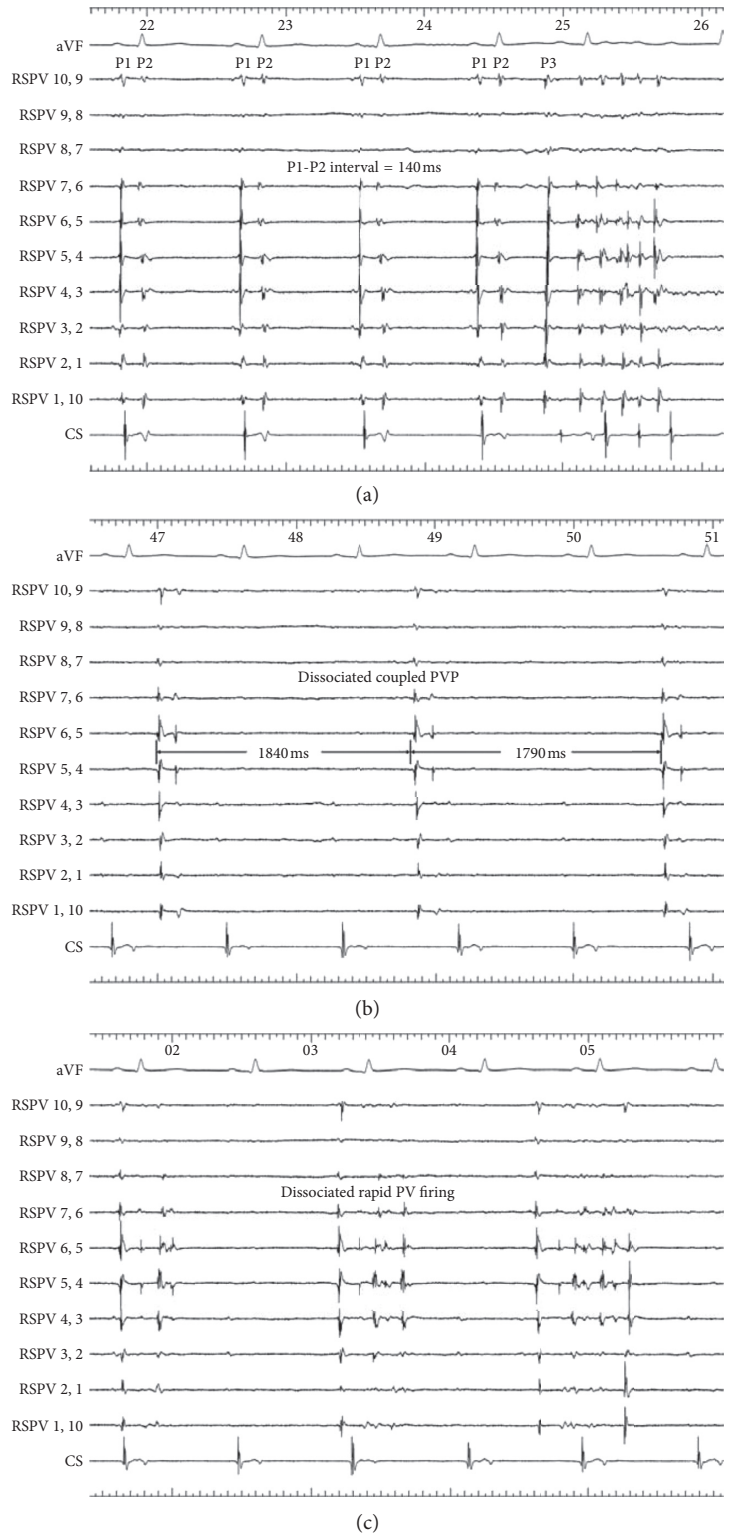

Figure 1: Tracings are from ECG lead aVF and intracardiac electrograms recorded from the electrode pairs of the circular mapping catheter positioned at the ostium of the right superior pulmonary vein (RSPV) and coronary sinus (CS). (a) There is a small atrial potential, followed closely by a pulmonary vein potential (PVP) (*P*1) and then by a second PVP (*P*2) during sinus rhythm. The concealed pulmonary vein bigeminy interval is 140 ms. Spontaneous rapid PV firing with the earliest potential *P*3 conducts out of the vein and activates the atrium. (b) After ipsilateral right pulmonary veins isolation, dissociated coupled pulmonary vein potential was confined in the RSPV. (c) After ipsilateral right pulmonary veins isolation, rapid PV firing was confined in the RSPV with dissociation of atrial potentials.

Table 1: Characteristics of cPVB.

	N (%)
Prevalence	
Total no. of patients (198)	22 (11%)
Total no. of veins (776)	27 (3.5%)
No. of cPVB/patient	1.2
PV potential location	
RSPV	9 (33%)
RIPV	3 (11%)
LSPV	10 (37%)
LIPV	5 (19%)
Wide double PV potentials interval	136 ± 16 ms
Relation to PV firing	24 (89%)

cPVB = concealed pulmonary vein bigeminy; PV = pulmonary vein; RSPV = right superior pulmonary vein; RIPV = right inferior pulmonary vein; LSPV = left superior pulmonary vein; LIPV = left inferior pulmonary vein.

Table 2: Patient demographics and procedural data.

Demographics	cPVB-positive (N = 22 patients)	cPVB-negative (N = 176 patients)	P value
Age (years)	65 ± 16	66 ± 20	0.462
Male (%)	64	62	0.917
Hypertension (%)	15	16	0.935
Diabetes (%)	5	4	0.856
AF history (years)	6.2 ± 5.1	6.9 ± 4.6	0.322
LA size (mm)	40 ± 6	41 ± 6	0.464
Weight (Kg)	76 ± 16	75 ± 12	0.605
Sleep apnea (%)	4	2	0.579
Structural heart disease (%)	6	6	0.943
Heart rate during sinus rhythm (beats/min)	72 ± 8	68 ± 8	0.002
RF number required for ipsilateral PVs isolation	21.6 ± 6.8	18.2 ± 5.6	0.024
AF recurrence (%)	27	17	0.032
Complication (%)	1	1	0.874
Follow-up (months)	33 ± 20	32 ± 20	0.713

cPVB = concealed pulmonary vein bigeminy; AF = atrial fibrillation; LA = left atrium; RF = radiofrequency.

compared with ipsilateral PVs without cPVB (21.6 ± 6.8 vs. 18.2 ± 5.6; $P = 0.024$) (Table 2).

The second PVP was coupled to the first PVP during SR and disappeared immediately after isolation of PV. Dissociated PVP originating from PV musculature was found in 290 (37%) PVs. All PVs exhibiting cPVB during SR prior to ablation showed dissociated PVP after isolation with a mean interval of 2.4 ± 0.6 second, and 50% of these PVs also showed coupled PVP and rapid PV firing (Figure 1). However, the incidence of dissociated PVP after isolation in PV not exhibiting cPVB prior to ablation was lower than that of PV exhibiting cPVB (35% vs. 100%; $P < 0.001$).

3.3. Follow-Up and Redo Ablation Procedure Findings. During a follow-up of 32 ± 20 months, 162 (82%) of the 198 patients were free of sustained atrial tachyarrhythmia lasting more than 30s. Eighty-two percent of the patients with cPVB were off antiarrhythmic drugs compared with 85% in the patients without cPVB, $P = 0.356$. All 36 patients with symptomatic recurrence presented with AF or atrial flutter. Compared to that of patients without cPVB, the recurrence rate of AF or atrial flutter in patients with cPVB was greater (27% vs. 17%; $P = 0.032$). Also, the basic clinical characteristics showed no difference between these two groups (Table 2).

Twenty of 36 patients underwent a redo procedure, and LA-PV reconnection was found in all patients with a mean of 2.4 ± 0.8 PV per patient (LSPV 25%, LIPV 25%, RSPV 23%, and RIPV 27%). Three patients with cPVB prior to the index procedure underwent a redo procedure. In all these 3 patients, 7 of 8 (88%) PVs with cPVB demonstrated LA-PV reconnection and at least one PV with cPVB showed LA-PV reconnection in each patient.

4. Discussion

To our knowledge, this is the first study to demonstrate that the cPVB is associated with PV firing. Such potential may indicate a more extensive PV muscular sleeve, which required a greater number of radiofrequency applications for isolation and is more susceptible to late reconnection. In addition, (1) cPVB was seen in about 11% of PAF patients during SR prior to ablation, (2) cPVB was seen in about 3.5% of PV targeted at the index procedure and more frequently originate from the superior PV, and (3) cPVB is a useful marker for PV firing.

4.1. cPVB during Sinus Rhythm Prior to Ablation. Generally, a single PV potential closely following or fused with an atrial potential in the ostium of the PVs is the most commonly observed pattern during SR [7]. This study demonstrated that cPVB with a mean interval of 136 ± 16 ms was seen in about 11% of PAF patients during SR prior to ablation. The underlying mechanism of cPVB is yet to be defined. The origin of the first potential of the cPVB is most probably activation of a spiral PV myocardial sleeve from the LA. The origin of the second potential of the cPVB is less obvious. Reithmann et al. reported eight patients with pulmonary vein bigeminy, and 3 of them showed cPVB [13]. They considered the triggered activity or automatic activity to be the mechanism. Kim et al. reported two patients with PAF who demonstrate widely split PV double potentials at the PV ostium during SR [11]. In their study, the authors considered that the second potential is a result of slow conduction deep into the PV that returns and either reactivates the same myocardial sleeve at the ostium, also what is often considered spontaneous PV ectopy may actually be a result from reentry in and out of a PV with reactivation of the PV ostium and, when manifest, reactivation of the LA. In this study, the second potential may be a concealed PV ectopy which did not reactivate the LA. Even the appearance of the second potential after PV isolation was all coupled to the dissociated PVP. In these patients, the second potential is a result of slow conduction from the LA into a second PV

fascicle is less likely. This dependent character strongly favors triggered activity as the arrhythmogenic mechanism. However, in some patients, we could not rule out a macroreentry in the vein or a slow conduction from the LA into a second PV fascicle may be the responsible mechanism because the second potential disappeared and never occurred again during the ostial ablation. The ostial ablation could partly destroy the circuit and prevent subsequent firing, eliminate the focus of PV ectopy, or interrupt the LA-PV conduction through the second PV fascicle.

4.2. cPVB and PV Firing. The current paradigm is that the PV sleeves primarily contribute to AF pathogenesis as a source of AF triggers [14]. In this study, 24 of 27 (89%) PVs with cPVB during SR showed focal firing spontaneously or with use of isoproterenol. When compared with PVs without cPVB during SR, the rate of focal firing was greater in PVs exhibiting cPVB (89% vs. 16%; $P < 0.001$). This observation may suggest that cPVB is a marker for PV firing during SR. In a postmortem anatomical study, Hassink et al. showed that patients with AF had significantly longer muscle sleeves present [15]. Similarly, muscle bundles in the superior veins were substantially longer than those in the inferior veins. Along with increased length, Guerra et al. using intravascular ultrasound found that patients with AF had considerably thicker PV myocardial tissue, and that PV firing could only be localized to these areas of thickening [16]. Nakagawa et al. also found that the incidence of PV firing increases with progressively wide LA-PV connections, and the number of radiofrequency applications required for isolation was greater in PVs exhibiting focal firing compared with PVs without firing [17]. In a study by De Greef et al., the incidence of preablation triggering PVs was higher in patients having recurrence of atrial fibrillation following the initial PV isolation compared with those who did not have a recurrence [18]. In this study, the distribution of the cPVB was more common in the upper veins compared with lower veins. The PV exhibiting cPVB also required a greater number of radiofrequency applications for isolation. All these characteristics are similar to that of dissociated pulmonary vein potentials after PV isolation reported by Lee et al. [19]. Compared to patients not exhibiting cPVB, the recurrence rate of AF in patients exhibiting cPVB was greater. All these similarities between cPVB and PV firing may indicate that cPVB itself is PV firing in a concealed manner which does not reactivate LA, that is to say "PV firing equals cPVB" to some extent. However, we could not rule out the role of the heart rate in the different results between patients with and without cPVB. When compared with the patients without cPVB, the higher heart rate in the patients with cPVB may represent the enhancement of the sympathetic nervous system which may cause a higher incidence of cPVB. The heart rate also can influence the contact between the catheter tip and myocardium which is important for lesion formation and may explain why a greater number of radiofrequency applications for isolation and a higher recurrence rate of AF in the patients with cPVB are needed. Jais et al. reported that the effective refractory periods of the PVs were shorter than that of the LA in AF patients but longer than LA in control patients, suggesting an association between short effective refractory periods in the PVs and the development of AF [20]. In this study, isoproterenol shortened the coupling interval of the cPVB, which is in accordance with clinical and experimental findings showing that isoproterenol shortens effective refractory periods and promotes the conduction in PV. Due to the relative shorter effective refractory periods in the PVs, the cPVB with possible mechanism of triggered activity or reentry mentioned above may be more susceptible to induce rapid PV firing. However, the activation sequence differed between cPVB and PV firing in all cases. The possible reason may be that cPVB and PV firing were conducted over the different PV fascicles. In addition, suppression of the second PV potentials by atrial pacing may explain the prevention of PAF by atrial pacing.

