

RISK STRATIFICATION STRATEGIES FOR CARDIAC RHYTHM ABNORMALITIES

EDITED BY: N A, Kamalan Jeevaratnam, Konstantinos Letsas, Tong Liu and
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RISK STRATIFICATION STRATEGIES FOR CARDIAC RHYTHM ABNORMALITIES

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Editorial: Risk Stratification Strategies for Cardiac Rhythm Abnormalities

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Editorial on the Research Topic

Risk Stratification Strategies for Cardiac Rhythm Abnormalities

INTRODUCTION

Cardiac rhythm abnormalities, such as atrial fibrillation (AF) and ventricular tachycardia (VT)/ventricular fibrillation (VF), account for a significant proportion of adverse cardiac events and mortality (1). In fact, most cardiovascular pathologies will lead to some form of rhythm abnormalities as the pathology worsens. In many instances such rhythm abnormalities precede the fatal event. Accurate risk stratification is central to early and timely treatment in high-risk patients and to avoid unnecessary invasive procedures. There are protocols and diagnostic algorithms currently in place that help clinicians to profile the risk associated with a condition. None of these tools is fully accurate despite ongoing efforts to improve risk stratification strategies (2). Current clinical practice involves a combination of clinical history, genetic testing, non-invasive electrocardiographic measurements (3) and invasive electrophysiological studies (4). The aim of this Research Topic is to (1) investigate the physiological mechanisms that underlie cardiac rhythm abnormalities, (2) examine current approaches used for risk stratification in different conditions and their impact on patient outcomes, and (3) explore the use of experimental models and computational and machine learning algorithms to facilitate the development of risk stratification tools.

PRE-CLINICAL MODELS FOR STUDYING CARDIAC ARRHYTHMIAS

Cellular, animal and computational models have been used to study the molecular and electrophysiological mechanisms underlying cardiac arrhythmias (5). Saadeh and Fazmin review the mechanisms by which age-related mitochondrial dysfunction promotes arrhythmic triggers and substrate. This provided insights into novel potential anti-arrhythmic pharmacological interventions that specifically target upstream mitochondrial function, and hence ameliorates the need for therapies targeting downstream changes which have constituted traditional antiarrhythmic therapy. Deng et al. reviews the key role of uric acid in mediating oxidative stress and inflammation, which underlie fibrotic change of the atria predisposing to AF. Fong et al.

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conducted a meta-analysis into modulated calcium homeostasis and calcium release events in AF, demonstrating higher sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA) expression in the between primary diseased AF group, but lower expression in the secondary AF groups. Costa et al. describe in-depth the molecular interactions between sex hormones and the cardiac ion channels, as well as the clinical implications of these interactions on the cardiac conduction system, in order to understand the link between these hormones and the susceptibility to cardiac arrhythmias.

Avula et al. presented APD dispersion data from two-dimensional optical mapping in a mouse model with F1759A SCN5A overexpression modeling long QT syndrome type 3. They then presented theoretical models for APD dispersion, methods for analysis and calculation of APD dispersion, and showed that APD dispersion in clustered patterns is the predominant configuration for spontaneous occurrence and persistence of AF and VF. Liu et al. conducted experiments to examine the effects of autonomic input in vein of Marshall-mediated AF. They found that electrical stimulation of the left superior ganglionated plexi shortened atrial refractoriness, increased APD dispersion and the vulnerability window. These effects were prevented by low level stimulation or ethanol ablation of the vein of Marshall, or muscarinic blockade by atropine. Acquired causes of VT/VF may arise from remodeling after myocardial infarction. In a mouse model with knockout of the CC chemokine receptor 9 (CCR9), abnormalities in ion currents, calcium handling, gap junction and action potential conduction were prevented, suggesting that the CCR9 can be a promising therapeutic target for reducing the likelihood of myocardial infarction-related arrhythmias (Huang Y. et al.).

RISK STRATIFICATION FOR CARDIAC ARRHYTHMIAS

AF is the commonest cardiac rhythm abnormality observed in clinical practice and accounts for significant morbidity and mortality *via* the development of ischemic stroke (6), dementia (7, 8) and heart failure (9). Shang et al. reviewed the clinical applications and utility of AF characteristics, cardiac imaging and electrocardiogram markers, arterial stiffness and atherosclerosis-related markers, circulating biomarkers, and novel genetic markers for the diagnosis of ischaemic stroke and non-valvular AF. Ning et al. reviewed the current literature on the etiology of atrial cardiomyopathy in embolic strokes of undetermined source. Liao L.-Z. et al. conducted a two-sample Mendelian randomization study demonstrating a causal inference between hypertension and AF. Hidru et al. conducted a cohort study of 9,618 hypertensive patients and identified serum uric acid levels and left atrial diameter as significant predictors of AF. Moreover, the modified Taiwan AF score consisting of age, male gender, hypertension, heart failure, coronary artery disease and end-stage renal disease predicted incident AF in a Chinese population with an area under the curve of 0.86 for 1-year follow-up, 0.83 for 5-year follow-up, 0.80 for 10-year follow-up, and 0.75 for 16-year follow-up (Liao J.-N. et al.).

One of the most devastating outcomes of AF is stroke for which the CHA2DS2-VASc score has been used for its risk stratification (10). A recent study explored low risk patients with CHA2DS2-VASc scores of 0 to 1 (Kim, Yu, Kim, Lee et al.), demonstrating that in patients with normal left ventricular ejection fraction, high H2FPEF score and increasing age were independently associated with the development of ischemic stroke. Song et al. found that a comprehensive evaluation of serum uric acid and B-type natriuretic peptide levels, left atrial diameter and left ventricular ejection fraction can stratify the risk of stroke in patients with non-valvular AF. A large retrospective cohort study conducted propensity score matching between with atrial flutter and AF (Wang H.-T. et al.). Among patients without history of stroke, the risk of dementia was higher in patients with AF than in patients with AFL.

The following studies investigated the impact of interventional and medical treatment on outcomes in patients with AF. Balloon-based catheter ablations, including hot balloon ablation and cryoballoon ablation, have rapidly emerged as alternative modalities to conventional catheter ablation owing to their procedural advantages and better clinical outcomes and safety profiles. A recent study found that patients undergoing hot balloon ablation had a higher incidence of touch-up ablation and longer procedural time, but with comparable clinical outcomes on mid-term follow-up (Peng et al.). patients with short episodes of AF <24 h were compared to those with 24 h or longer in a propensity score-matched analysis for cryoablation of pulmonary veins isolation (Jiang et al.). Higher success rate and lower incidence of stroke/transient ischaemic attacks were observed for the <24h group compared to the ≥ 24 h group during follow-up. Recurrence in AF remains a problem despite improvement in ablation technology. A prospective cohort study found that leukocyte telomere length predicted disease progression from paroxysmal to persistent AF following catheter ablation (Wang Q. et al.). Soluble Suppression of Tumorigenicity 2, was shown to be predictive of AF recurrence after radiofrequency ablation in patients with persistent AF (Tan et al.). A systematic review and meta-analysis found that the HAS-BLED score has moderate predictive abilities for bleeding risks in patients with AF regardless of type of oral anticoagulants (Gao et al.). For medical treatment of AF, beta blockers are frequently used. Interestingly, beta blockers consistently decreased long-term mortality in patients with high-burden and low-burden of premature atrial complexes, and this effect appears not to be mediated through a reduction in AF or new onset stroke (Huang T.-C. et al.). Recent work has explored traditional Chinese medicine as an adjunct therapy (11, 12). A systematic review and meta-analysis of randomized controlled trials on Zhigancao Decoction combined with metoprolol was performed, demonstrating good efficacy with few adverse events for different types of arrhythmias that include AF (Yang et al.).

A registry study from Shanghai, China investigated the impact of new onset AF in patients with acute myocardial infarction. Interestingly, the authors found that asymptomatic rather than symptomatic new onset AF was significantly predictive of cardiovascular mortality and all-cause mortality (Luo et al.). In a propensity score-matched study between patients undergoing

transcatheter and surgical aortic valve replacement, post-operative AF had a worse impact on heart failure-related hospital admissions and composite outcome of mortality, stroke and heart failure-related admissions (Jeong et al.). In a single center prospective study, machine learning models were developed using least absolute shrinkage and selection operator (LASSO) and random forest (RF) algorithms for variable selection (Chen Y. et al.). Their LASSO-Cox model showed an area under the curve of 0.84 for predicting 1-year mortality.

Age was identified as a risk factor of syncope recurrence in elderly patients with vasovagal syncope and positive head-up tilt test (Guo et al.). Another study from the same group examined a large cohort of 4,873 patients undergoing the head-up tilt test, finding that direct drug potentiation is safe and sensitive for diagnosing vasovagal syncope (Xu et al.). Patients with an overlap syndrome between chronic obstructive pulmonary disorder and obstructive sleep apnoea showed higher rates of cardiac arrhythmias (such as AF, premature atrial contraction, ventricular premature contraction, and atrioventricular or VT) compared to those with either condition alone (Tang et al.). Heart rate was shown to be predictive of all-cause mortality in patients suffering from multiple myeloma, but further work is needed to explore the prognostic role of cardiac arrhythmias in this condition (Wang J. et al.). Li and Zhang developed an autodetection algorithm using neural network combined with the attention-based bidirectional long-short term memory model for classifying nine different types of ECG patterns, including normal electrocardiogram, atrial fibrillation, atrioventricular block, left bundle branch block, right bundle branch block, premature atrial complexes, premature ventricular complexes, ST depression and ST elevation (Li and Zhang). Regarding AF treatment, the H2FPEF Score, which reflects the degree of left ventricular diastolic dysfunction, was improved following catheter ablation (Kim, Yu, Kim, Uhm et al.). Zhang et al. compared the catheter ablation to pacing in patients with tachycardia-bradycardia syndrome, reporting lower rates of the composite endpoint of cardiovascular-related hospitalization and thromboembolic events, as well as the progression of atrial fibrillation and heart failure.

VT/VF can arise from acquired causes, such as ischaemic heart disease, electrolyte disturbances and drugs. Tse, Li et al. reviewed the electrophysiological mechanisms that predispose to the development of atrial and ventricular arrhythmias by non-reentrant and reentrant mechanisms in hypokalaemia, a common electrolyte abnormality in hospitalized patients. Moreover, Zhou, Zhao et al. identified a non-linear relationship between body mass index and VT/VF in Chinese patients with implantable cardioverter-defibrillators (ICDs). Another study from the same group investigated whether the obesity paradox in all-cause mortality is present among the Chinese population with an ICD but did not demonstrate its presence (Zhou, Sun et al.). Sun et al. found that compared to night-time heart rate of ≤ 50 or ≥ 70 bpm, heart rate between 50 and 70 bpm was associated with lower risks of ventricular tachyarrhythmias, appropriate ICD shocks, inappropriate ICD shocks, and all-cause mortality. A retrospective study from Hong Kong, China investigated Chinese patients who were hospitalized for acute heart failure,

demonstrating that fragmented QRS was a significant predictor of VT/VF, sudden cardiac death and cardiovascular mortality (Chan et al.).

Alternatively, VT/VF can be due to inherited heart diseases such as cardiomyopathies (13) or ion channelopathies (14). Patients diagnosed with arrhythmogenic and dilated cardiomyopathy can harbor mutations in the phospholamban gene. In patients with phospholamban p.Arg14del mutational carriers, high procollagen type I carboxy-terminal propeptide to C-terminal telopeptide collagen type I ratios correlated with end-diastolic and end-systolic volumes, T-wave inversion and the presence of premature ventricular contractions (van der Voorn et al.). The commonest ion channelopathy globally is long QT syndrome, which is more common in Western compared to Asian populations (15). Nevertheless, Tse, Lee, Zhou et al. conducted a territory-wide study into the epidemiology of LQTS in a city of China, and found that the application of random survival forest technique significantly improved risk prediction for VT/VF compared to Cox regression.

Brugada syndrome (BrS) is characterized by coved or saddle-shaped ST segment elevation in the right precordial leads (16). It has a higher prevalence in Asia compared to western countries. However, risk stratification is difficult, especially in asymptomatic subjects (17). Both depolarization and repolarization abnormalities are hypothesized to underlie arrhythmogenesis in BrS. ECG indices that are manually measured have been explored for their ability to predict future arrhythmic events (18–20). Recent efforts have focused on the use of automated measurements to facilitate risk prediction in BrS (21). Thus, in a cohort of Chinese BrS patients, Tse, Lee, Li et al. identified ST slope as a novel predictor of ventricular arrhythmogenesis. The use of invasive programmed ventricular stimulation (PVS) has aided risk stratification although its sensitivity, specificity, positive predictive value and negative predictive value can differ depending on the protocols employed (22). Recent work has demonstrated that right ventricular outflow tract electro-anatomical abnormalities can predict VF inducibility (23).

Nevertheless, recent available evidence indicates that risk prediction is more accurate when a multi-parametric approach rather than rely on a single investigative method (24). Monasky et al. evaluated the role of genetic testing for risk stratification in BrS. They recommend that whole exome or whole genome testing and family segregation analysis should always be performed. Chen X. et al. studied the clinical and genetic characteristics of 104 probands with early repolarization syndrome, reporting its association with loss-of-function genetic defects in genes encoding the cardiac calcium channel. They identified a unique clinical entity characterized by decreased heart rate and QTc, as well as increased transmural dispersion of repolarization. In the case of the CACNA1C-P817S variant, impaired trafficking of the channel to the membrane contributes to the loss-of-function in the calcium channel.

Finally, several studies examined risk stratification strategies for other tachycardias and also bradycardias. For example, the role of baseline-corrected QT interval dispersion (QTcd) in predicting the effectiveness of metoprolol in pediatric postural

tachycardia syndrome was examined (Wang Y. et al.). The pre-treatment baseline QTcd were significantly longer in responders treated with metoprolol compared to non-responders and that it was negatively correlated with SS after metoprolol treatment. The prenatal management for immune-associated congenital heart block in fetuses was reviewed, in particular issues pertaining to clinical management, including the roles of autoantibodies in its pathophysiology, diagnosis and prognosis (Liao H. et al.). A study of multiple myeloma patients from Xi'an, China found that approximately half of the patients suffered from cardiac arrhythmias (Li et al.). In particular, those with sinus bradycardia had lower incidences of all-cause mortality compared to those without it.

CONCLUDING REMARKS

The articles collected under this Research Topic advance our understanding of risk stratification strategies for cardiac rhythm abnormalities, presenting recent progress on the pre-clinical, clinical and epidemiological studies on the different cardiac arrhythmias. There is a growing body of evidence supporting a more integrative approach by combining new and established computational and experimental/clinical approaches to improve our understanding and treatment of cardiac arrhythmias.

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A Study of Cardiogenic Stroke Risk in Non-valvular Atrial Fibrillation Patients

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Objectives: We attempted to develop more precisely quantified risk models for predicting cardiogenic stroke risk in non-valvular atrial fibrillation (NVAF) patients.

Methods: We conducted a case-control study, using data from hospitalized patients with AF who underwent transesophageal echocardiography at Shanghai Chest Hospital. A total of 233 high cardiogenic stroke risk patients with left atrial appendage thrombus (LAT) or left atrial spontaneous echo contrast (LA-SEC) and 233 controls matched for age, sex, AF type.

Results: AF history, LA diameter enlargement, larger left ventricular end diastolic diameter, lower ejection fraction, greater serum uric acid (SUA), and brain natriuretic peptide (BNP) levels showed association with high stroke risk. The multivariate logistic regression analysis revealed that AF duration, left atrial diameter (LAd), left ventricular ejection fraction (LVEF), SUA, and BNP were independent risk factors of the LAT/LA-SEC. We used LAd, LVEF, SUA, and BNP to construct a combined predictive model for high stroke risk in NVAF patients (the area under ROC curve: 0.784; sensitivity 66.1%; specificity 76.8%; 95% CI 0.744–0.825, $P < 0.001$).

Conclusion: Comprehensive evaluation of LAd, LVEF, SUA, and BNP may help stratify the cardiogenic stroke risk among non-valvular AF patients, guiding anticoagulation therapy.

Keywords: atrial fibrillation, cardiogenic stroke, left atrial appendage thrombus, LA-SEC, risk model

INTRODUCTION

Cardiogenic stroke is defined as the ischemic stroke caused by the shedding of a cardiogenic embolus and embolism corresponding to the cerebral artery. According to reports, it accounts for 14% of all ischemic strokes (1, 2). Atrial fibrillation (with or without other cardiovascular diseases) related stroke accounts for more than 79% of all cardiogenic stroke, which is the most important risk factor of cardiogenic stroke (3, 4). Compared with non-AF related stroke, AF related stroke has more severe symptoms, higher disability rate, higher mortality rate, and is easy to relapse; the mortality rate is twice as high as non-AF related stroke; the medical cost is 1.5 times as high as non-AF related stroke (5). Left atrial appendage thrombus (LAT) and left atrial spontaneous echo contrast (LA-SEC) caused by atrial fibrillation are high risk factors of cardiogenic stroke. The majority of AF is non-valvular AF. At present, esophageal ultrasound is still the gold standard for monitoring thrombus in left atrial appendage. Although there is evidence that standardized anticoagulation therapy can significantly improve the prognosis of patients with high risk of

thromboembolic events, in fact, most patients with atrial fibrillation do not use anticoagulation therapy. Strategies for identifying patients at risk for thromboembolism are commonly based on the basis of the CHA2DS2-VASc score (6), however study found biomarkers could further refine stroke risk differentiation among patients initially classified as low risk (7). Clinically, we also found patients with low CHA2DS2-VASc score still have a risk of thromboembolic events, more valuable forecast indicators of biomarkers in patients with AF seems to be necessary. Therefore, we aimed to develop a more precisely quantified risk models for predicting cardiogenic stroke risk in non-valvular atrial fibrillation (NVAf) patients.

METHODS

Study Population

The study population comprised 233 high cardiogenic stroke risk patients with LAT or LA-SEC and 233 controls matched for age, sex, AF type, between January 2017 and July 2019. AF was confirmed by a 12-lead surface electrocardiogram and Holter. Paroxysmal AF and non-paroxysmal AF were defined according to the published guideline. Stroke risk was then evaluated according to the Congestive Heart Failure, Hypertension, Age > 75 Years, Diabetes Mellitus, Stroke, Vascular Disease, Age 65–74 Years, Sex Category (CHA2DS2-VASc) score. All patients underwent echocardiography and TEE before catheter ablation, with written informed consent was obtained. LA thrombus was diagnosed by a well-circumscribed echogenic mass contrasted with the adjacent myocardium. LA-SEC was diagnosed by the presence of dynamic smog-like echoes in the left atrial cavity and left atrial appendage. The left atrial diameter (LAD) and left ventricular ejection fraction (LVEF) were measured by transthoracic two-dimensional echocardiography.

Data on the clinical baseline characteristics of all patients were collected from electronic medical records and analyzed. Patients were categorized into a thrombosis group and a normal group according to the TEE results. The Ethics Study Committee at Shanghai Chest Hospital approved the study protocols and agreed that informed consent was not necessary because of the observational nature of the study.

Statistical Analysis

All analyses were performed with SPSS software version 25.0 (IBM Inc., NY, USA). All continuous data are presented as the mean \pm SD deviation and were compared using Student *t*-test. Categorical variables were compared using Pearson's chi-square test or Fisher exact test whenever needed. The receiver operating characteristic (ROC) curve was constructed by plotting sensitivity vs. specificity used to discriminate the power of parameters in identifying the risk of stroke (LA/LAA thrombus, LA-SEC). Multivariable and univariable logistic regression was used to identify the risks of LAT or LA-SEC. All probability values were 2-sided and a *P* < 0.05 was considered statistically significant.

TABLE 1 | Baseline characteristics of the matched patient populations.

Variables	High-risk (<i>n</i> = 233)	Control (<i>n</i> = 233)	<i>P</i> -value
Age, years	68.2 \pm 7.9	68.2 \pm 7.9	1.000
Male sex, <i>n</i> (%)	152 (65.2)	152 (65.2)	1.000
Per-AF, <i>n</i> (%)	205 (88.0)	205 (88.0)	1.000
AF history, years	3.8 \pm 5.6	2.7 \pm 3.5	0.010
Hypertension, <i>n</i> (%)	144 (61.8)	129 (55.4)	0.592
Diabetes mellitus, <i>n</i> (%)	38 (16.3)	30 (12.9)	0.295
Congestive heart failure, <i>n</i> (%)	34 (14.6)	19 (8.2)	< 0.001
Previous stroke/TIA, <i>n</i> (%)	40 (17.2)	39 (16.7)	1.000
Coronary artery disease, <i>n</i> (%)	29 (12.4)	30 (12.9)	0.890
Age \geq 65	152 (65.2)	152 (65.2)	1.000
Age \geq 75	44 (18.9)	44 (18.9)	1.000
CHA2DS2-VASc score, <i>n</i> (%)			0.080
0	11 (4.7)	25 (10.7)	0.023
1	40 (17.2)	45 (19.3)	0.632
\geq 2	182 (78.1)	163 (70.0)	0.057
Serum uric acid, μ mol/L	406.4 \pm 116.2	358.2 \pm 78.2	< 0.001
Male	428.1 \pm 121.3	376.0 \pm 69.5	< 0.001
Female	365.7 \pm 93.8	324.8 \pm 83.1	0.004
Creatinine, μ mol/L	79.6 \pm 20.7	76.5 \pm 16.6	0.147
Hematocrit, %	44.2 \pm 10.0	43.0 \pm 4.8	0.159
Platelets, $10^3/\mu$ L	186.7 \pm 63.2	188.9 \pm 52.6	0.762
BNP, pg/mL	397.1 \pm 403.8	188.0 \pm 157.5	< 0.001
INR	1.31 \pm 0.57	1.23 \pm 0.54	0.358
INR \geq 2.0, <i>n</i> (%)	27 (11.6)	19 (8.2)	< 0.001
Warfarin use, <i>n</i> (%)	67 (28.8)	49 (21.0)	0.277
NOAC, <i>n</i> (%)	13 (5.6)	17 (7.3)	0.572
Aspirin, <i>n</i> (%)	7 (3.0)	19 (8.2)	0.025
LAd, mm	47.5 \pm 5.6	43.5 \pm 5.1	< 0.001
LVDd, mm	50.1 \pm 6.3	47.8 \pm 4.2	< 0.001
LVEF, %	57.1 \pm 9.5	62.2 \pm 4.1	< 0.001
> Mild MR, <i>n</i> (%)	71 (30.5)	67 (28.8)	0.761

RESULTS

Baseline Characteristics of the High Stroke Risk Group and Control Group

From January 1, 2017 to December 31, 2018, a total of 3,522 patients underwent TEE at the Shanghai Chest Hospital. After applying the exclusion criteria, 55 (1.56%) patients with non-valvular AF were LAT and 178 (5.05%) were LA-SEC. A case-control study was performed on 233 patients with LAT or LA-SEC and 233 age, sex, and AF-type matched control patients selected from a list of subjects who had undergone TEE. The baseline characteristics of patients in the high risk and control groups are summarized in **Table 1**.

As shown in **Table 1**, the patients in high risk group had greater proportion of congestive heart failure, larger

LA diameter, larger left ventricular end diastolic diameter, lower ejection fraction, greater SUA, and BNP than control group. The mean AF history (3.8 ± 5.6 vs. 2.7 ± 3.5 years, $P = 0.01$) was markedly longer in patients with LAT/LA-SEC. There were no statistically significant differences in hypertension, diabetes mellitus, previous stroke/TIA, coronary artery disease, CHA2DS2-VASc Score, and INR, more than moderate mitral regurgitation.

Factors Predict High Stroke Risk and ROC Curve Analysis

Compared with the normal group, the high risk group had longer AF history, higher serum uric acid and BNP levels, LA enlargement, LVD enlargement, lower LVEF in high risk patients than in control group. All the above differences were statistically significant ($P < 0.05$). However, the CHA2DS2-VASc were similar between the two groups.

ROC curve analysis was conducted to evaluate the diagnostic value of statistically significant parameters in high risk patients (Table 2). The best cut-off value of SUA was ≥ 429.5 $\mu\text{mol/L}$ (AUC 0.618, sensitivity 39.5%, specificity 83.7%, 95%CI 0.567–0.669, $P < 0.001$). The best cut-off value of BNP was ≥ 334.5 pg/mL (AUC 0.720, sensitivity 42.5%, specificity 88.8%, 95%CI 0.675–0.766, $P < 0.001$). The best cut-off value of LAd was ≥ 45.5 mm (AUC 0.705, sensitivity 61.8%, specificity 70.0%, 95%CI 0.659–0.752, $P < 0.001$). The best cut-off value of LVEF was $\leq 51.5\%$ (AUC 0.677, sensitivity 73.4%, specificity 50.2%, 95%CI 0.629–0.725, $P < 0.001$).

Multivariable Analysis for LAT or LA-SEC

Multiple candidate clinical predictors and echocardiography measurements was performed to identify the independent predictors for LAT/LA-SEC. Our results demonstrated that AF duration, LAd, LVEF, SUA, and BNP were significantly correlated with the presence of LAT/LA-SEC. Univariable and multivariable analysis showed that these parameters were found to be significantly predictive of high stroke risk in NVAf patients (Table 3).

Combined Predictive Model

We used SUC, BNP, LAd, and LVEF as independent variables for further multivariate logistic regression. The results show that the combined predictive mode had an excellent discriminatory capacity in predicting high stroke risk (AUC 0.784; sensitivity 66.1%; specificity 76.8%; 95% CI 0.744–0.825, $P < 0.001$, Figure 1).

Subgroup Analyses

Stratified analyses were performed to assess the predicted value of parameters in LAT and LA-SEC group. As shown in Table 4, the patients in LAT group had greater proportion of hypertension than LA-SEC group. There were no statistically significant differences in age, sex, AF type, AF history, diabetes mellitus, previous stroke/TIA, coronary artery disease, CHA2DS2-VASc Score, congestive heart failure, LAd, LVEDd, LVEF, creatinine, hematocrit, platelets, and use of anticoagulant or aspirin.

The SUA levels in LAT group were no statistically significant differences greater than in LA-SEC group. However, the mean male SUA level (467.8 ± 134.0 vs. 417.1 ± 115.7 $\mu\text{mol/L}$,

TABLE 2 | Receiver operating characteristic analysis of the risk factors.

	Sensitivity (%)	Specificity (%)	AUC	95% CI	P-value
AF history	20.6	87.6	0.527	0.474–0.580	0.313
SUA	39.5	83.7	0.618	0.567–0.669	<0.001
BNP	42.5	88.8	0.720	0.675–0.766	<0.001
LAd	61.8	70.0	0.705	0.659–0.752	<0.001
LVDd	50.2	65.7	0.595	0.544–0.647	<0.001
LVEF	73.4	50.2	0.677	0.629–0.725	<0.001

TABLE 3 | Univariable and multivariable logistic regression of cardiogenic stroke risk.

Variable	Univariable			Multivariable		
	OR	95% CI	P-value	OR	95% CI	P-value
SUA	1.005	1.003–1.007	<0.001	1.003	1–1.006	0.025
AF history	1.005	1.001–1.008	0.013	1.006	1.002–1.011	0.005
BNP	1.004	1.003–1.005	<0.001	1.002	1.001–1.003	0.006
LAd	1.160	1.113–1.209	<0.001	1.083	1.031–1.138	0.001
LVEDd	1.083	1.044–1.123	<0.001	0.969	0.916–1.025	0.275
LVEF	0.888	0.855–0.921	<0.001	0.9	0.858–0.944	<0.001

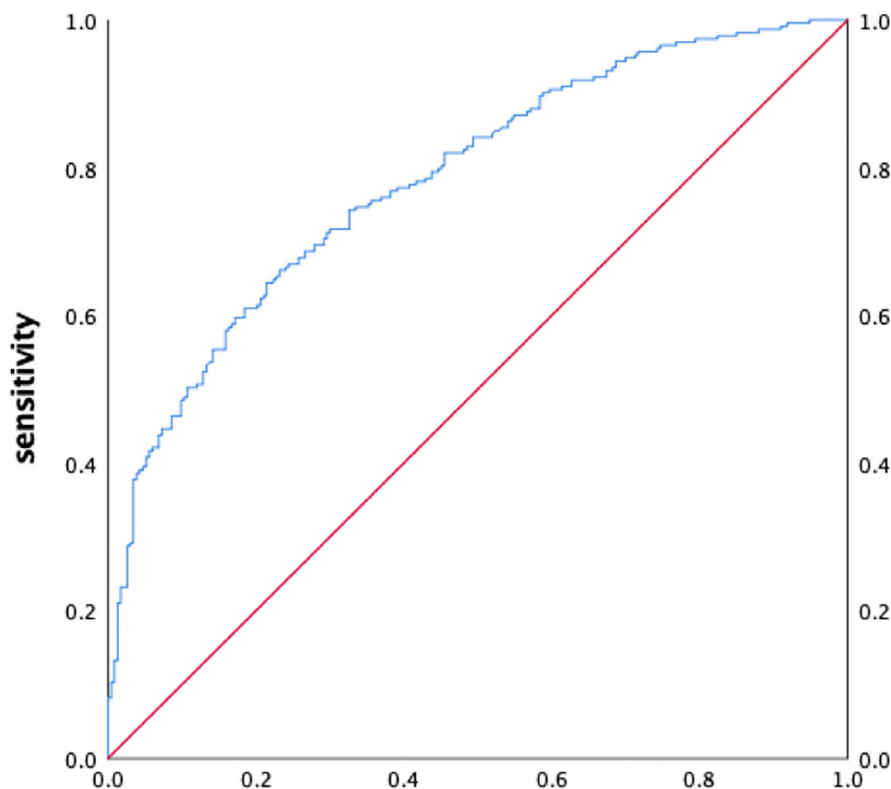


FIGURE 1 | ROC curves analysis for predictive value of combined predictive model.

$P = 0.033$) was significantly higher in patients with LAT than LA-SEC (Table 4).

The BNP level in LAT group were significantly greater than in LA-SEC group (592.2 ± 624.9 vs. 336.8 ± 281.3 pg/mL, $P = 0.005$). The corresponding AUC for BNP predicting LAT was 0.627 (95% CI: 0.539–0.715) and the best cut-off point for BNP predicting LAT was 627 pg/mL, the sensitivity and specificity were 34.5 and 89.3%, respectively (Figure 2).

DISCUSSION

Main Findings

In this case-control study, we demonstrated a significant positive association between SUA, BNP, LAd, LVEF and high stroke risk in non-valvular atrial fibrillation patients. The main findings were as follows: (1) Patients in the LAT/LA-SEC group had significantly higher SUA, BNP levels, LAd and lower LVEF than the control group; (2) Increased SUA and BNP, LA enlargement, LVEF reduction were independent risk factors and combining these four factors above is stronger than using any one single factor for predicting high stroke risk in non-valvular AF patients; (3) BNP levels in LAT group were significantly higher than LA-SEC group, which can be a modest predictor of higher stroke risk in AF patients with LA-SEC.

SUA is the final product of purine metabolism catalyzed by xanthine oxidase, which plays an important role in the

formation of free radical superoxide anion and oxidative stress, consequently resulting in calcium overload and decreasing sodium channels and aggravating cellular damage (8–10). These pathological processes promote electrical remodeling and structural remodeling of the left atrium, leading to an increase of its size and contribute to the occurrence and development of AF (11–15). High SUA level is an independent risk factor for stroke and cardiovascular death (16–19). Studies have shown that hyperuricemia is an important risk factor for stroke and may improve the clinical risk stratification of patients with atrial fibrillation (20). Although it is still unable to explain the mechanism of hyperuricemia and stroke. However, we found that patients with LAT and LA-SEC had higher SUA levels, which means hyperuricemia is associated with a high risk of cardiac stroke in patients with non-valvular atrial fibrillation. This may provide clues for screening high-risk groups and strengthening anticoagulation therapy.

BNP is a sensitive indicator reflecting the increase of cardiac pressure and volume load, and its level is related to the functional load of cardiac pump. When atrial fibrillation occurs, the left atrium cannot contract effectively, the damage of left ventricular diastolic function and the increase of left ventricular filling pressure can lead to left atrial blood stasis, presenting as SEC, and increase the risk of LAA thrombosis (21, 22). Studies have shown that BNP can predict the risk of atrial fibrillation (23), thromboembolism (22, 24–27), and general cardiovascular risk

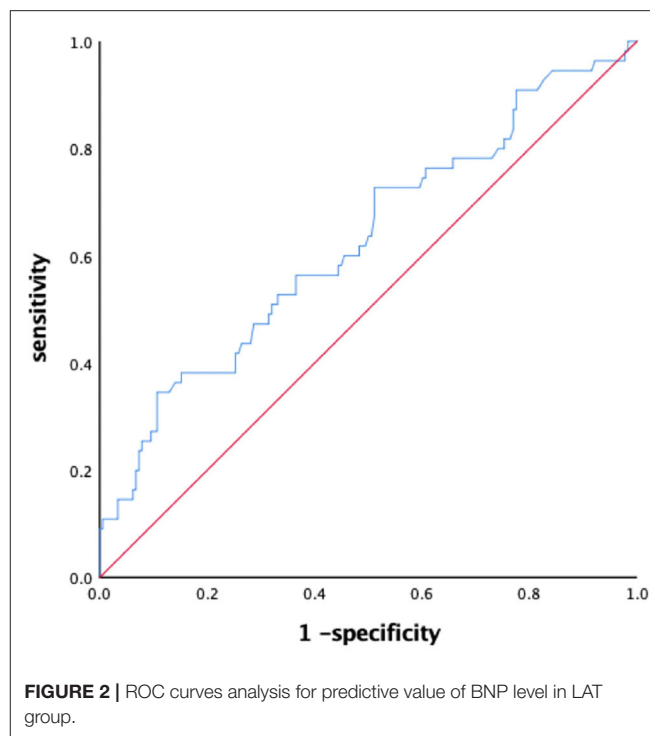
TABLE 4 | Baseline characteristics of the LAT and LA-SEC patient populations.