4.3. Clinical Implications. The present study was designed to obtain insight into the relation between cPVB and PV firing in PAF patients during SR and was not designed to define a new approach for ablation. The results of this study suggest that more extensive circumferential ablation may be required for the PAF patient exhibiting cPVB during SR.

5. Conclusion

cPVB during SR was observed prior to index ablation in 11% of PAF patients. Such potential itself may be a PV firing in a concealed manner which does not reactivate LA. The PV exhibiting cPVB required a greater number of radiofrequency applications for isolation. Compared to patients without cPVB, the recurrence rate of AF in patients with cPVB was greater.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

All the authors have read and approved the paper, and no part of this paper is being published or under consideration for publication elsewhere. There are no conflicts of interests for any of the authors.

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Clinical Study

The Impact of Left Atrial Size in Catheter Ablation of Atrial Fibrillation Using Remote Magnetic Navigation

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Objective. The objective of this study was to investigate the impact of left atrial (LA) size for the ablation of atrial fibrillation (AF) using remote magnetic navigation (RMN). *Methods*. A total of 165 patients with AF who underwent catheter ablation using RMN were included. The patients were divided into two groups based on LA diameter. Eighty-three patients had small LA (diameter <40 mm; Group A), and 82 patients had a large LA (diameter ≥40 mm; Group B). *Results*. During mapping and ablation, X-ray time (37.0 (99.0) s vs. 12 (30.1) s, $P < 0.001$) and X-ray dose (1.4 (2.7) gy·cm^2 vs. 0.7 (2.1) gy·cm^2, $P = 0.013$) were significantly higher in Group A. No serious complications occurred in any of the patients. There was no statistical difference in the rate of first anatomical attempt of pulmonary vein isolation between the two groups (71.1% vs. 57.3%, $P = 0.065$). However, compared with Group B, the rate of sinus rhythm was higher (77.1% vs. 58.5%, $P < 0.001$) during the follow-up period. More patients in Group A required a sheath adjustment (47/83 vs. 21/82, $P < 0.001$), presumably due to less magnets positioned outside of the sheath. *In vitro* experiments with the RMN catheter demonstrated that only one magnet exposed created the sheath affects which influenced the flexibility of the catheter. *Conclusions*. AF ablation using RMN is safe and effective in both small and large LA patients. Patients with small LA may pose a greater difficulty when using RMN which may be attributed to the fewer magnets beyond the sheath. As a result, the exposure of radiation was increased. This study found that having at least two magnets of the catheter positioned outside of the sheath can ensure an appropriate flexibility of the catheter.

1. Introduction

Remote magnetic navigation (RMN) has been widely used for atrial fibrillation (AF) ablation [1–6]. Compared with manual ablation, it has the advantages of less complications and X-ray exposure [1–6]. However, because it was cost prohibitive, the RMN system was not used widely in China; therefore, the application experience is limited. In 2013, Wuxi People's Hospital began to use RMN for AF ablation due to the advantages reported in the literature. By the end of 2017, more than 200 procedures had been completed, thus increasing this center's expertise with the RMN technology. During this experience, it was found that the left atrial (LA) size impacted the mapping and ablation procedure, and the reasonable hypothesis was that the number of magnets outside the sheath may play a role in the navigation in small LA. The aims of this study were to discuss the impact of different LA size on AF ablation using RMN and the methods to overcome this problem.

2. Materials and Methods

2.1. Study Population. From March 2014 to January 2018, a total of 165 patients with AF who underwent ablation therapy for first time using RMN in Wuxi People's Hospital were included in this analysis. Due to the good efficacy and safety, RMN ablation was performed in all AF ablation cases in our center. The baseline characteristics of patients are listed in Table 1. All patients failed at least one antiarrhythmic drug. Routine blood, liver and kidney function,

Table 1: Baseline characteristics of patients.

	Group A ($n = 83$)	Group B ($n = 82$)	Total ($n = 165$)	P value
Gender (male/female)	51/32	50/32	101/64	0.951
Age (years)	58.0 ± 11.5	61.1 ± 9.3	60.7 ± 10.9	0.054
Height (cm)	167.5 ± 6.8	167.1 ± 7.9	167.3 ± 7.5	0.652
Weight (kg)	66.5 ± 8.6	72.8 ±18.4	69.6 ± 14.7	0.006
BMI (kg/m^2)	23.6 ± 2.1	25.6 ± 3.0	24.6 ± 2.8	< 0.001
Paroxysmal/nonparoxysmal AF	71/12	42/40	113/52	< 0.001
Course of AF (month)	36.0 (48.0)	24.0 (60.0)	36.0 (48.0)	0.651
Basic diseases				
Hypertension	43	65	108	< 0.001
Diabetes	4	6	10	0.729
Coronary heart disease	10	15	25	0.263
Stroke	13	14	27	0.807
LVEF (%)	63.9 ± 4.2	62.3 ± 4.6	62.8 ± 4.5	0.113
LA diameter (mm)	35.0 ± 3.1	44.2 ± 3.2	39.6 ± 5.6	< 0.001
Four classical pulmonary vein ostia patterns/not	60/23	60/22	120/45	0.899

BMI: body mass index; AF: atrial fibrillation; LVEF: left ventricular ejection fraction. P values listed were calculated between Groups A and B.

coagulation index, electrocardiogram, and echocardiography were performed for each patient before operation, and esophageal echocardiography was performed 24 hours before operation to exclude LA thrombus. Patients were on either warfarin or dabigatran for anticoagulation therapy and were discontinued three days before the procedure and bridged with low molecular heparin according to the patient's weight. For patients taking warfarin, after 12 hours of the procedure, warfarin was administered orally until the INR was greater than 2.0, and then, the low molecular heparin was stopped. For patients taking dabigatran, low molecular heparin and dabigatran were given 12 h after the procedure simultaneously, and then, the low molecular heparin was discontinued. All patients signed informed consent before the procedure. The patients were divided into two groups based on echocardiographic LA size according to the recommendations of the American Society of Echocardiography and the European Association of Cardiovascular Imaging [7]. Group A was defined as the LA diameter <40 mm, and Group B was defined as the LA diameter ≥40 mm [7].

2.2. AF Ablation Using RMN. Two electrophysiological study catheters were positioned: a 6F quadrupole catheter (Inquiry, St. Jude Medical Inc., St. Paul, MN, USA) at the right ventricular apex and a 6F decapolar coronary sinus catheter (Inquiry, St. Jude Medical Inc., St. Paul, MN, USA) in the coronary sinus via the femoral vein. After a fluoroscopically guided single transseptal puncture, an SR0 sheath (St. Jude Medical Inc., St Paul, MN, USA) was advanced into the LA. After transseptal catheterization, intravenous heparin was injected 50~100 IU/kg and administered to maintain an activated clotting time of 250 to 350 seconds. A magnetic mapping and ablation catheter (Navistar Thermocool RMT, Biosense Webster Inc., Diamond Bar, CA, USA) was advanced through the sheath into the LA.

Carto RMT (Biosense Webster Inc., Diamond Bar, CA, USA) was used in conjunction with the RMN Niobe® II system (Stereotaxis, Inc., St. Louis, MO, USA) to perform stepwise remote-controlled magnetic 3D LA electroanatomic mapping. The Carto system transfers real-time catheter tip location and orientation to the RMN system, and the RMN system controls the direction of the catheter. The catheter-advancing system (QuikCAS®, Stereotaxis Inc., St. Louis, MO, USA) controls the advancement and retraction of the catheter. The voltage model of the LA was constructed under bipolar voltage mapping.

Radiofrequency ablation was performed in a temperature-controlled model. Radiofrequency delivered with a target temperature of 43-degree celsius. Power was limited to 35 W at the anterior LA wall and 30 W at the posterior LA wall with the irrigation rate set to 17 ml/min. Patients with paroxysmal AF were undergoing circumferential pulmonary vein (PV) ablation only. For patients with nonparoxysmal AF, a roof line and a mitral isthmus line ablation were performed. Radiofrequency current was applied for up to 30–60 seconds or until the maximal local electrogram amplitude reduced by 80%. The endpoint of the ablation was defined as bidirectional conduction block. This was verified by careful application of the Lasso electrode (Biosense Webster Inc., Diamond Bar, CA, USA) around the entire circumference of the PV ostia and pacing within the circumferential line at multiple sites. After the first anatomical attempt, in case of residual potentials at any location in the ablation line, a gap was suspected, and an additional ablation lesion was created.

2.3. Follow-up. All patients were followed at monthly visits for at least 6 months. All patients continued antiarrhythmic therapy at least 2 months. Holter monitoring was performed if necessary (symptoms of palpitation, etc.) in our hospital. In this study, after the three-month blanking period, the recurrence of AF was defined as atrial fibrillation documented by electrocardiogram or Holter examination. Patients were considered a success if they remained in sinus rhythm from the 3-month postoperative period to the end of the follow-up, with no evidence of AF. If the patient experienced AF during the blanking period, but no evidence of

AF was found after three months from the ablation, then they were considered a success. Antiarrhythmic drugs were terminated; otherwise, they would continue in patients without freedom from AF. Two patients underwent reablation, but they were not included in this study.

2.4. Complications. Complications were divided into two categories: serious and minor. Serious complications included acute myocardial infarction, stroke, major bleeding, PV stenosis, LA esophageal fistula, and pericardial effusion/cardiac tamponade. Minor complications included pericarditis and inguinal haematoma.

2.5. Adjunctive in Vitro Experiments. It was hypothesized that the number of magnets outside the sheath may play a role in the navigation in small LA. Therefore, *in vitro* experiments were designed to determine how the number of magnets beyond the sheath affects the flexibility of the RMN catheter. Figure 1(a) demonstrates the *in vitro* model of the RMN system simulating through a fixed SR0 sheath and varying catheter positions. Using the QuikCAS catheter-advancing system to control the advancement and retraction of the catheter, three different physicians controlled the RMN system at least three times, to confirm the maximum deflection of the catheter. This was performed with one magnet, two magnets, and all the magnets outside the sheath, and the maximum movement of the catheter was observed and recorded by X-ray.