Variables	LAT (<i>n</i> = 55)	LAECs (<i>n</i> = 178)	<i>P</i> -value
Age, years	67.1 ± 8.9	67.2 ± 8.4	0.946
Male sex, <i>n</i> (%)	33 (60.0)	119 (66.9)	0.418
Per-AF, <i>n</i> (%)	52 (94.5)	151 (84.8)	0.067
AF history, years	3.8 ± 5.5	3.8 ± 5.6	0.973
Hypertension, <i>n</i> (%)	26 (47.3)	118 (66.3)	0.017
Diabetes mellitus, <i>n</i> (%)	6 (10.9)	32 (18.0)	0.296
Congestive heart failure, <i>n</i> (%)	12 (21.8)	22 (12.4)	0.124
Previous stroke/TIA, <i>n</i> (%)	2 (3.6)	38 (21.3)	0.002
Coronary artery disease, <i>n</i> (%)	4 (7.3)	25 (14.0)	0.244
Age ≥ 65	35 (63.6)	117 (65.7)	0.871
Age ≥ 75	11 (20.0)	33 (18.5)	0.844
CHA2DS2-VASc score, <i>n</i> (%)	2.6 ± 1.4	3.0 ± 1.7	0.080
0	4 (7.3)	7 (3.9)	0.293
1	9 (26.4)	36 (20.2)	0.696
≥ 2	42 (76.4)	135 (75.8)	1.000
Serum uric acid, μmol/L	427.7 ± 120.1	399.8 ± 114.5	0.120
Male	467.8 ± 134.0	417.1 ± 115.7	0.033
Female	367.5 ± 58.2	365.0 ± 104.4	0.913
Creatinine, μmol/L	79.6 ± 22.4	79.6 ± 20.2	0.996
Hematocrit, %	45.7 ± 17.4	43.8 ± 6.2	0.219
Platelets (10 ³ /μL)	178.1 ± 68.5	189.3 ± 61.4	0.249
BNP	592.2 ± 624.9	336.8 ± 281.3	0.005
INR	1.25 ± 0.53	1.32 ± 0.58	0.452
INR ≥ 2.0, <i>n</i> (%)	6 (10.9)	21 (11.8)	1.000
Warfarin use, <i>n</i> (%)	14 (25.5)	53 (29.8)	0.611
NOAC, <i>n</i> (%)	1 (1.8)	12 (6.7)	0.310
Aspirin, <i>n</i> (%)	3 (5.5)	4 (2.2)	0.360
LAd, mm	48.8 ± 7.3	47.1 ± 5.0	0.122
LVDd, mm	51.5 ± 7.7	49.6 ± 5.7	0.095
LVEF, %	55.7 ± 12.4	57.5 ± 8.4	0.316
>Mild MR, <i>n</i> (%)	23 (41.8)	48 (27.0)	0.044

stratification in NVAF patients (28–30). Recent studies have suggested that BNP is not only a predictor of AF, but also an early predictor of cerebral embolism in patients with AF (31). Our study has demonstrated that BNP is associated with LAT and LA-SEC, and BNP levels in LAT patients are higher than those in SEC patients. BNP can predict the risk of cardiogenic stroke independently of CHADS 2 and CHA2DS 2-vasc scores, and a higher BNP value means a higher risk of stroke.

In our analysis, decreased LVEF was revealed to be a powerful and independent predictor of LAT/LA-SEC formation in AF patients, which means high cardiogenic stroke risk. Previous studies have suggested that incidence of LAT depending on LVEF, and severe LV systolic dysfunction (confirmed by echocardiography) was a strong predictor of stroke (32, 33).

Studies found that LA enlargement is association with LA-SEC and embolic events (34–36). Left atrial enlargement may lead to thrombotic stroke by promoting endothelial damage, atrial



blood stasis, and thrombosis (37). Atrial cardiomyopathy caused by fibrosis of the left atrium can lead to atrial fibrillation over time. LA enlargement is the manifestation of the severity of atrial cardiomyopathy, and co-exist with AF (38).

Despite that CHA2DS2-VASc score is mostly used to predict the stroke risk in atrial fibrillation (39). However, we found that there was no relationship between the score and LAT/LA-SEC formation. In this study, we found that there was an independent correlation between SUA, BNP, LAd, LVEF and LAT/LA-SEC in AF patients, and subgroup analysis found that the BNP level in LAT group was higher than that in LA-SEC group, with significant statistical difference, which may provide clues for high BNP to increase the risk of cardiogenic stroke and risk in non-valvular AF patients.

CLINICAL IMPLICATION

The main strength of our study was the generalization of the different features of the real-world non-valvular atrial fibrillation population in a matched cohort. Our present study found that a comprehensive evaluation of left atrial diameter, left ventricular ejection fraction, serum uric acid, and BNP may help stratify the cardiogenic stroke risk among non-valvular AF patients, which may help clinicians in the decision-guiding anticoagulation therapy.

LIMITATIONS

The present study had several limitations. The number of patients is relatively insufficient to determine the actual prediction value

of these parameters for LA-SEC. In addition, most of the study population met the criteria for catheter ablation of AF, so selection bias may limit the current statistical analysis, and the population in this study may not reflect all patients with non-valvular AF. Considering that this study is a retrospective study, further prospective clinical trials are necessary to verify the predictive value of these parameters on the risk of cardiogenic stroke caused by atrial fibrillation and the guiding significance of anticoagulation decision-making. The pathophysiological mechanism of LAT and LA-SEC has not been well-explored. The mechanism of combined predictive model for cardiogenic stroke risk in AF needs further study.

CONCLUSION

This study found that the combined predictive model has a moderate predictive value for cardiogenic stroke risk among non-valvular AF patients, which will help us strengthen the screening of high-risk populations and strengthen anticoagulation therapy.

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DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Does Serum Uric Acid Status Influence the Association Between Left Atrium Diameter and Atrial Fibrillation in Hypertension Patients?

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Objective: Both serum uric acid (SUA) levels and left atrium diameter (LAD) associate with AF. However, the influence of SUA status for the associated risk of AF related to LAD in hypertension patients is currently unknown.

Methods: We retrospectively analyzed a hospital-based sample of 9,618 hypertension patients. Standard electrocardiograms were performed on all patients and were interpreted by expert electro-physiologists.

Results: Overall 1,028 (10.69%) patients had AF out of 9,618 patients. In men >65 years of age, the prevalence of AF in the 1st, 2nd, and 3rd tertiles of SUA among those grouped in the third tertile of LAD were 9, 12.3, and 21.7%, respectively. In the hyperuricemia group, the OR (95% CI) of AF for the highest tertile of LAD in men ≤65 years of age was 3.150 (1.756, 5.651; $P < 0.001$). Similarly, the hyperuricemic men in the 3rd LAD tertile had a higher likelihood of AF than those belonging to the 1st tertile. The ORs and (95% CIs) were 3.150 (1.756, 5.651; $P < 0.001$) and 5.522 (2.932, 10.400; $P \leq 0.001$) for patients ≤65 and >65 years of age. An increase in SUA values was significantly associated with an increased likelihood of AF among women at the top tertiles of LAD, with the OR (95% CI) = 4.593 (1.857, 11.358; $P = 0.001$). Also, men > 65 years of age with large LAD, present at the third tertile of SUA, had a higher likelihood of AF, with the OR (95% CI) = 2.427 (1.039, 5.667; $P < 0.05$).

Conclusion: SUA levels and LAD are associated with AF in patients with hypertension and the risk of AF associated with LAD increases among those with hyperuricemia.

Keywords: uric acid, atrial fibrillation, hypertension, hyperuricemia, left atrial diameter (LAD)

INTRODUCTION

Atrial fibrillation (AF) is the most sustained arrhythmia, contributing to short and long-term cardiovascular complications such as hemodynamic instability, stroke, heart failure, and mortality risk (1–5). Considering the continuous rise in the average life expectancy in recent years and an increase in cardiac morbidity, the occurrence of AF has been increasing sharply in the past two decades. Despite advancements in the detection and management of AF, inadequate guidance persists, regarding primary prevention and risk stratification of this disease (6).

Several risk factors have been assumed to involve in the pathophysiology of atrial fibrillation, such as hyperuricemia, left atrial diameter (LAD), gender, high-sensitivity C-reactive protein, cystatin-C, obesity, and diabetes (7–9). Of those hypothesized risk factors, increased focus has been given to the possible mechanism by which hyperuricemia causes AF. As such, earlier studies have revealed that elevated serum uric acid (SUA) plays a role in the development of AF in the general population (9–12), as well as in patients with hypertension (13).

AF and hypertension often coexist, and AF patients who experience elevated systolic blood pressure experience increased adverse events (14). Moreover, left atrium volume, diameter, and strain were reported to correlate with new-onset AF in patients suffering from hypertrophic cardiomyopathy (15). Importantly, a piece of evidence also revealed that left atrium enlargement is a marker for increased risk of AF (16–20). Considering the direct effect of LAD in the occurrence and maintenance of AF, and the role of elevated SUA and hypertension in modifying the pathophysiology of AF, it is meaningful for the scientific community to analyze the interaction between SUA level and LAD in AF patients. We hypothesized that elevated SUA levels, in combination with widened LAD, could significantly estimate the risk of AF in patients with hypertension. Therefore, this study aimed to determine the association between SUA levels and LAD with AF and investigate their interaction among the Chinese population with hypertension.

MATERIALS AND METHODS

Population

Hypertension patients, aged 18 to 97 years, hospitalized between August 2015 and August 2018 at the First Affiliated Hospital of Dalian Medical University (FAHDMU) were included. Those with cardiomyopathy, valvular heart disease, myocardial infarction, heart failure, pericardial disease, undergoing dialysis of the kidney, chronic kidney diseases-4 (CKD4) /CKD5, and those patients missing key clinical covariates were excluded. Finally, the present study contained a total of 9,618 patients. **Figure 1** describes a brief overview of the selection of study participants. The research was conducted in accordance with the Helsinki declaration guidelines and was approved by the institutional review board of the FAHDMU. The informed consent provision was waived and all procedures listed here were carried out in compliance with the approved guidelines.

Clinical Measurements and Definition of Explanatory Variables

Demographic and clinical characteristics including age, gender, and major risk factors of hypertension including dyslipidemia, diabetes mellitus, arterial hypertension, alcohol, smoking, and other CVD comorbidities were ascertained from electronic health records. A sample of fasting blood from the brachial vein had been collected. The SUA concentrations were determined using an autoanalyzer using the Uricase-Peroxidase process (BECKMAN COULTER AU680 Chemistry Analyzer, USA). We performed comprehensive 2D transthoracic echocardiography for each patient. All the measurements, including fasting

glucose level, serum concentrations of triglycerides, total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol were performed at the FAHDMU laboratory using the standard protocols. Hypertension has been characterized as systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mm Hg or a self-reported history of hypertension with the active use of antihypertensive drugs. Diabetes mellitus (DM) has been defined as fasting 7.0 mmol/L plasma glucose or a self-reported history of diabetes mellitus and/or currently receiving antidiabetic treatments. Dyslipidemia was characterized as TC >240 mg/dL or LDL cholesterol >160 mg/dL or HDL cholesterol >40 mg/dL and/or lipid-lowering drug use (21). Participants were deemed current smokers if reported they are currently smoking or registered smoking at least 100 cigarettes during their lifetime (22, 23). The approximate glomerular filtration rate (eGFR) was determined using the Renal Disease equation for Diet Modification (24). We measured SUA and LAD when the AF incident was first diagnosed during hospitalization.

Identification of AF

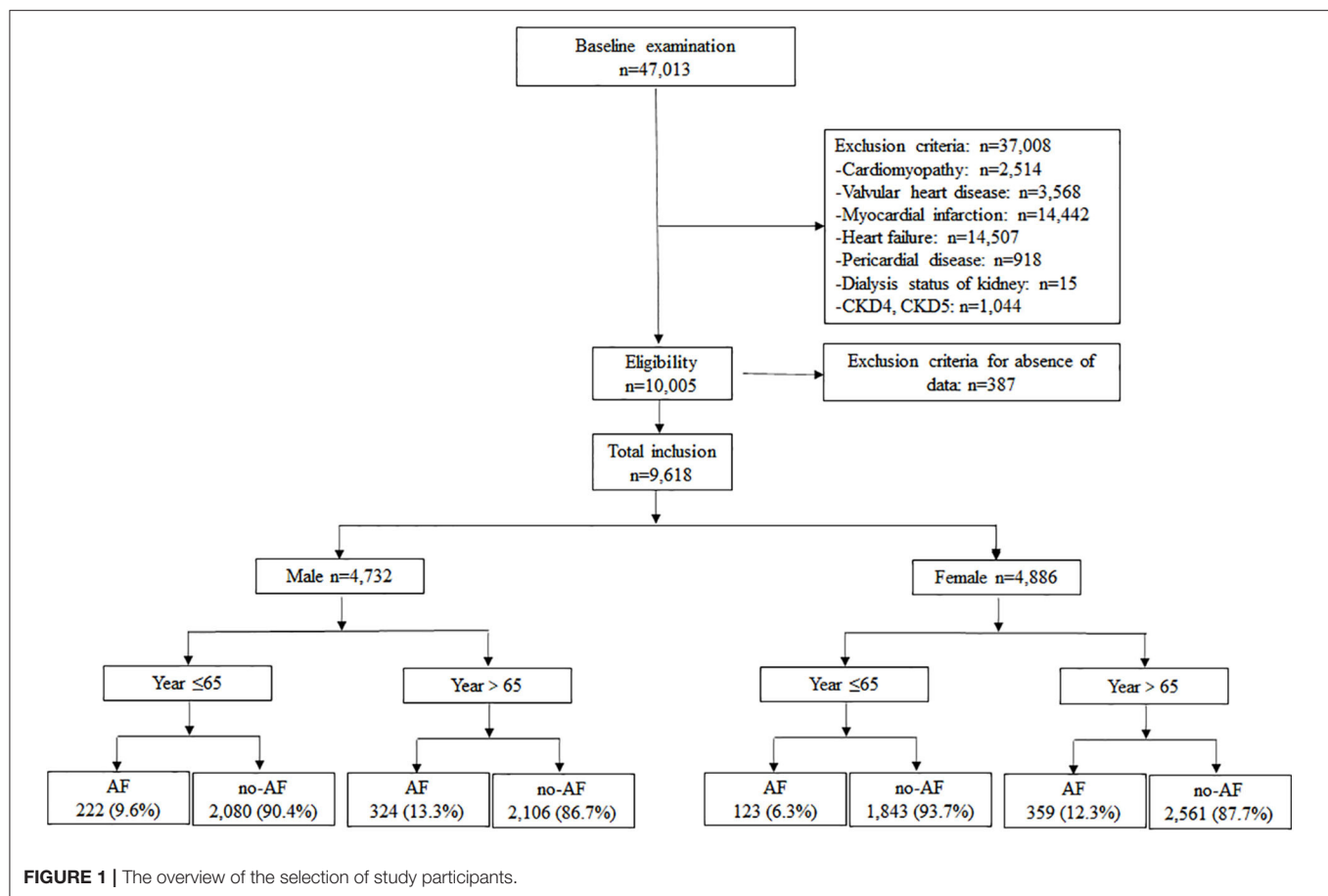
All subjects received echocardiographic examinations at rest in the left lateral decubitus position using the Vivid 7 ultrasound system (GE Vingmed Ultrasound, Horten, Norway). The Left atrium diameter was obtained based on the American Society of Echocardiography guidelines, a widely used approach to evaluate LAD (25). The LAD was assessed from a parasternal long-axis view at the end-systole (when the LA chamber is at its greatest dimension). Experienced radiologists who were blinded to the clinical data reviewed the echocardiography results.

Echocardiographic Assessment

All subjects received echocardiographic examinations at rest in the left lateral decubitus position using the Vivid 7 ultrasound system (GE Vingmed Ultrasound, Horten, Norway). The Left atrium diameter was obtained based on the American Society of Echocardiography guidelines, a widely used approach to evaluate LAD (26). The LAD was assessed from a parasternal long-axis view at the end-systole (when the LA chamber is at its greatest dimension). Experienced radiologists who were blinded to the clinical data reviewed the echocardiography results.

Statistical Analysis

All statistical analyses were conducted using SPSS version 21. Patients were categorized based on age into two groups including, under 65 years of age and above 65 years of age. SUA levels and LAD were stratified into tertiles (T) separately for men and women for the two groups (≤ 65 and >65 years). The respective cut-off of SUA and LAD values for T1, T2, and T3 for men and women are given in the footnote of each table. All categorical variables were expressed as counts and percentiles and continuous variables were expressed as mean \pm SD. Variables were compared for differences between AF and non-AF patients using two independent sample *t*-test and χ^2 test for continuous and categorical data, respectively. Binary logistic regression models were estimated, and the odds ratios (OR) at 95% confidence interval (CIs) were used to approximate the



associated risk for AF according to tertiles of SUA levels and LAD, with the lowest tertile serving as the reference category. Model 1 was adjusted for age. Model 2 was adjusted for age, SBP, serum creatinine, and smoking. Model 3 was adjusted for the covariates in model 2, followed by dyslipidemia and DM, statin, and antihypertensive agents. Further, we ran a sub-analysis to estimate the associated AF risk across the tertiles of LAD in hyperuricemic and normouricemic patients, and the associated AF risk across tertiles of SUA in patients with normal and widened LAD. All statistical analyses were two-sided, and a $P < 0.05$ was considered statistically significant.

RESULTS

Baseline Characteristics of the Participants

Among the 47,013 patients who were included, a total of 9,618 patients (4,732 men and 4,886 women) were selected in the final analysis. The mean \pm SD ages of AF and non-AF patients were 69.51 ± 9.20 and 66.30 ± 10.49 , respectively. In total, 546 of the 4,732 men (11.54%) had AF, which accounted for 53.11% of the AF population. In the entire population, the AF patients had a higher mean age, SUA, and LAD than the non-AF group. However, patients with AF had a lower mean SBP compared

with non-AF patients. The proportion of patients, who were in diuretic and β -blocker use, was higher in AF patients compared to the non-AF group (Table 1).

Unlike younger female patients, male patients under 65 years of age in the AF group were more likely to have DM and dyslipidemia. Conversely, women patients who were above 65 years of age, were more likely to have DM and dyslipidemia. Regardless of the age group, patients in the AF group were more likely to use β -blockers. Demographic data for the patients in the two groups separately are shown for men and women in Supplementary Tables 1, 2, respectively.

The Prevalence of AF

Overall, 1,028 (10.69%) patients had AF out of 9618 patients. 222/2302 AF occurred in men patients who were under 65 years of age, whereas 324/2430 AF occurred in male subjects over 65 years of age. In the group of ≤ 65 years of age, the prevalence of AF was 9.6% in men and 6.3% in women.

The prevalence of AF in patients ≤ 65 years of age, categorized at the 3rd SUA tertile, was 10.5% compared with the prevalence of AF in those belonging to 2nd and 1st tertile (8.7 and 5.1%, respectively). In the group of ≤ 65 years of age, the prevalence of AF was significantly increased from 3.5% in the low tertile to 6.7 and 14.0% in the middle and high tertiles of LAD, respectively. Similarly, the prevalence of AF in old aged patients

TABLE 1 | Baseline characteristics of the participants.

Variables	AF (n = 1,028)	no-AF (n = 8,590)	P-value
Male (n/%)	546 (53.1%)	4,186 (48.7%)	0.008
Age (year)	69.51 ± 9.20	66.30 ± 10.49	<0.001
Blood pressure readings			
SBP (mm Hg)	141.54 ± 19.50	146.29 ± 20.96	<0.001
DBP (mm Hg)	82.65 ± 13.31	82.90 ± 12.81	0.566
DM (n/%)	264 (25.7%)	2,502 (29.1%)	0.021
Dyslipidemia (n/%)	793 (77.1%)	7,078 (82.4%)	<0.001
Lipid panel			
TC (mg/dL)	179.67 ± 40.27	186.28 ± 42.50	<0.001
TG (mg/dL)	132.70 ± 74.56	149.18 ± 102.96	<0.001
HDL (mg/dL)	47.25 ± 10.47	47.25 ± 11.24	0.977
LDL (mg/dL)	104.96 ± 28.97	104.97 ± 30.24	0.995
Smoking (n/%)	219 (21.6%)	1,989 (24.6%)	0.037
Alcohol (n/%)	145 (14.6%)	1,242 (15.7%)	0.351
SUA (mol/L)	356.59 ± 90.99	340.73 ± 89.63	<0.001
Scr (umol/L)	75.24 ± 42.02	70.59 ± 35.85	<0.001
LAEDD (mm)	39.57 ± 5.05	36.58 ± 3.42	<0.001
Antihypertensive agent			
ACEI/ARB (n/%)	569 (55.4%)	4,779 (55.6%)	0.862
β-blocker (n/%)	675 (65.7%)	4,211 (49.0%)	<0.001
CCB (n/%)	625 (60.8%)	5,490 (63.9%)	0.500
Statin (n/%)	719 (69.9%)	6,567 (76.4%)	<0.001
Diuretic (n/%)	179 (17.45)	1,197 (13.9%)	0.003

AF, Atrial fibrillation; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; DM, Diabetes mellitus; TC, Total Cholesterol TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SUA, Serum uric acid; Scr, Serum creatinine; LAEDD, Left atrial end-diastolic diameter; ACEI, Angiotensin-Converting Enzyme Inhibitors; ARB, angiotensin-converting enzyme receptor blockers; and CCB, Calcium channel blockers.

was significantly increased from 7.8% in the low to 10.1 and 22.9% in the middle and high tertiles of LAD, respectively.

Relationship Between SUA/LAD and AF

The association between the SUA levels and the risk of AF among patients above 65 years old is presented in **Table 2**. When the values of SUA were treated as continuous data, the adjusted OR and 95% CI for AF in men ≤ 65 years of age was 1.002(1.010, 1.004, $P = 0.001$). This association persisted when SUA values were divided into three tertiles. In the ≤65-year-old group, the third tertile of SUA concentrations was significantly associated with AF in both men and women. In the fully adjusted multivariate analysis, the ORs and 95% CIs of AF for the patients in tertile 3 compared to patients in the first tertile of SUA were 2.098 (1.393, 3.161; $P < 0.001$) and 1.805 (1.070, 3.044; $P < 0.05$), respectively.

The result of this study also shows a positive association between the higher tertiles of LAD and the presence of AF in men and women in both age groups after adjusting for age, SBP, serum creatinine, and smoking (**Supplementary Table 3**). In the men ≤65-year-old group, compared with the first tertile of LAD, the multivariate-adjusted OR and 95% CI of AF for the second and third tertiles were 1.581 (1.029, 2.429); $P < 0.05$, and

4.473 (2.991, 6.688; $P < 0.001$), respectively. In the group of >65 years, the OR associated with AF in those belonging to the third tertile of LAD was increased by nearly four-folds compared to the first tertile after adjustment for multiple confounding variables (adjusted OR = 3.992, 95% CI: 2.871, 5.549; $P < 0.001$). **Table 3** presents the prevalence and ORs of AF among men grouped by the tertiles of SUA levels and LAD.

Women in the third tertile had a higher AF prevalence compared with those in the first and second LAD tertiles. This relationship persisted even after adjusting for potential confounders including, age, SBP, serum creatinine, smoking, dyslipidemia, DM, statin use, and antihypertensive agents such as ACEI, ARB, CCB, and β-blocker use. In patients ≤65 years of age, the OR (95% CI) of AF for the women in T3 compared to the first tertile of LAD was 2.628 (1.627, 4.243; $P < 0.001$). Among women older than 65 years of age, the third tertile of LAD was associated with a nearly three-fold increased risk of AF compared to those in the first tertile of LAD after adjustment for multiple confounding factors [the adjusted OR (95% CI) = 3.178 (2.366, 4.269; $P < 0.001$)].

The Effect of SUA and LAD Interaction in AF

To compute the interaction effect of elevated SUA and enlarged LAD, we calculated the prevalence of AF and estimated the OR and 95% CI of AF among those patients grouped in different tertiles of SUA along their corresponding LAD tertiles. The prevalence of AF increases across SUA and LAD tertiles, implying the patients in higher tertiles of SUA and LAD had a higher prevalence of AF than in lower tertiles. An increase in LAD shows a progressively higher prevalence of AF across the tertiles of SUA (**Figure 2**). With an increase in tertiles of LAD, the men under the age of 65 years had a higher prevalence of AF across the tertiles of SUA (6.35 vs. 9.4 vs. 17.6%, respectively). Moreover, those men >65 years in the 3rd tertile of SUA grouped by LAD tertiles had a higher prevalence of AF than those belonging to 2nd or 1st tertile (9, 12.3, and 21.7% for the 1st, 2nd, and 3rd tertiles of SUA, respectively). The prevalence of AF in the 1st, 2nd, and 3rd tertiles of SUA for those women >65 years of age grouped in the third tertile of LAD were 18.3, 19.8, and 23.1%, respectively.

Those patients at higher tertiles of SUA grouped by LAD had a higher risk of AF, with patients in T3 accounting for the highest risk of AF (OR = 10.49 in men ≤65 and 4.62 in men >65, respectively). Similarly, the estimated risk of AF in young aged women was significantly increased from 3.17 to 4.69% across the first to third tertile of LAD (in those patients grouped by SUA tertiles), respectively. At the same time, the women at higher tertiles of LAD grouped by SUA had a higher risk of AF in the old aged population, with patients in T3 accounting for the highest risk of AF (OR = 2.33, 2.68, and 2.95 for T1, T2, and T3, respectively). **Figure 3** describes the risk of AF based on LAD tertiles in patients grouped by SUA tertiles.

Table 4 presents the odds ratios associated with an increase in SUA among participants grouped by tertiles of LAD. With an increase in SUA levels, the regression analysis confirmed that men under the age of 65 years in the third tertile of LAD had an

TABLE 2 | The relationship between SUA/LAD and Atrial fibrillation.

Men	Unadjusted model				Adjusted model			
	Age ≤65 (n = 2,302)		Age >65 (n = 2,430)		Age ≤65 (n = 2,302)		Age >65 (n = 2,430)	
	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
SUA	1.002 (1.001, 1.004)	0.001	1.020 (1.010, 1.040)	0.017	1.003 (1.001, 1.004)	0.003	1.000 (0.998, 1.001)	0.934
LAEDD	1.229 (1.188, 1.273)	<0.001	1.177 (1.143, 1.212)	<0.001	1.25 (1.206, 1.304)	<0.001	1.189 (1.152, 1.226)	<0.001

Women	Age ≤65 (n = 1,966)		Age >65 (n = 2,920)		Age ≤65 (n = 1,966)		Age >65 (n = 2,920)	
	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
SUA	1.003 (1.001, 1.005)	0.007	1.001 (1.000, 1.002)	0.090	1.001 (0.999, 1.004)	0.290	1.000 (0.998, 1.001)	0.778
LAEDD	1.227 (1.166, 1.293)	<0.001	1.208 (1.173, 1.245)	<0.001	1.195 (1.134, 1.260)	<0.001	1.212 (1.173, 1.252)	<0.001

Adjusted for age, systolic blood pressure, serum creatinine, smoking, dyslipidemia and diabetes mellitus, statin, ACEI, ARB, CCB, β -blocker.

TABLE 3 | The prevalence of AF and the risk estimate for the atrial fibrillation based on the tertiles of SUA/LAD.

Age ≤65 (n = 4,268)				Age >65 (n = 5,350)		
Tertiles of serum uric acid levels						
	T1 (n = 1,428)	T2 (n = 1,417)	T3 (n = 1,423)	T1 (n = 1,785)	T2 (n = 1,799)	T3 (n = 1,766)
No. of AF(%)	73 (5.1%)	123 (8.7%)	149 (10.5%)	198 (11.1%)	241 (13.4%)	244 (13.8%)
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Men	Ref.	1.892 (1.267, 2.825) [†]	2.098 (1.393, 3.161) [‡]	Ref.	1.044 (0.764, 1.426)	1.061 (0.774, 1.454)
Women	Ref	1.546 (0.923, 2.591)	1.805 (1.070, 3.044)*	Ref.	0.868 (0.642, 1.172)	1.009 (0.753, 1.353)
Tertiles of LAD						
	T1 (n = 1,421)	T2 (n = 1,425)	T3 (n = 1,422)	T1 (n = 2,109)	T2 (n = 1,754)	T3 (n = 1,487)
No. of AF(%)	50 (3.5%)	96 (6.7%)	199 (14.0%)	165 (7.8%)	177 (10.1%)	341 (22.9%)
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Men	Ref.	1.581 (1.029, 2.429)*	4.473 (2.991, 6.688) [‡]	Ref.	1.713 (1.201, 2.444) [†]	3.992 (2.871, 5.549) [‡]
Women	Ref	1.136 (0.647, 1.997)	2.628 (1.627, 4.243) [‡]	Ref.	1.056 (0.755, 1.479)	3.178 (2.366, 4.269) [‡]

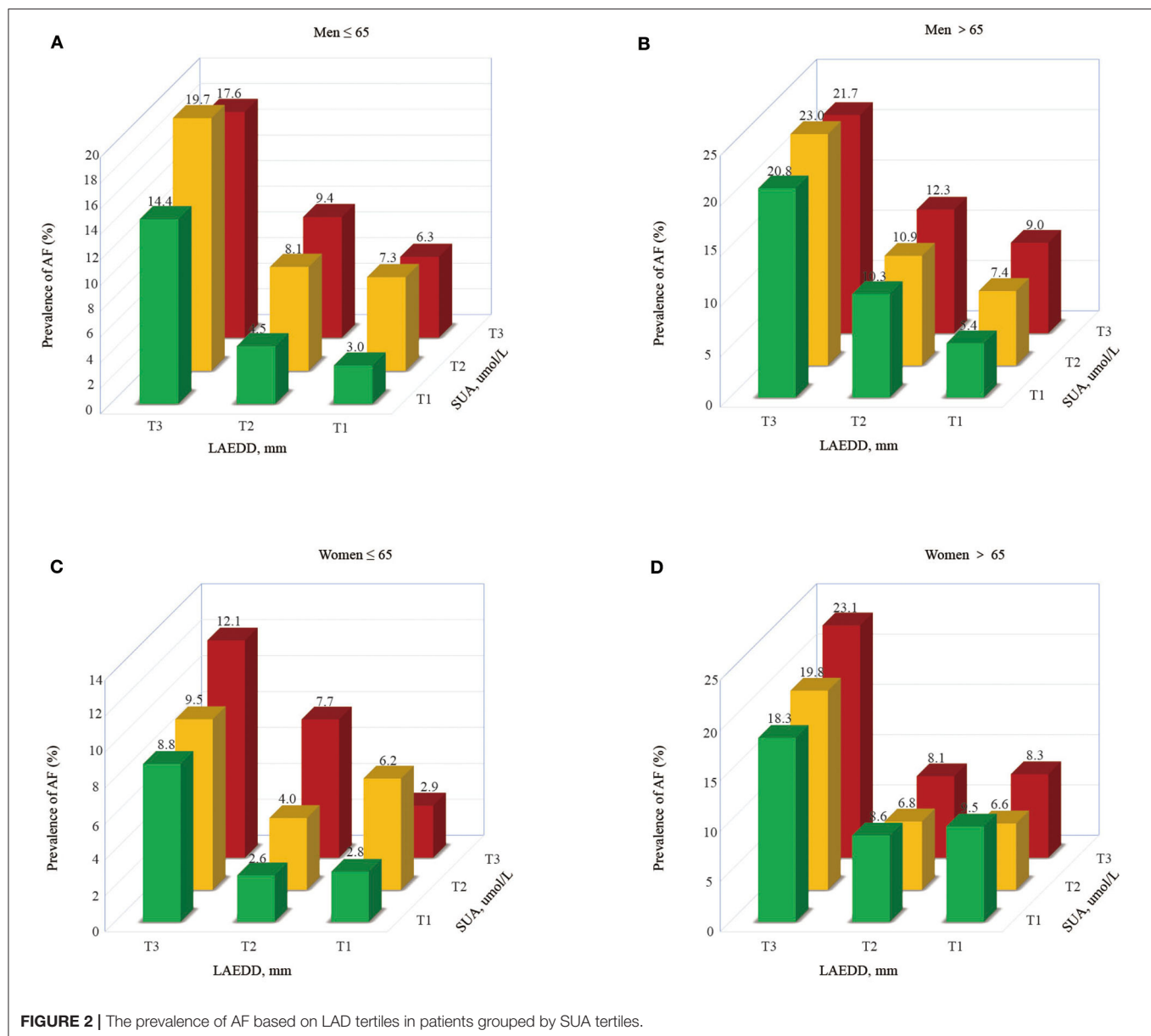
^{*} $P < 0.05$, [†] $P < 0.01$, [‡] $P < 0.001$. Age ≤65 men = Tertile 1, ≤303.00 $\mu\text{mol/L}$; SUA-Tertile 2, 303.00–376.93 $\mu\text{mol/L}$, and SUA-Tertile 3, >376.93 $\mu\text{mol/L}$; LAEDD-Tertile 1, ≤35.90 mm, LAEDD-Tertile 2, 35.90–37.26 mm, and LAEDD-Tertile 3, >37.26 mm. Age >65 men = SUA-Tertile 1, ≤298.00 $\mu\text{mol/L}$, SUA-Tertile 2, 298.00–366.00 $\mu\text{mol/L}$, and SUA-Tertile 3, >366.00 $\mu\text{mol/L}$; LAEDD-Tertile 1, ≤36.00 mm, LAEDD-Tertile 2, 36.00–38.00 mm, and LAEDD-Tertile 3, >38.00 mm. Adjusted for age, SBP, Serum creatinine, smoking, dyslipidemia and DM, statin, diuretic, ACEI, ARB, CCB, β -blocker.

independent increase in risk for AF. The ORs (95% CIs) for the 1st, 2nd, and 3rd tertiles of SUA were 3.403 (1.787, 6.478), 4.596 (2.355, 8.968), and 6.614 (2.870, 15.245), respectively. Similarly, an increase in SUA values in women was significantly associated with an increased likelihood of AF among those of the highest tertiles of LAD, with the OR (95% CI) = 4.593 (1.857, 11.358; $P = 0.001$). Also, in the population above 65 years (both men and women), an increase in SUA values was markedly associated with an increased risk for AF among those of the highest tertiles of LAD, suggesting a substantial AF risk was present among those patients classified at the highest tertile of SUA levels and LAD.

The Impact of Left Atrium End-Diastolic Diameter in Atrial Fibrillation Patients With Normouricemia and Hyperuricemia

To investigate whether there was a possibility that the hyperuricemic status might have influenced the predictive power

of LAD values for AF among the patients with hypertension, we calculated the multivariable-adjusted ORs and 95% CIs in two separate groups, normouricemia ($n = 7,196$) and hyperuricemic ($n = 2,422$). Those men with a large LAD value, regardless of the hyperuricemic status, had a greater likelihood of having AF. The OR (95% CI) of AF for the highest tertile of LAD was 3.150 (1.756, 5.651; $P < 0.001$) in hyperuricemic middle-aged men. Likewise, those hyperuricemic old aged men in the 3rd tertile of LAD had a higher likelihood of AF than those belonging to the 1st tertile, the OR (95% CI) was 5.522 (2.932, 10.400; $P \leq 0.001$). Similar findings were observed in the normouricemic group. Compared to the first tertile of SUA, the OR and 95% CI of AF for men younger and older than 65 years of age in the highest tertile were 4.976 (3.044, 8.136; $P < 0.001$) and 4.150 (2.832, 6.081; $P < 0.001$), respectively. In the group of >65 years of age, the risk of AF was significantly increased in the third tertiles of LAD in both hyperuricemic and normouricemic women (OR = 2.947 and 4.336, respectively). The impact of LAD in atrial fibrillation in

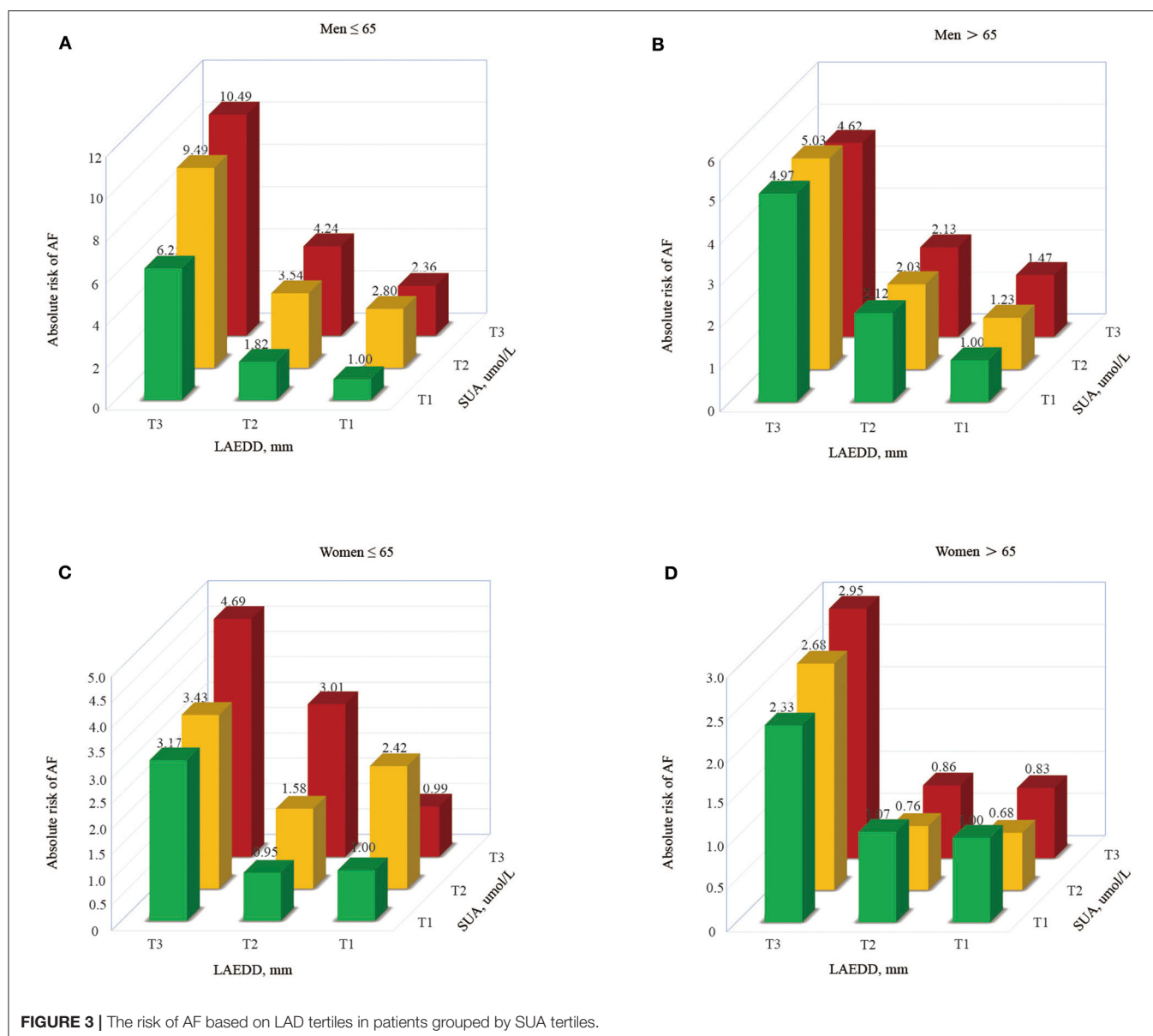


patients with normouricemia and hyperuricemia is summarized in Table 5.

The Impact of Serum Uric Acid in Atrial Fibrillation in Patients With Normal and Enlarged Left Atrium Diameter

We performed sub-analyses by applying recently published LAD criteria (27). The left atrium was considered enlarged when left atrial diameter exceeded 4.2 cm in men and 3.8 cm in women, thus the patients were grouped into two categories based on their LAD size: normal LAD ≤ 4.2 cm and large LAD > 4.2 cm in men, and normal LAD ≤ 3.8 cm and large LAD > 3.8 cm in women. When patients were broken down based on the size of LAD, elevated levels of SUA was associated with greater odds

of AF in those patients with normal LAD in the younger age group. The effect of SUA appeared most pronounced among men diagnosed with AF categorized under the age group of ≤ 65 years of age. The ORs (95% CI) for the middle and the highest tertiles of SUA compared to the lowest tertile were 2.139 [1.361, 3.361; $P < 0.01$] and 2.228 (1.397, 3.552; $P = 0.01$), respectively. Moreover, those patients > 65 years of age with large LAD, present at the third tertile of SUA, had a higher likelihood of AF, with the OR (95% CI) = 2.427 (1.039, 5.667; $P < 0.05$). The AF risk was lower in the normal LAD group in women above 65 years of age compared with those patients in the first tertile [Adjusted OR (95% CI) = 0.656 (0.446, 0.966; $P < 0.05$]. However, there was no significant risk of AF associated with an increase in SUA in the normal LAD and large LAD groups in the women population. Table 6 presents the impact



of SUA in AF among hypertension patients with normal and enlarged LAD.

DISCUSSION

In this cross-sectional study, conducted in 9,618 hypertension patients from the hospital registry, elevated SUA and LAD were independently associated with an increased prevalence of AF. Also, the interaction analysis shows that patients in the highest SUA and LAD tertile had a significantly increased risk of AF. This association of the SUA concentrations and LAD with AF remains consistent even after adjusting for potential confounders, which confirmed that SUA levels and LAD could predict the presence of AF.

Hypertension, a well-recognized public health burden worldwide, is associated with an increased risk of AF (28). Furthermore, AF and hypertension often coexist in hyperuricemic patients. For instance, earlier evidence suggested that increased SUA level positively associates with AF prevalence in patients with chronic systolic heart failure (29). A recent study, which enrolled patients aged ≥ 35 years in the rural Liaoning province of China, proposed an independent association between SUA and AF in the total population and men after adjusting for conventional CVD risk factors (30). Likewise, the present data suggested that elevated SUA was associated with AF in individuals with large LAD. In our study, the prevalence of AF increased from 5.1 to 10.5% across T1-T3 of SUA levels in individuals ≤ 65 -year-old and from 11.1 to 13.8% across T1-T3 of SUA levels in aged patients (> 65 -year-old). These results

TABLE 4 | The prevalence of AF according to baseline SUA tertile grouped by LAD in men and women.

Age ≤65 Years						Age >65 Years					
SUA	T1 (N = 772)	T2 (N = 842)		T3 (N = 688)		T1 (N = 818)	T2 (N = 802)		T3 (N = 810)		
		OR (95% CI)	P-value	OR (95% CI)	P-value		OR (95% CI)	P-value	OR (95% CI)	P-value	
Tertile of LAD in men (N = 4,732)											
T1	Ref.	1.812 (0.747, 4.398)	0.189	3.403 (1.787, 6.478)	<0.001	Ref.	2.188 (1.156, 4.141)	0.016	3.188 (1.867, 5.441)	<0.001	
T2	Ref.	1.282 (0.654, 2.514)	0.469	4.596 (2.355, 8.968)	<0.001	Ref.	1.624 (0.876, 3.011)	0.124	4.082 (2.297, 7.255)	<0.001	
T3	Ref.	1.839 (0.888, 3.806)	0.101	6.614 (2.870, 15.245)	<0.001	Ref.	1.450 (0.790, 2.664)	0.231	4.790 (2.586, 8.872)	<0.001	
Age ≤65 Years						Age >65 Years					
SUA	T1 (n = 712)	T2 (n = 599)		T3 (n = 655)		T1 (n = 973)	T2 (n = 973)		T3 (n = 974)		
		OR (95% CI)	P-value	OR (95% CI)	P-value		OR (95% CI)	P-value	OR (95% CI)	P-value	
Tertile of LAD in women (N = 4,868)											
T1	Ref.	0.862 (0.279, 2.658)	0.796	3.347 (1.244, 9.006)	0.017	Ref.	1.054 (0.618, 1.798)	0.846	2.331 (1.412, 3.847)	0.001	
T2	Ref.	0.604 (0.240, 1.521)	0.285	1.488 (0.674, 3.289)	0.326	Ref.	1.164 (0.621, 2.182)	0.637	3.622 (2.190, 5.991)	<0.001	
T3	Ref.	3.577 (1.233, 10.372)	0.019	4.593 (1.857, 11.358)	0.001	Ref.	1.037 (0.557, 1.931)	0.909	4.210 (2.364, 7.495)	<0.001	

Adjusted for age, SBP, Serum creatinine, smoking, dyslipidemia, DM, statin, diuretic, ACEI, ARB, CCB, and β -blocker.

TABLE 5 | The impact of left atrium diameter in atrial fibrillation patients with normouricemia and hyperuricemia.

Men					Age >65				
Age ≤65									
Tertiles of LAD									
	T1 (n = 772)	T2 (n = 842)	T3 (n = 688)		T1 (n = 818)	T2 (N = 802)	T3 (n = 810)		
AF (n, %)	41 (5.3%)	61 (7.2%)	120 (17.4%)		58 (7.1%)	89 (11.1%)	177 (21.9%)		
	OR (95% CI)	OR (95% CI)	OR (95% CI)	P-trend	Ref.	OR (95% CI)	OR (95% CI)	P-trend	
Normouricemia	Ref.	1.532 (0.908, 2.586)	4.976 (3.044, 8.136) [‡]	<0.001	Ref.	1.824 (1.217, 2.735) [†]	4.150 (2.832, 6.081) [‡]	<0.001	
Hyperuricemia	Ref.	1.049 (0.502, 2.193)	3.150 (1.756, 5.651) [‡]	<0.001	Ref.	1.521 (0.765, 3.023)	5.522 (2.932, 10.400) [‡]	<0.001	
Women									
	T1 (n = 712)	T2 (n = 599)	T3 (n = 655)		T1 (n = 973)	T2 (n = 973)	T3 (n =9 74)		
AF (n, %)	28 (3.9%)	26 (4.3%)	69 (10.5%)		80 (8.2%)	76 (7.8%)	203 (20.8%)		
	OR (95% CI)	OR (95% CI)	OR (95% CI)	P-trend		OR (95% CI)	OR (95% CI)	P-trend	
Normouricemia	Ref.	1.075 (0.552, 2.094)	2.593 (1.491, 4.511) [†]	0.001	Ref.	1.015 (0.689, 1.496)	2.947 (2.094, 4.147) [‡]	<0.001	
Hyperuricemia	Ref.	1.125 (0.416, 3.045)	1.668 (0.720, 3.863)	0.227	Ref.	1.190 (0.638, 2.217)	4.336 (2.488, 7.557) [‡]	<0.001	

[†]P < 0.01, [‡]P < 0.001. In men: ≤420 μ mol/L considered normal SUA levels; and >420 μ mol/L level was considered as hyperuricemia. In women: Normal: ≤360 μ mol/L and High: >360 μ mol/L. Men Age ≤65 = LAD-Tertile 1, ≤36.00 mm; LAEDD-Tertile 2, 36.00–38.00 mm and LAD-Tertile 3, >38.00 mm. Men Age >65 = LAEDD-Tertile 1, ≤36.00 mm, LAD-Tertile 2, 36.00–38.09 mm, and LAEDD-Tertile 3, >38.09 mm. Women Age ≤65 = LAEDD-Tertile 1, ≤35.00 mm, LAD-Tertile 2, 35.00–36.82 mm; and LAD-Tertile 3, >36.82 mm. Women Age >65 = LAD-Tertile 1, ≤35.69 mm; LAD-Tertile 2, 35.69–37.36 mm; LAEDD-Tertile 3, >37.36 mm. Adjusted for age, SBP, Scr, smoking, dyslipidemia, DM, statin, diuretic, ACEI, ARB, CCB, and β -blocker.

demonstrate a substantial increase in the proportion of AF patients with an increase in SUA levels.

According to the present study, the mean LAD was significantly higher in hypertension patients with AF than their counterparts without AF. This finding is in line with the previous observations among the general population (7, 20). Thus, the findings of the present study consolidated the association between the LAD and the presence of AF in hypertension patients. As per our results, those patients with an increased SUA level and LAD had a higher likelihood of AF. It has been previously demonstrated that hyperuricemia contributes to atrial remodeling, large left atrial size (7), ionic channel remodeling, metabolic syndrome (31), endothelial dysfunction (32), and arterial stiffness (33). Conversely, the use of allopurinol,

a medication that lowers uric acid via xanthine oxidase inhibition mechanism, is associated with a lower risk of AF (34). Hence, the possible biological explanations for the link between serum urate and risk of AF could be attributed to the mechanism that involves xanthine oxidase-mediated oxidative stress.

Several biological speculations have been suggested for the link between SUA and the risk of AF. Putative mechanisms through which SUA participates in AF development can be summarized as follows: First, an elevated SUA level is indeed an independent marker of various cardiovascular events. In many instances, there is a mutual relationship between SUA and cardiovascular risk factors (insulin resistance, chronic kidney disease conditions, metabolic syndrome, overweight/obesity); and subsequently teasing out the distinct influence of individual

TABLE 6 | The impact of serum uric acid in atrial fibrillation patients with normal and wide left atrium diameter.

Age ≤65					Age >65			
Tertiles of SUA								
Men	T1 (n = 767)	T2 (n = 771)	T3 (n = 764)		T1 (n = 818)	T2 (n = 803)	T3 (n = 809)	
AF (n, %)	47 (6.1%)	86 (11.2%)	89 (11.6%)		92 (11.2%)	109 (13.6%)	123 (15.2%)	
	OR (95% CI)	OR (95% CI)	OR (95% CI)	P-trend	OR (95% CI)	OR (95% CI)	OR (95% CI)	P-trend
Normal LAEDD	Ref.	2.139 (1.361, 3.361) [†]	2.228 (1.397, 3.552) [†]	0.001	Ref.	1.116 (0.792, 1.574)	1.094 (0.722, 1.552)	0.639
Large LAEDD	Ref.	1.364 (0.549, 3.389)	2.239 (0.901, 5.567)	0.081	Ref.	1.595 (0.738, 3.450)	2.427 (1.039, 5.667)*	0.041
Women	T1 (n = 659)	T2 (n = 663)	T3 (n = 644)		T1 (n = 977)	T2 (n = 972)	T3 (n = 971)	
AF (n, %)	27 (4.1%)	43 (6.5%)	53 (8.2%)		113 (11.6%)	103 (10.6%)	143 (14.7%)	
	OR (95% CI)	OR (95% CI)	OR (95% CI)	P-trend	OR (95% CI)	OR (95% CI)	OR (95% CI)	P-trend
Normal LAEDD	Ref.	1.565 (0.860, 2.848)	1.612 (0.862, 3.014)	0.148	Ref.	0.703 (0.485, 1.019)	0.656 (0.446, 0.966)*	0.033
Large LAEDD	Ref.	2.074 (0.850, 5.060)	1.534 (0.590, 3.984)	0.381	Ref.	1.739(1.075, 2.812) *	1.600 (0.964, 2.658)	0.086

* $P < 0.05$, [†] $P < 0.01$. In men: ≤ 42 mm considered normal LAD levels; and > 42 mm was considered as left atrial enlargement. In women: ≤ 38 mm was considered normal LAD and > 38 mm was considered as left atrial enlargement. Age ≤ 65 men: SUA-Tertile 1, ≤ 337.59 $\mu\text{mol/L}$; SUA-Tertile 2, 337.59–409.00 $\mu\text{mol/L}$; SUA-Tertile 3, > 409.00 $\mu\text{mol/L}$. Age > 65 men: SUA-Tertile 1, ≤ 323.00 $\mu\text{mol/L}$; SUA-Tertile 2, 323.00–393.00 $\mu\text{mol/L}$; SUA-Tertile 3, > 393.00 $\mu\text{mol/L}$. Age ≤ 65 Women: SUA-Tertile 1, ≤ 272.00 $\mu\text{mol/L}$; SUA-Tertile 2, 272.00–337.00 $\mu\text{mol/L}$; SUA-Tertile 3, > 337.00 $\mu\text{mol/L}$; Age > 65 women: SUA-Tertile 1, ≤ 279.00 $\mu\text{mol/L}$; SUA-Tertile 2, 279.00–342.00 $\mu\text{mol/L}$; SUA-Tertile 3, > 342.00 $\mu\text{mol/L}$. Adjusted for age, SBP, Scr, smoking, dyslipidemia, DM, statin, diuretic, ACEI, ARB, CCB, and β -blocker.

factors has proven a challenge to the research community. In this regard, an increased SUA level may be considered as an epiphenomenon of co-existing cardio-metabolic risk or a correlate of cardiovascular risk factors. Second, SUA is a product of xanthine-oxidoreductase activity (XOR), which is known to be one of the most essential courses of reactive oxygen species (ROS) in an organism. XOR *per se* has extensive implications in CVD and is closely interrelated to another key ROS producer, the enzyme NADPH oxidase (35). It is also well-established that XOR activity associates with risk factors for CVD and inflammatory markers (36). Furthermore, SUA may represent an endogenous signal of cell injury activating the cellular immune response. In fact, SUA has been associated with systemic inflammatory markers [such as c-reactive protein (CRP), interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor- α (TNF- α)] (37). These inflammatory cytokines (IL-6, IL-8, IL-1 β , and TNF- α) exert differential effects on vascular inflammation and dysfunction in patients with gout, a condition characterized by hyperuricemia. Additionally, uric acid increases the expression of angiotensin II in vascular endothelial cells (38) and activates the intrarenal renin-angiotensin system in humans (39). Likewise, experimental studies showed that uric acid promotes vasoconstriction and vascular smooth muscle cell proliferation (40), which could further increase the risk of vascular injury (due to inflammation and endothelial damage), vascular resistance, and cardiac hypertrophy. As such, SUA induced inflammation, oxidative stress, and endothelial dysfunction are factors increasing the risk of AF. Third, the electrophysiological hypothesis is also one of the conceivable mechanisms for the SUA induced AF. According to the electrophysiological hypothesis, uric acid enters atrial cells through uric acid transporters and stimulates Kv1.5 protein expression which could further contribute to an increased Kv1.5 ion activity and channel/I_{Kur} current that reduce the action potential duration of atrial cardiomyocytes (41). Overall, the possible explanation for the connection between SUA and AF

may support the pathophysiology milieu of vascular function injury and electrophysiologic theory.

The interaction analysis among those patients grouped in the different tertiles of SUA and their corresponding tertiles of LAD demonstrated that most patients at the top tertiles of SUA level and LAD had a higher likelihood of AF. These results provide support for the speculated link between SUA and LAD with the prevalence of AF. Though the underlying mechanisms of elevated SUA relating to the risk of enlarged LAD are poorly understood, some studies intend to elaborate on the mechanism that involves xanthine oxidase-mediated oxidative stress and inflammation. Xanthine oxidase activity alters several important physiological functions, including vessel diameter modulation, remodeling, and lesion formation. The consequences of inflammation and oxidative stress can lead to cardiac remodeling and atrial fibrosis, which may increase the susceptibility of AF. Of note, chronic inflammation is well-known for its contribution to endothelial damage, enhanced activity of the platelet, and up-regulated fibrinogen expression (42). The left atrium has been reported for its sensitivity to oxidative stress (43), and the xanthine oxidase enzyme present in the left atrium seems to boost atrial oxidative stress in patients with AF (44). In a recent experimental study, the enzymatic activity of xanthine oxidase in left atrial appendages was 4.4 times higher in the AF group compared to the controls (45). Also, documented evidence revealed that SUA can result in overloading calcium and reducing sodium channels, and exacerbating the cellular injury. These pathological processes endorse left atrial electrical remodeling (46). Besides, SUA has a direct effect on the activation of the local renin-angiotensin system, endothelial dysfunction, smooth muscle cell proliferation (47), and decreasing nitric oxide production (48). It should be noted that SUA not only promotes inflammation through the release of pro-inflammatory cytokines (37) but also via localized stimulation of the renin-angiotensin system (49). Therefore, the ROS and inflammation associated with the up-regulation

of xanthine oxidase with hyperuricemia may participate in the mechanism to explain the observed association.

The development of AF as a result of elevated SUA among hypertension subjects has not been well-investigated, nor have mechanisms of such consequences been fully illuminated. The recent literature suggests a prominent hypertension risk associated with SUA, presumably because of the decrease in renal blood flow that would further stimulate urate reabsorption (45). Consequently, the higher levels of SUA may intensify the existing inflammation and vascular remodeling that result from underlying hypertension and influences the process of normal blood flow hemostasis adversely. Such physiological alterations due to high SUA levels could contribute to the activation of the renin-angiotensin system and endothelial dysfunction, which could eventually lead to AF (50) in hypertension patients. Thus, the link between SUA and risk of AF in hypertensive patients could be attributed to abnormal accumulation of SUA associated with low renal blood flow during the hypertension phase.

When we carried out a secondary analysis to investigate whether there was a possibility that the uricemic status might have influenced the predictive power of LAD values for AF in normouricemic and hyperuricemic group, the findings of the present study clarified that the estimated risk of AF associated with SUA varies depending on age groups and gender. Compared to the lowest tertile, the risk of AF was increased by two-folds in the highest tertiles of LAD and SUA in normouricemic women. Those patients with high LAD values, regardless of the hyperuricemic status, had a greater likelihood of having AF. Also, when patients were stratified by the size of LAD, SUA was associated with greater odds of AF in those patients present with large LAD but not in those patients with normal LAD, implying wide LAD tends to link more with AF patients than elevated SUA levels. Moreover, the estimated risk for AF in the highest tertile of LAD in >65 years women were lower in normouricemia compared to the hyperuricemia group. The incidence of AF normally increases with age, and similarly, the SUA levels increase with age. In males and females, the pathophysiology of AF can vary slightly. This is not extensively investigated, but in several studies, the risk factors for AF showed different strengths and qualities of association in men and women (10). From the viewpoint of our results, the assortment of such results depending on the hyperuricemic status, and size of LAD may resolve the previous conflicting results produced from other studies. Therefore, the findings can be used to guide the study designs to strictly classify AF patients based on uricemic status, and LAD size, in addition to age and gender grouping, to improve the reliability and robustness of the future studies.

Limitation

The present study has a couple of strengths and limitations. The sample size of this study was relatively large. To our knowledge, no study investigated the interaction between the SUA and LAD in hypertension with AF in depth. Thus, it has been unknown up to now whether the interaction between elevated SUA levels and LAD amplifies the risk of AF in patients with hypertension. However, this study has several limitations. First, the cross-sectional design restricted the cause and effect relationship

between the SUA/LAD and AF. Similarly, since a clear timeline of diagnosis and events are not in place, the cause and effect nature of LAD and SUA cannot be adequately determined. Second, the study involved hospitalized patients in Dalian, Northeast China, a region known for consuming seafood, therefore, the lifestyle and diet customs may significantly influence the SUA metabolism. Third, our study sample lacks national or regional representation that limits the ability to generalize the results at an international level. Thus, the results of this study may require replication for consistency from other parts of the country or other regions of the world. Fourth, our study didn't include data on left ventricular volume index and left atrial strain, and the lack of Holter monitoring for some patients in our study and the use of ECG to determine the presence of AF may negatively influence the accuracy of AF prevalence as some patients with paroxysmal AF may escape from ECG. Fifth, our study recruited only patients with hypertension therefore, further study is required to replicate and extend the results in the general population. Sixth, we were unable to rule out the influence of antihypertensive agents on our findings due to the retrospective design and ethical reasons that oppose the withdrawal of these medications. Nevertheless, in the multivariate model, we have considered antihypertensive treatment to reduce the confounding effect of antihypertensive use. Seventh, our study does not provide detailed data on the duration of AF, how long the patients have been on treatment for and how well-managed are the patients' co-morbidities (if there was any co-morbidity that did not fulfill the exclusion criteria).

CONCLUSION

In conclusion, elevated SUA and LAEDD were independently associated with an increased prevalence of AF. The effect of SUA appeared most pronounced among those with AF in younger men. LAD associates independently with AF incidence in men and women alike. Those patients with high LAD values, regardless of the hyperuricemic status, had a greater likelihood of having AF. The risk of AF was increased by 2 folds in the top tertiles of LAD and SUA in normouricemic middle-aged women, suggesting that the interaction between SUA and LAD modifies the estimated risk of AF in women with hypertension. Further longitudinal studies are needed to prove whether lowering the SUA level may or may not be necessary to prevent AF in hypertension patients.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by First Affiliated Hospital of Dalian Medical University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Non-linear Association Between Body Mass Index and Ventricular Tachycardia/Ventricular Fibrillation in Patients With an Implantable Cardioverter-Defibrillator or Cardiac Resynchronization Therapy Defibrillator: A Multicenter Cohort Study

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Background: Results from studies on the effects of obesity on sudden cardiac death (SCD) or ventricular tachycardia/ventricular fibrillation (VT/VF) in patients with an implantable cardioverter-defibrillator/cardiac resynchronization therapy defibrillator (ICD/CRT-D) are inconsistent. Our study aimed to explore the impact of BMI on VT/VF in patients with an ICD/CRT-D.

Methods: We retrospectively analyzed the data from the Study of Home Monitoring System Safety and Efficacy in Cardiac Implantable Electronic Device-implanted Patients in China. Nine hundred and seventy ICD/CRT-D patients were enrolled. The outcome was the first occurrence of VT/VF requiring appropriate ICD/CRT-D therapy. A general linear model and general additive model were used to assess the relationship between BMI and VT/VF.

Results: After a median follow-up of 5.17 years, 352 (36.3%) patients experienced VT/VF requiring appropriate ICD/CRT-D therapy. BMI, whether as a continuous variable or a categorical variable classified by various BMI classification criteria, had no significant effect on VT/VF according to a multivariable Cox proportional hazards model with adjustment for potential confounders. However, a non-linear association between BMI and VT/VF was identified using a cubic spline function model and smooth curve fitting. The inflection point for the curve was found at a BMI level of 23 kg/m². The hazard ratios (95% confidence intervals) for VT/VF were 1.12 (1.01–1.24) and 0.96 (0.90–1.02) to the left and right of the inflection point, respectively.

Conclusions: BMI is related to VT/VF in a non-linear manner in patients with an ICD/CRT-D. Our research suggests a complicated role of BMI in VT/VF with different impacts at different ranges.

Keywords: body mass index, sudden cardiac death, ventricular tachycardia, implantable cardioverter-defibrillator, non-linearity

INTRODUCTION

Sudden cardiac death (SCD) is a global public health concern, accounting for up to 50% of all cardiovascular deaths (1). The exact definition of SCD is sudden and unexpected death occurring within an hour of the onset of symptoms or occurring in patients found dead within 24 h of being asymptomatic presumably due to a cardiac arrhythmia or hemodynamic catastrophe (2). Fatal ventricular tachycardia/ventricular fibrillation (VT/VF) plays vital roles in the development of SCD and results in hemodynamic collapse with cessation of cardiac mechanical activity (2). Current clinical practice guidelines recommend the implantation of an implantable cardioverter-defibrillator/cardiac resynchronization therapy defibrillator (ICD/CRT-D) to treat possible ventricular VT/VF in the management of patients at high risk of SCD (2–4). For patients with an ICD/CRT-D, VT/VF requiring appropriate ICD/CRT-D therapy is a commonly used surrogate for SCD (5, 6).

Additionally, as a global health problem, obesity, which is usually assessed by body mass index (BMI) in clinical practice, has been recognized as a risk factor for SCD in the general population (7). However, the results from the few studies on the effects of obesity on SCD or VT/VF in patients who had received an ICD/CRT-D due to their high risk of SCD have been controversial (6, 8–10).

Fully understanding the effect of obesity on the occurrence of SCD or VT/VF in patients with an ICD/CRT-D is conducive to risk stratification and helpful for guiding proper treatment for these patients. Our study intended to explore the impact of BMI on VT/VF requiring appropriate ICD/CRT-D therapy in patients with an ICD/CRT-D.

METHODS

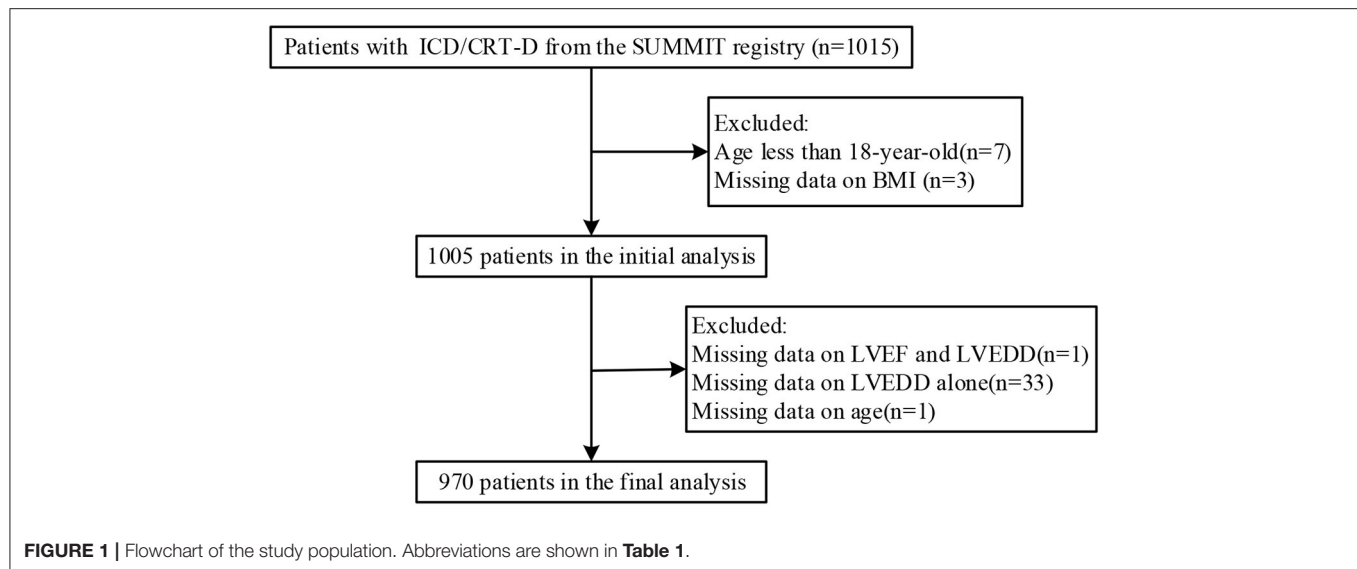
Study Design, Setting, and Population

Study of Home Monitoring System Safety and Efficacy in Cardiac Implantable Electronic Device-implanted Patients (SUMMIT) was a prospective, observational, multicenter registry used to evaluate the safety and efficacy of a cardiac implantable electronic device with a home monitoring (HM) system in China. We performed a retrospective cohort study based on data from the SUMMIT registry. A total of 1,015 patients who underwent ICD or CRT-D implantation with an HM system (Biotronik, Berlin, Germany) between May 2010 and May 2015 from the SUMMIT registry were included. Next, we excluded patients meeting any of the following criteria: (1) patients younger than 18 years ($n = 7$); (2) patients with missing body mass index (BMI) data ($n = 3$); (3) patient with missing data on left

ventricular ejection fraction (LVEF) and left ventricular end-systolic dimension (LVEDD) ($n = 1$); (4) patients with missing data on LVEDD alone ($n = 33$); and (5) patient with missing data on age ($n = 1$). Thus, 970 patients were enrolled in the final analysis. The flowchart of the study population is shown in **Figure 1**. According to the clinical practice guidelines (2–4), all patients were satisfied with indications of primary prevention or secondary prevention of SCD. Primary prevention of SCD refers to the use of ICDs in individuals who are at risk for but have not yet had an episode of sustained VT, VF, or resuscitated cardiac arrest; secondary prevention refers to the prevention of SCD in patients who have survived a prior sudden cardiac arrest or sustained VT or VF (3). A total of 394 patients satisfied the secondary prevention of SCD in our study. Among these patients, 98 (25%) had documented VF and resuscitated SCD, 236 (60%) had a history of documented sustained VT and 60 (15%) had a history of unexplained syncope and could be induced to VT or VF during electrophysiological study. The study protocols were approved by Ethics Committee of Fuwai Hospital, Chinese Academy of Medical Sciences (the chief institute) and all other participating organizations (Zhongshan Hospital, Fudan University et al.), and were in accordance with the Declaration of Helsinki. All patients signed informed consent forms before the study. All reporting followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (11).

Clinical Data Collection

BMI was calculated as weight (kg) divided by the square of the patient's height (m^2) and shown as kg/m^2 . Because the BMI cutoff points for overweight and obesity vary under World Health Organization (WHO) criteria (12), Asian criteria (13), or Chinese criteria (14), and/or different BMI cutoff points were used in previous studies, we divided the BMI values into tertiles, which is a common and convenient method (15). Other baseline clinical characteristics, including age at implantation, gender, systolic blood pressure, diastolic blood pressure, indication of primary or secondary prevention, New York Heart Association (NYHA) class, implantation of ICD or CRT-D, ischemic cardiomyopathy, dilated cardiomyopathy, hypertrophic cardiomyopathy, Long QT syndrome, hypertension, diabetes, stroke, atrial fibrillation (AF), preimplant syncope, LVEF, LVEDD, β -blockers, amiodarone, angiotensin-converting enzyme inhibitor or angiotensin receptor blocker, loop diuretic, and aldosterone antagonists were acquired from the patients' medical records before ICD/CRT-D implantation. LVEF was calculated by using the modified Simpson's biplane rule.



Device Settings and Outcome

The protocol of device programming settings was consistent with those of our previous study (16). The detailed protocol of the device programming settings is shown in **Supplementary Material 1**. The outcome was the first occurrence of VT/VF requiring appropriate ICD/CRT-D therapy based on data automatically transmitted to the HM system and as confirmed by two or more cardiologists through reviewing the intracardiac electrograms. Inappropriate events, VT with a heart rate slower than 140 bpm and non-sustained VT were excluded. Routine follow-ups were conducted, and patient status was confirmed via phone calls in the event that the transmission of their data was disrupted. The HM system recorded the interval from ICD/CRT-D implantation to the first occurrence of VT/VF requiring appropriate ICD/CRT-D therapy. The last time we assessed the VT/VF events in the HM system was June 2018.