2.6. Statistical Analysis. SPSS v19.0 statistical software was used for analysis. Continuous variables with normal distribution were expressed as mean±standard deviation. Continuous variables without normal distribution were reported as median (interquartile range). Categorical variable is expressed as a percentage. The independent sample Student's t test was performed for comparison of normal distribution variables, and the nonparametric test (Mann–Whitney) was performed for nonnormal distribution variables. The comparison of rates was confirmed by the chi-squared test. Age, gender, BMI <24 kg/m^2, paroxysmal AF, LA diameter <40 mm, basic diseases, without four classical PV ostia patterns, procedure duration ≥141.6 min, duration from mapping begin to ablation finish ≥98.0 min, and total X-ray time ≥432.0 s were used for multivariate analysis with logistic regression to estimate if these parameters are associated with the using of X-ray during mapping and ablation. The Kaplan–Meier survival function was used to analyze the event-free survival rate, and differences between groups were assessed using the logrank test. P value <0.05 was considered to be statistically significant.

3. Results

3.1. Baseline Patients' Characteristics. Baseline patients' characteristics are shown in Table 1. No statistical differences were found between the two groups of patients for gender, age, height, AF duration, left ventricular ejection fraction, the proportion of diabetes, coronary heart disease, and stroke, and four classical PV ostia patterns ($P > 0.05$). Compared with Group B, body weight, body mass index, the proportion of hypertension, and the proportion of nonparoxysmal AF were lower in Group A ($P < 0.05$).

3.2. Ablation Procedure Data. Ablation procedure data are shown in Table 2. There were no statistical differences in procedure duration and total X-ray time between the two groups ($P > 0.05$). However, the total X-ray dose was higher in Group B ($P < 0.001$). The X-ray time and dose during mapping and ablation of Group A patients were significantly higher than those in Group B (37.0 (99.0) s vs. 12.0 (30.1) s, $P < 0.001$, and 1.4 (2.7) gy·cm^2 vs. 0.7 (2.1) gy·cm^2, $P = 0.013$). More patients in Group A (80/83 patients) used X-ray guidance during mapping and ablation than Group B (68/82 patients) ($P = 0.01$). In Group A, 47/83 of the patients had the SR0 sheath adjustment at least once, and 13/83 patients needed the SR0 sheath adjusted multiple times. In Group B patients, only 21/82 patients had a single SR0 sheath adjustment, and only 2/82 patients had the SR0 sheath adjusted more than one time. No serious complications occurred in either group.

3.3. Outcomes and Follow-up Data. Outcomes and follow-up data are shown in Table 3 and Figure 2. The total rate of first anatomical attempt of PV isolation is 64.2% with no statistical difference found between the two groups (71.1% vs. 57.3%, $P = 0.065$). However, the success rate of first anatomical attempt in paroxysmal AF patients was higher than nonparoxysmal AF patients (73.5% vs. 44.2%, $P < 0.001$). By June 2018, the average follow-up time of the patients in this study was 24.0 (20.0) months. Compared with Group B, patients in Group A had lower recurrence rate, and a higher proportion of patients remained in sinus rhythm ($P = 0.009$). Subgroup analysis showed that the recurrence rate of paroxysmal AF in Group A was lower than that in Group B ($P = 0.036$), but the recurrence rate of patients with nonparoxysmal AF in Group A was similar to that in Group B ($P = 0.978$). From another perspective, patients with paroxysmal AF have lower recurrence rate than patients with nonparoxysmal AF (24.8% vs. 48.1%, $P = 0.002$).

3.4. Logistic Regression Analysis. The results of logistic regression analysis are shown in Table 4. Age, gender, BMI <24 kg/m^2, paroxysmal AF, LA diameter <40 mm, basic diseases, without four classical PV ostia patterns, procedure duration ≥141.6 min, duration from mapping begin to ablation finish ≥98.0 min, and total X-ray time ≥432.0 s were analyzed to determine if they were predictors of X-ray application for mapping and ablation. According to the result of logistic regression analysis, only LA diameter <40 mm proved to be an independent predictor in multivariate analysis associated with the use of X-ray during mapping and ablation (OR, 7.3; 95% CI, 1.7–31.0).

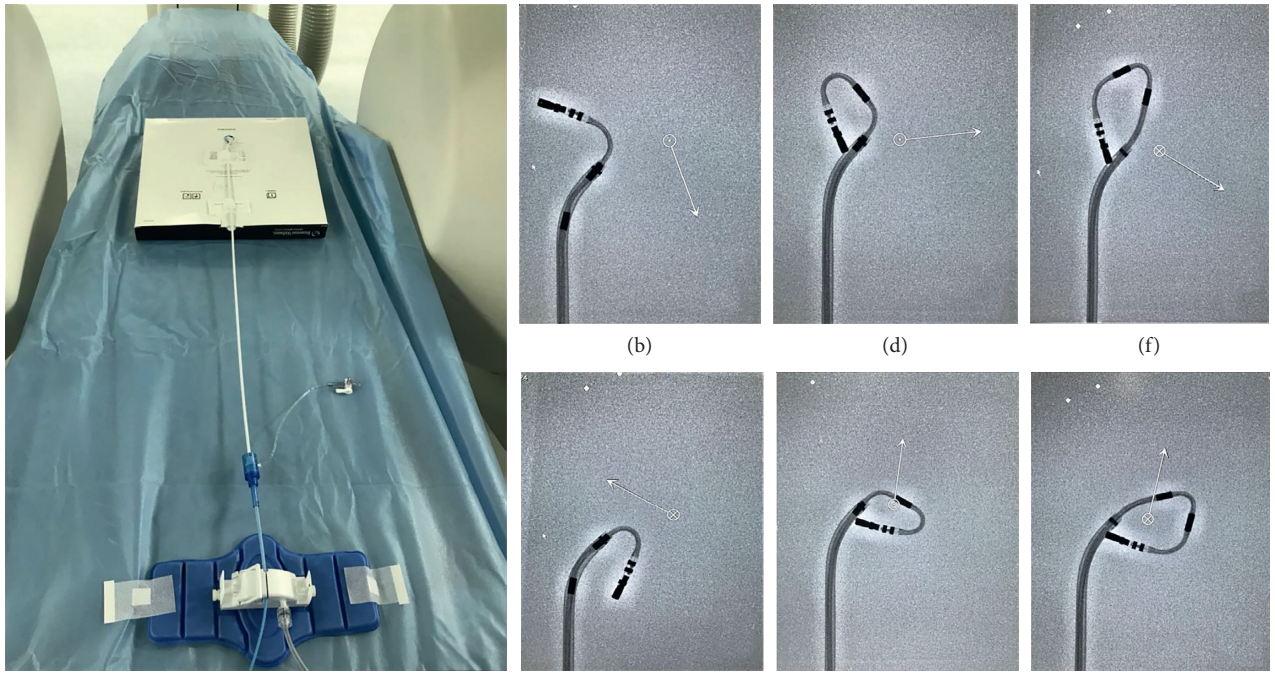


Figure 1: *In vitro* experiments showed the effect of different amounts of magnets deployed beyond the sheath tip on the flexibility of the catheter. (a) The work position of the RMN, catheter, and SR0 sheath. (b) and (c) The maximum catheter reach in two different orientations with one magnet deployed. (d) and (e) The maximum catheter reach in two different orientations of two magnets deployed. (f) and (g) The maximum catheter reach in two different orientations with all three magnets deployed.

Table 2: Procedural parameters.

	Group A (n = 83)	Group B (n = 82)	Total (n = 165)	P Value
Procedure duration (min)	143.9 ± 31.5	139.3 ± 25.1	141.6 ± 28.5	0.295
Duration from mapping begin to ablation finish (min)	100.3 ± 30.1	95.7 ± 25.9	98.0 ± 28.4	0.289
Total X-ray time (sec)	422.0 (228.0)	468.0 (190.0)	432.0 (193.5)	0.399
Total X-ray dose (gy·cm^2)	17.1 (11.0)	23.9 (19.4)	20.9 (18.4)	<0.001
X-ray time during mapping and ablation (sec)	37.0 (99.0)	12.0 (30.1)	23.0 (58.0)	<0.001
X-ray dose during mapping and ablation (gy·cm^2)	1.4 (2.7)	0.7 (2.1)	1.0 (2.2)	0.013
Patients using X-ray during mapping and ablation	80	68	148	0.01
Patients adjust sheath during mapping and ablation	47	21	68	<0.001
Patients adjust sheath for several times during mapping and ablation	13	2	15	0.007
Times of adjust sheath during mapping and ablation	1.0 (1.0)	0.0 (1.0)	0.0 (1.0)	<0.001
Heparin	6500.0 ± 1095.4	6073.2 ± 1331.3	5960.6 ± 1334.3	0.283
Complications (minor)	0	0	0	–
Complications (major)	0	0	0	–
LA volume mapped by C3 (ml)	91.8 ± 15.7	129.3 ± 35.6	109.9 ± 35.2	<0.001

C3: Carto 3 system; LA: left atrial. P values listed were calculated between Groups A and B.

3.5. In Vitro Experiments. Figures 1(b)–1(g) illustrate the sheath position, the catheter deflection, reach, pivot position, and curve shape in each RMT catheter relative to the catheter length deployed from sheath. The flexibility of the catheter was represented by the distance between catheter tip and sheath. To demonstrate the catheter flexibility, attempts were made to touch the distal tip of the catheter to the sheath by moving the tip inferiorly. Attempts to touch the sheath with one magnet positioned outside of the sheath were not possible; however, when 2 or 3 magnets were positioned outside of the sheath, the catheter tip could reach the sheath. This experiment suggests that having one magnet outside the sheath limits the flexibility of the catheter.

4. Discussion

This is the first study focusing on the impact of LA size in AF ablation using RMN. Our results indicate that the small LA may bring challenges in navigating the LA using RMN; however, this can overcome with sheath maneuvers that

Table 3: Long-term follow-up.

	Group A ($n = 83$)	Group B ($n = 82$)	Total ($n = 165$)	P value
Follow-up (month)	27.0 (21.0)	20.0 (18.3)	24.0 (20.0)	0.003
Success of first anatomical attempt				
Total	59	47	116	0.065
Paroxysmal	52	31	83	0.947
Nonparoxysmal AF	7	16	23	0.262
Recurrence				
Total	19	34	53	0.009
Paroxysmal	13	15	28	0.036
Nonparoxysmal AF	6	19	25	0.978
Long-term success (%)				
Total	77.1	58.5	67.9	0.009
Paroxysmal	81.7	64.3	75.2	0.036
Nonparoxysmal AF	50.0	52.5	51.9	0.978

AF: atrial fibrillation. P values listed were calculated between normal group and abnormal group.

allow at least two magnets to be exposed but at the cost of an increase in X-ray exposure. *In vitro* experiments confirmed that with only one magnet deployed, the flexibility of the catheter is reduced. Despite the limitations, patients in our study with small LA had lower recurrence rate and the higher rate of sinus rhythm in follow-up. There were no serious complications reported in either group; thus, the additional sheath adjustments did not affect the safety profile in this study.

AF ablation using RMN is safe and effective [1–6]. Previous studies have confirmed that the RMN system can provide more accurate and stable catheter tissue contact, potentially increasing the efficiency of the ablation [8]. However, many studies have shown that the success rate of AF ablation using RMN is not higher than that of manual procedure, and the recurrence rate is similar during period of 6 to 18 months follow-up [2, 3, 5, 6, 9]. Further studies reported that the RMN system has unique advantages. Firstly, using RMN can obviously reduce severe complications such as death and cardiac tamponade [1, 5, 10]. Secondly, RMN ablation can significantly reduce the X-ray time and dose of patients and operators [1, 5, 10]. However, because it was cost prohibitive, the RMN system was not used widely in China; therefore, the application experience is limited.

As found in other reports, this study has shown that mapping and ablation of AF using RMN is not only safe and effective but also reduces the rate of complications and the radiation exposure [6]. Similar results are shown in this study, despite challenges of navigating the RMT catheter in smaller LAs. This was especially true in some locations such as the lower right PV, where the catheter during mapping and ablation point became unstable, and the tip of the catheter often jumped away or had difficulty during movements when the magnetic field direction was adjusted. In this study, the data of patients with the LA diameter <40 mm were analyzed and compared with patients with the LA diameter ≥40 mm. The results showed that during mapping and ablation, the catheter was more challenging to navigate in a small LA. However, this was remedied by making sheath adjustments under X-ray guidance to ensure the flexibility of the ablation catheter. This results in a significant increase in X-ray time and dose during mapping and ablation and was only a small portion of the overall dose; however, it did not affect the overall X-ray time for the procedure, which was slightly longer for Group B patients. Due to the differences in sheath maneuvers between the two groups, we hypothesized that the flexibility of the catheter may be compromised; therefore, the *in vitro* experiments were designed to determine how the number of magnets beyond the sheath affects the flexibility of the RMN catheter.

The RMN system is designed to steer the magnetic catheter by the three magnets located in the flexible distal portion of the catheter. The combined adjustments of the catheter length and the magnetic field direction are used to make precise movements and establish stable focal contact. This unique characteristic allows the physician to maintain control of the electrode position independent of the complexity of the path. However, during the procedure in the patients with a small LA, the operation space of magnetic catheter is also limited, thus reducing the flexibility of catheter, inducing the instability of catheter, and increasing the difficulty in navigation.

The logistic regression analysis showed that only LA diameter <40 mm proved to be an independent predictor associated with the using of X-ray during mapping and ablation, while other factors, including the variation of PV anatomy, were not related. This suggests that other factors did not increase the difficulty of RMN catheter operation.

In order to overcome these difficulties, we used some techniques to compensate for the navigation challenges of the RMN catheter in the small LA. The puncture point should be selected in the anterior region of the fossa ovalis, so that the ostium of sheath can be as far away as possible from the PV. For improving the fine movement of the catheter, the position of the sheath can be adjusted during the procedure as follows. First, "point to the light and ablation the right PV," which means the ostium of sheath points to the opposite direction of the target, while the direction of the magnetic field points to the ablation target (Figure 3(a)). Second, retract the sheath from the LA to the

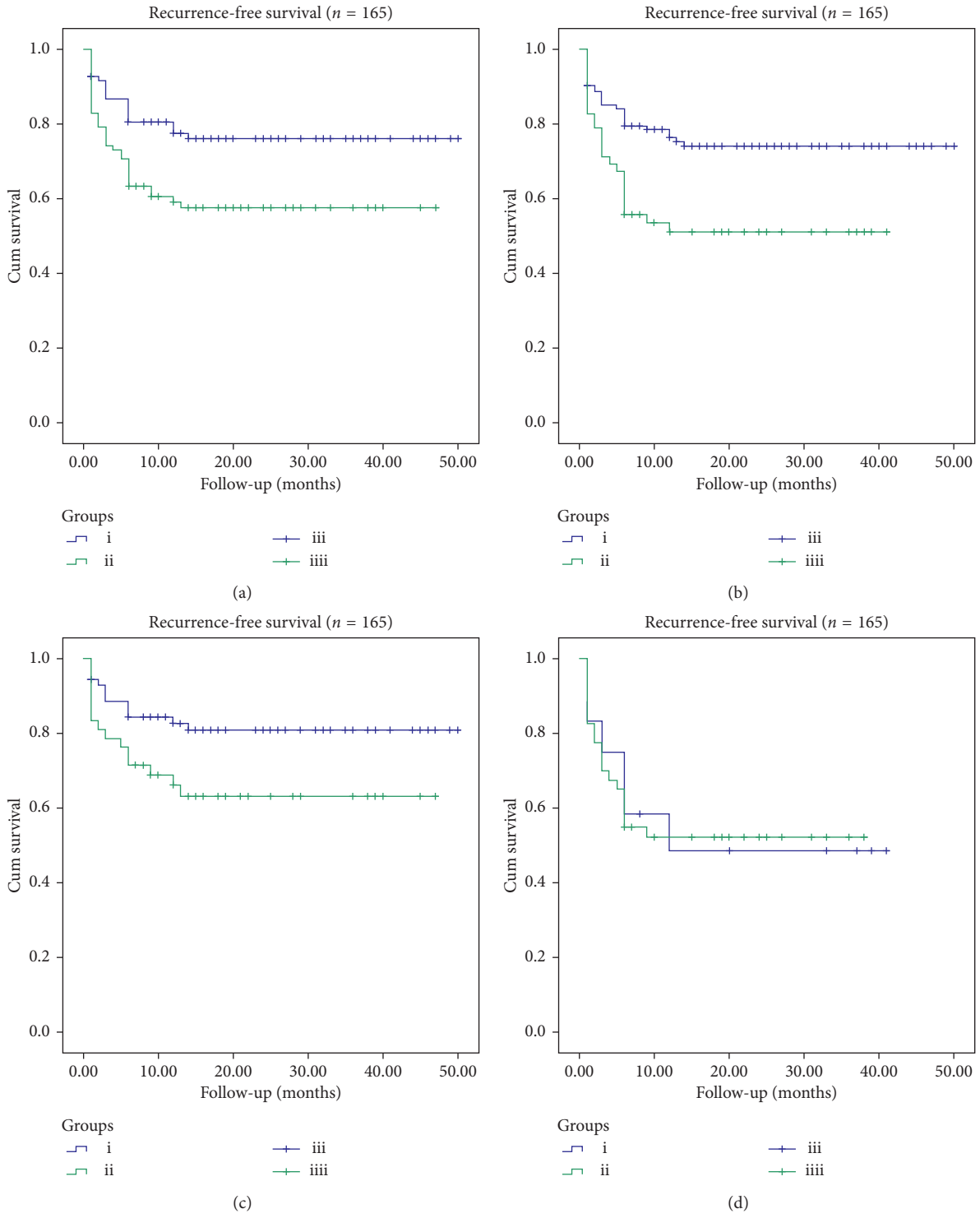
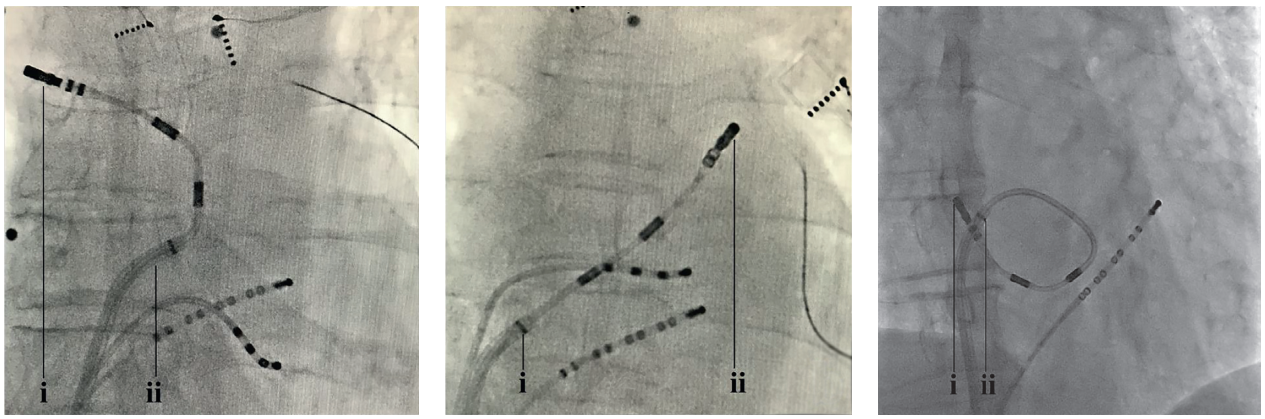


Figure 2: Kaplan–Meier curves of recurrence-free survival after primary successful ablation. (a) Kaplan–Meier curves demonstrate recurrence-free survival for patients with small left atrial (LA) group and large LA group. (i) Patients with LA diameter <40 mm, (ii) patients with LA diameter ≥40 mm, (iii) patients with LA diameter <40 mm censored, and (iv) patients with LA diameter ≥40 mm censored. (b) Kaplan–Meier curves demonstrate recurrence-free survival for patients with paroxysmal atrial fibrillation (AF) group and nonparoxysmal AF group. (i) Patients with paroxysmal atrial fibrillation, (ii) patients with nonparoxysmal atrial fibrillation, (iii) patients with paroxysmal atrial fibrillation censored, and (iv) patients with nonparoxysmal atrial fibrillation censored. (c) Kaplan–Meier curves demonstrate recurrence-free survival for patients with paroxysmal AF in small LA group and large LA group. (i) Paroxysmal atrial fibrillation patients with LA diameter <40 mm, (ii) paroxysmal atrial fibrillation patients with LA diameter ≥40 mm, (iii) paroxysmal atrial fibrillation patients with LA diameter <40 mm censored, and (iv) paroxysmal atrial fibrillation patients with LA diameter ≥40 mm censored. (d) Kaplan–Meier curves demonstrate recurrence-free survival for patients with nonparoxysmal AF in small LA group and large LA group. (i) Nonparoxysmal atrial fibrillation patients with LA diameter <40 mm, (ii) nonparoxysmal atrial fibrillation patients with LA diameter ≥40 mm, (iii) nonparoxysmal atrial fibrillation patients with LA diameter <40 mm censored, and (iv) nonparoxysmal atrial fibrillation patients with LA diameter ≥40 mm censored.

Table 4: Multivariate analysis with logistic regression.

Variables		OR	95% C.I		P
			Lower	Upper	
Gender	Male vs. female	1.2	0.4	3.6	0.771
Age (years)	<60 vs. ≥60	0.9	0.3	3.4	0.904
BMI (kg/m^2)	<24 vs. ≥24	1.9	0.6	6.3	0.301
Paroxysmal AF	Yes vs. No	1.8	0.5	6.7	0.357
LA diameter (mm)	<40 vs. ≥40	7.3	1.7	31.0	0.007
Basic diseases	Yes vs. No	0.8	0.2	4.0	0.815
Four classical pulmonary vein ostia patterns	Yes vs. No	0.5	0.2	1.6	0.234
Procedure duration (min)	<141.6 vs. ≥141.6	3.1	0.6	14.6	0.163
Duration from mapping begin to ablation finish (min)	<98.0 vs. ≥98.0	2.4	0.5	10.7	0.248
Total X-ray time (sec)	<432.0 vs. ≥432.0	0.7	0.2	2.3	0.515

BMI: body mass index; AF: atrial fibrillation; LA: left atrial; OR: odds ratio; CI: confidence interval.



(a) (b) (c)

Figure 3: Fluoroscopic views in anteroposterior projection showing the special position of the sheath and RMT catheter. (a) The sheath (ii) points to the left and the catheter (i) points to the right. (b) The sheath (i) drags from the left atrial (LA) while the catheter (ii) remains in the LA. (c) The sheath (ii) extends to the LA roof and the catheter (i) makes a circular.

right atrium while the ablation catheter remains in the LA (Figure 3(b)). Third, when the ablation of the inferior PV is performed, the ostium of sheath is extended to the LA roof (Figure 3(c)). The purpose of these maneuvers is to leave enough space for deploying the three magnets of the RMN catheter from the sheath.

The results showed that, after applying the navigation technique above, patients with a small LA had successful electrical isolation of the PV. The results of follow-up showed that the proportion of patients maintained sinus rhythm with small LA that remains high, which was consistent with previous studies [11–13]. However, these techniques significantly increase X-ray time and dose during mapping and ablation. In some centers, the V-drive™ system can be used to robotically move the sheath in the control room without manual maneuvers [14, 15], however, at an increased cost for Chinese patients. The total X-ray time was not different in the two groups because the X-ray time was mainly used in the placement of catheter, transseptal puncture, and PV imaging. These processes "dilute" the differences in X-ray time during mapping and ablation; however, the difference in fluoro time was 37 s and 12 s between Group A and Group B, respectively. The X-ray dose increased in the large LA group because this group had higher body weight, thus needing the increased radiation. The follow-up time was longer in the small LA group, likely due to the proportion of patients maintaining sinus in the small LA group being high, so the patients had better compliance, while some patients in the large LA group often lost follow-up after recurrence.