Statistical Analysis and Sensitivity Analysis

Data are presented as the means \pm standard deviation or proportions. Chi-square test (categorical variables) or one-way analysis of variance with Bonferroni *post-hoc* test (continuous variables) was used to calculate differences between different BMI groups (tertiles). To investigate the association between BMI and VT/VF requiring appropriate ICD/CRT-D therapy, our statistical analyses consisted of 4 main steps.

Step 1: We plotted Kaplan-Meier curves to compare the outcomes of different BMI groups (log-rank test). Step 2: We used a generalized linear model such as the standard Cox proportional hazards model to assess the association between BMI and VT/VF. We constructed 4 Cox proportional hazards models: model 1, adjusted for none; model 2, adjusted for age and gender; model 3, adjusted for variables in model 2 plus variables that had a statistically significant effect on VT/VF at the 0.05 level in the

univariate Cox model; and model 4, adjusted for all covariates presented in **Table 1**. Step 3: To address the non-linearity of the relation between BMI and an outcome, a generalized additive model was used. We conducted a cubic spline function model and smooth curve fitting (penalized spline method) to further explore the association between BMI and VT/VF. If non-linearity was detected, we first calculated the inflection point using a recursive algorithm and then constructed a 2-piecewise Cox proportional hazards model on both sides of the inflection point. We determined the best fit model (1-line Cox proportional hazards model vs. piecewise Cox proportional hazards model) based on the *P*-values for the log likelihood ratio test. Step 4: The subgroup analyses were performed using the Cox proportional hazards model. For continuous variables, we first converted them to categorical variables according to the clinical cutoff point and then performed an interaction test. Tests for effect modification by subgroup were based on interaction terms between subgroup indicators followed by likelihood ratio test.

To ensure the robustness of the data analysis, we performed the following sensitivity analysis. (1) We compared the complete dataset and missing dataset, and the results demonstrated that nearly all variables were similar, showing that the selection bias was relatively small (**Supplementary Table 1**). (2) We converted the BMI into a categorical variable by tertiles and calculated the *P* for trend. The purpose was to verify the results of BMI as a continuous variable and to observe the possibility of non-linearity. (3) We performed the same analysis in steps 1 and 2 using the BMI classification based on WHO criteria, Asian criteria or Chinese criteria.

All analyses were performed using R version 4.0.0 (R Foundation for Statistical Computing, Vienna, Austria) and Empower (R) (X&Y Solutions, Inc., Boston, MA). All *P* < 0.05 (two-sided) were considered statistically significant.

TABLE 1 | Baseline characteristics of study population according to BMI.

Characteristics	Total (n = 970)	Tertile of BMI			P-value
		Tertile 1 (<22.2 kg/m ²) (n = 317)	Tertile 2 (22.2–24.4 kg/m ²) (n = 312)	Tertile 3 (>24.4 kg/m ²) (n = 341)	
Age at implantation, years	60.3 ± 13.5	60.4 ± 14.6	60.3 ± 12.6	60.3 ± 13.4	0.988
Male	707 (72.9%)	198 (62.5%)	241 (77.2%)	268 (78.6%)	<0.001
SBP, mmHg	124.5 ± 17.4	124.0 ± 17.8	123.0 ± 16.5	126.4 ± 17.7	0.151
DBP, mmHg	76.9 ± 10.9	75.6 ± 11.3	76.5 ± 9.9	78.4 ± 11.2	0.002
Primary prevention	576 (59.4%)	187 (59.0%)	188 (60.3%)	201 (58.9%)	0.930
NYHA, class III/IV	484 (49.9%)	172 (54.3%)	146 (46.8%)	166 (48.7%)	0.148
CRT-D	266 (27.4%)	89 (28.1%)	91 (29.2%)	86 (25.2%)	0.503
Ischemic cardiomyopathy	324 (33.4%)	96 (30.3%)	98 (31.4%)	130 (38.1%)	0.069
Dilated cardiomyopathy	238 (24.5%)	83 (26.2%)	73 (23.4%)	82 (24.0%)	0.695
Hypertrophic cardiomyopathy	37 (3.8%)	9 (2.8%)	12 (3.8%)	16 (4.7%)	0.463
Long QT syndrome	12 (1.2%)	5 (1.6%)	3 (1.0%)	4 (1.2%)	0.777
Hypertension	305 (31.4%)	89 (28.1%)	92 (29.5%)	124 (36.4%)	0.049
Diabetes mellitus	101 (10.4%)	24 (7.6%)	34 (10.9%)	43 (12.6%)	0.101
Stroke	18 (1.9%)	3 (1.0%)	4 (1.3%)	11 (3.2%)	0.076
Atrial fibrillation	104 (10.7%)	38 (12.0%)	33 (10.6%)	33 (9.7%)	0.629
Pre-implant syncope	194 (20.0%)	67 (21.1%)	60 (19.2%)	67 (19.7%)	0.820
LVEF, %	42.5 ± 14.9	41.6 ± 15.0	42.9 ± 15.0	42.8 ± 14.8	0.476
LVEDD, mm	58.8 ± 13.1	58.1 ± 12.8	58.8 ± 13.5	59.6 ± 13.0	0.301
β-Blocker	566 (58.4%)	177 (55.8%)	181 (58.0%)	208 (61.0%)	0.402
Amiodarone	290 (29.9%)	91 (28.7%)	104 (33.3%)	95 (27.9%)	0.266
ACEI or ARB	360 (37.1%)	128 (40.4%)	100 (32.1%)	132 (38.7%)	0.073
Loop diuretic	280 (28.9%)	84 (26.5%)	93 (29.8%)	103 (30.2%)	0.523
Aldosterone antagonists	363 (37.4%)	125 (39.4%)	105 (33.7%)	133 (39.0%)	0.246

Continuous data and categorical data were given as mean ± SD and number (percentage), respectively.

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CRT-D, cardiac resynchronization therapy defibrillator; DBP, diastolic blood pressure; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-systolic dimension; NYHA, New York Heart Association; SBP, systolic blood pressure.

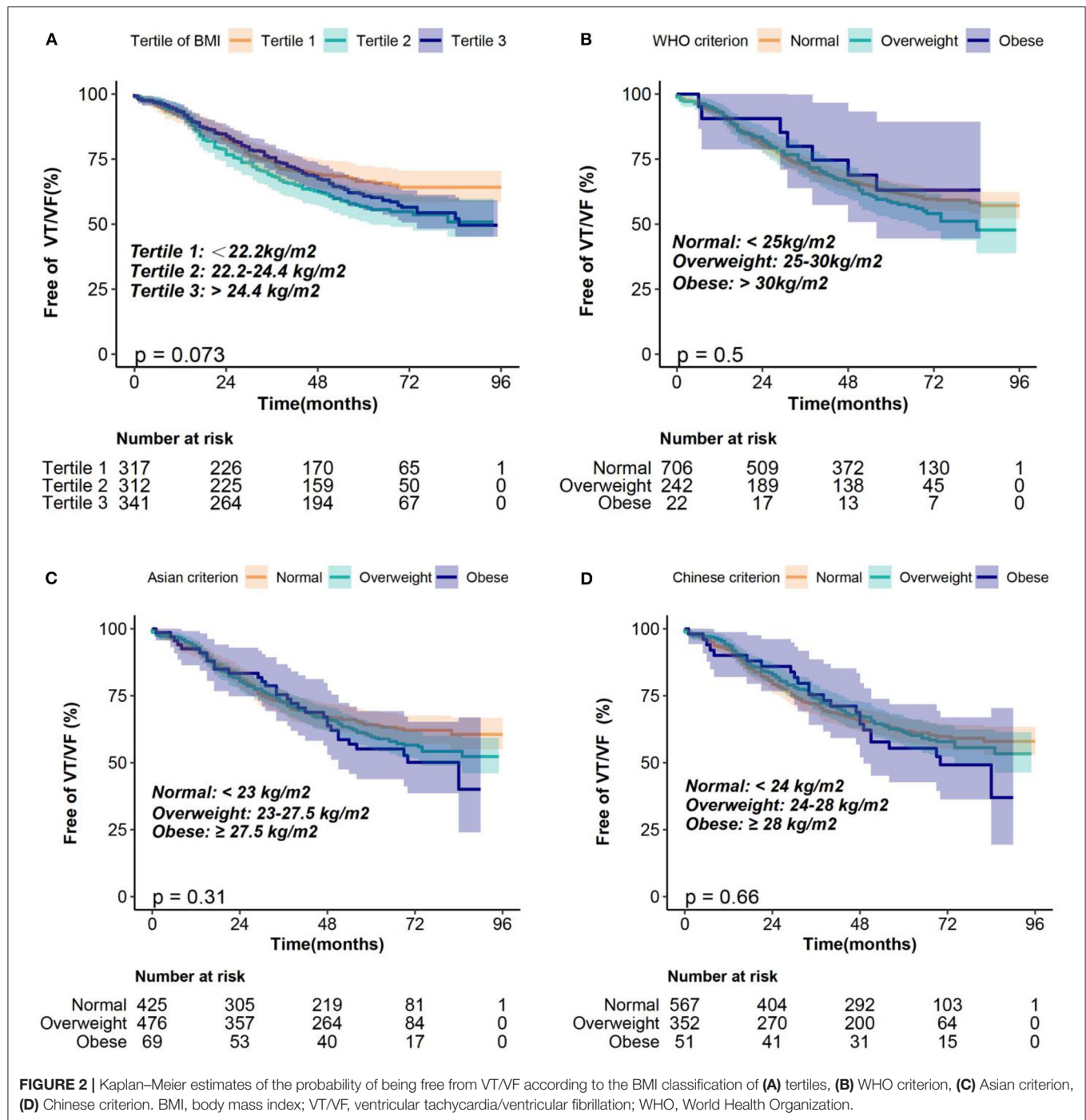
RESULTS

Baseline Characteristics of the Participants

After exclusions, the final analysis dataset consisted of 970 adults (mean age at implantation: 60.34 ± 13.50 years, 72.89% male). The distribution of the baseline characteristics of the study population according to BMI tertiles is shown in **Table 1**. The ranges of BMI for tertiles 1 through 3 were <22.2, 22.2–24.4, and >24 kg/m², respectively. There were nearly no significant differences between BMI tertiles for all included characteristics except for gender, diastolic blood pressure and presence of hypertension. Compared with those in T1 and T2 of the BMI, the participants in T3 were more likely to be male, have a higher diastolic blood pressure and present with hypertension (all $p < 0.05$).

Association Between BMI and VT/VF Using Kaplan-Meier Curves and the Cox Proportional Hazards Models

The median follow-up duration was 5.17 (interquartile, 4.3–5.83) years. During the follow-up, 352 (36.3%) patients experienced VT/VF requiring ICD/CRT-D therapy. Kaplan-Meier survival curves were plotted to determine the probability of patients being VT/VF free according to BMI tertile (**Figure 2**). The results showed that the probability of being free from VT/VF for patients in the BMI tertiles was not significantly different (log-rank, $p = 0.073$). Additionally, we plotted the Kaplan-Meier survival curves using different BMI classification criteria for clinical applications, and the results were consistent with those using BMI tertiles (**Figure 2**). Univariate Cox proportional hazards models of VT/VF are shown in **Supplementary Table 2**. Older age, male, AF, lower



LVEF and wider LVEDD were associated with higher risk of VT/VF in the univariate Cox proportional hazards models ($P < 0.05$). Then, we constructed 4 Cox proportional hazards models to analyze the independent role of BMI in VT/VF. The HRs and 95% CIs for these 4 models are listed in Table 2. In the unadjusted model (model 1), each 1 kg/m² BMI increase was associated with a 4% increased risk of VT/VF. However, in model 2, after adjusting for age and gender, the association between BMI and the risk of VT/VF was

not statistically significant. Additionally, after adjusting for additional covariates in model 3 (adjusting for age, gender, AF, LVEF, and LVEDD) and all covariates presented in Table 1 in model 4, the results negligibly changed. We also converted BMI from a continuous variable to a categorical variable (tertiles). Compared with participants in T1 of the BMI, there was no significant increased risk of VT/VF for patients in either T2 or T3 in the adjusted models (models 2–4). The P trend value was not significant in any of the models,

TABLE 2 | Association of BMI with VT/VF in different models.

BMI (kg/m ²)	No. of VT/VF	Model 1		Model 2		Model 3		Model 4	
		HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Continuous	352	1.04 (1.00, 1.07)	0.0364	1.03 (0.99, 1.07)	0.1315	1.03 (0.99, 1.06)	0.1662	1.03 (0.99, 1.07)	0.1510
Tertiles									
<22.1	96	Reference		Reference		Reference		Reference	
22.1–24.4	126	1.36 (1.04, 1.77)	0.0233	1.28 (0.98, 1.67)	0.0702	1.29 (0.99, 1.69)	0.0616	1.30 (0.99, 1.71)	0.0565
>24.4	130	1.20 (0.92, 1.57)	0.1687	1.14 (0.87, 1.48)	0.3517	1.13 (0.87, 1.48)	0.3541	1.11 (0.84, 1.46)	0.4643
<i>P</i> _{trend} -value			0.1899		0.3975		0.4072		0.5405
WHO criterion									
<25	245	Reference		Reference		Reference		Reference	
25–30	100	1.13 (0.90, 1.43)	0.3017	1.11 (0.88, 1.40)	0.3786	1.11 (0.88, 1.41)	0.3662	1.08 (0.85, 1.38)	0.5123
≥30	7	0.84 (0.40, 1.79)	0.6598	0.83 (0.39, 1.75)	0.6170	0.73 (0.34, 1.56)	0.4177	0.80 (0.36, 1.78)	0.2562
<i>P</i> _{trend} -value			0.6169		0.7284		0.8864		0.8235
Asian criterion									
<23	138	Reference		Reference		Reference		Reference	
23–27.5	183	1.14 (0.92, 1.42)	0.2406	1.08 (0.87, 1.36)	0.4791	1.09 (0.87, 1.37)	0.4384	1.06 (0.84, 1.34)	0.6014
≥27.5	31	1.29 (0.88, 1.91)	0.1943	1.24 (0.84, 1.84)	0.2749	1.22 (0.83, 1.81)	0.3177	1.25 (0.83, 1.87)	0.2848
<i>P</i> _{trend} -value			0.1280		0.2544		0.2691		0.3018
Chinese criterion									
<24	197	Reference		Reference		Reference		Reference	
24–28	131	1.01 (0.81, 1.26)	0.9188	0.98 (0.79, 1.22)	0.8613	0.97 (0.78, 1.21)	0.8057	0.94 (0.75, 1.18)	0.6017
≥28	24	1.22 (0.80, 1.86)	0.3657	1.17 (0.77, 1.80)	0.4565	1.13 (0.74, 1.73)	0.5802	1.20 (0.77, 1.88)	0.4246
<i>P</i> _{trend} -value			0.5240		0.7202		0.8452		0.8012

Model 1: adjusted for none. Model 2: adjusted for age, gender. Model 3: adjusted for variables in Model 2 plus atrial fibrillation, LVEF, LVEDD. Model 4 adjusted for all covariates presented in **Table 1**. CI, confidence interval; HR, hazard ratio; VT/VF, ventricular tachycardia /ventricular fibrillation; WHO, World Health Organization; other abbreviations are shown in **Table 1**.

indicating a possible non-linear association between BMI and VT/VF. Moreover, we also performed sensitivity analyses using different BMI classification criteria for clinical applications, and the results were nearly the same as those based on the BMI tertiles.

Association Between BMI and VT/VF Using the Cubic Spline Function Model and Smooth Curve Fitting

We conducted a cubic spline function model and smooth curve fitting (penalized spline method) to visualize the relationship between BMI and VT/VF. The fully adjusted smooth curve fitting showed a non-linear association between BMI and VT/VF (Figure 3). We further conducted a threshold effect analysis of BMI on VT/VF. We fitted the association between BMI and VT/VF using a 1-line Cox proportional hazards model and a 2-piecewise Cox proportional hazards model, respectively. The *P*-value for the log likelihood ratio test was <0.05 , indicating that the 2-piecewise Cox proportional hazards model was more suitable for fitting the association between BMI and VT/VF. As shown in Figure 3, a non-linear association between BMI and VT/VF was found (*P* for non-linearity = 0.035). The inflection point that we detected for the BMI was 23 kg/m^2 . When the BMI was $\leq 23 \text{ kg/m}^2$, the hazard ratio (HR) per unit (kg/m^2) of higher BMI was 1.12 [95% confidence interval (CI) 1.01–1.24]. However, when the BMI was $>23 \text{ kg/m}^2$, the higher BMI did not add to the risk of VT/VF but showed a trend of decreased risk of VT/VF (HR 0.96, 95% CI 0.90–1.02).

Subgroup Analysis

We further investigated the role of other covariates between BMI and VT/VF. As shown in Figure 4, the association between BMI and VT/VF was consistent in the following subgroups: sex, age, NYHA, primary prevention, CRT-D, ischemic cardiomyopathy, hypertension, diabetes mellitus, AF, syncope, LVEF and LVEDD (all *P*-values for these interactions >0.05).

DISCUSSION

The major findings of this study are as follows: (1) BMI, whether as a continuous variable or a categorical variable classified by various BMI classification criteria, had no significant impact on VT/VF in the ICD/CRT-D patients according to Kaplan-Meier curves and Cox proportional hazards models. (2) A non-linear association between BMI and VT/VF was identified using a cubic spline function model and smooth curve fitting. When the BMI was $\leq 23 \text{ kg/m}^2$, a higher BMI was associated with a higher risk of VT/VF. When the BMI was $>23 \text{ kg/m}^2$, a higher BMI did not increase the risk of VT/VF but showed a trend of decreased risk of VT/VF.

Several studies have illustrated the effect of BMI on the risk of VT/VF in ICD/CRT-D patients, but the results have been inconsistent (6, 8–10). Pietrasik et al. conducted a retrospective analysis of non-diabetic patients with ischemic left ventricular dysfunction using data from the Multicenter Automatic Defibrillator Implantation Trial-II (MADIT II) and

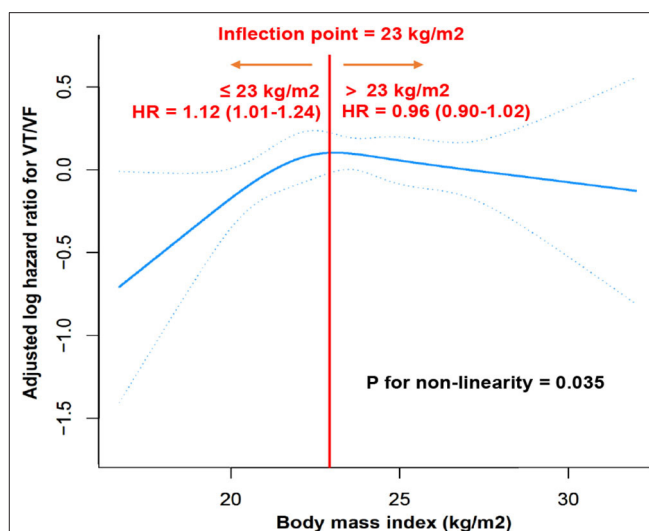


FIGURE 3 | Dose response relationship of BMI and VT/VF. A non-linear association between BMI and VT/VF was found (*P* for non-linearity = 0.035) in a generalized additive model. The solid blue line and dashed blue line represent the estimated values and their corresponding 95% CI. Adjustment factors included all covariates presented in Table 1. The inflection point detected for BMI was 23 kg/m^2 . When BMI was $\leq 23 \text{ kg/m}^2$, HR per unit (kg/m^2) higher BMI was 1.12 (95% CI 1.01–1.24). However, When BMI was $> 23 \text{ kg/m}^2$, higher BMI did not add risk of VT/VF but showed a trend of decreased risk of VT/VF (HR 0.96, 95% CI 0.90–1.02). BMI, body mass index; CI, confidence interval; HR, hazard ratio; VT/VF, ventricular tachycardia/ventricular fibrillation.

demonstrated that a higher rate of VT/VF was detected in obese patients ($\text{BMI} \geq 30 \text{ kg/m}^2$) compared with the rate in non-obese patients (8). However, a *post-hoc* analysis of the ICD/CRT-D patients from the Multicenter Automatic Defibrillator Implantation trial with Cardiac Resynchronization Therapy (MADIT-CRT) showed that BMI had no impact on VT/VF (9). Another study from Spain also found that BMI was not associated with VT/VF in ICD patients for the purpose of primary prevention (10). These contradictory results may be the result of differences in the population characteristics, sample sizes, racial groups, and the adjustment of confounders. Moreover, these studies only investigated the linear relationship between BMI and VT/VF and did not address non-linear relationships. Gandhi et al. found an interesting phenomenon in their study that suggested an inverted U-shaped relationship between BMI and risk of VT/VF among ICD patients with systolic heart failure, with the highest risk found for the overweight BMI group ($\text{BMI } 25\text{--}30 \text{ kg/m}^2$) and the lower risk found in the normal group ($\text{BMI } 18.5\text{--}25 \text{ kg/m}^2$) and obese group ($\text{BMI} \geq 30 \text{ kg/m}^2$) (6). However, this study did not investigate the non-linear relationship between BMI and VT/VF. The U-shaped relationship for BMI and VT/VF was based on the segmentation effect, which was not intuitive.

Our study used the Kaplan-Meier curve/Cox proportional hazards model or cubic spline function model/smooth curve fitting to explore the relationship between BMI and VT/VF. The results showed that BMI had a non-linear association

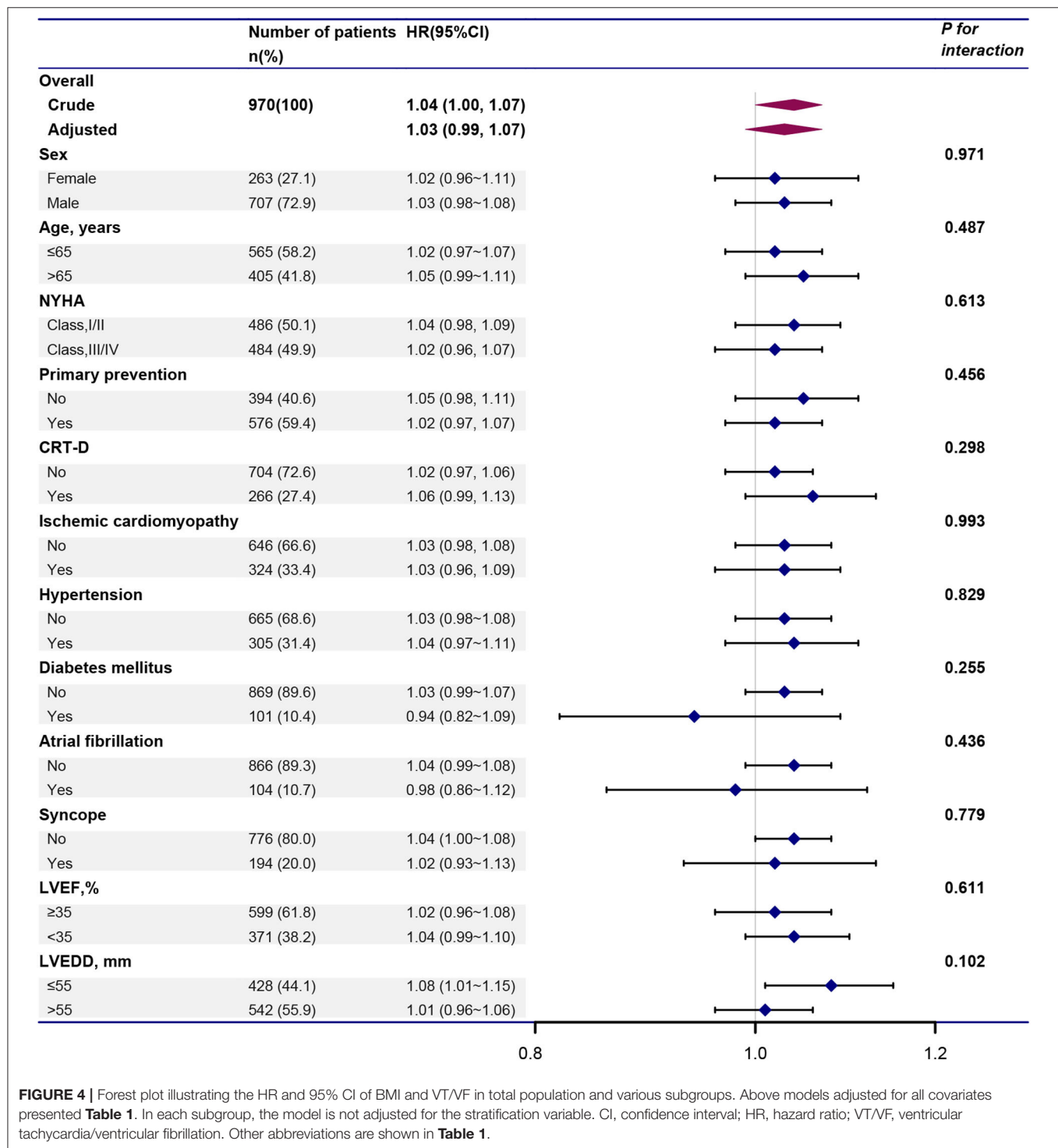


FIGURE 4 | Forest plot illustrating the HR and 95% CI of BMI and VT/VF in total population and various subgroups. Above models adjusted for all covariates presented **Table 1**. In each subgroup, the model is not adjusted for the stratification variable. CI, confidence interval; HR, hazard ratio; VT/VF, ventricular tachycardia/ventricular fibrillation. Other abbreviations are shown in **Table 1**.

with VT/VF. Had we used only a generalized linear model to identify the association between BMI and VT/VF, the way we classified the BMI would not have mattered: the result would be negative. Once the non-linear relationship is identified, it is not appropriate to use a generalized linear model to analyze the correlation. The cubic spline function model and smooth curve fitting were helpful in detecting the non-linear relationship

between BMI and VT/VF. Additionally, the inflection point was identified to show the role of BMI in different intervals.

The mechanism resulting in this non-linear association is unclear. In the general population, obesity is associated with cardiac structural changes (i.e., cardiac hypertrophy and myocardial abnormalities such as fibrosis or fatty infiltration), electrical abnormalities (i.e., QT prolongation) and sleep apnea,

which could lead to an increased risk of ventricular arrhythmias (17–20). However, the obesity paradox suggesting a positive association between a higher BMI and positive outcomes has been found in several cohorts, although the reason remains unclear (21–23). We suggest that there is a complicated interaction between the impact of obesity-related cardiac structural/electrical changes and obesity-related risk factors compared to the factors accounting for improved outcomes with obesity in patients at high risk of SCD. The non-linear association between BMI and VT/VF shows that the impact of BMI is quite different in different ranges. Perhaps when BMI is in a certain range ($\text{BMI} \leq 23 \text{ kg/m}^2$ in our study), and the BMI is increased, then the harm caused by BMI dominates, which would lead to the increased risk of VT/VF; however, when BMI exceeds a certain range ($\text{BMI} > 23 \text{ kg/m}^2$ in our study), the benefits conferred by the BMI play a leading role, and the risk of VT/VF exhibits a decreasing trend.

Our study has several strengths. First, our research is a multicenter study with a relatively large sample size and good generalizability. Second, we used both a generalized linear model and generalized additive model to explore the relationship between BMI and VT/VF to the maximum extent. Our results showed an interesting non-linear relationship between BMI and VT/VF. Third, we performed sensitivity analyses to enhance the robustness of the results. However, there are some limitations to our study. First, our study was an observational study that was subject to selection bias because patients without complete data were excluded. However, we sincerely compared the complete dataset and the missing dataset, and the results demonstrated that nearly all variables were similar, showing that the selection bias was relatively small. Second, our study did not collect data on obstructive sleep apnea and laboratory parameters which may have influence on the effect of BMI on VT/VF. We could not adjust for these substantial confounders; therefore, a prospective study collecting more variables may be necessary to validate our results. Third, the number of obese patients in our study was relatively small, which limited the generalizability of our results. Finally, we only had baseline BMI information, and data on changes in BMI were not collected during the follow-up. Higher fluctuations in BMI were related to adverse outcomes in patients with coronary heart disease (24). In the future, we will perform a prospective study enrolling a larger sample size to ensure the sample balance of each group and collect the change in BMI to better illustrate the influence of BMI at the baseline and upon dynamic changes in BMI on VT/VF in patients with an ICD/CRT-D.

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CONCLUSIONS

Our study identifies a non-linear relationship between BMI and VT/VF in patients with an ICD/CRT-D. Our research suggests a complicated role of BMI in VT/VF with different impacts at different ranges. Prospective studies are needed to better illustrate the role of BMI in VT/VF as well as the potential mechanisms accounting for this role.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The study protocols were approved by ethics committee of Fuwai Hospital, Chinese Academy of Medical Sciences (the chief institute), and all other participating organizations (Zhongshan Hospital, Fudan University et al.), and were in accordance with the Declaration of Helsinki. All patients signed informed consent forms before the study. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SZhang conceived and designed the research. MT, KC, WH, YS, JY, ZL, and WX conducted the ICD/CRT-D implantation. SZhao collected the data. BZ analyzed the data and wrote the manuscript. SZhang revised the manuscript. All authors read and approved the final manuscript.

FUNDING

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcvm.2020.610629/full#supplementary-material>

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Automated Electrocardiogram Analysis Identifies Novel Predictors of Ventricular Arrhythmias in Brugada Syndrome

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Background: Patients suffering from Brugada syndrome (BrS) are at an increased risk of life-threatening ventricular arrhythmias. Whilst electrocardiographic (ECG) variables have been used for risk stratification with varying degrees of success, automated measurements have not been tested for their ability to predict adverse outcomes in BrS.

Methods: BrS patients presenting in a single tertiary center between 2000 and 2018 were analyzed retrospectively. ECG variables on vector magnitude, axis, amplitude and duration from all 12 leads were determined. The primary endpoint was spontaneous ventricular tachycardia/ventricular fibrillation (VT/VF) on follow-up.

Results: This study included 83 patients [93% male, median presenting age: 56 (41–66) years old, 45% type 1 pattern] with 12 developing the primary endpoint (median follow-up: 75 (Q1–Q3: 26–114 months). Cox regression showed that QRS frontal axis > 70.0 degrees, QRS horizontal axis > 57.5 degrees, R-wave amplitude (lead I) < 0.67 mV, R-wave duration (lead III) > 50.0 ms, S-wave amplitude (lead I) < –0.144 mV, S-wave duration (lead aVL) > 35.5 ms, QRS duration (lead V3) > 96.5 ms, QRS area in lead I < 0.75 Ashman units, ST slope (lead I) > 31.5 deg, T-wave area (lead V1) < –3.05 Ashman units and PR interval (lead V2) > 157 ms were significant predictors. A weighted score based on dichotomized values provided good predictive performance (hazard ratio: 1.59, 95% confidence interval: 1.27–2.00, *P*-value < 0.0001, area under the curve: 0.84).

Conclusions: Automated ECG analysis revealed novel risk markers in BrS. These markers should be validated in larger prospective studies.

Keywords: Brugada syndrome, automated ECG, risk stratification, depolarization, repolarization

INTRODUCTION

Brugada syndrome (BrS), originally described in 1992, is an electrical disease that is associated with higher risks of life-threatening ventricular tachycardia (VT)/ventricular fibrillation (VF) and sudden cardiac death (SCD). Symptoms (1, 2), ECG markers (3) and invasive tests such as electrophysiological studies (4–6) have been used for risk stratification, but prediction remains difficult (7), especially in asymptomatic patients (8). In prior studies, ECG markers have been determined manually, but these measurements are limited by inter-observer variability and have subjective bias. By contrast, automated measurements have not been used for risk prediction, yet they may reveal useful information that is difficult to extract manually (9, 10). In this study, we extracted raw ECG data files, exported the automated measurements and tested the hypothesis a score system based on these variables can predict spontaneous VT/VF in a cohort of BrS patients.

METHODS

Study Population

This retrospective study received Ethics approval from The Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee and is based on datasets that have already been made available in an online repository (<https://zenodo.org/record/3266172>; <https://zenodo.org/record/3266179>; <https://zenodo.org/record/3351892>). The diagnosis of BrS is made based on the 2017 ACC/AHA/HRS Guideline (11), after reviewing documented patient history, and confirmed by analysis of all documented ECG by S.L. and G.T. Type 1 Brugada pattern is defined as a coved-shape ST segment with elevation of >2 mm followed by a negative T-wave, and type 2 pattern is defined as convex ST segment with >0.5 mm elevation followed by variable T-wave, resulting in a saddleback-shaped morphology (12). The study inclusion criteria were: (1) BrS diagnosis and (2) raw ECG data were available for automated ECG analysis.

Baseline Characteristics and ECG Measurements

Clinical data was extracted from electronic health records. The following baseline clinical data were collected: (1) sex; (2) age of initial Brugada pattern presentation; (3) follow-up period; (4) type of Brugada pattern and presence of fever at initial presentation; (5) family history of BrS and VF/SCD; (6) manifestation of syncope and if present, the number of episodes; (7) manifestation of VT/VF and if present, the number of episodes; (8) sodium channel blocker challenge test and results; (9) concomitant presence of other arrhythmia; (10) implantation of ICD. Patients presented with two or more episodes of VT/VF were defined to be of high VT/VF burden. Automatically measured parameters from ECG related to the P, Q, R, S and T-wave were extracted. The full list of variables is shown in **Supplementary Table 1**.

Primary Outcome, Statistical Analysis, and Creation of a Score-Based System for Risk Prediction

The primary outcome was new occurrences of spontaneous VT/VF after diagnosis of BrS. The outcome was assessed by review of inpatient and outpatient case records. Cox regression was used to identify ECG variables that were significant predictors of the primary outcome. The following steps were undertaken to create a score system for risk stratification: (1) the variables related to Q, R, S and T waveforms that achieved P -values < 0.10 were identified, (2) related variables were discarded, (3) the location out of all 12 leads with the lowest P -values was selected, (4) optimum cut-off was calculated from receiver operating characteristic analysis, (5) each variable was dichotomized based on the cut-off, (6) calculation of beta coefficient and ORs for each dichotomized variable, (7) weight-adjusted score by proportion of beta coefficients and P -values.

RESULTS

A total of 83 patients were included [93% male, median presenting age: 56 (41–66) years old] were included. The clinical characteristics of this cohort are shown in **Table 1**. The prevalence of an initial type 1 Brugada pattern on presentation was 45%. Twelve patients developed spontaneous VT/VF with a median follow-up of 74 (Q1–Q3: 26–114) months. Automated measurements of the ECG variables were extracted from the raw data (**Figure 1**).