5. Limitations

There are still some limitations in our research. First, although the *in vitro* experiment assessed the flexibility of the catheter, there was no reliable method to measure the contact force of the catheter. Second, the result of *in vitro* test cannot fully represent the situation in LA. Third, the number of patients with nonparoxysmal AF in this study, especially in the large LA group, is limited, which may affect the results.

6. Conclusions

AF ablation using RMN is safe and effective. The small LA can bring challenges to navigation using RMN due to only one magnet beyond the distal sheath, thus increasing the

need to use X-ray to guide the operator. We recommend that at least two magnets be deployed beyond the sheath tip during mapping and ablation therapy. Despite these navigation challenges, small LA patients had lower AF recurrence rate compared with large LA patients.

Data Availability

The figure and table data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Efficacy of Wenxin Keli Plus Amiodarone versus Amiodarone Monotherapy in Treating Recent-Onset Atrial Fibrillation

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Background. Use of amiodarone (AMIO) in atrial fibrillation (AF) has significant side effects over prolonged periods. Wenxin Keli (WXKL), a Chinese herb extract, has been shown to be effective in atrial-selective inhibiting peak I_{Na} and hence beneficial in treating atrial arrhythmias, including atrial fibrillation. The aim of this randomized controlled trial was to evaluate potential effects of AMIO plus WXKL on conversion rate and time in patients with recent-onset AF. *Methods*. A total of 41 patients (71 ± 12 years, 44% male) with recent-onset (<48 h) AF eligible for conversion were randomized to receive either intravenous amiodarone (loading dose 5 mg/kg in 1 hour followed by 50 mg/h; $n = 21$) or amiodarone with same dosage plus oral WXKL 18 g thrice daily ($n = 20$) for 24 hours. *Results*. Conversion rate at 24 hours was of no difference between the two groups (75.0% vs. 81.0%, $P = 0.72$); however, conversion time was markedly shorter in the AMIO + WXKL group compared to the AMIO group (291 ± 235 minutes vs. 725 ± 475 minutes, $P = 0.003$). There were no serious adverse events during the study. *Conclusion*. Administration of amiodarone plus WXKL for recent-onset AF conversion was safe and effective, with faster sinus rhythm restoration compared with amiodarone alone.

1. Introduction

Atrial fibrillation (AF), the most common cardiac arrhythmia, is independently responsible for a five-fold increased risk of stroke, a three-fold increased risk of heart failure, and causes significant mortality [1]. By 2030, 14–17 million individuals living in the European Union are predicted to suffer from AF. Restoration and maintenance of sinus rhythm is a major strategy for AF management [2]. The 2012 European Society of Cardiology (ESC) guidelines for the management of AF recommend that amiodarone (AMIO) be used for conversion in patients with ischemic or structural heart disease [2]. However, AMIO has extracardiac toxic effects, only moderate efficacy, and a delayed onset of action [1]. By contrast, Wenxin Keli (WXKL), a traditional Chinese medicine composed of Nardostachys, Codonopsis, Notoginseng, Amber, and Rhizoma, is an approved preparation that has demonstrated efficacy and safety in the treatment of several forms of cardiac arrhythmia. In isolated canine, arterially perfused right atrial preparations, Wenxin Keli significantly prolonged effective refractory period (ERP) and induced postrepolarization refractoriness

(PRR) despite abbreviating the action potential duration in an atrial-selective manner. It also reduced maximum rate of rise of action potential upstroke, prolonged P wave duration such that sodium channel current (I_{Na}) dependent parameters were depressed, and prevented induction of persistent AF in 100% of preparations tested [3]. Burashnikov et al. reported that WXKL has anti-AF properties due to its atrial-selective depression of I_{Na}-dependent parameters in canine-isolated coronary-perfused preparations [3]. The atrial selectivity of WXKL likely contributes to its usefulness for effective management of AF with minimal effects on the ventricular electrophysiology [3–5]. Atrial-selective I_{Na} block occurred following trains of pulses elicited over a range of pulse durations and interpulse intervals in canine myocytes. This was due to a combination of the more negative voltage of steady-state inactivation, the less-negative resting membrane potential, and the shorter diastolic intervals in atrial cells at rapid activation rates [6]. When used in combination with AMIO, WXKL has been found to shorten conversion time, decrease the required dosage of AMIO, and prevent adverse drug reactions [7]. The aim of this pilot study was to evaluate the effects of adding WXKL to AMIO on conversion rate and time within 24 hours after administration in patients with recent-onset AF.

2. Methods

2.1. Study Protocol. This was a single-center, randomized, open-label prospective clinical trial in the Second Hospital of Tianjin Medical University. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and the CONSORT recommendations and was approved by the Institutional Committee on Human Research of the Second Hospital of Tianjin Medical University. Agents used in this trial were approved by the State Food and Drug Administration (SFDA) and have become available in the market for the treatment of cardiac arrhythmias. All patients provided their informed consent. Eligible patients, in accordance with the random number table, were divided into two groups (random numbers 1 to 21 for AMIO group, random numbers 22 to 41 for AMIO + WXKL group) and assigned to receive either intravenous AMIO (loading dose 5 mg/kg in 1 hour followed by 50 mg/h) or AMIO with the same dosage plus oral WXKL 18 g three times daily for a maximum of 24 hours. All of the patients received appropriate anticoagulation therapy before and during conversion as per recommended guidelines. After conversion to sinus rhythm, both groups continued to receive AMIO orally 200 mg three times a day for a week, 200 mg twice a day for the next week, and once a day afterwards, or as according to the physician's prescription. Beta-receptor blockers were allowed in these two groups to achieve rate control. Furthermore, patients underwent careful 24-hour electrocardiogram (ECG) monitoring during the course of the treatment. The trial continued until one or more of the following events were encountered: new onset sustained ventricular tachycardia, ventricular fibrillation, or torsades de pointes; QTc >550 ms; heart rate <40 bpm or symptomatic bradycardia; systolic blood pressure <90 mmHg; acute hyperthyroidism; allergic shock; and severe superficial phlebitis. In cases of AF persisting beyond 24 hours, electrical conversion or radiofrequency ablation was attempted directly (without atrial thrombus) or after 4 weeks of anticoagulation administration.

2.2. Patient Selection and Exclusion Criteria. All patients who developed recent-onset (duration <48 hours) AF and presented from February 2016 to December 2016 were eligible for our study. All patients were fit for pharmacological conversion as well as adequate anticoagulation therapy in accordance with guidelines. Exclusion criteria were acute coronary syndrome, cardiogenic shock, atrial flutter, symptomatic bradycardia, history of sick sinus syndrome or atrioventricular block, severe valvular diseases, thyroid disorders, renal failure, and administration of Classes I or III antiarrhythmic drugs within 24 hours before enrolling into the study.

2.3. Endpoints and Clinical Parameters. The primary endpoint was the conversion rate of AF to SR within 24 hours in the AMIO group and the AMIO + WXKL group. The secondary endpoint was the conversion time in these two groups within 24 hours. In terms of electrocardiography (ECG), QT interval was measured at baseline, at the time of conversion to SR (success in conversion), or at 24 hours (failure in conversion), and then corrected for heart rate (QTc) using Bazett's formula. Transthoracic echocardiographic (TTE) parameters were recorded at baseline, including left atrial diameter (LAD), left ventricular end diastolic diameter (LVEDD), left ventricular systolic diameter (LVSDD), interventricular septal (IVS) thickness, and left ventricular ejection fraction (LVEF). In addition, biochemical parameters, including serum potassium, serum sodium, serum chlorine, serum creatinine, blood uric acid, and blood nitrogen, were assessed at baseline.

2.4. Statistical Analyses. Statistical analyses were performed with IBM SPSS statistics 22.0. Continuous variables were summarized as mean ± SD or median (quartiles) and compared using unpaired Student's t-test or the Mann–Whitney test. Categorical variables were presented as absolute numbers and percentages and compared by the chi-square test. Time to conversion to SR was assessed via Kaplan–Meier analysis. We used the log-rank test to compare cumulative progression curves for AF conversion in the two groups. Outcomes were considered statistically significant at P level <0.05.

3. Results

From February 2016 to December 2016, 41 consecutive patients were considered eligible for this study based on our inclusion criteria and were consequently randomly allocated to either the AMIO group ($n = 21$) or the AMIO + WXKL group ($n = 20$). These two groups were similar in terms of demographic, clinical, and echocardiographic characteristics, as summarized in Tables 1 and 2. Most patients in both groups

Table 1: Demographics and clinical characteristics of enrolled patients.

Variables	AMIO group ($n = 21$)	AMIO + WXKL group ($n = 20$)	P value
Male	10 (47.6%)	8 (40.0%)	0.62
Age (years)	72 ± 13	71 ± 12	0.73
Smoke	5 (23.8%)	5 (25.0%)	0.93
New-onset/recurrent AF	6/15	3/17	0.29
Onset of AF (hours)*	4.00 (0.50–29.50)	2.75 (0.31–13.75)	0.72
HTN	14 (66.7%)	15 (75%)	0.56
T2DM	3 (14.3%)	9 (45.0%)	0.03
Medications			
ACEI/ARBs	12 (57.1%)	14 (70.0%)	0.39
β-blockers	12 (57.1%)	11 (55.0%)	0.89
CCBs	4 (19.0%)	13 (65.0%)	<0.05
Statins	16 (76.2%)	15 (75.0%)	0.93
Digoxin	1 (4.8%)	5 (25.0%)	0.07

Data are expressed as numbers (percentage) and mean ± standard deviation. AMIO = amiodarone; WXKL = Wenxin Keli; HTN = arterial hypertension; T2DM = type 2 diabetes mellitus; ACEI/ARBs = angiotensin converting enzyme inhibitiors/angiotensin receptor blockers; CCBs = calcium channel blocker.
*"Onset of AF" means the time from the onset of AF to the record of AF.

Table 2: Clinical parameters, echocardiographic (ECHO), and ECG characteristics of patients after admission.

Variables	AMIO ($n = 21$)	AMIO + WXKL ($n = 20$)	P value
ECHO			
LAD (mm)	40.5 ± 5.9	40.9 ± 5.5	0.85
LVEDD (mm)	46.7 ± 4.0	48.4 ± 4.8	0.21
LVESD (mm)	30.0 ± 5.4	31.4 ± 6.3	0.44
IVS (mm)	9.8 ± 1.6	9.6 ± 2.5	0.75
LVEF (%)	58.1 ± 8.7	61.3 ± 7.3	0.22
ECG			
QTc_1 (ms)	437 ± 29	456 ± 25	0.03
QTc_2 (ms)	446 ± 34	439 ± 34	0.53
P value*	0.26	0.002*	
Clinical parameters			
K+ (mmol/L)	4.0 ± 0.4	4.1 ± 0.5	0.60
Na+ (mmol/L)	142.4 ± 5.0	141.5 ± 3.7	0.51
Cl− (mmol/L)	104.4 ± 3.8	104.1 ± 4.7	0.84
Cr (μmol/L)	69.7 (60.5–79.4)	75.0 (63.6–105.3)	0.19
UA (μmol/L)	327.4 ± 97.4	344.9 ± 105.5	0.58
BUN (mmol/L)	6.4 ± 2.0	7.1 ± 2.7	0.35

Data are expressed as mean ± standard deviation or median (P_{25}–P_{75}). QTc_1 means the QTc interval at baseline. QTc_2 represents the QTc interval at the end of the study. *P value at the end of the study compared with prior to drug administration in the AMIO group and the AMIO + WXKL group, respectively. AMIO = amiodarone; WXKL = Wenxin Keli; LAD = left atrial diameter; LVEDD = left ventricular end diastolic diameter; LVESD = left ventricular end systolic diameter; IVS = interventricular septum; LVEF = left ventricular ejection fraction; HR = heart rate; QTc = heart rate corrected QT interval; K+ = serum potassium; Na+ = serum sodium; Cl− = serum chlorine; Cr = serum creatinine; UA = blood uric acid; BUN = blood urea nitrogen.

exhibited recent-onset AF with arterial hypertension. However, there were more patients with type 2 diabetes mellitus in the AMIO + WXKL group compared with the AMIO monotherapy group ($P = 0.03$). QTc interval prior to administration was longer in the AMIO + WXKL group than in the AMIO group (456 ± 25 ms vs. 437 ± 29 ms, $P = 0.03$). The mean heart rate of the patients following conversion showed no significant difference in the AMIO group than in the AMIO + WXKL group (67.4 ± 14.1 beats per minutes vs. 62.3 ± 9.2, $P = 0.24$). The echocardiographic parameters, including LAD, LVEDD, LVESD, IVS, and LVEF, did not differ significantly between the two groups. None of the patients in either group had previously undergone cardiac surgery.

3.1. Effects of Amiodarone Plus Wenxin Keli. The proportion of patients converted to sinus rhythm within 24 hours in the AMIO group was not significantly different from that of the AMIO + WXKL group (81.0% vs. 75.0%, $P = 0.72$) (Figure 1). In the AMIO group, there was no significant difference in the conversion rate (83.3% vs. 80.0%, $P > 0.99$) or conversion time (808 ± 447 vs. 691 ± 501 minutes, $P = 0.66$) between patients presenting with first episodes of AF and those with recurrent AF episodes. Similarly, in the AMIO + WXKL group, no significant differences in the conversion rate (66.7% vs. 76.5%, $P > 0.99$) but marked differences in the conversion time (646 ± 319 minutes vs. 236 ± 178 minutes, $P = 0.02$) were observed. In the AMIO group, the conversion rate within 24 hours for male patients had a tendency to be higher than that of female patients (100.0% vs. 63.6%, $P = 0.09$). Comparatively, in the AMIO + WXKL group, these proportions were 100.0% and 58.3%, respectively ($P = 0.06$). Figure 2 shows that the secondary endpoint, conversion time, was markedly shortened in the AMIO + WXKL group compared with the AMIO-only group (291 ± 235 minutes vs. 725 ± 475 minutes, $P = 0.003$). Cumulative conversion progression in the two groups is shown in Figure 3.

3.2. Electrocardiography. Baseline QTc interval was significantly different in the AMIO and AMIO + WXKL groups (437 ± 29 ms vs. 456 ± 25 ms, $P = 0.03$). The QTc interval at the time of conversion was not significantly different between the AMIO and AMIO + WXKL groups (446 ± 34 ms vs. 439 ± 34 ms, $P = 0.53$). The QTc interval tended to be increased nonsignificantly in the AMIO group (437 ± 29 ms to 446 ± 34 ms, $P = 0.26$), but was significantly shortened in

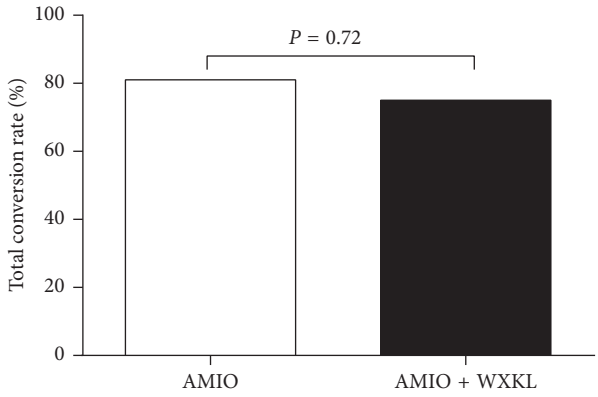

Figure 1: Effect of the combination of AMIO plus WXKL ($n = 20$) versus AMIO monotherapy ($n = 21$) on conversion rate.

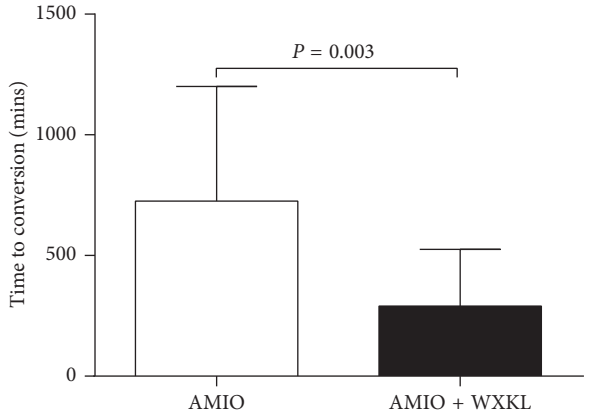

Figure 2: Effect of the combination of AMIO plus WXKL ($n = 20$) versus AMIO monotherapy ($n = 21$) on conversion time.

the AMIO + WXKL group (456 ± 25 ms to 439 ± 34 ms, $P = 0.002$). The PR interval after conversion was not significantly different in the AMIO + WXKL group from the AMIO group (159 ± 33 ms vs. 145 ± 29 ms, $P = 0.31$). No serious adverse events, including substantial QTc prolongation (defined as QTc interval >550 ms), were observed during the 24 h in either group.

4. Discussion

The main finding of this randomized controlled trial is that the combination of Wenxin Keli (18 g three times daily) and amiodarone (loading dose 5 mg/kg in 1 hour followed by 50 mg/h) was safe and superior to amiodarone monotherapy in chemical conversion of recent-onset AF. Conversion time to restore sinus rhythm was markedly shortened in patients receiving the combination therapy (291 ± 235 minutes) compared with amiodarone alone (725 ± 475 minutes) despite similar results for conversion rate within 24 hours between the two groups (75.0% vs. 81.0%, respectively). In addition, the QTc interval shortened from baseline to 24 hours in the combination group (456 ± 25 ms to 439 ± 34 ms), with an opposite result in the amiodarone

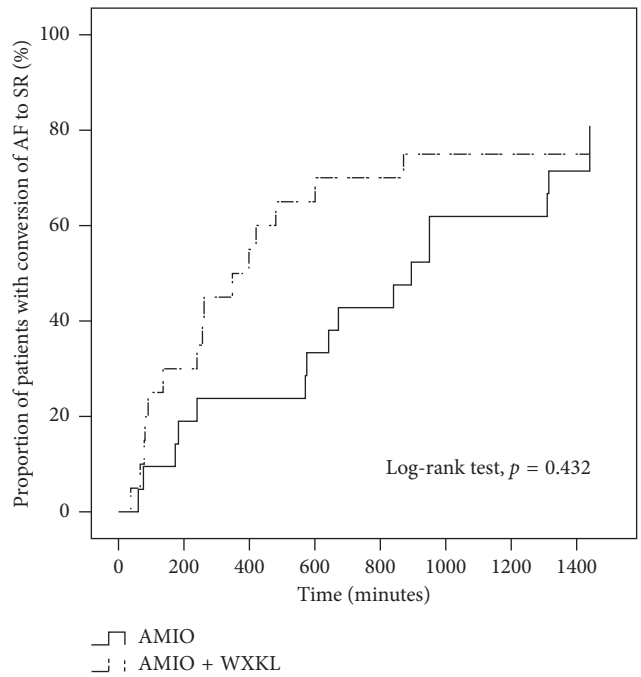

Figure 3: Cumulative progression of atrial fibrillation conversion to sinus rhythm in the AMIO plus WXKL group ($n = 20$) versus the AMIO monotherapy group ($n = 21$) during the initial 24 hours of treatment.

monotherapy group (437 ± 29 ms to 446 ± 34 ms). No serious adverse effects occurred during the study. The females had a tendency to be older than the males in both the AMIO group (75 ± 9 years vs. 68 ± 16 years, $P = 0.22$) and the AMIO + WXKL group (73 ± 12 years vs. 68 ± 10 years, $P = 0.37$). Furthermore, the mean LAD in the females was larger with no significant difference than that in the males in both the AMIO group (40.9 ± 6.6 mm vs. 40.1 ± 5.4 mm, $P = 0.79$) and the AMIO + WXKL group (41.6 ± 5.8 mm vs. 39.8 ± 5.3 mm, $P = 0.49$). To the best of our knowledge, the age and the LAD are the independent risk factors of AF, which may explain the lower conversion rates of the females of older ages with larger LADs than the males in the two groups.

Wenxin Keli, a traditional Chinese medicine composed of Nardostachys, Codonopsis, Notoginseng, Amber, and Rhizoma, is a formally approved drug that has proven effective and safe for treatment of several forms of cardiac arrhythmia, such as atrial fibrillation and premature ventricular contractions [8, 9]. In isolated rabbit left ventricular myocyte models, Wenxin Keli preferentially inhibited late sodium current while the QRS duration remained less affected at different pacing rates. At higher concentrations (>1 mg/mL), this drug also prevented QT prolongation, suppressed early and delayed postdepolarizations, and subsequent triggered activity with no significant effects on the L-type calcium current ($I_{Ca,L}$) and gave a positive staircase pattern in contractility [10]. This is a possible explanation as to why we found a significant decrease in QTc interval at the end of the study compared with the QTc at baseline in the Wenxin Keli plus amiodarone group. Wenxin

Keli attenuated ischemia-induced ventricular arrhythmias in rats *in vivo* by significantly reducing the amplitude of $I_{Ca,L}$ via decreasing the rate of activation, while also inhibiting the transient outward potassium current (I_{to}) by increasing the rate of deactivation [11].

According to the results from a prospective, randomized study on paroxysmal atrial fibrillation (PAF) due to hyperthyroidism, sinus rhythm was observed in 91.2% of patients with Wenxin Keli and 89.9% of patients with sotalol after three months [12]. This suggests equal efficacies of both drugs in sinus rhythm conversion. The rate of maintenance of sinus rhythm in the Wenxin Keli-Western medicine group (84.6%) exceeded that of the Western medicine group (62.7%), revealing a significant beneficial effect in the combination group. Despite inadequate data to definitively indicate the efficacy of Wenxin Keli on PAF, this meta-analysis provides encouraging evidence for the effects of Wenxin Keli on sinus rhythm [4]. Our results showed that there was no significant difference in the conversion rate within 24 hours between the AMIO group and the combination group, but this does not necessarily contradict the aforementioned meta-analysis. This is because, to the best of our knowledge, diabetes mellitus is an independent risk factor for AF. In a diabetic rabbit model, high glucose and H_2O_2 stimulation promoted atrial fibroblast proliferation [13]. In some cases, atrial fibrillation is considered a complication of hypoglycemia [14, 15]. As such, due to numerous metabolic abnormalities, atrial structural, electrical, and electromechanical remodeling take place [16]. In the combination group, there were more diabetic patients than in the AMIO monotherapy group. As a result, the conversion rate of the combination group may be greatly reduced. Studies of larger sample sizes are therefore warranted to eliminate confounding factors, such as diabetes mellitus.