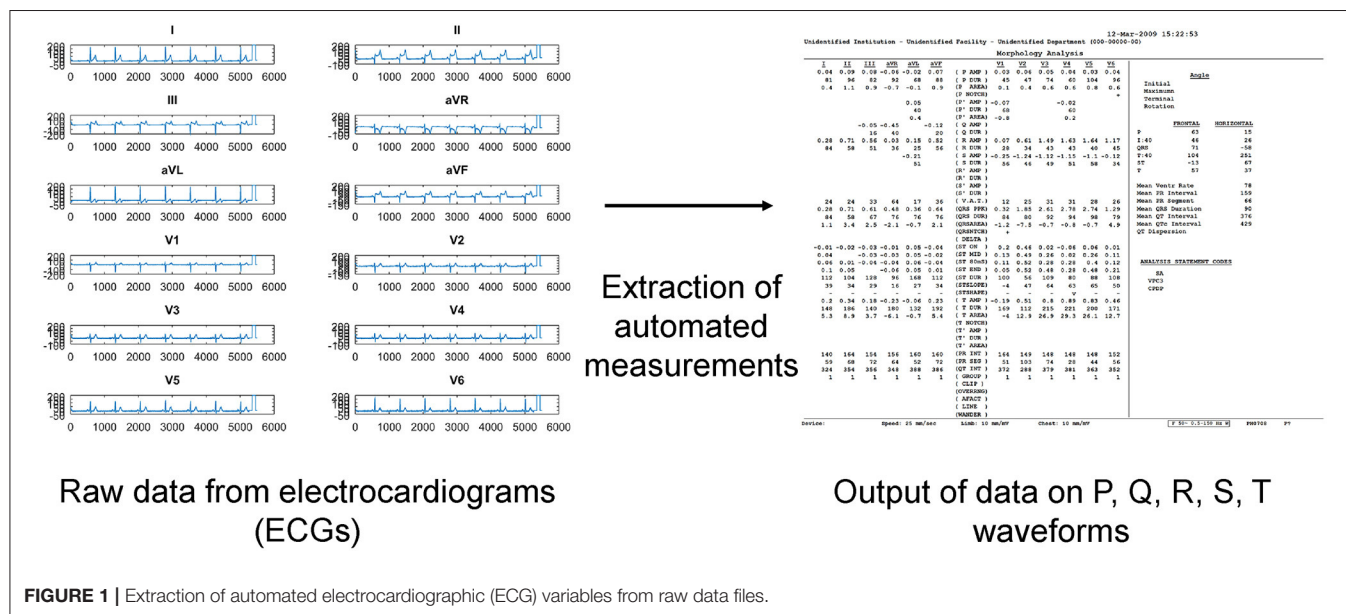
A weighted score system for risk stratification was created, as illustrated in **Figure 2**. Briefly, ECG variables related to Q, R, S and T waveforms, which achieved significance of P -values < 0.10 on Cox regression, were identified. Their median values (Q1–Q3) and hazard ratios (HR) with 95% confidence intervals (CIs) for classifying incident spontaneous VT/VF are shown in **Table 2**, whereas optimum cut-off values and area under the curve (AUC) from receiver operating characteristic (ROC) analysis are shown in **S2**. For each variable, the lead with the lowest P -value was selected. This selection process yielded 11 ECG variables: vector magnitude of the initial 40 ms of the transverse QRS signal, QRS horizontal axis, ST horizontal axis, R-wave amplitude in lead I, R-wave duration in lead III, S-wave amplitude in lead I, S-wave duration in lead aVL, QRS duration in lead V3, QRS area in lead aVL, ST slope in lead I, T-wave area in lead V1 and PR interval in lead V2. Vector magnitude of the initial 40 ms of the transverse QRS signal was not processed further as not all ECGs had this variable reported.

The remaining ECG variables were then dichotomized based on the optimum cut-off values from receiver operating characteristic (ROC) analysis. The dichotomized ECG variables were weighted based on the beta coefficients and P -values. After dichotomization, two variables lost significance for prediction (highlighted in red in **Supplementary Table 3**) and therefore the final score had a total of eight ECG and three clinical variables (**Supplementary Table 4**). A histogram plot for this *weighted score* is shown in **Supplementary Figure 1**. This weighted score provided good predictive performance when

TABLE 1 | Baseline characteristics of the Brugada patients ($n = 83$) included in this study.

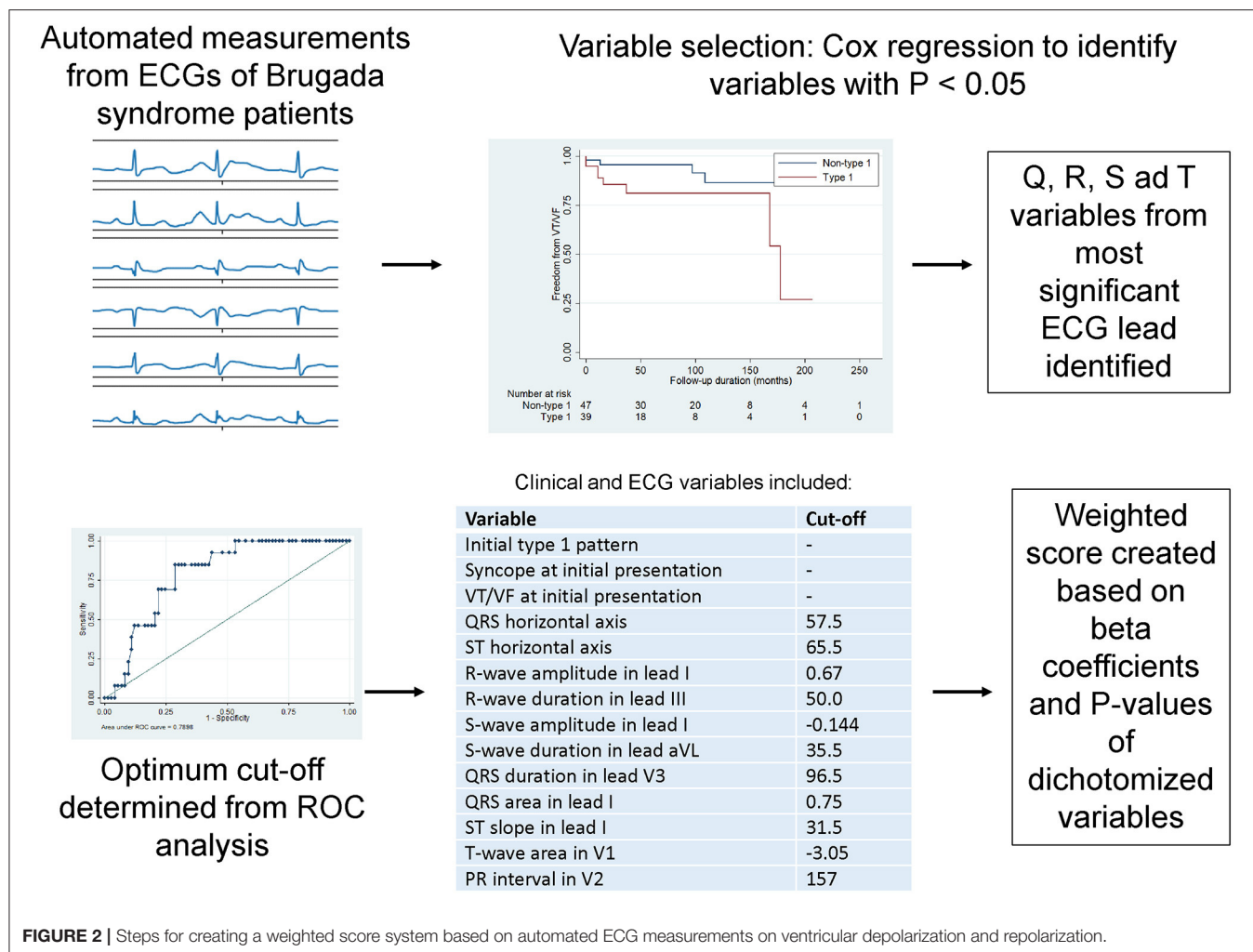
Characteristics	Count	Hazard ratio (HR) [#]	95% CI	P-value	Hazard ratio (HR) [^]	95% CI	P-value
Female gender	6 (7)	1.10	0.14–8.50	0.931			
Age of Initial Presentation	56 (41–66)	0.99	0.95–1.02	0.455	0.98	0.95–1.02	0.383
Initial Type 1 BrP	37 (45)	3.64	1.08–12.30	0.037	3.02	0.91–10.04	0.072
Type 1 BrP	52 (63)	1.96	0.53–7.25	0.313	1.94	0.53–7.18	0.319
Evolution	29 (35)	0.46	0.12–1.73	0.251	0.54	0.15–1.99	0.355
Fever-induced type 1	11 (13)	1.49	0.33–6.85	0.607	1.50	0.33–6.86	0.599
FH BrS	3 (4)	2.03	0.25–16.24	0.503	3.14	0.41–24.33	0.273
Family History of VF/SCD	6 (7)	1.02	0.13–7.92	0.985	1.10	0.14–0.50	0.929
Syncope at initial presentation	29 (35)	5.24	1.05–26.20	0.044	4.72	0.95–23.39	0.057
Syncope at any point	43 (52)	5.62	1.22–25.94	0.027	4.66	1.02–21.29	0.047
# syncope	65 (83)	–	–	–	–	–	–
VT/VF at initial presentation	9 (11)	7.14	2.23–22.85	0.001	7.80	2.47–24.58	<0.0001
VT/VF at any point	16 (19)	–	–	–	–	–	–
High VT/VF Burden	6 (7)	18.96	5.69–63.13	<0.0001	19.38	6.25–60.14	<0.0001
Drug Challenge Performed	51 (61)	0.90	0.27–3.00	0.858	1.03	0.31–3.42	0.966
Drug Positive*	49 (96)	0.18	0.02–1.60	0.125	0.26	0.03–2.08	0.202
ICD	29 (35)	–	–	–	–	–	–
Other Arrhythmia	12 (14)	0.45	0.06–3.48	0.440	0.50	0.07–3.90	0.512

Hazard ratios for predicting incident spontaneous VT/VF from Cox regression.

*Denominator only included patients undergoing testing. Variables with $P < 0.05$ are shown in bold text. [#]Breslow methods for ties. [^]Parametric model with Weibull distribution.

analyzed as a continuous variable [hazard ratio (HR): 1.59, 95% confidence interval (CI): 1.27–2.00, P -value < 0.0001 , area under the curve (AUC): 0.84; **Supplementary Figure 3**] or a dichotomized variable (HR: 14.88, 95% CI: 3.99–55.50, P -value

< 0.0001 , AUC: 0.81) (**Supplementary Table 5**). A simplified algorithm was generated using decision tree learning for potential clinical application (AUC: 0.93, **Supplementary Tables 6, 7; Supplementary Figure 3**).



DISCUSSION

The main findings of this study is that (i) automated measurements from raw ECG data can be extracted and used for risk stratification, (ii) ST slope was identified as a novel risk marker, and (iii) a weighted score system based on QRS frontal axis, R-wave duration (lead III), S-wave duration (lead I), QRS duration (lead I) and ST slope (lead I) predicted incident spontaneous VT/VF with an AUC of 0.95.

Previously, investigators have commented that manual measurements may be susceptible to variations and errors (13). Indeed, accuracy and reproducibility of measurements made manually have not been examined (14). Our study provides the proof-of-concept that the axis of the QRS vector, depolarization and repolarization variables extracted automatically from raw ECG data can predict arrhythmic events with good fidelity. In BrS, both depolarization and repolarization abnormalities are posited to play important roles in ventricular arrhythmogenesis (15, 16). ECG indices related depolarization (13), such as QRS duration, QRS dispersion, R-wave and S-wave durations have been identified as useful predictors in this condition (3, 17–21).

For Brugada syndrome, QRS vector magnitude was identified as a predictor of ventricular arrhythmias (22). In keeping with their findings, our study similarly demonstrated that the magnitude of initial 40 ms transverse QRS signal was borderline predictive of VT/VF (P -value = 0.051). However, the magnitude of the maximum transverse QRS vector or of its terminal portion were not significant predictors.

By contrast, repolarization abnormalities, as reflected by alterations in the ST segment, QT or T_{peak} - T_{end} intervals, are also important arrhythmogenic substrates in BrS (23–26). Our novelty is the demonstration that the slope of ST segment is significantly associated with arrhythmic risk. Whilst the angle between the R' wave and the vertical line has been used to distinguish Brugada pattern from other causes with similar morphology, such as right bundle branch block (27), we are not aware of any previous study demonstrating the use of R or ST angles for risk stratification. Moreover, T_{end} , and sometimes T_{peak} , can be difficult to determine with a degree of certainty with different methods of determining its location (28). Recently, an automated algorithm calculated a global T_{peak} based on the root mean square average of T_{peak} from individual leads

TABLE 2 | Significant ECG variables of Q, R, S, and T waves for predicting incident spontaneous VT/VF from univariate Cox regression.

Characteristics	Median (Q1–Q3)	Hazard ratio (HR) [#]	95% CI	P-value	Hazard ratio (HR) [^]	95% CI	P-value
Vector magnitude of the initial 40 ms transverse QRS signal (deg)	0.43 (0.30–0.70)	8.42	1.05–67.41	0.045	8.21	1.03–65.17	0.046
QRS horizontal axis (deg)	11 (–8 to 36)	1.01	1.001–1.012	0.024	1.01	1.001–1.012	0.030
ST wave horizontal axis (deg)	71 (53–83)	0.98	0.96–0.99	0.009	0.97	0.95–0.99	0.003
R-wave amplitude in lead I (mV)	0.50 (0.33–0.72)	0.06	0.004–0.86	0.038	0.12	0.01–1.35	0.086
R-wave duration in lead III (ms)	48 (28–60)	1.02	1.002–1.03	0.030	1.02	1.01–1.04	0.006
S-wave amplitude in lead I (mV)	–0.15 (–0.26 to –0.07)	0.006	0.0002–0.21	0.005	0.004	0.0001–0.13	0.002
S-wave duration in aVL (ms)	24 (0–48)	1.03	1.01–1.05	0.001	1.04	1.02–1.06	<0.0001
QRS duration in V3 (ms)	96 (88–104)	1.03	1.003–1.06	0.029	1.04	1.01–1.07	0.004
QRS area in lead I (ms.mV)	1.4 (–0.4 to 3.6)	0.67	0.54–0.84	0.001	0.69	0.56–0.84	<0.0001
ST slope in lead I (deg)	18 (9–33)	1.05	1.01–1.10	0.015	1.06	1.02–1.10	0.005
T-wave area in V1 (ms.mV)	–2.5 (–4.2 to –0.8)	0.82	0.73–0.93	0.002	0.80	0.70–0.90	<0.0001
PR interval in lead V2 (ms)	156 (144–176)	1.02	1.001–1.03	0.036	1.01	1.0002–1.03	0.046

Median (Q1–Q3) and hazard ratios (HRs) with 95% confidence intervals (CIs) are presented. For each variable, only the lead with the lowest P-value across all 12 leads was shown.

[#]Breslow methods for ties. [^]Parametric model with Weibull distribution.

with a similar methodology for determining T_{end} (29). Whether these measurements provide more accurate risk stratification than manual measurements in BrS and other disease cohorts remain to be tested. Other than outcome prediction, other investigators have used automated ECG variables for disease detection and tracking (30). Future studies should examine whether serial changes in ECG variables can improve disease detection especially in type 2 Brugada subjects and be used to track disease progression in BrS.

From our predictive analysis, we generated a simple algorithm based on decision tree learning method for potential clinical application, as we have done so previously for other cohorts (31, 32). For Brugada syndrome, other decision tree-type algorithms have been proposed (33–35). These algorithms should be compared for their ability to predict arrhythmic outcomes. Previously, other groups have developed useful clinical risk scores for risk stratification in BrS. For example, Subramanian et al. proposed a score based on four variables: the presence of spontaneous type 1 pattern, QRS fragmentation in the inferior leads, S-wave upslope duration ≥ 0.8 and $T_{\text{peak}}-T_{\text{end}}$ intervals ≥ 100 ms with an excellent AUC of 0.95 (36). As not all of the above variables were obtained from the automated ECG outputs in our study. Future studies should develop novel algorithms to automatically identify the presence or absence of QRS fragmentation and to determine $T_{\text{peak}}-T_{\text{end}}$ intervals to allow comparisons of between the different risk scores.

LIMITATIONS

Several limitations of our study should be noted. Firstly, the size of our cohort is relatively small. Our findings should be validated in larger prospective studies. Secondly, the majority of patients with detected VT/VVF events had ICDs implanted. Therefore, we cannot exclude ascertainment bias, where silent VT/VF events were missed in those without ICDs. Secondly, our extraction did not enable us to determine the $T_{\text{peak}}-T_{\text{end}}$ interval. Future work should focus on modifying existing algorithms to determine T_{peak} and T_{end} , which would allow us to determine to extent to which repolarization abnormalities contribute to the arrhythmic substrate in BrS. Thirdly, the ECG predictors identified in this study may not be exclusive for BrS and may also be useful for risk stratification in other cardiovascular diseases such as myocardial infarction. This remains to be elucidated in future studies.

CONCLUSIONS

Automated ECG measurements related to depolarization and repolarization are useful for risk stratification in BrS. These markers should be validated in larger prospective studies. If the predictability of automated measurements is verified, they have the potential to open the gate for the wide application of advanced machine learning models to facilitate risk stratification and clinical decision making in BrS and other diseases.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article are available in the datasets already made available in an online repository (<https://zenodo.org/record/3266172>; <https://zenodo.org/record/3266179>; <https://zenodo.org/record/3351892>).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

GT: study conception, study supervision, project planning, data interpretation, statistical analysis, manuscript drafting,

and critical revision of manuscript. SL, DC, GL, AL, and JZ: data analysis, data interpretation, statistical analysis, manuscript drafting, and critical revision of manuscript. TL and QZ: data analysis, data interpretation, statistical analysis, study supervision, and critical revision of manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcvm.2020.618254/full#supplementary-material>

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Arrhythmogenic Mechanisms in Hypokalaemia: Insights From Pre-clinical Models

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Potassium is the predominant intracellular cation, with its extracellular concentrations maintained between 3.5 and 5 mM. Among the different potassium disorders, hypokalaemia is a common clinical condition that increases the risk of life-threatening ventricular arrhythmias. This review aims to consolidate pre-clinical findings on the electrophysiological mechanisms underlying hypokalaemia-induced arrhythmogenicity. Both triggers and substrates are required for the induction and maintenance of ventricular arrhythmias. Triggered activity can arise from either early afterdepolarizations (EADs) or delayed afterdepolarizations (DADs). Action potential duration (APD) prolongation can predispose to EADs, whereas intracellular Ca^{2+} overload can cause both EADs and DADs. Substrates on the other hand can either be static or dynamic. Static substrates include action potential triangulation, non-uniform APD prolongation, abnormal transmural repolarization gradients, reduced conduction velocity (CV), shortened effective refractory period (ERP), reduced excitation wavelength ($\text{CV} \times \text{ERP}$) and increased critical intervals for re-excitation (APD-ERP). In contrast, dynamic substrates comprise increased amplitude of APD alternans, steeper APD restitution gradients, transient reversal of transmural repolarization gradients and impaired depolarization-repolarization coupling. The following review article will summarize the molecular mechanisms that generate these electrophysiological abnormalities and subsequent arrhythmogenesis.

Keywords: hypokalaemia, potassium, cardiac arrhythmia, conduction, repolarization

INTRODUCTION

Hypokalaemia is the most common electrolyte abnormality found in hospitalized patients (1) and therefore represents an important cause of arrhythmias and associated mortality observed in clinical practice (2). It is commonly observed in patients with pre-existing heart conditions (3–5). Hypokalaemia manifests, in order of decreasing likelihood, due to (i) increased K^+ loss, (ii) transcellular K^+ shift into cells or (iii) reduced dietary K^+ intake. Increased loss of K^+ mostly occurs secondary to the use of diuretics or laxatives, or from diarrhea. Transcellular shift of K^+ into cells can be caused by medications, such as β_2 receptor agonists (6), hormonal abnormalities, or metabolic alkalosis (7). Decreased intake can develop in conditions such as anorexia, dementia or reduced appetite from malignancy.

The following features are observed on the electrocardiogram (ECG) during hypokalaemia: ventricular premature complexes (VPCs), prolonged QT interval, ST segment depression and the appearance of a U wave (8). Extracellular potassium concentration ($[K^+]_o$) is negatively correlated with the development VPCs, with each unit decrease in $[K^+]_o$ (mM) corresponding to a 28% increased risk of VPCs (9, 10). A potentially life-threatening form of ventricular tachycardia (VT) termed *torsade de pointes* (TdP) also manifests in hypokalemia (11), which in turn can degenerate into ventricular fibrillation (VF) and sudden cardiac death (12). Other cardiac rhythm abnormalities induced by hypokalaemia include atrial fibrillation (13) and atrial flutter (14).

Animal models, particularly guinea pigs (15–21) and mice (22–24), have provided much insight into the detailed mechanisms underlying hypokalaemia-induced arrhythmogenicity. In these models, arrhythmic activity has been observed during regular pacing (Figure 1A), programmed electrical stimulation that delivers S1S2 pacing (increasing premature S2 stimuli delivered following trains of regular S1 stimuli) (Figure 1B) and dynamic pacing (trains of regular S1 stimuli of decreasing basic cycle length) (Figure 1C). The review article aims to consolidate pre-clinical findings on the electrophysiological mechanisms underlying hypokalaemia-induced arrhythmogenicity.

BASIC ELECTROPHYSIOLOGY: PRE-CLINICAL LESSONS FROM SMALL ANIMAL MODELS (MICE, RABBIT, AND GUINEA PIGS)

Whether serving as a disease model for pharmaceutical purposes or toxicology, the use of animal models as fundamental building blocks has enabled rapid advances in biomedical knowledge (27). This is no different in cardiology, with mice, rabbit and guinea pigs considered to be the most frequently used animal models in experimental cardiac electrophysiology (28). However, despite similarities in cardiac ion channel distribution, salient differences in electrophysiological results are still observed between small animal species, especially within the context of hypokalaemia.

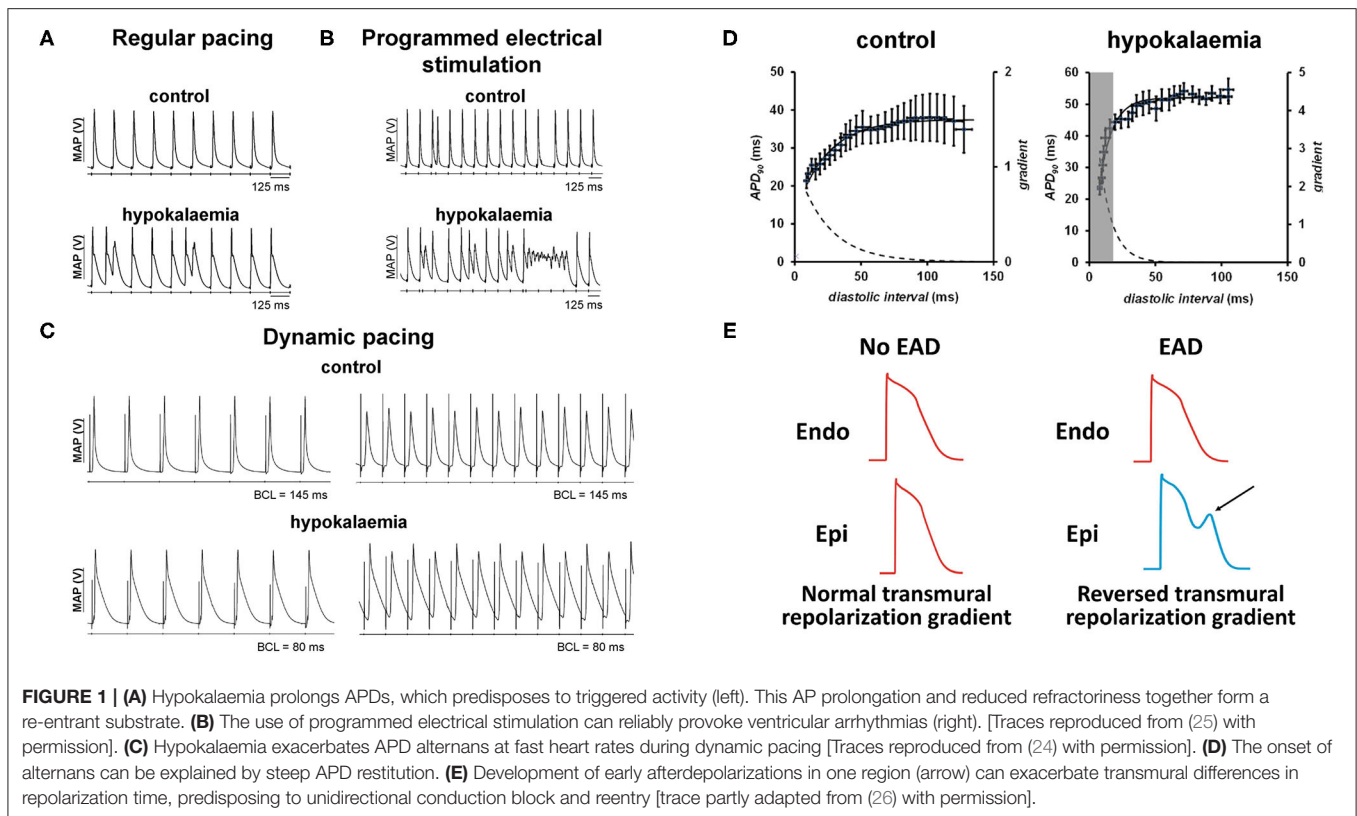
Triggered Activity Can Arise From Afterdepolarizations

At the cellular level, reduction in $[K^+]_o$ is expected to shorten the time course of repolarization by increasing the K^+ electrochemical gradient across the cell membrane. Recent experiments conducted in rabbit hearts showed that hypokalaemia activated the apamin-sensitive small-conductance calcium-activated potassium current (I_{KAS}) to shorten action potential durations (APD), thereby preserving repolarization reserve (29). However, prolonged APDs are observed because of I_{Kr} , I_{K1} , I_{Ks} , and I_{to} inhibition (30–34). These repolarization abnormalities explain the electrocardiographic QT interval prolongation observed in clinical practice (35). In a similar hypokalaemic *in vivo* rabbit model, prolonged exposure to reduced $[K^+]_o$ was also found to be significantly correlated with decreased HERG channel density due to its internalization and subsequent degradation, which may play a major role in APD prolongation (36). Recently, reduced Na^+/K^+ -ATPase currents have been identified as a contributory mechanism toward prolonged repolarization (37, 38). Normally, Na^+ and Ca^{2+} handling is closely coupled via the sodium-calcium exchanger (NCX), which uses the electrochemical gradients of both ions to exchange three Na^+ for 1 Ca^{2+} .

A change in the morphology of the action potential, such as in triangulation reflected by an increase in the APD_{90} - APD_{50} difference, is thought to increase the likelihood of inward current re-activation that in turn produces triggered activity over the terminal phases of action potential repolarization (16, 39). More severe reductions in $[K^+]_o$ can induce Ca^{2+} overload due to a combination of suppressed Na^+ - K^+ -ATPase activity, reversal of transport by the NCX, and reduced intracellular ATP concentrations (40, 41).

Afterdepolarizations refer to the oscillations in the membrane potential before the next action potential. They can occur early (early afterdepolarizations, EADs) or late (DADs, delayed afterdepolarizations). EADs can be subdivided into those that occur during phase 2 and phase 3. Hypokalaemia can generate both EAD types by distinct mechanisms. APD prolongation increases the susceptibility to phase 2 EADs because of a wider window over which the L-type Ca^{2+} channels can be re-activated (42, 43). Ca^{2+} overload can promote EADs during phase 3 of the action potential (and during phase 2 in some species), thereby activating the NCX to mediate Na^+ entry (44, 45). Recent experiments in rabbit hearts showed that when combined with increased beta-adrenergic drive, I_{KATP} can be activated, leading to heterogeneous APD shortening and the subsequent generation of late phase 3 EADs in the presence of enhanced Ca^{2+} . Intracellular Ca^{2+} accumulation can promote DADs. Isolated, perfused ventricular muscle in guinea pig (46) and rabbit (47) hearts have exhibited DADs in severe, experimental hypokalaemia.

Both EADs and DADs can lead to triggered activity (Figure 1A), thereby initiating arrhythmic activity and producing a sustained tachycardia upon encountering favorable reentrant substrates (48, 49). Such substrates can be revealed by programmed electrical stimulation (PES) (Figure 1B) or



dynamic pacing (**Figure 1C**). Dynamic pacing can unmask APD alternans at short basic cycle lengths (BCLs), which can be explained by steep restitution in hypokalaemia compared to control conditions (**Figure 1D**). EADs, DADs or triggered activity can themselves increase the spatial heterogeneity in repolarization as well as areas of slowed conduction. In other words, triggers of arrhythmias may themselves create the substrates for re-entry (50), as demonstrated recently in modeling studies (51). Normally, endocardial APD is longer than epicardial APD resulting in a normal repolarization gradient (**Figure 1E**, left). When an EAD (arrow) develops, epicardial APD will be longer than endocardial APD, causing a reversal in the transmural repolarization gradient (**Figure 1E**, right) that is potentially arrhythmogenic (16, 17, 20).

Reentry Is Due to Static and Dynamic Abnormalities in Repolarization, Refractoriness and Conduction

Numerous static and dynamic re-entrant substrates contribute to increased arrhythmogenicity in hypokalaemia (52).

Repolarization: Steep Spatial Gradients

The most important experimental finding consistently observed across the different species during hypokalaemia is non-uniform prolongation of repolarization, be it when comparing the left (LV) and right ventricle (RV), epicardium and endocardium, or apex and cardiac base (16, 53). Spatial differences in

repolarization are thought to increase the risk of unidirectional conduction block, a prerequisite for circus-type or spiral wave reentry (54). Such spatial variations in repolarization may be present during regular pacing and further exacerbated following triggered activity, thereby enhancing arrhythmic risk. In guinea pig hearts, greater APD₉₀ prolongations were seen in the RV epicardium relative to the LV epicardium (16, 53). These APD differences were attributed to differing expression patterns and levels of ion channels, in particular higher density of I_{K1} channels in the LV compared to in the RV (55, 56). The consequence of RV APD₉₀ prolongation during hypokalaemia is an increased RV-LV transepical APD₉₀ difference compared to control during both regular and S1S2 pacing (16), which partly underlies the capacity of VPCs to induce sustained VT (57, 58).

In addition to transepical repolarization gradients, transmural gradients may contribute to arrhythmogenesis in hypokalaemia. Experimental data obtained from mouse hearts have been conflicting, as pointed out previously (16). LV epicardial and LV endocardial APD₉₀ difference was found to be either unaltered (59) or reduced (23, 43). In guinea pig hearts, there was no demonstrable APD₉₀ difference between the epicardium and endocardium under either normokalaemic or hypokalaemic conditions. Moreover, transient alterations in transmural repolarization gradients have been explored in mouse hearts (60). It was shown that the S2 stimulus proportionally decreased epicardial and endocardial APD₉₀. After the following S3 stimulus, endocardial APD₉₀ decreased

more sharply than did epicardial APD₉₀, albeit the former recovered after S4 stimulation.

Repolarization: Steep APD Restitution Gradients and APD Alternans

The relationships between APD, the diastolic interval (DI) and basic cycle length are detailed in **Figure 2A**. The relationship $BCL = APD + DI$ can be shown graphically as a straight line with a gradient of -1 . The original descriptions of alternans were based on a graphical method that related them to restitution of APD (61). APD restitution is the APD abbreviation that occurs when heart rate is increased and reflects an adaptive response to maintain a period of diastole, allowing blood to refill in the cardiac chambers. In a normal APD restitution curve (**Figure 2B**), APD is plotted against the previous DI. This relationship can be represented by the equation $APD_{n+1} = f(DI_n)$, where f is the function relating the new APD to its previous DI. When DI shortens, APD also shortens to accommodate. The region for long DIs is almost flat, whereas the region at short DIs is steep.

The restitution gradient reflects the recovery of the different ion channels that are activated during action potential generation. Na⁺ channels show the fastest inactivation kinetics and recover quickly, and their effects on restitution are observed mostly at the shortest DIs. The Ca²⁺ channels recover at a slower rate compared to Na⁺ channels, and their effects are observed at longer DIs. Because these channels mediate much of the transmembrane currents during the action potential plateau, they affect APD restitution greatly. K⁺ channels have the slowest recovery rates compared to Na⁺ and Ca²⁺ channels and their effects are therefore mostly observed at long DIs. An important property of K⁺ channels is their reverse use dependence, in which increasing use leads to a lower level of channel blockade (62). As hypokalaemia inhibits K⁺ channels, its effects are most prominent at long DIs, which may occur during a compensatory pause following an ectopic beat, and bradycardia. In hypokalaemia, due to the APD prolongation, the DIs can engage the steeper portion of the restitution curve even when heart rate is normal.

Cobweb plots can be used to illustrate the stability of beat-to-beat alternations in APD (**Figures 2C,D**). In the original formulation, it is assumed that the DI depends on the preceding APD. The line of the equation, $DI = BCL - APD$, represents the feedback mechanism, where DI is inversely related to APD. If APD is longer, then the next DI is shorter. The APD equilibrium point at each BCL is located at the intersection between this line and the restitution curve. A sudden increase in heart rate, as reflected by a decrease in BCL, leads to shortening of APD. Under normal conditions, the restitution gradient is <1 . With a perturbation leading to a small decrease in DI, the next APD decreases. For the next beat, the DI increases, but to a value smaller than the original DI. Each iteration leads to a smaller beat-to-beat difference in APD and DI, until eventually a stable point is reached (**Figure 2C**). In hypokalemia, the restitution gradient is steeper at the same range of DIs (63). Each iteration leads to a successive increase in the beat-to-beat variation in APD, leading to 2:1 block (**Figure 2D**). A special case occurs if the

restitution gradient is exactly 1, in this case, alternans do not converge or diverge, and become stable.

The appearance of APD alternans has been associated with steeper APD restitution. However, it should be stressed that restitution is not the only factor that determines the presence or absence of alternans. Thus, other factors such as electronic and memory effects can suppress APD alternans even when the APD restitution gradient is >1 (64). Moreover, normally APD is closely coupled to the effective refractory period (ERP). Yet, APD is prolonged but ERP is shortened in hypokalaemia. Thus, APD restitution may not accurately predict the onset of alternans in this situation and VERP restitution may be a better indicator (18). Conversely, APD alternans can occur when the APD restitution gradient is <1 when restitution-dependent mechanisms are present (65). However, these effects have not been studied in detail for hypokalaemia. Finally, the relationships between repolarization dynamics, membrane excitability and cardiac memory are complex and warrants further study (66–71).