Amiodarone is widely used for conversion in patients with abnormal left ventricular function or ischemia, but it is limited by its delayed onset of action and lower conversion rate compared with other antiarrhythmic agents. Wenxin Keli is a type of Chinese medicine composed of five different components: Nardostachys jatamansi DC (Gansong), Radix Notoginseng (Sanqi), Succinum (Hupo), Polygonatum sibiricum (Huangjing), and Codonopsis pilosula (Dangshen) [7, 17–20]. Previous experiments have demonstrated the effects of these components on ion channel function in the atria. WXKL shortens atrial action potential durations and blocks the sodium channels [3]. It shifts the steady-state availability of sodium channels to more negative potentials in atrial than in ventricular cells in a dose-dependent manner [6]. These would be expected to prolong atrial effective refractory periods and induce post-repolarization refractoriness. The consequence would be a reduction in the critical interval for reexcitation given by APD-ERP [21], reflecting a lower likelihood of reentry [3, 22]. Our findings are in keeping with previous clinical studies reporting the similar efficacy between Wenxin Keli and sotalol for rhythm control in paroxysmal AF associated with hyperthyroidism [12]. In a prospective randomized pilot study, addition of a single dose of ranolazine, an anti-anginal and anti-ischemic agent with atrial-selective inhibition of late sodium channel current, substantially increased AF conversion rate at 24 hours by 23%, in addition to significantly accelerating SR restoration by >4 hours compared with amiodarone treatment [23]. In another study, the efficacy benefit of ranolazine plus amiodarone was more accentuated in patients with left atrial (LA) enlargement (>46 mm) determined by transthoracic echocardiography, whereas amiodarone alone was as highly efficient as combination therapy in patients with smaller LA size [24]. Moreover, QTc was not one of the matching variables as QTc interval has not been widely accepted to be an independent risk factor for AF or AF conversion but we cannot exclude that this could have modified the risk of incident AF or the substrate of AF. There was modest QT prolongation in both groups. However, in our study, the QTc interval shortened from baseline to 24 hours in the combination group, which may be due to the synergistic effect of Wenxin Keli [10]. In another prospective, randomized clinical trial, mean time of conversion was significantly shorter in the ranolazine-amiodarone group, suggesting a superior antiarrhythmic effect against postcoronary artery bypass graft (CABG) AF compared to amiodarone alone [25, 26].

An important limitation in our study lies in the small sample size restricted to a single center. This was in part due to the cost of running the trial. Nevertheless, we were able to demonstrate statistical significance for the effects of Wenxin Keli in reducing conversion time. However, the study was underpowered in detecting the difference in the conversion at 24 hours or meaningful changes in the electrocardiographic parameters. Furthermore, the QTc before onset of atrial fibrillation was not included in the study. In our study, effects of WXKL on ventricular conduction and side effects or interactions with our drugs administrated can not be excluded, and there exist no sufficient data supporting atrial selectivity on I_{Na}+ exclusively. Although our result of shortened QTc with Wenxin Keli-amiodarone therapy proves intriguing and safe compared with trials with other drug combinations in terms of outcomes, confirmation from larger studies is required prior to clinical application.

In conclusion, addition of Wenxin Keli to amiodarone markedly shortened conversion time as well as the QTc interval, despite similar results for conversion rates within 24 hours, with no adverse reactions or proarrhythmic effects. A comparison using a larger sample size with varied echocardiographic parameters in the presence of underlying heart conditions would be merited to confirm the superiority of combination therapy in conversion of recent-onset AF. In this study, we explored the acute effects of WXKL as an adjunct therapy to amiodarone. In future studies, the next step will be to explore whether WXKL is effective in converting AF into sinus rhythm beyond 24 hours.

Data Availability

The raw data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

NZ and TL conceived and designed the study. NZ, YY, and MG were involved in collection and assembly of data. NZ was involved in data analysis and interpretation. All authors were involved in writing and final approval of the manuscript.

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Research Article

A Pilot Study on Parameter Setting of VisiTag™ Module during Pulmonary Vein Isolation

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Objectives. To identify optimal predefined criteria (OPC) for filters of the VisiTag™ module in the CARTO 3 system during pulmonary vein isolation (PVI). *Methods*. Thirty patients with atrial fibrillation (AF) who experienced PVI first were enrolled. PVI was accomplished by using a Thermocool SmartTouch catheter. Ablation lesions were tagged automatically as soon as predefined criteria of the VisiTag™ module were met. OPC should be that ablation with the setting resulting in the conduction gap (CG) as few as possible, while contiguous encircling ablation line (CEAL) without the tag gap (TG) on the 3D anatomic model as much as possible. *Result(s)*. When ablation with parameter setting is being catheter movement with a 3 mm distance limit for at least 20 s and force over time (FOT) being off, there were 60 CEAL without TG on the 3D anatomic model. However, 26 CGs were found. After changing FOT setting to be a minimal force of 5 g with 50% stability time, 22 TGs were displayed. Of them, 20 TGs were accompanied by CGs. On reablation at sites of TG with changed parameter setting, 18 CGs were eliminated when 20 TGs disappeared. When reablation with FOT is being a minimal force of 10 g with 50% stability time, 6 remaining CGs were eliminated. However, there was no CEAL. With a mean of follow-up 10.93 months, 2 patients with persistent AF suffered AF recurrence. *Conclusion*. A 3 mm distance limit for at least 20 s and FOT being a minimal force of 5 g with 50% stability time might be OPC for the VisiTag™ module.

1. Introduction

Since pulmonary vein isolation (PVI) has been verified to be the cornerstone for treatment of atrial fibrillation (AF) with radiofrequency ablation, making a durable PVI has always been the goal we are chasing. Despite advances in technology, resumption of conduction between the PV and the left atrium is not uncommon, which plays a critical role for AF recurrence [1, 2]. This should be partly attributed to the lack of an effective method to quantify the efficacy of each ablation application during procedure of PVI. As we all know, without the help of the intelligent assistant system many factors affecting the efficacy of ablation cannot be objectively judged by operators during procedures, such as contact force (CF) and catheter stability. With the advent of the CF sensing catheter and automated ablation lesion tagging software, annotation of ablation lesion can be tagged automatically as soon as the predefined criteria for tagging are met. For this reason, a better result of ablation might be achieved if the tagging system is employed with reasonable predefined criteria.

VisiTag™ module (Biosense Webster, Inc.) is an automated ablation lesion tagging software on the CARTO 3 system (Biosense Webster, Inc.), which can continuously store, track, and quantify ablation catheter positions along with the electrophysiological parameters acquired during RF applications. It contains settable constraints for the catheter status according to user preference, including maximal range of catheter drift, minimal time of catheter-tissue connection, and force over time (FOT) which means

percentage of minimal time with CF consistently above certain number of grams, impedance drop, and target temperature. Once all preset values for constraints are met, an annotation of ablation lesion would be automatically put on the 3D electroanatomic model during each application of ablation. Thus, quantifying the efficacy of each ablation application might be reflected indirectly and objectively. To our knowledge, however, there is no any recommendation for predefined criteria of VISITAG™ module for initial ablation of PVI. In this study, we hypothesize that optimal predefined criteria (OPC) should meet the ablation with the setting and should result in both conduction gap (CG) and tag gap (TG) on contiguous encircling ablation line (CEAL) as few as possible.

2. Methods

2.1. Patient Population. From January 2017 to September 2017, thirty patients with drug refractory paroxysmal or persistent AF underwent catheter ablation with the 3D navigation CARTO 3 system were recruited. Antiarrhythmic drugs except amiodarone were discontinued at least 5 half-lives before the procedure. All these patients provided a written informed consent before procedures.

2.2. VisiTag™ Module Setting. VisiTag™ module was employed in all ablation procedures for automated tagging of ablation lesion. The filter of respiration adjustment was ticked for avoiding the effects of breathing on tags. The filters, impedance drop and target temperature, were not ticked because these two filters were not well predicted according to the suggestion by Johnson & Johnson. For local drift of the ablation catheter during ablation, 3 mm was used to define the maximal moving range of the catheter according to the outcome of study by Ullah et al. [3]. Twenty-second was used to define the minimal stability time of the ablation catheter based on the study by Chikata et al. [4]. These two parameters, 3 mm and 20 s, were constant in the study. As for FOT, 3 different settings were used during the procedure. At the beginning, the values of the filters in FOT, time, and minimum force, were zero until accomplishment of isolation line around ipsilateral PVs. After that, 50% for time in conjunction with 5 g for minimum force or 50% for time in conjunction with 10 g for minimum force was used as a FOT setting successively. Diameter of lesion tag size was 4 mm.

2.3. Ablation Procedure. All patients underwent the procedure under conscious sedation. Transesophageal echocardiography or computed tomography was performed to rule out thrombus in the left atrium before the procedure. After dual transseptal catheterizations, 3D electroanatomical maps of the LA and PVs were reconstructed with a 20-pole mapping catheter LASSO (Biosense Webster, Inc.) or PentaRay (Biosense Webster, Inc.). PVI was performed in a wide area circumferential ablation pattern using a Thermocool SmartTouch irrigated-tip contact-force sensing radiofrequency ablation catheter (Biosense Webster, Inc.). The lesions were created by sequential point-by-point application of radiofrequency. The default power setting was 15–25 W for the posterior wall and 30–40 W for other regions of the left atrium. For each ablation application, duration of ablation was 40–60 s with a stable contact of catheter-tissue. The ablation lesions were tagged automatically based on predefined criteria on the VisiTag module. After accomplishment of isolation line around ipsilateral PVs, reablation would be applied at the sites with conduction gap (CG) which indicated electric connection between PV and left atrium.

2.4. Pulmonary Vein Isolation. Firstly, a wide-area circumferential ablation around ipsilateral PVs was accomplished with automated tagging criteria being catheter movement with a 3 mm distance limit for at least 20 s without FOT setting. After that, electric conversion would be implemented if it was not sinus rhythm. Then, CGs were identified by the mapping catheter. The value of the FOT filters was set to hide the tags which did not conform to FOT criteria. Thus, tag gaps (TGs) would emerge on the 3D electroanatomic map. TG was defined as the distance between two neighboring tag points more than 7 mm from the center point to the center point. Afterwards, PVI was achieved as following strategy: if CG and TG were located at the same site of ablation line when FOT setting is being a minimal force of 5 g or 10 g with 50% stability time, elimination of CG was attempted by putting new tags on TG when reablation with FOT setting. If CG still existed after reablation with FOT setting being a minimal force of 10 g with 50% stability time, ablating the corresponding part of pulmonary vein ostium was performed. If TG existed without CG, no reablation was delivered. Bidirection block would be verified after ablation.

2.5. Data Analysis. For the purpose of analysis, the ablation line around each ipsilateral PV was divided into 8 distinct segments as previous study [5]. The following data were analyzed after accomplishment of PVI, including force time integral (FTI) and impedance drop (ID) in each ablation application, the distribution of CGs after initial ablation around ipsilateral PVs, and the relationship between CG and TG.

2.6. Follow-Up. A 24-hour Holter recording was obtained at the 3 month and 6 month after the procedure. A 12-lead electrocardiogram was assessed at every follow-up. Chronic clinical success at the 6-month follow-up was defined as the absence of sustained AF, atrial flutter, or atrial tachycardia for over 30 s after the blanking period no matter with or without the antiarrhythmic drug.

2.7. Statistical Analysis. Continuous variables are expressed as mean and standard deviation. Categorical variables are presented as frequency or percentage. All statistical analysis was performed using SPSS (version 22, IBM Corp., Armonk, NY).

3. Results

Of these 30 patients, 24 were male with mean age 58.40 ± 12.11 years. Paroxysmal AF was diagnosed in 23 patients. The anteroposterior diameter of the left atrium on transthoracic echocardiography was 39.54 ± 5.06 mm. The procedure lasted an average time of 161.34 ± 34.29 minutes, with an average ablation time of 57.78 ± 12.65 min, and a mean fluoroscopy time of 11.48 ± 9.02 min. There were no severe cardiac complications, such as pericardial tamponade and atrioesophageal fistula. With a mean follow-up of 10.93 ± 2.10 months, 2 patients with persistent AF suffered AF recurrence after the 3-month blanking period.

3.1. Contact Force, Force Time Integral, and Impedance Drop. A total of 2496 ablation lesions were delivered during the PVI procedures (83.20 ± 10.76 lesions per patient). Mean values of CF, FTI, and ID in initial ablation were shown in Table 1. At this moment, automated tag criteria in the VisiTag™ module was catheter movement with a 3 mm distance limit for at least 20 s without the FOT setting. After reablation for elimination of CG while adding FOT setting minimal force 5 g with 50% stability time to previous criteria for automated tagging, recalculated mean values of CF, FTI, and ID were shown in Table 2.