Electrical restitution can generally be assessed by two stimulation protocols: dynamic pacing and S1S2 pacing measure the steady-state response and the intermediate response, respectively, of the myocardium to a change in the basic cycle length (BCL). S1S2 pacing has the advantage of safety because pacing at a high heart rate is not required (19, 72, 73), albeit this method cannot assess beat-to-beat variations, that is, alternans, in action potential properties. In contrast, dynamic pacing can induce myocardial ischaemia (74, 75), but can be used experimentally to quantify alternans. In mice, greater amplitudes of epicardial APD₉₀ alternans associated with increased maximum APD₉₀ restitution gradients were observed during dynamic pacing in hypokalaemia compared to in normokalaemia (**Figure 1D**) (63). Endocardial APD₉₀, maximum APD₉₀ restitution gradients and DI_{crit} were not altered (23, 63). However, guinea pig hearts showed significant differences, such as increased endocardial APD₉₀ restitution gradients (18) and APD₉₀ alternans despite shallower APD₉₀ restitution gradients (18). Recent experiments in mouse hearts have further separated the roles of abnormal electrical restitution from other electrophysiological substrates in hypokalaemia (24). Moreover, these data provide the proof-of-concept that restitution can be assessed by both dynamic and S1S2 pacing procedures with largely agreeable restitution parameters.

Reduced Refractoriness and Steep ERP Restitution

The refractoriness of the myocardium, which can be measured experimentally as the effective refractory period (ERP), is an important determinant of the likelihood of reentry for the following reasons. Firstly, a decrease in the excitation wavelength, λ [conduction velocity (CV) \times ERP] increases the number of reentry circuits available within the myocardium (**Figure 2E**) (76). Secondly, an increase in the critical interval given by APD–ERP would prolong the time window during which re-excitation can take place, potentially by reactivation of inward Na⁺ and Ca²⁺ currents (59). Furthermore, reduced ERP can decrease the core size around which a spiral wave can meander (77). Shortening of ERP is observed during hypokalaemia despite concomitant APD prolongation. Studies in mouse and

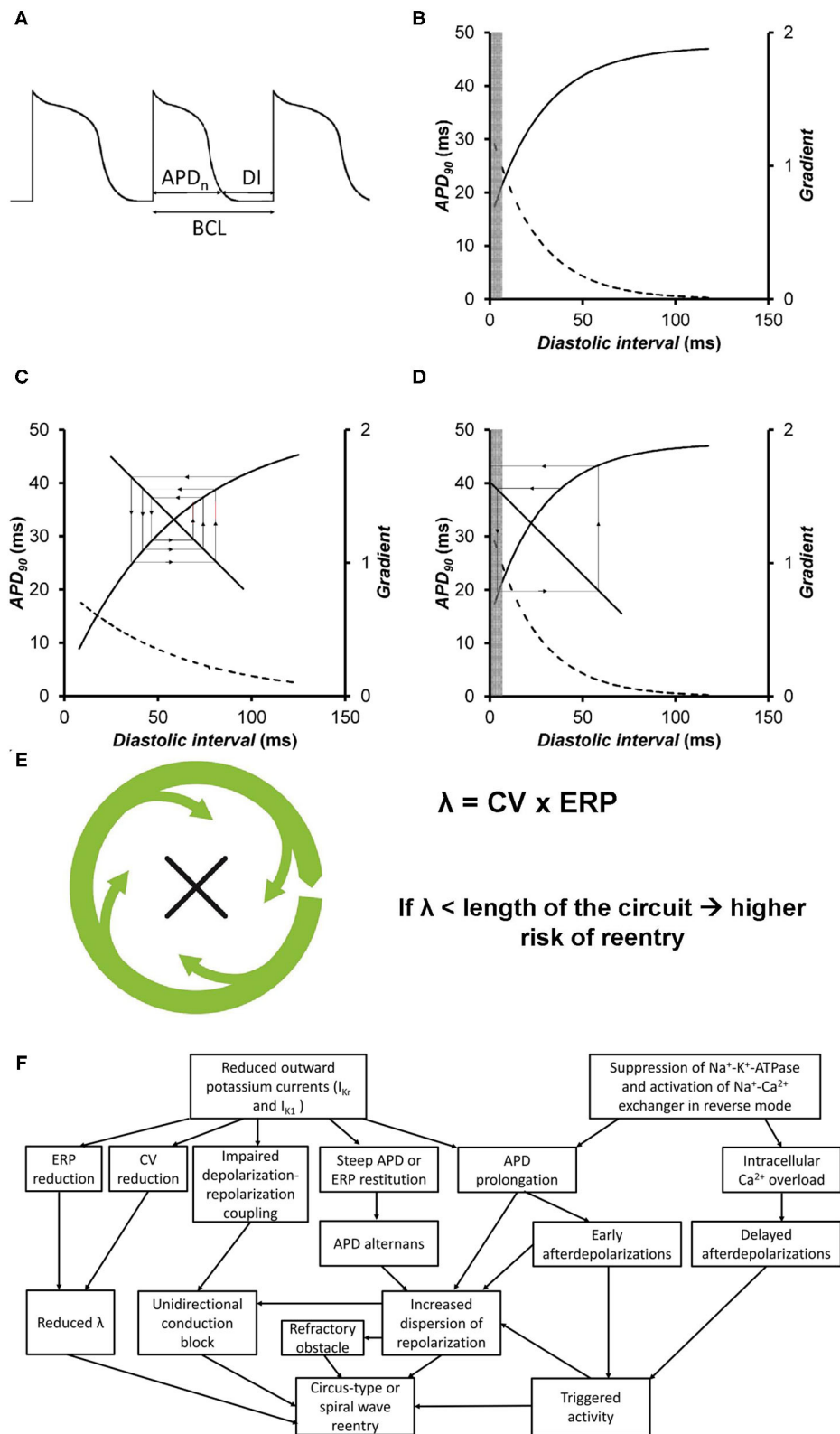


FIGURE 2 | (A) Voltage trace showing the relationships between action potential duration (APD), diastolic interval (DI), and basic cycle length (BCL). **(B)** An APD restitution curve describes the relationship between the APD and the previous diastolic interval (solid line). The gradients of the curve are represented by the broken line. (Continued)

FIGURE 2 | line. The values of DIs at which such gradients are >1 are represented by the gray box. **(C)** APD restitution curve plotting APD against the previous DI (solid line) along with their gradients (broken line). The values of DIs with gradients >1 are represented by the gray box. The cobweb plot shows that when the APD restitution gradient is <1 , a stable equilibrium point is produced on successive beats. **(D)** APD restitution curve plotting APD against the previous DI (solid line) along with their gradients (broken line). The values of DIs with gradients >1 are represented by the gray box. The cobweb plot shows that when the APD restitution gradient is >1 , an unstable equilibrium point is produced on successive beats, eventually leading to conduction block. Reproduced from (52) with permission. **(E)** Circus-type reentry depends on the wavelength of excitation, given by the product of conduction velocity and effective refractory period [Figures adapted from (26) with permission]. **(F)** Summary of different electrophysiological mechanisms that are responsible for triggered activity and reentry in hypokalaemia.

guinea pig hearts showed that LV epicardial and endocardial ERPs were decreased by similar extents (16, 23). Though debatable, this ERP shortening was found to be associated with excessive hyperpolarization of the resting membrane potential in ventricular cardiomyocytes. This subsequently results in increased activation of fast Na^+ channels, leading to a more pronounced action potential amplitude and an increased upstroke velocity during the depolarization phase (17). Under normokalaemic conditions, the critical opening for LV re-excitation is narrow, rendering the induction of re-excitation highly unlikely. Therefore, it is no surprise that prolongation of the critical interval from reduced $[\text{K}^+]_o$ is associated with an increased likelihood of sustained triggered activity over terminal repolarization (16). Recent experiments in guinea pigs demonstrate a contributory role of steep ERP restitution in predisposing the tissues to the generation of alternans and reentry (18).

Conduction Slowing

Conduction velocity (CV) is governed by Na^+ channels and gap junctions (78). Hypokalaemia is known to decrease CV in the atria, atrioventricular node, Purkinje fibers and the ventricles (16, 79). The underlying mechanism is thought to involve depressed membrane excitability from membrane depolarization, increased threshold potential for Na^+ channel activation and increased membrane resistance (80, 81). Enhanced stimulation threshold, decreased LV to RV transepical and LV epicardial to endocardial transmural CVs were all observed in guinea pigs during both regular and S1S2 pacing (16). In contrast, local epicardial and endocardial CV as well as transmural CV were not altered in hypokalaemic mouse hearts (59).

Impaired Activation-Repolarization Coupling and Other Arrhythmogenic Factors

Activation-repolarization coupling is an intrinsic property of the myocardium, allowing local APD values to be adjusted to conduction slowing at different myocardial sites along the path of the propagating action potential (21). This effect has been attributed to modulation of APD in neighboring cardiomyocytes by gap junction conduction, which would reduce regional differences in APD (82). Normally, the APD difference between the RV and LV is minimized by delayed LV activation, an effect that is impaired by hypokalaemia (21).

It is worth noting that arrhythmogenicity is stimulation site-dependent. Experiments in guinea pig hearts showed that ventricular arrhythmias were readily inducible upon LV stimulation, whereas RV stimulation failed to induce arrhythmic

events (15). This observation can be attributed to interventricular differences in ion channel expression. Thus, larger I_{K1} is found in the LV compared to in the RV, which would be expected to shorten APDs and therefore ERPs to greater extents in the LV. A steep repolarization gradient between the epicardium and endocardium, and between the LV and RV, can lead to a block of an action potential, favoring reentry. All of the above electrophysiological mechanisms underlying arrhythmogenesis in hypokalaemia are summarized in **Figure 2F**.

BASIC ELECTROPHYSIOLOGY: LARGER ANIMAL MODELS—CANINE, CAT AND SHEEP

It is important to note the fundamental relationship and differences between body weight and various cardiovascular parameters across all types of laboratory animals. An equation encapsulating this concept was coined in 1979 as heart weight ($\text{HW (g)} = 6.0 \times \text{BM}^{0.98}$) and P-R interval ($\text{PR (ms)} = 53 \times \text{BM}^{0.24}$) where BM is body mass in kg (83). Such differences are reinforced in electrophysiology, where small rodents are found with significantly shorter APD than humans due to lack of a prominent plateau phase found in cardiomyocytes (84–86). Therefore, the rabbit myocardium presents a more representative model of the human heart. Despite this similarity, important inter-species variations remain especially when K^+ handling is examined. Cardiac K^+ channel expression is significantly different between rabbits, guinea pigs and humans, accounting for the increased susceptibility to ventricular fibrillation in rabbit hearts, as well as the reduced transient outward current and large slow component of the delayed rectifier current in guinea pigs (87).

Furthermore, it is imperative to consider the potential usage of other relevant cardiovascular animal models. Similar to rabbit models, canine heart models show similar cardiac ion channel distribution with human hearts, making them suitable for the study of ion-channel-related mechanisms (e.g., repolarization and depolarization mechanics) and arrhythmic drug effects. Moreover, canine heart models have a much more comparable APD, sino-atrial node activity, Purkinje fiber distribution and activation sequence to humans (88–90). In contrast, goat and horse models have also shown to be suitable for the study of atrial fibrillation given the ease of obtaining ECG recordings (28, 91, 92). Regardless, mainly canine, cat and sheep models have been used to investigate electrophysiological changes in hypokalaemia.

Canine and sheep models were similar to smaller animal models with regards to an observed reduction in conduction

velocity during hypokalaemia across the cardiac conduction system (atria, atrioventricular node, Purkinje fibers and the ventricles) (93, 94). The underlying mechanism was thought to involve depressed membrane excitability from membrane depolarization, increased threshold potential for Na^+ channel activation and increased membrane resistance (80). However, further experiments have shown differing effects of hypokalaemia on epicardial vs. endocardial APD parameters (95) as well as regional differences in repolarization in canine hearts, due to greater I_{Ks} and I_{to} in RV compared to in the LV (96, 97). This shows that both the interlayer restitution gradient and transepical APD difference constitute viable pathways for arrhythmogenesis.

DIFFERENTIAL EFFECTS OF HYPOKALAEMIA ON DISTINCT CELL TYPES

Arrhythmogenic mechanisms in atrial and ventricular cell types can differ. For example, EADs in ventricular cardiomyocytes and tubulated atrial cardiomyocytes are attributed to Ca^{2+} overload (98). However, phase 3 EADs in untubulated atrial cardiomyocytes are instead linked to the reactivation of non-equilibrium Na^+ current and are driven by membrane hyperpolarization and short action potential configurations (98). Furthermore, hypokalaemia induces Ca^{2+} overload in ventricular cardiomyocytes by reduced pumping rate of the Na^+ - K^+ -ATPase leading to subsequent Na^+ accumulation (37). Moreover, structurally and functionally different small conductance Ca^{2+} -activated K^+ -channel (KCa2) inhibitors, ICA, AP14145, and AP30663, exerted anti-arrhythmic effects in hypokalaemic guinea pig hearts (99). In contrast, KCa2 blockade was found to be pro-arrhythmic in rabbit hearts (29), the reasons for which may be attributed to species differences or variations in the pharmacological agents used (ICA, AP14145, and AP30663 vs. apamin) (99). Both AP14145 and AP30663 can inhibit the late Na^+ current at higher concentrations (100). Indeed, the increase in intracellular Ca^{2+} can activate Ca^{2+} -calmodulin-dependent kinase to increase the activity of the late Na^+ channel (38). Hypokalaemia can also cause conduction abnormalities in the cardiac conduction system, although not to the same extent as hyperkalaemia. Thus, it can cause slowed conduction of action potentials through the atrioventricular node in canine (94, 101) and rabbit hearts (81), an abnormality that has also been reported in humans (102).

BRIDGING OVER FROM BASIC TO CLINICAL ELECTROPHYSIOLOGY

Human cardiac models tend to have differences in repolarization reserve when compared to animal models, depending on cardiac miRNA levels for ion channel subunit production (103). Utilizing human induced pluripotent stem cell-derived engineered heart tissue can overcome this human-to-animal model gap to better simulate physiological outcomes in humans (104). While there is a limited understanding specifically on the implications of

steep AP restitution gradients within the context of human hypokalaemia, the heterogeneity of APD restitution slopes have been proposed as a substrate for arrhythmogenesis in a whole-heart modeling study (105). This phenomenon was subsequently confirmed by the introduction of the Regional Restitution Instability Index (R2I2) by Nicholson and colleagues (106, 107).

HYPOKALAEMIA IN THE CLINICAL CONTEXT

The importance of understanding the underlying mechanisms during hypokalaemia resides in its relationship with the development cardiac arrhythmias in various clinical conditions. Hypokalaemia is associated with increased risks of atrial fibrillation amongst hospitalized patients (108). Moreover, hypokalaemia is common in patients presenting with VT/VF, and those with severe hypokalaemia have found to be associated with preceding gastrointestinal illness, higher doses of diuretics (109), use of drugs such as anti-depressants (110), as well as post-operative settings (111). In patients with implantable cardioverter-defibrillators (ICDs), hypokalaemia but not hyperkalaemia has been linked with increasing risk of recurrent ventricular tachyarrhythmias and appropriate ICD therapies (112). However, it should be stressed that the relationship between hypokalaemia and adverse outcomes is complex, in that it may or may not be an independent predictor of mortality (113) and that its correction may not lead to better outcomes in hospitalized patients (114). Moreover, altered repolarization correlates with prolonged QTc and $T_{\text{peak}}-T_{\text{end}}$ intervals in pre-clinical experimental studies (99). Both ECG indices have been reported to provide predictive value for arrhythmic risk stratification in the clinical context of acquired long QT syndrome for humans (115). Indeed, in a Chinese cohort of patients with acquired long QT syndrome, random survival forest analysis identified hypokalaemia as the second most important variable after cancer for predicting all-cause mortality (116).

In heart failure, the use of diuretics and activation of the renin-angiotensin system are the predominant causes of hypokalaemia (117). Ventricular arrhythmias, particularly non-sustained VT, are common (118, 119), involving both triggered and re-entrant arrhythmias have been described (120–122). A recent meta-analysis suggested a strong inverse association between serum K^+ channel concentration and ventricular arrhythmias in patients with myocardial infarction (123). In a large animal model of chronic post-myocardial infarction fibrosis, hypokalaemia revealed vulnerable electrophysiological substrates, which highlighted the importance of conduction slowing over repolarization instability in its arrhythmogenesis (124). Thus, clinical decision-making should take into consideration hypokalaemia as a common side effect of diuretics in patients with prior myocardial infarction (125, 126). In emergency settings, serum K^+ concentrations on admission alone or together with the co-existing Thrombolysis in Myocardial Infarction (TIMI) risk score was shown to predict more accurately short- and long-term risk of malignant ventricular

arrhythmias respectively (127, 128). Moreover, hypokalaemia is not only a risk factor for VT/VF in the acute phase of ST-segment-elevation myocardial infarction (STEMI), but is also associated with VF before primary percutaneous coronary intervention (129). Finally, hypokalaemia exerts pro-arrhythmic effects in congenital long QT syndrome, such as in the context of salt-wasting nephropathy (130). In otherwise silent mutational carriers, it can reveal a long QT phenotype (131, 132). In such patients, K⁺ supplement can protect congenital LQTS patients or silent carriers against the development of VT/VF (133, 134).

CONCLUSION

This article reviewed the electrophysiological mechanisms of triggered and re-entrant arrhythmogenesis in hypokalaemia, in which the data were largely derived from pre-clinical animal models. Prolonged repolarization can cause EADs, and Ca²⁺ handling can lead to the development of both EADS and DADs, leading to triggered activity. Reduced conduction velocity,

prolonged repolarization, increased dispersion of repolarization, reduced refractoriness, steep APD restitution gradients, transient reversal of transmural repolarization gradients and impaired depolarization-repolarization coupling, all collectively contribute to reentrant arrhythmogenesis.

AUTHOR CONTRIBUTIONS

GT and WW: drafting of manuscript, revision of manuscript, preparation of figures, and data interpretation. All other authors: drafting of manuscript, revision of manuscript, and data interpretation.

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Territory-Wide Chinese Cohort of Long QT Syndrome: Random Survival Forest and Cox Analyses

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Introduction: Congenital long QT syndrome (LQTS) is a cardiac ion channelopathy that predisposes affected individuals to spontaneous ventricular tachycardia/fibrillation (VT/VF) and sudden cardiac death (SCD). The main aims of the study were to: (1) provide a description of the local epidemiology of LQTS, (2) identify significant risk factors of ventricular arrhythmias in this cohort, and (3) compare the performance of traditional Cox regression with that of random survival forests.

Methods: This was a territory-wide retrospective cohort study of patients diagnosed with congenital LQTS between 1997 and 2019. The primary outcome was spontaneous VT/VF.

Results: This study included 121 patients [median age of initial presentation: 20 (interquartile range: 8–44) years, 62% female] with a median follow-up of 88 (51–143) months. Genetic analysis identified novel mutations in KCNQ1, KCNH2, SCN5A, ANK2, CACNA1C, CAV3, and AKAP9. During follow-up, 23 patients developed VT/VF. Univariate Cox regression analysis revealed that age [hazard ratio (HR): 1.02 (1.01–1.04), $P = 0.007$; optimum cut-off: 19 years], presentation with syncope [HR: 3.86 (1.43–10.42), $P = 0.008$] or VT/VF [HR: 3.68 (1.62–8.37), $P = 0.002$] and the presence of PVCs [HR: 2.89 (1.22–6.83), $P = 0.015$] were significant predictors of spontaneous VT/VF. Only initial presentation with syncope remained significant after multivariate adjustment [HR: 3.58 (1.32–9.71), $P = 0.011$]. Random survival forest (RSF) model provided significant improvement in prediction performance over Cox regression (precision: 0.80 vs. 0.69; recall: 0.79 vs. 0.68; AUC: 0.77 vs. 0.68; c-statistic: 0.79 vs. 0.67). Decision rules were generated by RSF model to predict VT/VF post-diagnosis.

Conclusions: Effective risk stratification in congenital LQTS can be achieved by clinical history, electrocardiographic indices, and different investigation results, irrespective of underlying genetic defects. A machine learning approach using RSF can improve risk prediction over traditional Cox regression models.

Keywords: long QT syndrome, risk stratification, genetic variants, machine learning, random survival forest

INTRODUCTION

Long QT syndrome (LQTS) is characterized by an abnormally long QT interval on the electrocardiogram, which predisposes affected individuals to life-threatening ventricular tachycardia/fibrillation (VT/VF) and sudden cardiac death (SCD). They can result from a decrease in repolarizing currents or an increase in depolarizing currents at the cellular level and can have either congenital or acquired causes. Today, more than 16 genetic subtypes of congenital LQTS have been described. However, the overall aggregate risk of arrhythmogenesis depends on not only the genotypes but also on interacting clinical risk factors, leading to difficulty in accurate risk stratification.

The clinical and genetic epidemiology of congenital LQTS has been described in detail in Western populations. For example, differences in electrocardiographic variables have been observed between LQTS types 1, 2, and 3 (1). Bradycardia is a common feature regardless of subtype (1) and early-onset atrial fibrillation may be present (2). In Asia, several large-scale studies have been conducted in Japan. It was recently reported that pathogenic variants affecting the pore-forming regions of the ion channels led to more arrhythmic phenotypes within a particular LQTS subtype, and that gender-specific differences are seen in LQTS types 1 and 2, but not type 3 (3). However, the epidemiological and genetic data in Chinese patients are much less well-defined. A single-center study of 58 Chinese pediatric patients with congenital LQTS described the clinical course, confirming the presence of other arrhythmias such as sinus node dysfunction, atrioventricular block and atrial tachy-arrhythmias in addition to VT/VF (4). It also reported that LQTS type 3 was the most common, followed by Jervell and Lange-Nielsen syndrome type 1, LQTS types 1, 8, 2, and 4. The main aims of this territory-wide study from Hong Kong are (1) to provide a description of the local epidemiology of LQTS, (2) to identify significant risk factors of ventricular arrhythmias in this cohort, and (3) to compare the performance of traditional Cox regression with that of random survival forests. In doing so, we describe several novel genetic mutations that have not been previously identified in cohorts from other geographical regions.

METHODS

Study Population

This retrospective study was approved by The Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee (study approval number: 2019.338). The relevant datasets have been made available in an online repository. The inclusion criteria were patients diagnosed

with congenital LQTS between 1997 and 2019 identified from searching the electronic health records from the Hospital Authority of Hong Kong. This system was previously used by our team to study other ion channelopathies such as Brugada syndrome (5, 6). Congenital LQTS was diagnosed if any of the following criteria were met: (i) Schwartz LQTS score ≥ 3.5 , (ii) an unequivocally pathogenic mutation in one of the LQTS genes, (iii) corrected QT interval of ≥ 500 ms on repeated 12-lead ECG in the absence of a secondary cause for QT prolongation, in accordance with the 2013 Heart Rhythm Society Expert Consensus Statement (7). Those with unclassified variants were also included in the present analysis if there is a high clinical suspicion of LQTS or if prior clinical or functional studies have reported an arrhythmogenic phenotype.

Extraction of Clinical and Electrocardiographic Data

Clinical data of included patients were extracted from their electronic health records. The following baseline clinical data were collected: (1) sex; (2) presentation age; (3) follow-up period defined as the time between presenting date and the date of last follow-up or death, whichever was earlier; (4) family history of LQTS and VT/VF/SCD; (5) initial presentation with syncope or spontaneous VT/VF; (6) development of syncope or VT/VF on follow-up and the number of episodes, if any; (7) electrophysiological study (EPS), 24-h Holter study, genetic testing and results; (8) performance of treadmill test and their effects on QTc prolongation on recovery, if present; (9) concomitant presence of other cardiac arrhythmias; (10) implantable-converter defibrillator (ICD) insertion; and (11) dosage regimen on the prescription of beta-adrenergic blockers and mexiletine.

Automatically measured parameters from baseline ECGs were extracted, including (1) heart rate; (2) P-wave duration; (3) PR interval; (4) QRS duration; (5) QT and QTc interval; (6) P-wave, QRS and T-wave axis; (7) S-wave amplitude in lead V1 and (8) R-wave amplitude in lead V5.

Statistical and Survival Analyses

All statistical analysis was performed using Stata MP (Version 13.0). Categorical variables were expressed as total number (percentages). Continuous variables were expressed as mean \pm standard deviation. The primary outcome of this study was spontaneous VT/VF. The above clinical and ECG variables were analyzed as risk factors for survival analysis. Cox regression with Efron's method for ties was used to identify independent predictors for shorter time to the first post-diagnosis VT/VF event. Variables achieving P -value < 0.10 were entered into

TABLE 1 | Baseline clinical characteristics and electrocardiographic variables of the included subjects.

Variable	Overall (n = 121)	VT/VF on follow-up (n = 25)	No VT/VF on follow-up (n = 96)	P-value
Female gender	76 (62%)	17 (68%)	59 (61%)	0.221
Age of Initial Presentation	20 (8–44)	42 (21–53)	17 (8–37)	0.003
Follow-up Period (months)	88 (51–143)	131 (38–163)	85 (51–133)	0.332
Family history of LQTS	47 (39%)	7 (28%)	40 (42%)	0.358
Family history of sudden cardiac death	18 (15%)	2 (8%)	16 (17%)	0.355
Initial presentation with syncope	63 (52%)	18 (72%)	45 (47%)	0.005
Syncope	69 (57%)	19 (76%)	50 (52%)	0.006
Stress syncope	12 (10%)	2 (8%)	10 (10%)	0.828
Palpitations	22 (18%)	6 (24%)	16 (17%)	0.275
Premature ventricular complexes	21 (17%)	8 (32%)	13 (14%)	0.014
Initial presentation with VT/VF	31 (26%)	12 (48%)	19 (20%)	0.001
Other arrhythmias [#]	28 (23%)	6 (24%)	22 (23%)	0.710
EPS performed	6 (5%)	3 (12%)	3 (3%)	0.047
EPS positive	4 (67%)	1 (33%)	3 (100%)	0.083
Schwartz score	4 (4–5)	5 (4–5)	4 (4–5)	0.010
Heart rate	71 (60–92)	69 (59–80)	76 (61–93)	0.300
P-wave duration	102 (93–111)	111 (102–135)	102 (91–110)	0.218
PR interval	158 (146–175)	167 (156–184)	156 (138–172)	0.029
QRS duration	90 (84–104)	98 (86–116)	89 (83–100)	0.141
QT interval	447 (402–490)	448 (428–510)	440 (396–482)	0.121
QTc interval	489 (460–516)	493 (467–522)	486 (456–508)	0.380
P-wave axis	61 (37–73)	67 (51–78)	57 (37–72)	0.123
QRS axis	68 (30–83)	60 (20–82)	69 (44–85)	0.360
T-wave axis	53 (23–75)	38 (11–136)	55 (26–73)	0.769
R-wave amplitude in V5	1.07 (0.79–1.52)	1.04 (0.49–1.58)	1.07 (0.82–1.52)	0.739
S-wave amplitude in V1	0.60 (0.38–0.93)	0.60 (0.25–1.54)	0.60 (0.41–0.84)	0.993

[#] Other arrhythmias include any brady- or tachy-arrhythmias of non-ventricular origin, sinus node dysfunction and atrio-ventricular block. P-values less than 0.05 are shown in bold text.

multivariate analysis. Duration from the date of initial LQTS presentation to the first post-diagnosis VT/VF event for patient subgroups was compared qualitatively by Kaplan-Meier survival curve and intergroup differences were compared using the log-rank test.

Random Survival Forest (RSF) analysis was used to examine the relative importance of different risk predictors. In RSF, statistical methods are used to estimate the hazard function under the framework of a random forest (8) without making any assumptions about the individual hazard function (9), and ranks the significance of predictors for spontaneous VT/VF. Features and samples are randomly selected for a tree, and log-rank splitting is used to grow the trees. At the end of each branch, a cumulative hazard function is calculated for the selected individual tree. Finally, the ensembled estimated cumulative hazard function is computed by averaging the results of all the trees.

The `rfsrc()` function of `rfsrc` package and `rpart()` function of `rpart` package in RStudio (Version 1.1.456) was used to fit a RSF model. Sensitivity analysis on the number of trees and out-of-bag (OOB) prediction performance of the RSF model were then assessed. Survival estimates were calculated using the Brier score (0 = perfect, 1 = poor, and 0.25 = guessing) based

on the inverse probability of censoring weight (IPCW) method (10). The cohort was stratified into four groups based on 0–25, 25–50, 50–75, and 75–100 percentile values of incident VT/VF (Figure 4).

RESULTS

Baseline Characteristics, Genetic Testing, and Pharmacotherapy

This study included 121 consecutive congenital LQTS patients [median age of initial presentation: 20 (interquartile range: 8–44) years, 62% female] with a median follow-up of 88 (51–143) months. The baseline characteristics of the cohort are shown in Table 1. The spontaneous VT/VF incidence rate per 1,000 person-year is 26.2. Family history of LQTS and SCD was present in 39 and 15% of the cohort, respectively. Of the cohort, 69 (52%) and 31 (26%) patients had syncope or spontaneous VT/VF as the initial complaint (of these, 21 patients presented with both syncope and spontaneous VT/VF). EPS studies were rarely conducted (6/121 patients) of which four tested positive. Forty-six (38%) patients underwent 24-h Holter study. Of these, abnormal heart

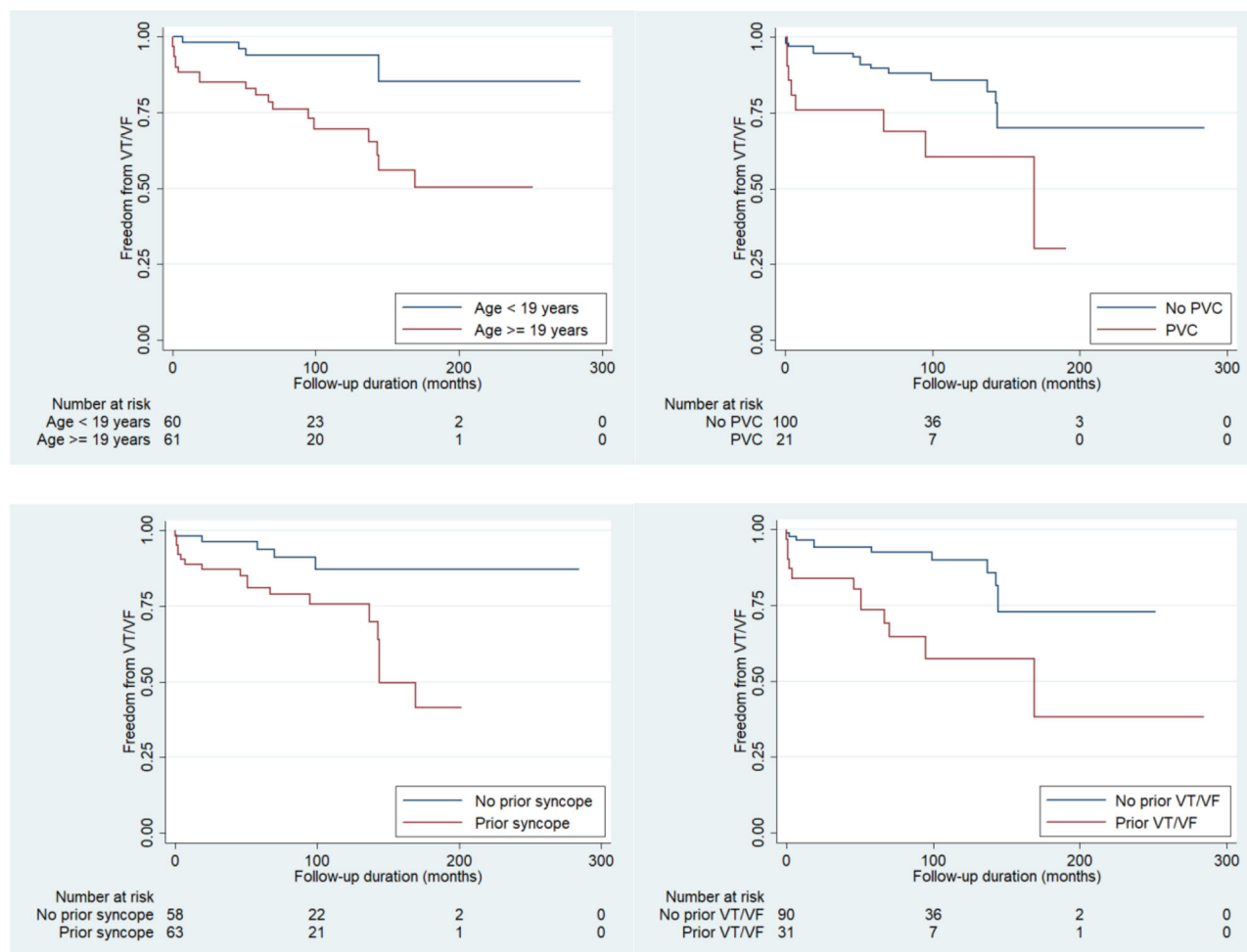


FIGURE 1 | Kaplan-Meier survival curves demonstrating freedom from spontaneous ventricular tachycardia/ventricular fibrillation (VT/VF) during follow-up stratified by age ≥ 19 years of age (top left), PVC (top right), initial presentation with syncope (bottom left), initial presentation with VT/VF (bottom right). All showed significant difference between the two groups by the log-rank test.

rhythms (sinus arrhythmia, sinus bradycardia, atrioventricular block, progressive cardiac conduction defect, premature atrial complexes, atrial tachycardia or fibrillation, supraventricular arrhythmias, ventricular couplets) were detected in 28 (23%) patients. premature ventricular complexes (PVCs) were seen in 21 (17%) of the patients. Treadmill exercise tolerance test was performed in 40 (33%) patients. ICDs were implanted in 48 (40%) patients.

Genetic tests were performed for 61% of the study cohort (**Supplementary Table 1**). Positive test results, defined as identification of pathogenic, likely pathogenic or variant of uncertain significance if supported by evidence of abnormal ion channel function from functional or clinical studies, were found in 81% of the tested individuals. Five patients had normal genetic tests and the remainder did not undergo testing. The novel mutations not described in cohorts from other geographical regions are marked in **Supplementary Table 1**. KCNQ1, KCNH2, SCN5A, KCNE1, CACNA1C mutations were

identified in 23, 24, 4, 4, and 6 patients, confirming LQTS subtypes 1, 2, 3, 5, and 8, respectively. Single mutations in CAV3 (c.277G>A), AKAP9 (c.6065A>G) and CALM3 (c.286G>C) were found, which corresponded to LQTS types 9, 11 and 16.

The following six patients had compound mutations. The first had c.782A>G in KCNQ2 and c.328G>A in SCN3B. The latter has been described in a Japanese cohort of Brugada Syndrome (11). However, our patient did not have any Brugada pattern on the ECG. The second patient had the c.31G>A mutation in KCNQ1 and c.56T>C KCNH2. The third patient had the c. 1046C>G mutation in KCNQ1 and c.253G>A mutation in KCNE1. The fourth patient had a variant of uncertain significance and a low clinical significance variant in SCN10A. She had recurrent syncope with ICD implantation but no VT/VF. The final two patients are siblings whose mother died of SCD, with c.1186G>C mutation in CACNA1C

TABLE 2A | Univariate Cox regression analysis for shorter time to VT/VF post-diagnosis.

Variable	Hazard ratio (HR)	95% confidence intervals (CIs)	P-value
Female gender	1.53	0.60–3.90	0.372
Age	1.02	1.01–1.04	0.007
Initial QTc interval	1.08	1.00–1.02	0.107
Family history of LQTS	0.59	0.24–1.45	0.252
Family history of sudden cardiac death	0.63	0.15–2.71	0.537
Family history of LQTS or SCD	0.61	0.26–1.44	0.261
Initial presentation with syncope	3.86	1.43–10.42	0.008
Syncope	4.21	1.43–12.45	0.009
Stress syncope	0.76	0.18–3.23	0.705
Palpitations	1.60	0.63–4.07	0.323
Premature ventricular complexes	2.89	1.22–6.83	0.015
Initial presentation with VT/VF	3.68	1.62–8.37	0.002
Other arrhythmias	0.92	0.36–2.35	0.869
EPS performed	3.77	1.10–12.87	0.034
EPS positive	–	–	–
Schwartz score	1.39	0.88–2.21	0.157
Heart rate	0.99	0.97–1.01	0.398
P-wave duration	1.01	0.96–1.05	0.819
PR interval	1.01	0.995–1.031	0.149
QRS	1.01	0.997–1.028	0.104
QT interval	1.01	0.998–1.012	0.128
QTc interval	1.00	0.995–1.012	0.382
P-wave axis	1.00	0.99–1.01	0.857
QRS axis	1.00	0.99–1.01	0.921
T-wave axis	1.00	0.99–1.01	0.936
R-wave amplitude in V5	0.76	0.33–1.76	0.523
S-wave amplitude in V1	1.30	0.37–4.56	0.679

P-values less than 0.05 are shown in bold text.

TABLE 2B | Multivariate Cox regression analysis for shorter time to VT/VF post-diagnosis.

Variable	Hazard ratio (HR)	95% confidence intervals (CIs)	P-value
Age	1.02	0.998–1.04	0.073
Initial presentation with syncope	3.58	1.32–9.71	0.011
Initial presentation with VT/VF	2.22	0.87–5.64	0.093
Premature ventricular complexes	2.00	0.77–5.21	0.156

P-values less than 0.05 are shown in bold text.

(pathogenic) and c.1627G>A mutation in ANK2 (variant of uncertain significance).

In terms of pharmacotherapy, 107 patients (79.9%) were administered beta-adrenergic receptor blockers. Amongst the 107 patients, the following beta-adrenergic receptor blockers (most common dosage, daily dose) were prescribed: (1) atenolol ($n = 29$, 225 ± 452 mg); (2) bisoprolol ($n = 8$, 1.22 ± 1.37 mg); (3) carvedilol ($n = 4$, 12.9 ± 11.9 mg); (4) labetalol ($n = 1$, 600 ± 0 mg); (5) metoprolol ($n = 55$, 112 ± 89.6 mg); (6) nadolol ($n = 19$, 229 ± 915 mg); (7) nebivolol ($n = 2$, 5 ± 0 mg); (8) propranolol ($n = 46$, 43.1 ± 27.2 mg); sotalol ($n = 1$, 160 ± 0 mg). Mexiletine was prescribed to 12 of the 107 patients (364 ± 113 mg).

Follow-Up and Predictors of Spontaneous VT/VF Outcomes Post-diagnosis

In total, 23 patients developed VT/VF during follow-up. Kaplan-Meier curves demonstrating freedom from spontaneous VT/VF stratified by age ≥ 19 years old, PVC, initial presentation with syncope or VT/VF status are shown in **Figure 1** (top left, top right, bottom left and bottom panels). Significant differences were found between all groups by the log-rank test ($P = 0.002$, $P = 0.011$, $P = 0.004$ and $P = 0.001$, respectively).

Univariate Cox regression analysis was performed (**Table 2A**), revealing that age [hazard ratio (HR): 1.02 (1.01–1.04), $P = 0.007$; optimum cut-off: 19 years], presentation with syncope [HR: 3.86 (1.43–10.42), $P = 0.008$] or VT/VF [HR: 3.68 (1.62–8.37),

TABLE 3 | Variable importance ranking to predict VT/VF post-diagnosis with RSM model.

	Importance	Relative importance
Age	0.0815	1.0000
Schwartz score	0.0057	0.0705
Family LQTS	0.0035	0.0427
Sex	0.0022	0.0269
Family SCD	0.0018	0.0223
Initial QTc interval	-0.0030	-0.0369
Other arrhythmias	-0.0045	-0.0551
Initial VT/VF	-0.0091	-0.1120

$P = 0.002$] and the presence of PVCs [HR: 2.89 (1.22–6.83), $P = 0.015$] were significant predictors of spontaneous VT/VF. Only initial presentation with syncope remained significant after multivariate adjustment [HR: 3.58 (1.32–9.71), $P = 0.011$; Table 2B].

Random Survival Forest (RSF) Analysis and Comparisons With Cox Proportional Hazard Model

Next, RSF analysis was applied to the present dataset. The data input into the model and relative importance values of the included variables for outcome prediction are shown in Table 3. Sensitivity analysis based on tree number in the RSF model and the derived variable importance ranking were also obtained (Figure 2, left panel and right panel). The prediction error becomes smaller when the number of trees in the RSF model increases, indicating that the model learns better when the forest structure becomes more complex. However, this is offset by the disadvantage that more trees take more time for model training and potentially lead to over-fitting. The sensitivity analysis provides a guidance for choosing the optimum number of trees to yield an acceptable prediction error without overcomplicating the model. Marginal effects reveal how a dependent outcome variable varies when the independent variable changes. The survival curve and cumulative hazard function generated by the RSF model is detailed in Figure 3 (left panel and right panel).

Survival estimates from the RSF model are shown in Figure 4. A survival function was determined for each LQTS patient. In the top left panel, the red line illustrates the overall ensemble survival, whereas the green line shows the Nelson-Aalen estimator. The top right panel shows Brier score (0 = perfect, 1 = poor, and 0.25 = guessing) stratified by ensemble spontaneous VT/VF based on the inverse probability of censoring weight (IPCW) method (10). The cohort was stratified into four groups of 0–25, 25–50, 50–75, and 75–100 percentile for the occurrence of spontaneous VT/VF (the overall non-stratified Brier score is shown by the red line). The bottom left panel shows the continuous rank probability score (CRPS) given by the integrated Brier score divided by time, whereas the bottom right panel shows a plot of VT/VF postdiagnosis of each patient against time.

Finally, comparative analysis showed that the RSF model showed an improved performance compared to Cox regression model as illustrated by the higher values in precision, recall, AUC and Harrell's C index with a 5-fold cross validation approach (Table 4). Decision rules were generated by RSF model to predict VT/VF post-diagnosis as shown in Figure 5. ROC and AUC of RSF model to predict VT/VF post-diagnosis were presented in Figure 6.

DISCUSSION

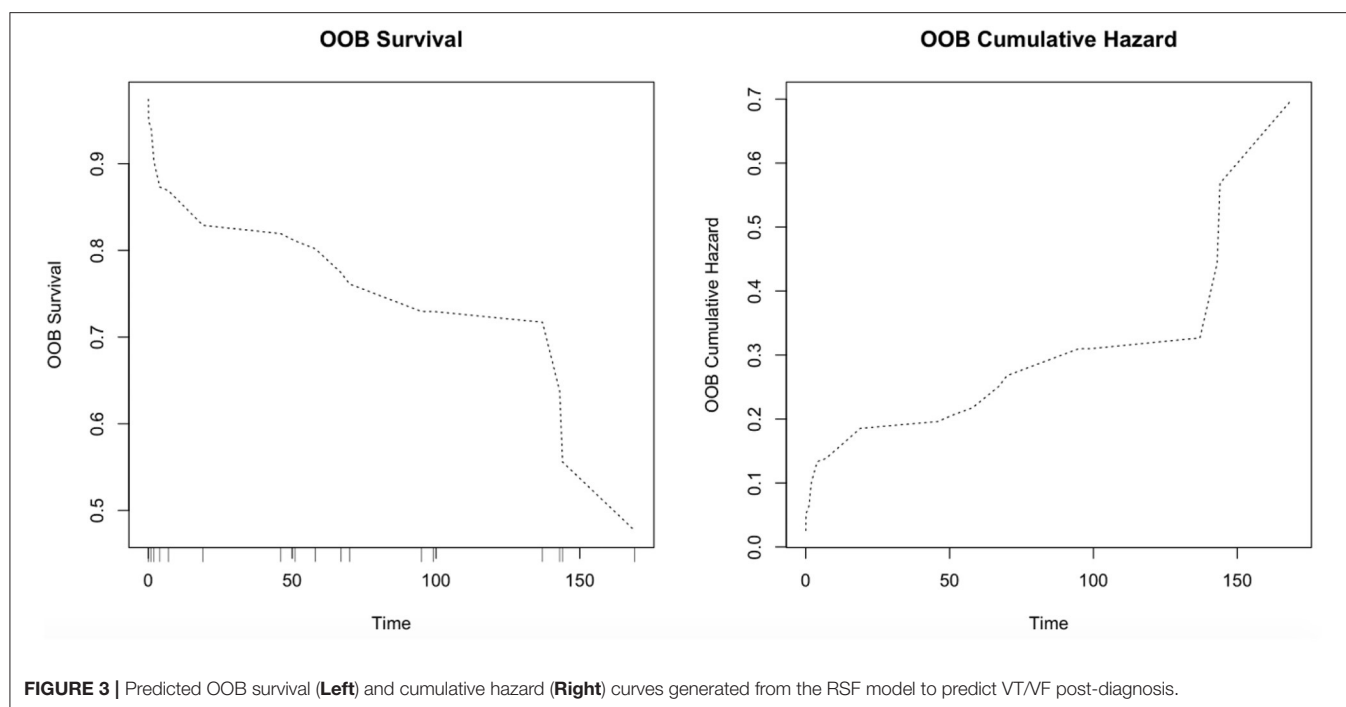
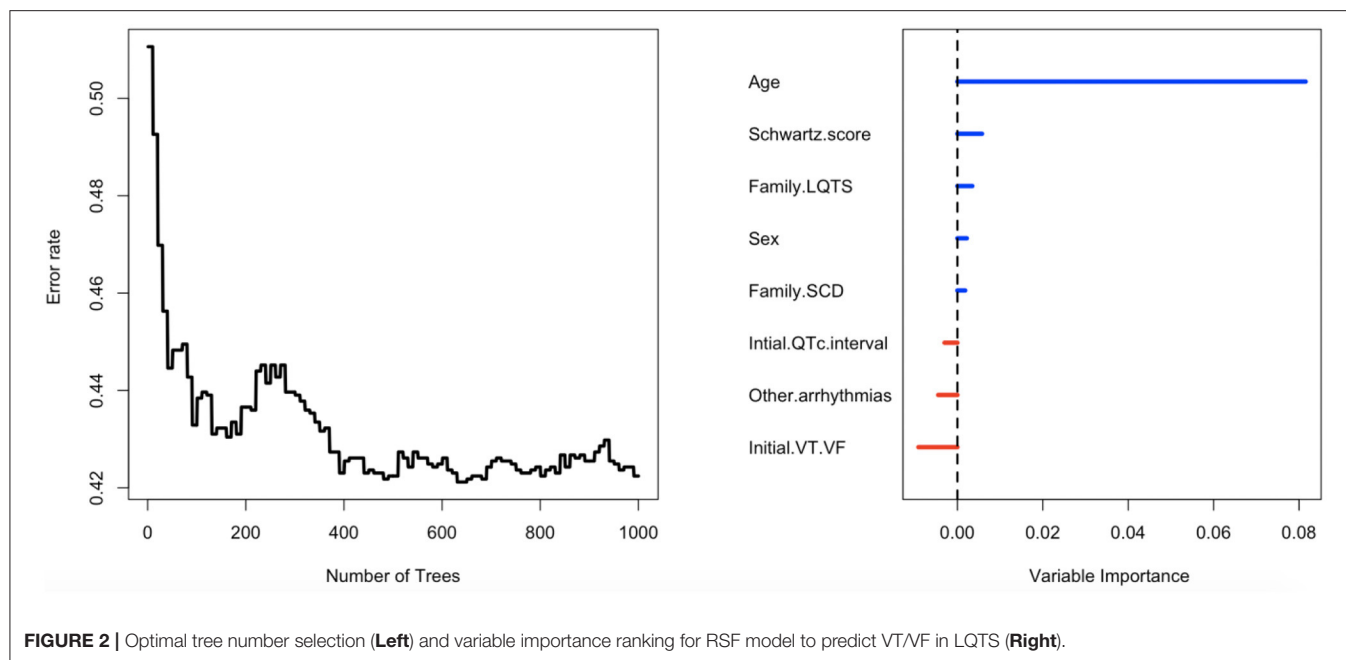
In this territory-wide study of congenital LQTS patients, the main findings are: (i) the identification of novel mutations in a number of putative ion channel genes, (ii) family history of LQTS or SCD, initial presentation with syncope or VT/VF, the presence of PVCs, QTc interval and QRS duration were significant predictors of spontaneous VT/VF on univariate Cox regression and only prior presentation with VT/VF remained significant after multivariate adjustment; (iii) RSF model provided significant improvement in risk prediction over Cox regression.

Genetic Heterogeneity in a Chinese Cohort of Congenital LQTS

Loss-of-function mutations in various potassium channel subunits are responsible for LQTS types 1 (KCNQ1), 2 (KCNH2), 5 (KCNE1), 6 (KCNE2), 7 (KCNJ2), and 13 (KCNJ5). Gain-of-function mutations in sodium channel subunits lead to LQTS types 3 (SCN5A) and 10 (SCN4B), and in the L-type calcium channel produces LQT type 8 (CACNA1C, Timothy syndrome). Mutations in supporting proteins are responsible for the LQT type 4 (ANKB), 9 (CAV3), 11 (AKAP9), and 12 (SNTA1) phenotypes. The underlying mechanisms can be due to a direct reduction in gating properties, or altered expression, localization or trafficking of these ion channel proteins affecting repolarization or late depolarization. Genetic analysis identified novel mutations in KCNQ1, KCNH2, SCN5A, ANK2, CACNA1C, CAV3, AKAP9, and HCN4.

The following novel mutations in KCNQ1 were identified. The c.31G>A mutation in exon 1 leads to E11K variant, altering the secondary structure of this subunit. *In silico* analysis predicts this mutation to be probably damaging to channel function. The Human Gene Mutation Database has reported two mutations in nearby regions, A2V, P7S, in the context of LQTS (12). The c.782A>G mutation in exon 6 affects the S4/S5 region and is predicted to be likely pathogenic. The c.1018T>C mutation in exon 7 affecting the S5-pore-S6 region and c.1831G>A in exon 16 affecting the C-terminus are pathogenic. Three novel KCNH2 mutations were found. Firstly, c.211G>T in exon 2 affecting the N-terminus is pathogenic. A different missense variant affecting the same codon, c.211G>C has been reported previously in LQTS patients (13, 14). The c.1738G>A in exon 7 affects the S5-pore-S6 region. The c.1738G>C mutation affecting the same codon was reported to be likely pathogenic (VCV000191223.1).

The c.1627G>A mutation in ANK2 leads to a change in amino acid from valine to methionine in the membrane-binding domain and has not been described in LQTS. It has been classified



as a variant of uncertain significance, but the valine is located at a moderately conserved region (VCV000526909.1). In the two siblings harboring this mutation, the pathogenic variant c.1186G>C in CACNA1C was also found. It was therefore not possible to examine the relative contributions of these variants to the electrophysiological phenotype.

Moreover, a mutation in CAV3, c.277G>A leading to p.Ala92Thr, was identified in a neonatal patient who presented with supraventricular tachycardia associated with prolonged QTc of values between 450 and 480 ms. CAV3 encodes for the scaffolding protein caveolin-3, which is

the main component of caveolae. Previously, autosomal recessive c.277G>A mutation was associated with rippling electromyographic discharges with muscular dystrophy (15), whereas heterozygotes were asymptomatic with normal cardiac function but electrocardiographic findings were not reported (16). Nevertheless, the p.Ala85Thr and p.Phe97Cys mutations were linked to a persistent late sodium current in LQTS (17). Given that caveolin-3 and the SCN5A subunit co-localize in the cell membrane, the CAV3 mutation in our patient may increase the QTc interval by increasing the late sodium current, but this remains to be elucidated in functional studies.

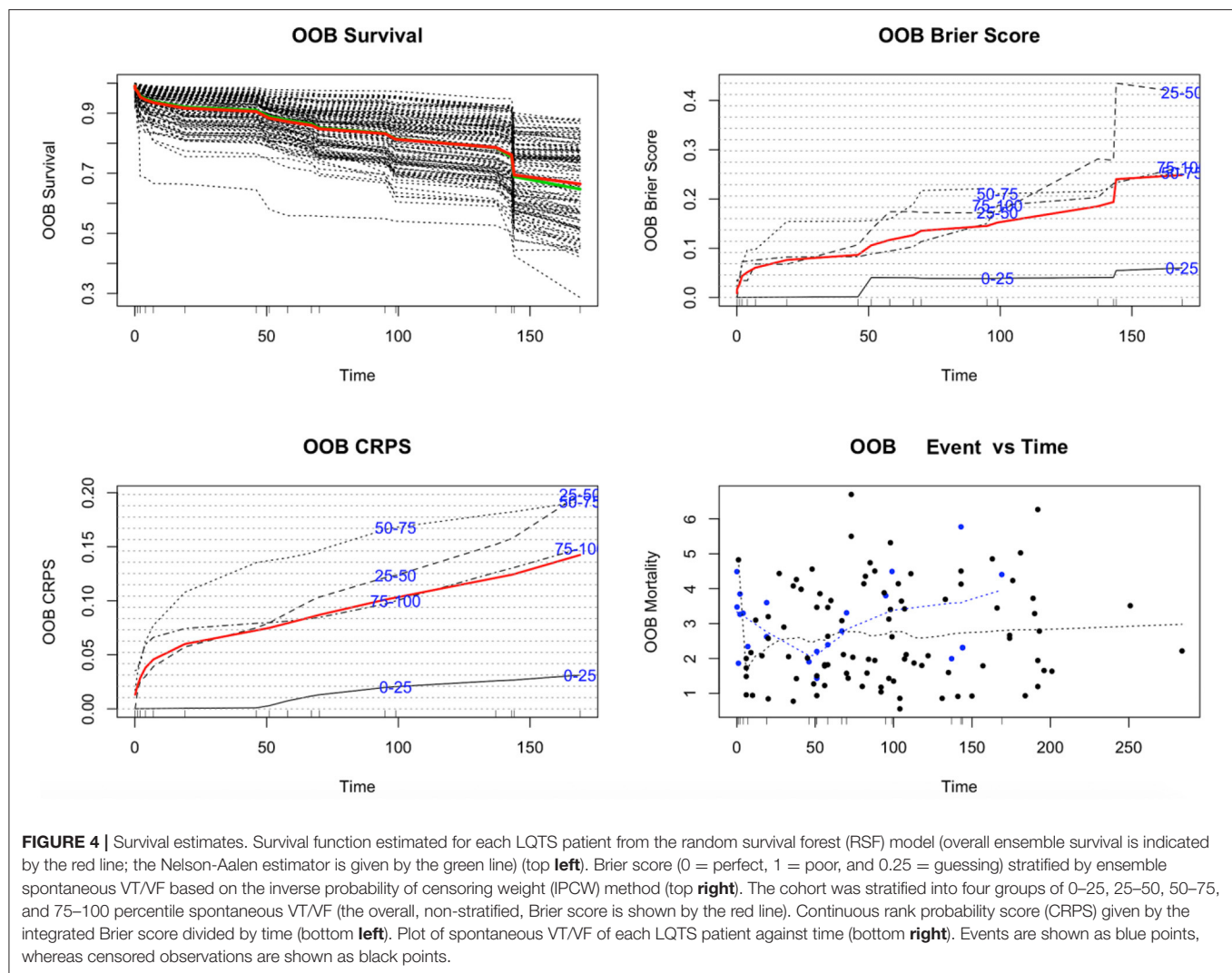


TABLE 4 | Performance comparisons of RSF and multivariate Cox models to predict VT/VF post diagnosis with 2-fold cross validation approach.

Model	Precision	Recall	Brier score	AUC	Harrell's C index
RSF	0.80	0.79	0.10	0.77	0.79
Multivariate Cox	0.69	0.68	0.16	0.68	0.67

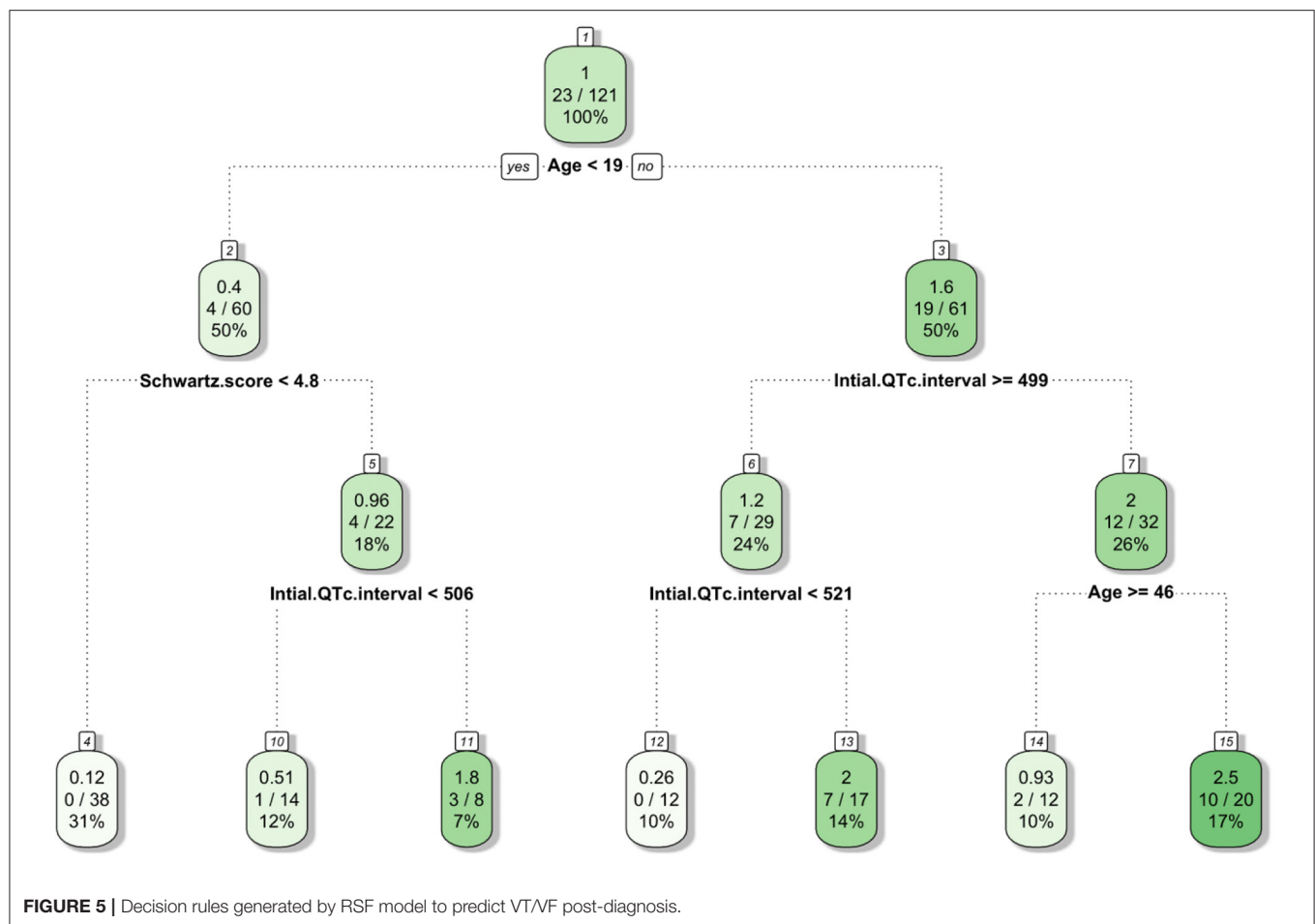
A mutation in AKAP9 was detected in an asymptomatic young boy with ECG findings of QTc prolongation to 485 ms, slow rising T-waves, T-wave inversion in V1–V3 and notched waves in V4–V6. He initially presented with seizures and had a diagnosis of XL creatine transporter deficiency. AKAP9 encodes for the kinase-anchor protein-9 and is recognized as a genetic modifier of congenital LQTS (18). Its loss-of-function mutations have been associated with congenital LQTS type 11 (19).

In addition to the novel mutations described above, our study also identified pathogenic variants in KCNQ1, KCNH2, SCN5A, and KCNE1. Moreover, the D96V mutation in CALM3

(c.286G>C leading to p.Asp96His) was also found in one patient. This mutation was previously associated with severe QTc prolongation to 690 ms with 2:1 atrioventricular block and T-wave alternans, recurrent VF and aborted SCD events accompanying cerebral seizures (20).

Mechanisms of Ventricular Arrhythmogenesis in Congenital LQTS

Univariate Cox regression findings using clinical electrocardiographic data demonstrate that PVCs and prolonged QTc intervals predicted incident spontaneous VT/VF. They therefore support the trigger-substrate hypothesis in LQTS (21). Significant predictors of spontaneous VT/VF were syncope at initial presentation or occurring at follow-up, in accordance findings from previous studies investigating congenital LQTS cohorts (22, 23). Family history of LQTS was identified as a protective factor. The reason is that family members of the probands who were tested positive for the genetic mutations, but without spontaneous VT/VF



events, were also included. As many were silent carriers, their inclusion meant that the hazard ratios were skewed to lower values.

Improved Prediction of Spontaneous VT/VF Using Random Forest Analysis Compared to Cox Regression

RSF builds hundreds of trees and generates outcome prediction by voting method for analyzing right censored survival data (8). The advantage is that unlike the Cox proportional hazard model, it does not make assumptions about the individual hazard function (9) and ranks the significance of predictors for spontaneous VT/VF. Randomization is introduced in two forms: a randomly drawn bootstrap sample of data for growing the tree, and nodes splitting on randomly selected predictors for growing the tree learner. The boosting tree structure in RSF can capture the nonlinear effects and complex interactions among the variables, which can reduce prediction variance and bias as well as significantly improve learning performance (9). Moreover, RSF can handle the effects of the treatments and predictor variables, whereas traditional methods using Cox or Kaplan Meier analysis utilize a linear combination of attributes (24). RSF has been applied to

improve prediction of all-cause mortality, heart failure-related hospitalizations, cost and home days loss in heart failure (25) in addition to mortality prediction in heart failure patients undergoing cardiac resynchronization therapy (26). Moreover, it successfully predicted inpatient mortality following cardiac arrest after admission to intensive care (27), sudden cardiac arrest in the Left Ventricular Structural (LV) Predictors of Sudden Cardiac Death (SCD) Registry (28) and all-cause mortality prediction in acquired long QT syndrome (29). Our study demonstrates for the first time that RSF model can significantly improve spontaneous VT/VF prediction in inherited LQTS.

LIMITATIONS

Several limitations should be noted. Firstly, this was a retrospective study. Nevertheless, for most patients, six-monthly to annual follow-ups were available. In Hong Kong, all public hospitals have linked electronic health records, meaning that if patients are admitted to another hospital, the case records and investigation results can be traced back and viewed electronically. Secondly, the predictive value of investigations was limited by the relatively small sample size of this cohort. Thirdly, only

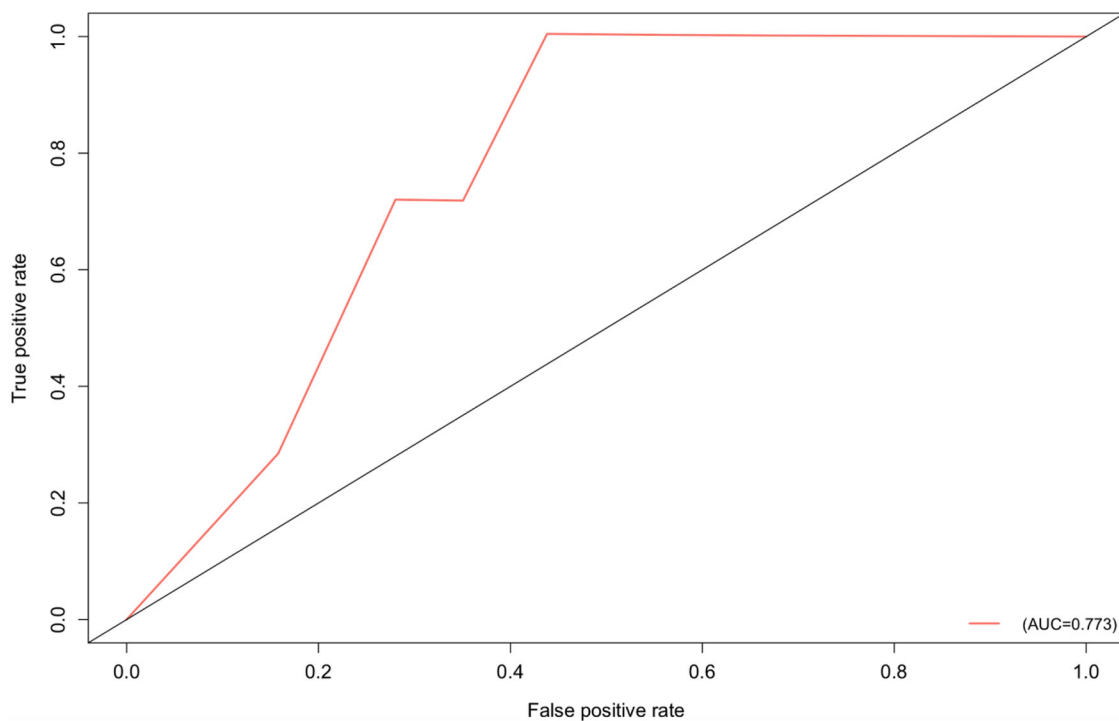


FIGURE 6 | ROC and AUC of RSF model to predict VT/VF post-diagnosis.

scanned ECGs were available and therefore the ECG variables summarized were averaged from the 12 leads. The raw ECG files could not be obtained and therefore it was not possible to extract the measurement from each lead. Future work should explore the possibility of converting scanned images to electronic ECG files for detailed analyses, for example to investigate whether the incorporation of novel indices such as T-wave morphology can further enhance diagnosis or risk prediction (30, 31). Fourthly, for some patients, only Sanger sequencing of targeted genes was performed, without next generation sequencing (NGS) of their entire genomes. Therefore, contributions from mutations in other genes cannot be excluded. Because genetic tests were not performed in all of the LQTS patients included, our risk model did not include genetic results as a predictive variable. Other studies have reported that genotype is an important determinant of arrhythmic risk (32–34), and prospective studies should be conducted to identify genetic risk factors. Finally, the family history of LQTS was low because the medical records for the relatives of probands were often not accessible, unless the attending physicians specifically noted down the identity details or coded them with ICD-9 codes.

CONCLUSIONS

Effective risk stratification in congenital LQTS can be achieved by clinical history, electrocardiographic indices, and different investigation results, irrespective of underlying

genetic defects. A machine learning approach using RSF can improve risk prediction over traditional Cox regression models.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://doi.org/10.5281/zenodo.3465850>, Zenodo.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Joint Chinese University of Hong Kong–New Territories East Cluster Clinical Research Ethics Committee. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

GT, SL, and JZ: data collection, clinical data analysis, manuscript drafting, and manuscript critical revision. TL and IW: data analysis and manuscript critical revision. CM, NM, and KJ: data collection, genetic analysis and interpretation, ECG analysis, and manuscript critical revision. QZ, SC, and WTW: genetic

results interpretation, manuscript critical revision, and study supervision. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcvm.2021.608592/full#supplementary-material>

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Mitochondrial Dysfunction Increases Arrhythmic Triggers and Substrates; Potential Anti-arrhythmic Pharmacological Targets

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Incidence of cardiac arrhythmias increases significantly with age. In order to effectively stratify arrhythmic risk in the aging population it is crucial to elucidate the relevant underlying molecular mechanisms. The changes underlying age-related electrophysiological disruption appear to be closely associated with mitochondrial dysfunction. Thus, the present review examines the mechanisms by which age-related mitochondrial dysfunction promotes arrhythmic triggers and substrate. Namely, via alterations in plasmalemmal ionic currents (both sodium and potassium), gap junctions, cellular Ca^{2+} homeostasis, and cardiac fibrosis. Stratification of patients' mitochondrial function status permits application of appropriate anti-arrhythmic therapies. Here, we discuss novel potential anti-arrhythmic pharmacological interventions that specifically target upstream mitochondrial function and hence ameliorates the need for therapies targeting downstream changes which have constituted traditional antiarrhythmic therapy.

Keywords: arrhythmias, mitochondrial dysfunction, ROS, aging, ion channels

INTRODUCTION

Aging is the progressive decline in the fitness of an organism due to cumulative organ-specific physiological deterioration (1, 2). The advancement of modern medicine is thus reflected in increasing human life expectancy (3). However, an aging population offers novel medical challenges with increasing prevalence of a number of conditions including cardiovascular, oncological, and neurological diseases. The incidence of cardiovascular diseases increases exponentially in the elderly population (4, 5). In the aging population, cardiovascular diseases are the leading cause of morbidity and mortality (3, 5, 6). Thus, cardiovascular diseases have a prevalence of 82.6 million (36.2%) in the United States (4) carrying a greater financial burden than any other group of diseases including cancer and benign neoplasms (4). In 2007, 33.6% of all deaths (~814,000 people) in the United States had cardiovascular disease as the underlying cause of death (4). It is estimated that eliminating mortality from cardiovascular diseases would add between 5.5 and 7 years to mean life expectancy (4, 7). As the aging population continues to increase, with the number of elderly people predicted to double in the next 25 years in the United States age related cardiovascular diseases will continue to represent a major public health concern (5, 8). As such, it is increasingly important to be able to stratify risk of cardiovascular diseases by age and understand their underlying age-related molecular mechanisms in order to develop effective pharmacological therapies.

Within cardiovascular diseases, cardiac arrhythmias arise due to disruption in the orderly sequence of cardiomyocyte action potential activation and recovery through successive regions of the myocardium compromising cardiac function (9, 10). Of atrial arrhythmias, atrial fibrillation (AF) is the most common type. It is associated with major morbidity by increasing the risk of stroke and heart failure, as well as all-cause mortality (11–13). Ventricular arrhythmias such as ventricular tachycardia often degenerating into ventricular fibrillation (VF) are also a major public health concern. They constitute the primary cause of sudden cardiac death (SCD), which accounts for 4–5 million deaths/year worldwide (14) representing over 5% of overall mortality (15).