3.2. The Relationship between CGs and TGs Based on Different Tagging Criteria. Contiguous encircling ablation line (CEAL) consisted of tag points one by one was achieved in all the patients when automated tag criteria being catheter movement with a 3 mm distance limit for at least 20 s without FOT setting. In such situation, there was no TG on CEAL. However, CGs were verified in 26 segments out of 17 pairs of ipsilateral PVs in 11 patients. The distribution of CGs was 2 in the left superior, 6 in the left anterior superior, 7 in the left anterior, 2 in the left anterior inferior, 1 in the left inferior, 3 in the right superior, 1 in the right posterior superior, 2 in the right posterior inferior, 1 in the right inferior, and 1 in the right anterior inferior, respectively. Once FOT setting minimal force 5 g with 50% stability time was included into tagging criteria, 22 TGs displayed on encircling ablation line. Among them, 20 TGs were localized at the sites of CG. They were 1 in the left superior, 4 in the left anterior superior, 5 in the left anterior, 1 in the left anterior inferior, 1 in the left inferior, 3 in the right superior, 1 in the right posterior superior, 2 in the right posterior inferior, 1 in the right inferior, and 1 in the right anterior inferior, respectively. The other two TGs were localized at 1 in the left posterior inferior and 1 in the right anterior inferior, respectively. After reablation with adjusted tagging criteria, 18 CGs were eliminated while 20 TGs were filled by new tag points. When FOT setting was changed to minimal force 10 g with 50% stability time, almost half of the tag points disappeared on the 3D electroanatomic map, and there was no CEAL in all patients (Figure 1). Reablating at such tagging criteria, 6 out of 8 CGs were eliminated with annotation of new tag points. They were localized at 1 in the left superior, 3 in the left anterior superior, and 2 in the left anterior, respectively. The remaining 2 CGs were eliminated when ablation was delivered at the areas of the left anterior carina.

4. Discussion

PVI has been verified playing very important role in treatment of AF with radiofrequency ablation [6, 7]. However, duration of PVI was unsatisfactory since recovery of PV conduction had not been an uncommon phenomenon. Multiple reasons might contribute to recovery of PV conduction, including unstable tissue-catheter contact during the ablation and traditional method of annotation of ablation lesion. Without assistance of the intelligent tool, it is hard for the operator to objectively judge the stability of the catheter during ablation application. In addition, traditional ablation lesion tagging bears a strong tinge of subjectivity. Ablation lesion would be annotated as soon as ID or morphological change of the electrogram met the operator's target. After that, the ablation catheter would be rolled to other areas. As a result, inadequate damage for each ablation might be produced. With the employment of the VisiTag™ module, an automated tagging of ablation lesion is realized, which can uniform the standard of ablation lesion annotation and reduce the influence of subjectivity. As a result, a better outcome of PVI could be expected when the VisiTag™ module is employed during the procedure of PVI. In study by Tanaka et al., successful PVI at completion of the initial anatomical line was more frequent when the VisiTag™ module was used for ablation annotation [8]. Similarly, a lower recurrent rate at follow-up was proved when comparison in patients underwent AF ablation with or without employment of the VisiTag™ module [9]. However, a 66.3% successful rate of PVI at the initial procedure and a percentage of 77.5% freedom from atrial tachyarrhythmias at a 12-month follow-up in aforementioned studies might indicate suboptimal parameter settings for filters of the VisiTag™ module, respectively. In study by Fujiwara et al., although the ablation lesions with the acute conduction block was realized at a mean ablation time 12.5 s, the maximum value of FTI in these lesions was 213 g·s, which was almost half of an accepted reference value 400 g·s [10]. For these reasons, what is OPC for filters of the VisiTag™ module is still to be tested. A lenience setting might prevent the endurance of PVI, whereas a strict setting might increase the complication of ablation. To test OPC for the automated tagging system VisiTag™ module during the initial procedure of PVI, we did this study.

According to the results of previous studies, 3 mm maximal moving range of the catheter and 20 s minimal stability time of the ablation catheter were constant in this study. As for FOT, 3 different settings were tested. When ablation with FOT setting is being zero, a CEAL consisting of tag points one by one around ipsilateral PVs was shown on the 3D electroanatomic map in all patients. However, CGs were verified in 26 segments (5%) out of 480 segments in 30 patients. Once FOT setting minimal force 5 g is added with 50% stability time to tagging criteria, 22 segments with TG were displayed. Of these segments, 20 segments localized at the same sites where CGs existed. After reablation with

Table 1: Mean values of CF, FTI, and ID in each ablation segment after the initial wide-area circumferential ablation around ipsilateral PVs without FOT setting.

Segments	CF (g)	FTI (g·s)	ID (Ω)
Left superior	11.88 ± 5.90	512.49 ± 172.83	13.16 ± 6.16
Left anterior superior	8.91 ± 3.73	418.03 ± 148.63	12.15 ± 5.67
Left anterior	10.39 ± 4.18	427.16 ± 163.99	14.51 ± 6.39
Left anterior inferior	9.06 ± 3.49	427.26 ± 153.31	12.55 ± 6.19
Left inferior	11.38 ± 4.44	441.65 ± 152.46	14.02 ± 5.33
Left posterior inferior	14.04 ± 5.68	495.35 ± 174.51	13.13 ± 4.84
Left posterior	11.76 ± 5.15	445.90 ± 143.90	11.24 ± 4.89
Left posterior superior	11.83 ± 5.33	477.17 ± 152.47	12.13 ± 5.58
Right superior	14.29 ± 4.68	552.94 ± 140.85	15.46 ± 6.21
Right anterior superior	14.02 ± 4.63	575.31 ± 149.48	17.67 ± 5.71
Right anterior	17.89 ± 5.65	615.66 ± 153.57	15.85 ± 450
Right anterior inferior	11.56 ± 4.96	514.95 ± 213.81	14.22 ± 8.50
Right inferior	11.81 ± 5.51	482.34 ± 181.52	14.91 ± 6.85
Right posterior inferior	11.92 ± 5.83	511.77 ± 203.11	11.50 ± 4.78
Right posterior	12.96 ± 5.09	517.56 ± 153.14	10.78 ± 4.48
Right posterior superior	13.77 ± 6.92	545.55 ± 239.42	12.00 ± 5.81

CF: contact force; FTI: force time integral; ID: impedance drop.

Table 2: Mean values of CF, FTI, and ID in each ablation segment after the initial wide-area circumferential ablation around ipsilateral PVs with FOT setting being minimal force 5 g with 50% stability time.

Segments	CF (g)	FTI (g·s)	ID (Ω)
Left superior	12.28 ± 5.58	537.32 ± 148.71	13.58 ± 5.61
Left anterior superior	10.06 ± 3.08	489.00 ± 141.33	12.89 ± 5.16
Left anterior	11.19 ± 3.57	491.10 ± 137.84	15.41 ± 5.51
Left anterior inferior	9.68 ± 3.01	467.32 ± 144.73	13.00 ± 5.66
Left inferior	11.65 ± 4.17	456.23 ± 137.10	14.21 ± 5.04
Left posterior inferior	14.04 ± 5.68	495.35 ± 174.51	13.13 ± 4.84
Left posterior	11.76 ± 5.15	445.90 ± 143.90	11.24 ± 4.89
Left posterior superior	11.83 ± 5.33	477.17 ± 152.47	12.13 ± 5.58
Right superior	14.53 ± 4.22	557.19 ± 130.73	15.61 ± 5.96
Right anterior superior	14.02 ± 4.63	575.31 ± 149.48	17.67 ± 5.71
Right anterior	17.89 ± 5.65	615.66 ± 153.57	15.85 ± 450
Right anterior inferior	11.90 ± 4.65	536.34 ± 197.24	14.46 ± 8.27
Right inferior	12.36 ± 5.03	514.32 ± 157.16	15.36 ± 6.26
Right posterior inferior	12.96 ± 4.79	559.48 ± 167.37	12.19 ± 4.26
Right posterior	12.96 ± 5.09	517.56 ± 153.14	10.78 ± 4.48
Right posterior superior	14.09 ± 6.63	565.96 ± 229.08	12.27 ± 5.53

CF: contact force; FTI: force time integral; ID: impedance drop.

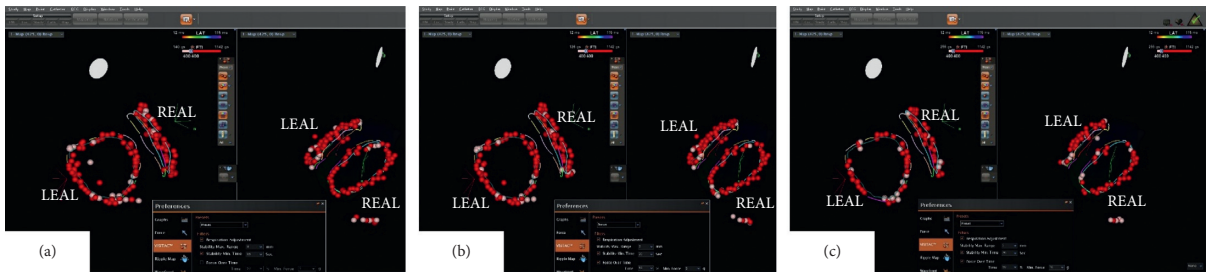

Figure 1: An example of different parameter settings of the VisiTag™ module for pulmonary vein isolation: a 3 mm maximal moving range of the catheter and 20 s minimal stability time of the ablation catheter were constant. (a) FOT setting being zero; (b) FOT setting being minimal force 5 g with 50% stability time; (c) FOT setting being minimal force 10 g with 50% stability time. Different color lines indicated different segments of ablation line. White arrow indicated a TG at the situation of FOT setting being a minimal force of 5 g with 50% stability time, which did not display when FOT setting is zero. FOT: force over time; LEAL: left encircling ablation line; REAL: right encircling ablation line.

adjusted tagging criteria, 18 CGs were eliminated as soon as TGs were filled by new tag points appeared on the 3D electroanatomic map. So far, PVI was achieved in 26 patients (86.7%) with CEAL on the 3D electroanatomic map. For remaining 8 CGs, 6 of them were eliminated while new tag points were put in TGs during reablation with FOT setting being a minimal force of 10 g with 50% stability time. However, there was no CEAL on the 3D electroanatomic map in all patients at this automated tagging setting. Almost one-third of tag points disappeared from the 3D electroanatomic map. According to this result, we can infer that it is difficult to achieve a CEAL when ablation with tagging criteria being 3 mm maximal moving range of the catheter and 20 s minimal stability time of the ablation catheter with FOT setting being minimal force 10 g with 50% stability time. To many inexperienced operators, however, achievement of CEAL around ipsilateral PVs is the first step for achieving PVI. For this reason, repeated ablations might be implemented by part of operators because it has been proven that human behavior are influenced by the visual effect [11]. If so, unnecessary overablation would be performed, which could increase the possibility of complication. Thus, with consideration of both efficacy of ablation and effect of automated tagging, the following values might be OPC for the automated tagging system VisiTag™ module during the initial procedure of PVI: 3 mm for maximal moving range of the catheter, 20 s for minimal stability time of the ablation catheter, 5 g for minimal force of FOT, and 50% for stability time of FOT, respectively.

4.1. Limitations. This study had some limitations. First, the results are based on a relatively small size of 30 patients. Second, this is the report of a single operator. Third, the ablation procedures were performed under sedation and not general anesthesia with jet ventilation. Forth, an accumulated efficacy for eliminating CG cannot be evaluated due to technical limitations when reablating at sites with CG. Finally, the period of follow-up was not long enough.

5. Conclusion

A 3 mm maximal moving range of the catheter and 20 s minimal stability time of the ablation catheter in addition to FOT setting being minimal force 5 g with 50% stability time might be OPC for the automated tagging system VisiTag™ module in the initial ablation procedure of PVI.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

Yu-Chuan Wang and Bo Huang are the co-first authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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