Incidence of cardiac rhythm abnormalities increases exponentially with age (6, 16, 17). Hence, incidence of AF in the general population increases 23-fold from the 20–24 to the 55–59 years age group (18, 19) and reaches a prevalence of over 13% in the >80 years age group (20). Similarly, incidence of VF in the general population increases 18-fold from the 20–24 to the 55–59 years age group (21).

Primary electrical abnormalities due to congenital channelopathies represent an important cause of arrhythmias and SCD (15, 22, 23). These include long QT syndrome 3 (LQT3) arising from a gain-of-function mutation in the cardiac sodium $\text{Na}_v1.5$ channel gene *SCN5A*, Brugada Syndrome (BrS) arising from a loss-of-function mutation in the *SCN5A* gene, and catecholaminergic polymorphic ventricular tachycardia (CPVT) arising from a gain-of-function mutation in *RyR2* gene or loss-of-function mutation in *CASQ2* gene encoding cardiac calcium homeostasis proteins (23, 24). Proarrhythmic inherited channelopathies demonstrate how each component of the cardiac electrophysiological system contributes to arrhythmogenesis. Thus, studying those channelopathies has been crucial to elucidating the mechanisms underlying arrhythmogenesis in the general and aging population.

Interestingly, arrhythmic risk in individuals with many inherited channelopathies, such as BrS and LQT3, increases markedly with age, despite these individuals carrying the proarrhythmic mutation from birth (25). For example, LQT3 patients show significantly increased arrhythmic risk after the 40 years of age (26, 27). In CPVT however, patients are usually diagnosed in the first or second decade of life with the mean age of onset of symptoms, usually a syncopal episode, is between age seven and 12 years (28). Therefore, select channelopathies demonstrate an excellent paradigm to study the effects of age-related molecular changes on susceptible hearts with inherent proarrhythmic tendency. This will elucidate the molecular mechanisms underlying proarrhythmic changes with age and hence offer novel anti-arrhythmic pharmacological targets.

AGING AND ENERGETIC DYSFUNCTION

It has long been established that central to the aging process of any organ is energetic dysfunction giving rise to free radical reactive oxygen species (ROS) that cause damage to cellular macromolecules, accumulation of this damage leads to the physiological compromise seen in aging (5, 29). Current evidence suggests that mitochondrial dysregulation is the cause

and primary target of energetic dysfunction and free radical production (5, 30). Thus, transgenic mice overexpressing the cellular antioxidant catalase targeted to the mitochondria had a reduced ROS-induced damage of the mitochondria and significantly increased lifespan (31).

A clear link exists between aging and mitochondrial dysfunction, occurring through various mechanism which include mitochondrial DNA damage, clonal expansion of deleterious mutations in mitochondrial DNA and deficiencies in the enzymes of the mitochondrial respiratory chain, such as cytochrome-c-oxidase (32–36). This phenomenon of aging driving mitochondrial genetic instability has thus been observed not just in humans but several other mammalian species, including in mice, rats, and rhesus monkeys (37–39). The link between aging and mitochondrial dysfunction appears to be bidirectional. For example, increased levels of mitochondrial DNA mutations are associated with a premature aging syndrome in mice (34, 40). Therefore, it is apparent that understanding the biology of mitochondrial instability via mitochondrial DNA mutations and enzyme deficiencies is key to understanding cellular- and tissue-level changes that underlie aging-related pathology.

As such, damaged and dysfunctional mitochondria result in production of high levels of ROS, disrupted mitochondrial membrane potentials, reduced ATP production capacity, and altered cellular redox potential (5, 41–44). The consequent aberrant mitochondrial signaling predisposes the myocardium to arrhythmias (9, 43).

This is demonstrated clinically and experimentally. Mitochondria from human AF patients are abnormal in terms of morphology and function and show DNA damage (45–48). Abnormal mitochondria are also seen in animal models of AF and ventricular arrhythmia (49–51). Additionally, inherited errors of metabolism involving mitochondria such as Kearns-Sayre syndrome manifest symptomatically as fatal rhythm abnormalities (52). Detailed electrophysiological studies in peroxisome proliferator-activated receptor gamma coactivator 1- α (*Pgc-1 α*) and *Pgc-1 β* knockout models of mitochondrial dysfunction yield similar overt arrhythmic phenotypes whilst also yielding information on the ionic basis of these arrhythmias. For example, *Pgc-1 β* ^{-/-} mice show decreased atrial and ventricular conduction velocity, which may be attributed to reduced voltage gated inward Na^+ currents (53–60).

The present review separates the pro-arrhythmic molecular changes in aging into multiple pathways. However, this is largely to make the topic more accessible and easier to conceptualize. In reality these pathways are dependent upon and interact with each other through complex feedback loops. Physiological interactions which are important to the arrhythmic process are also highlighted.

MICE MODELS

Animal models have been pivotal in studying arrhythmias, permitting experimentation on the cellular and system level. Mice, often with electrophysiologically stable 129/Sv or C57BL/6 genetic backgrounds, have thus far represented the main transgenic system for modeling arrhythmic syndromes (61,

62) typically via well-defined mutations strategically positioned to reflect the genotypes associated with these syndromes and reliably reflecting their phenotype (9, 23, 63). From a practical aspect, mice are inexpensive, easily maintained, and reproduce rapidly thus allowing provision of aged mice over relatively short periods (25). Mice also reflect the human aging process such that they complete their growth before reproduction commences (1, 64). Furthermore, confounding risk factors which influence cardiovascular health (e.g., smoking and hypercholesterolaemia) are absent in murine models and as such their hearts reflect intrinsic cardiac aging (1). Together, these features of murine models make them valuable in the study of the mechanisms underlying cardiac aging.

Despite differences with regards to heart rate, heart size, as well as calcium- and potassium-mediated repolarization currents, which limits their ability to model conditions such as LQT1 and LQT2, murine and human hearts show significant structural and physiological resemblances (65). Structural similarities include similar conducting, sinoatrial and atrioventricular nodes, His-Purkinje systems and contracting atrial and ventricular chambers (25, 65, 66). Important electrophysiological similarities exist especially with respect to their action potential (AP) waveforms where they both share the same role of the inward sodium current in mediating phase 0 depolarization (25, 65) as well as similar transmural differences in AP duration and AP conduction velocities (65, 67, 68). These similarities are critical in permitting mice to effectively model LQT3 and BrS (9).

MITOCHONDRIAL DYSFUNCTION AND DISRUPTED SURFACE MEMBRANE IONIC CURRENTS

Mitochondrial Dysfunction and Sodium Currents

ROS promote both arrhythmic triggers and substrates and hence exert numerous proarrhythmic actions through modulation of intracellular and cell surface ion channels. Firstly, ROS modifies the expression and function of voltage gated Na^+ carrying channel, $\text{Na}_v1.5$, causing a decrease in the fast depolarizing component of the sodium current (I_{Na}) but an increase in the late sodium current ($I_{\text{Na-L}}$) (9, 69–72). Thus, in human embryonic kidney (HEK) cells and C57BL/6 murine cardiomyocytes, application of cytosolic NADH and mitochondrial ROS-generating molecules, such as the complex III inhibitor Anti-mycin A, reduced I_{Na} (69, 70). However, this effect was blocked by application of mitoTEMPO a specific scavenger of mitochondrial superoxide (69, 70). In murine hearts modeling mitochondrial dysfunction, increased age and *Pgc-1 β* ^{-/-} genotype interacted to decrease atrial $\text{Na}_v1.5$ channel expression (36). Furthermore, the A280V mutation in glycerol-3-phosphate dehydrogenase 1-like (GPD1-L) protein, which causes Brugada syndrome, reduces I_{Na} via increasing cytosolic NADH and mitochondrial ROS levels (73, 74). Additionally, through oxidation of the Ca^{2+} -calmodulin-dependent kinase

II (CaMKII), ROS has also been shown to enhance $I_{\text{Na-L}}$ (75–78). Together, these alterations in the cardiomyocyte sodium current promote arrhythmogenesis through increased triggered activity and arrhythmic substrate. These findings are summarized in **Figure 1**.

Increased $I_{\text{Na-L}}$ prolongs membrane repolarization and as such allows the development of early-after depolarizations (EADs) through reactivation of voltage-gated Ca^{2+} channels (VGCC), and in turn, EADs can trigger arrhythmic events (79, 80). In addition, repolarization defects caused by increased $I_{\text{Na-L}}$ promote spatiotemporal heterogeneity and transmural dispersion of repolarization arrhythmic substrate (79, 80). The changes in I_{Na} have profound consequences on ordered action potential propagation through the myocardium. Cardiac conduction velocity is largely determined by the maximum rate of membrane depolarization $(dV/dt)_{\text{max}}$, which in turn is determined by I_{Na} and conducted by the $\text{Na}_v1.5$ channel. Reduced conduction velocity forms the arrhythmic substrate associated with re-entrant arrhythmias (81, 82). Interestingly, these findings may explain the change in phenotype with age in certain channelopathies. For example, an overlap syndrome in aging LQT3 patients describes the emergence of Brugada syndrome patterns on surface ECGs in addition to the prolonged QT interval indicative of LQT3 (83, 84). Similarly, electrophysiological studies on murine LQT3 models report decreased conduction velocity in aged, but not young, hearts (85, 86). Therefore, age-related mitochondrial dysfunction and ROS generation may account for the activation abnormalities that appear later in life in LQT3 patients and associated with increased arrhythmic risk. Another important consideration is the close link between intracellular Na^+ and Ca^{2+} regulation. Hence, the increase in $I_{\text{Na-L}}$ causing increased $[\text{Na}^+]_i$ has been shown to increase $[\text{Ca}^{2+}]_i$ largely through reversing the activity of the sodium-calcium exchanger (NCX) (87, 88). In turn, as discussed later, elevated $[\text{Ca}^{2+}]_i$ promotes proarrhythmic electrophysiological changes including inhibition of I_{Na} (89, 90).

Mitochondrial Dysfunction and Gap Junctions

Cardiac conduction velocity is also influenced by the axial resistance (r_a) to local current flow between cells as determined by intercellular gap junction channels formed by connexin (Cx) proteins (82, 91). ACE8/8 mice are produced by placing the angiotensin-converting enzyme (ACE) gene under the control of the α -myosin heavy chain promoter using targeted homologous recombination. This results in significantly increased cardiac ACE and angiotensin II levels. Studies on ACE8/8 mice demonstrated that increased ROS production through renin-angiotensin system (RAS) activation, increased expression and activation of the redox-sensitive tyrosine kinase cSrc in ventricular cardiomyocytes resulting in reduced Cx43 function and expression (74, 92, 93). This reduced conduction velocity and increased risk of ventricular arrhythmias (74, 94). Similarly, *Pgc-1 β* ^{-/-} transgenic mice reflecting mitochondrial dysfunction showed reduced atrial Cx protein expression (36). The latter finding may represent a direct consequence of ROS induced

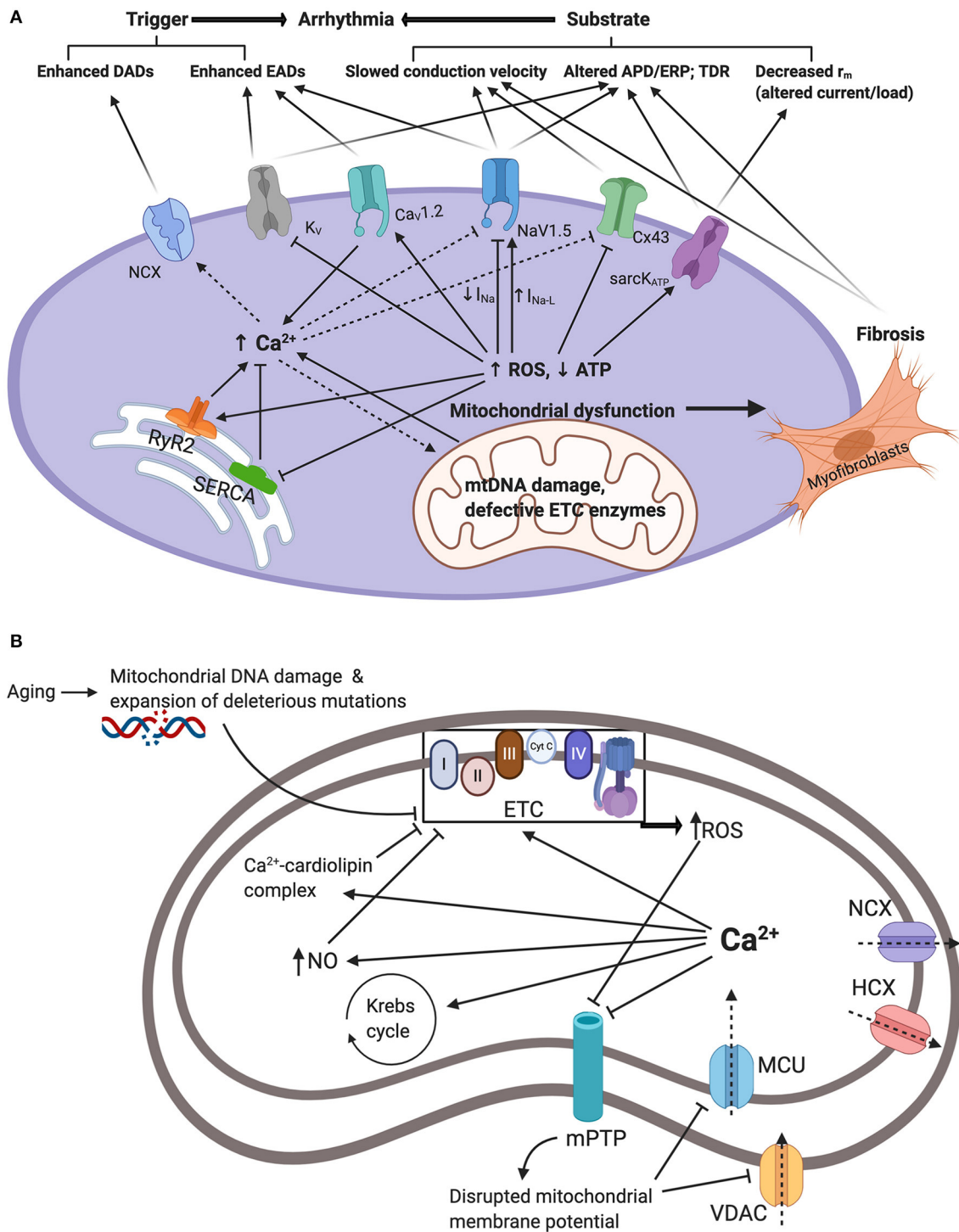


FIGURE 1 | (A) Summarizes the interacting mechanisms by which mitochondrial dysfunction promotes arrhythmogenesis. Mitochondrial dysfunction driven by mitochondrial DNA damage and defective electron transport chain (ETC) enzymes results in reduced ATP and increased reactive oxygen species (ROS) production. In turn, this modifies function and/or expression of sarcolemmal ATP-sensitive K^{+} channels (sarcoATP), gap junction proteins (Cx43), cardiac sodium channels (NaV1.5), cardiac L-type voltage gated Ca^{2+} channels (Cav1.2), and cardiac voltage gated potassium channels (K_v). Moreover, ROS modify endoplasmic reticulum Ca^{2+} homeostasis proteins ryanodine receptor (RyR) and sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA) resulting in elevated cellular Ca^{2+} levels which drives the depolarizing Na^{+} - Ca^{2+} exchanger (NCX). Together, these changes increase arrhythmic triggers and substrates. APD/ERP: action potential duration/effective refractory period ratios; DADs: delayed after-depolarizations; EADs: early after-depolarizations; TDR: transmural dispersion of repolarization. **(B)** Summarizes mitochondrial Ca^{2+} (Continued)

FIGURE 1 | handling and its relation to mitochondrial dysfunction and elevated cytosolic Ca^{2+} . Ca^{2+} enters the mitochondria via voltage dependent anion channels (VDAC) and mitochondrial calcium uniporter (MCU). This is driven by the negative mitochondrial membrane potential ($\sim -180\text{mV}$). Increased cytosolic Ca^{2+} through mechanisms demonstrated in (A) will result in increased uptake and concentration of mitochondrial Ca^{2+} . Mitochondrial Ca^{2+} overload contributes to mitochondrial dysfunction. Firstly, Ca^{2+} stimulation of the Krebs cycle and the oxidative phosphorylation electron transport chain increasing electron leakage and ROS by-products. Secondly, Ca^{2+} -cardiolipin complexation disrupting mitochondrial lipid and protein arrangement causing proteins such as cytochrome c dislocation and inhibition of the electron transport chain and ROS production. Thirdly, through activation of nitric oxide synthase generating NO radicals which inhibit components of the ETC and promote ROS production. Increased ROS and cytosolic Ca^{2+} also inhibit mitochondrial permeability transition pores (mPTP) impairing Ca^{2+} uptake and contributing to increased cytosolic Ca^{2+} . Ca^{2+} is also extruded through the hydrogen-calcium exchanger (HCX) and the mitochondrial sodium-calcium exchanger (NCX).

pathophysiology, although it may also be linked to the increased cardiac fibrosis which is seen in these mice, and discussed later in this review.

Mitochondrial Dysfunction and Potassium Currents

Voltage gated potassium (K_V) channels are regulated by cellular metabolism. K_V channels give rise to the transient outward K^+ current (I_{to}) underlying phase 1 repolarization, and the delayed rectifier K^+ current (I_{K}) underlying repolarization during phases 2 and 3 of the action potential (95). Electrophysiological studies on diabetic rats have demonstrated repolarization abnormalities resulting from downregulation K_V currents (96, 97). Experimentally, increased ROS has been shown to reduce I_{to} and I_{K} (including I_{K_r} , I_{K_s} and $\text{I}_{\text{K}_{ur}}$) currents (74, 98, 99). This inhibition can be reversed through application of cellular antioxidant glutathione (74, 97, 100). ROS reduces K_V currents through reducing channel mRNA and protein expression levels (74, 99, 101). Peroxisome proliferator-activated receptor α (PPAR α) upregulation during metabolic dysfunction has specifically been associated with reduced transcription of K_V channels (102). Additionally, ROS modulates K_V channel function by altering their phosphorylation status particularly acting through PKC and PKA (74, 103). Reduced K_V currents results in repolarization abnormalities resulting in prolonged action potential duration (APD) promoting EAD arrhythmic triggers (9, 95). Furthermore, altered APD/effective refractory period (ERP) ratios result in spatiotemporal heterogeneity in activation and repolarization hence furnishing an arrhythmic substrate for re-entry arrhythmias (9, 81, 95).

Another group of K^+ channels conduct an inwardly rectifying K^+ current (K_{ir}). These include sarcolemmal ATP-sensitive K^+ channels. (sarco K_{ATP}) predominantly formed by Kir6.2 and SUR2A and are important in the electrophysiological response to stresses such as ischemia (104). These are activated by a reduced ATP/ADP ratio during metabolic stress (105). The high density of sarco K_{ATP} channels means that only 1% of those channels need to open to significantly shorten the APD and hence the ERP and action potential wavelength (9, 106). Furthermore, opening of a large number of channels drives the membrane potential toward E_{K} causing the cardiomyocyte to become hyperpolarized and unexcitable (107). Thus, opening of sarco K_{ATP} channels generates a “current sink” which can slow or block action potential propagation (108). Together, these changes promote re-entrant arrhythmias (9, 106, 108, 109).

MITOCHONDRIAL DYSFUNCTION AND DISRUPTED CALCIUM HOMEOSTASIS

With a 10,000-fold transmembrane gradient, Ca^{2+} is the most tightly regulated intracellular ion being utilized virtually ubiquitously in cellular signaling pathways (110, 111). Cardiomyocyte Ca^{2+} homeostasis is heavily influenced by cellular metabolism with increased ROS levels increasing cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) (74, 112). These findings are summarized in **Figure 1**. The addition of H_2O_2 generating ROS in guinea pig ventricular myocytes resulted in increased current through the L-type voltage gated Ca^{2+} channels (I_{CaL}) and hence significantly increased $[\text{Ca}^{2+}]_i$ (113). This, however, was reversed by application of the mitochondrial inhibitor myxothiazol or the L-type channel inhibitor nisoldipine (113). CAMKII activated by ROS has been shown to increase I_{CaL} via phosphorylation of the $\text{Ca}_v1.2$ subunit (114) and similar accentuating effects on I_{CaL} were induced by oxidized LDL in rat ventricular cardiomyocytes (115). Furthermore, L-type channel appear to undergo direct redox modification and glutathionylation at cysteine residues in the alpha interacting domain (116, 117). Interestingly, the effect of ROS accentuating I_{CaL} has been challenged by other findings obtained under different experimental conditions which reported reduced I_{CaL} following oxidative stress (118).

In addition to sarcolemmal Ca^{2+} entry, ROS modulates intracellular Ca^{2+} handling proteins. Both canine and rat cardiomyocytes show increased opening of RyR2 in response to elevated ROS which triggers RyR2 Ca^{2+} sparks and accentuated Ca^{2+} efflux from the sarcoplasmic reticulum (119–121). Similarly, old rabbit hearts had more depolarized mitochondria membrane potential and increased rate of ROS production associated with increased RyR activity and Ca^{2+} leak which was accentuated under conditions of β -adrenergic stimulation (122). Treatment with antioxidant dithiothreitol reduced RyR-mediated SR Ca^{2+} leak to levels of young hearts highlighting the role of thiol-oxidation of RyR in underlying pathological SR Ca^{2+} release (122). This response also appears to depend on calmodulin as a functional mediator of ROS-triggered Ca^{2+} release (119). In contrast to RyR2, the sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA) activity is reduced in response to ROS (123, 124). SERCA inhibition by oxidative stress appears to arise through multiple mechanisms including reduced ATP supply (125), direct oxidation of thiol groups by ROS (123), and CAMKII-dependent phosphorylation (74, 126). Interestingly, adult rat ventricular myocytes expressing redox-insensitive SERCA where C674 is replaced by serine (C674S) decreased

basal SR calcium content, attenuated the rise in mitochondrial Ca^{2+} , and prevented cytochrome c release and apoptosis (127). Furthermore, beyond ROS generation, dysfunctional mitochondria contribute to disrupted Ca^{2+} homeostasis through reduced Ca^{2+} storage capacity. Mitochondria function as an important cellular Ca^{2+} store with Ca^{2+} ions entering the inner mitochondrial membrane via the mitochondrial Ca^{2+} uniporter (MCU) (128). However, under conditions of metabolic stress, mitochondrial Ca^{2+} handling is disrupted (101). This results in increased size and frequency of cytosolic Ca^{2+} transients resulting in arrhythmogenic Ca^{2+} alternans (129). For example, in rat ventricular myocytes, stress induced by thoracic aortic banding enhanced mitochondrial Ca^{2+} accumulation and hence disrupted global Ca^{2+} handling, increased spontaneous Ca^{2+} waves, shortened RyR refractoriness and decreased SR Ca^{2+} content (130). These effects were inhibited by MCU inhibitor Ru360 which normalized RyR oxidation state, improved intracellular Ca^{2+} homeostasis and reduced triggered activity (130). However, other studies have produced contradicting evidence (130–133). In rabbit atrial myocytes MCU inhibitor Ru360 increased the severity of Ca^{2+} alternans whereas stimulation of Ca^{2+} uptake was protective (133). In fact, diabetic cardiomyopathy has been associated with abnormal mitochondrial Ca^{2+} handling with altered MCU expression and reduced mitochondrial Ca^{2+} levels (134, 135). As such, the mechanisms by which mitochondrial dysfunction contributes to abnormal Ca^{2+} transients remain controversial with further experiments required to clarify this relationship particularly in the context of the pro-arrhythmic aging heart.

Intriguingly, mitochondrial Ca^{2+} overload itself contributes to mitochondrial dysfunction and ROS generation (136, 137). This perpetuates a positive feedback cycle of ROS-induced Ca^{2+} overload, Ca^{2+} -induced ROS generation, and ROS-induced ROS release (74, 138, 139). This occurs via multiple mechanisms. Firstly, Ca^{2+} stimulation of the Krebs cycle and the electron transport chain increasing electron leakage and ROS by-products (140, 141). Secondly, Ca^{2+} -cardiolipin complexation disrupting mitochondrial lipid and protein arrangement causing proteins such as cytochrome c dislocation and inhibition of the electron transport chain and ROS production (142, 143). Thirdly, through activation of nitric oxide synthase generating NO radicals which itself has been shown to disrupt Ca^{2+} handling proteins (144, 145) but also to inhibit components of the respiratory chain and promote ROS production (146, 147).

Therefore, age-related mitochondrial dysfunction results in disrupted cellular Ca^{2+} handling causing elevated $[\text{Ca}^{2+}]_i$. The pro-arrhythmic consequences of elevated $[\text{Ca}^{2+}]_i$ are evident in CPVT hearts occurring due to mutations in cellular Ca^{2+} handling components, typically RyR2 or calsequestrin (148–150). This leads to potentially fatal ventricular arrhythmic episodes, often biventricular or polymorphic ventricular tachycardia and VF (151, 152). Interestingly, compared to aging mice where mitochondrial dysfunction disrupts multiple aspects of Ca^{2+} homeostasis, 129/Sv mice modeling CPVT demonstrated that altered function of only one of the Ca^{2+} handling proteins is sufficient to result in the proarrhythmic phenotype (150, 153).

Disrupted cardiomyocyte Ca^{2+} homeostasis develops a number of pro-arrhythmic pathways. Firstly, elevated $[\text{Ca}^{2+}]_i$ promotes the activity of the electrogenic NCX resulting in the generation of delayed after-depolarizations (DAD) which act as arrhythmic triggers (154, 155). As such, ROS causing cytosolic Ca^{2+} overload has been shown to stimulate NCX activity in guinea pig ventricular myocytes (112, 156). Secondly, dysregulation of Ca^{2+} handling allows pathological Ca^{2+} cycling which has been associated with APD alternans and spatiotemporal heterogeneities in repolarization (9, 157, 158). Thirdly, cytosolic Ca^{2+} interacts with surface membrane $\text{Nav}1.5$ and Cx channels causing reduced conduction velocity. Thus, Ca^{2+} regulates $\text{Nav}1.5$ and reduces I_{Na} through (1) directly binding to the EF hand motif, (2) associating with calmodulin and binding to the IQ domain, and (3) CAMKII-mediated phosphorylation (89, 90, 159). Inhibition of Cx function occurs through activating calcineurin-dependent Cx phosphorylation (160). Finally, increased cytosolic Ca^{2+} , through increased ROS production, promotes tissue fibrosis which is associated with slowed conduction velocity (161).

MITOCHONDRIAL DYSFUNCTION AND CARDIAC FIBROSIS

Aging is associated with increased cardiac fibrosis. Histological analysis of human hearts also demonstrates age-related progressive increase in collagen content and myocardial fibrosis (162, 163). Clinically, this is reflected in echocardiographic studies in both males and females which showed increased left ventricular wall thickness representing increased left ventricular hypertrophy (LVH) with age even in the absence of cardiovascular risk factors such as hypertension (5, 164). As such, age-related myocardial fibrosis has been shown to reduce ventricular elasticity, compromise left ventricular filling, and cause diastolic dysfunction (164, 165). Similarly, experimental mouse models also demonstrate increased collagen deposition in the aging myocardium (166). Transgenic premature aging ($\text{Polg}^{m/m}$) mice show increased interstitial and subendocardial fibrosis along with greater amyloid deposition, vacuolization of cytoplasm and hyaline cytoplasmic change (5, 167).

Increased cardiac fibrosis with age reflects a disruption in the equilibrium of extracellular matrix (ECM) synthesis and degradation. ECM synthesis is stimulated by fibrogenic growth factors, such as transforming growth factor (TGF)- β which induce fibroblast production of matrix proteins and protease inhibitors such as tissue inhibitors of metalloproteinases (TIMPs) (168). However, ECM degradation is dependent on tumor necrosis factor (TNF)- α and interleukin (IL)-1 β stimulating fibroblast production of matrix metalloproteinases (MMPs) (168). Hence, reduced MMP expression and inhibited ECM degradation appears to play a pivotal role in increased tissue fibrosis. As such, aging in murine models was associated with reduced MMP-1 and MMP-2 transcription and activity (169, 170).

With age, elevated ROS generation increases TGF- β and its downstream effector connective tissue growth factor (CTGF)

(168). TGF- β in turn activates Smad2/3 signaling inducing fibroblast proliferation, differentiation into myofibroblasts, and the production of ECM components such as fibrillar collagen, fibronectin, and proteoglycans (168, 171). This is supported by studies in C57BL/6 mice which found increased cardiac fibrosis under conditions of immune dysregulation and tissue inflammation known to promote ROS production (172). Additionally, mice overexpressing catalase targeted to the mitochondria showed reduced cardiomyocyte hypertrophy and significantly diminished cardiac fibrosis (167). Similarly, knock-out of SIRT3, which deacetylates the regulatory component of the mitochondrial permeability transition pore (mPTP), resulted in mitochondrial dysfunction, increased ROS production and accelerated signs of cardiac hypertrophy and fibrosis (173). Cardiac fibrosis also appears to be regulated by the renin-angiotensin system (RAS) signaling (174, 175). Angiotensin II (ANG II) activates the pro-fibrotic Smad2/3 signaling directly by acting on the ANG II type 1 receptor (AT1) and indirectly by promoting TGF- β production (168, 176). Furthermore, RAS has been shown to increase ROS levels through mechanisms including activation of NADPH oxidase (168, 177). ROS in turn have been found to promote the pro-fibrotic effects of ANG II which were suppressed through the application of antioxidants and AT1 antagonist losartan (177). Consistent with this, aged rat hearts demonstrate significantly increased angiotensin converting enzyme (ACE) and ANG II concentrations (167, 178, 179). Hence, mice carrying a gain-of-function mutation in the Ang II receptor type 1A developed early and progressive cardiac fibrosis (180).

Clinical studies strongly associate fibrosis with increased arrhythmic risk. For example, the origin of arrhythmia post-myocardial infarction is often mapped to the fibrotic border of the infarcted zone in patients undergoing ablation for recurrent VT (181). Similarly, most cases of AF are thought to originate from the atrial myocardial sleeve extending into the pulmonary veins. Histological analysis of pulmonary veins of patients with AF demonstrates increased myocardial content characterized by severe hypertrophy and fibrosis (182). Isolated Langendorff-perfused explanted human hearts with extensive infarction or dilated cardiomyopathy demonstrated increased vulnerability to triggering of VT due to cardiac fibrosis facilitating re-entry mechanisms (183, 184). Correspondingly, aged (24 months) Kunming mice had greater electrocardiographic abnormalities and inducibility of AF compared to young (2 months) mice which was associated to age-related increase in atrial fibrosis (185). Furthermore, transgenic Mkk4 knockout mice had dysregulated MMP function and upregulated TGF- β signaling causing increased susceptibility to atrial tachyarrhythmias (186). Importantly, these effects were more prominent in aged than young mice (186).

Interestingly, despite the primary biophysical defect of Nav1.5 haploinsufficiency being present from birth, BrS symptoms occur mainly in adulthood with mean age of SCD in BrS patients being 40 years (187, 188). The increased arrhythmogenicity later in age has thus been attributed to age-related structural changes primarily cardiac fibrosis (25, 188). Hence, old Scn5a^{+/-} BrS mice demonstrated reduced conduction

velocity and increased myocardial fibrosis compared to young mice (189, 190).

Age-related cardiac fibrosis increases arrhythmic tendency through a variety of mechanisms. Firstly, fibrosis causes slowed cardiac conduction velocity (82). Fibrosis creates strands of cardiomyocytes which are electrically isolated from each other by collagenous septa (191). Thus, this forces the action potential waves to follow a “zigzag” pattern, conducting circuitously from one strand to the other resulting in slowed conduction velocity (181, 192). Fibrosis also results in Cx-mediated cardiomyocyte-fibroblast coupling which increases cardiomyocyte membrane capacitance (C_m) slowing down action potential propagation (193, 194). Additionally, fibrosis reduces myocyte-myocyte coupling by decreasing Cx expression and promoting their redistribution away from the intercalated disc and hence increasing axial resistance resulting in slowed conduction velocity (195–198). Secondly, spatial heterogeneity in cardiac fibrosis and hence in compromised Cx function and altered ionic currents, including reduced Na⁺ current density, promotes APD alternans and dispersions of refractoriness causing unidirectional conduction block arrhythmic substrate (199–202). Additionally, patchy or interstitial fibrosis creates cardiomyocyte strands that are electrically coupled to nonfibrotic regions. Hence, creating a situation that reflects a 1 dimensional cable entering a 3 dimensional syncytium at which the interface acts as a “current sink” generating a “current-sink mismatch” due to the unequal transfer of depolarizing charge (191). Thus, if charge transfer to the syncytium is insufficient to depolarize the syncytium then action potential propagation fails (203). On the other hand, conduction from the syncytium to the 1-dimensional cable will succeed as the source-to-sink ratio is reversed. Therefore, this establishes a unidirectional conduction block facilitating arrhythmic re-entry circuits (191, 204).

TARGETED PHARMACOLOGICAL THERAPY

Elucidation of the mechanisms by which age-related mitochondrial dysfunction and ROS generation increases arrhythmic risk offers a number of potential anti-arrhythmic pharmacological targets. Some of these targeted therapies, differentiated from non-targeted antioxidant therapies, are highlighted in Table 1.

Antioxidant Therapy

Since mechanisms of ionic current dysregulation, disrupted Ca²⁺ homeostasis, and increased fibrosis all occur downstream of mitochondrial dysfunction, then it is likely that targeting upstream mitochondrial dysfunction and ROS generation will result in significant anti-arrhythmic effects.

Non-targeted Antioxidant Therapy

The first attempts to counteract oxidative damage in aging has been with the administration of non-targeted antioxidants such as vitamins E and C and β -carotene. While initial small studies indicated some protective effects of non-targeted antioxidants on cardiac function, meta-analysis of larger clinical randomized

TABLE 1 | Potential targeted therapeutics which may alleviate arrhythmogenic mitochondrial dysfunction.

Mitochondria-targeted pharmacological therapy	Mechanism	Therapeutic effect
Antioxidants		
TPP ⁺ conjugated antioxidants	Highly lipophilic antioxidants conjugated to strongly positive cations accumulate in mitochondria	Reduce ROS production Reduce mitochondrial component oxidation Reduce ROS-induced apoptosis and necrosis
Szeto-Schiller peptides	Cationic tetrapeptides, accumulate in the inner mitochondrial membrane	Scavenge ROS Reduce lipid peroxidation Reduce Ca ²⁺ induced mitochondrial swelling Reduce reperfusion injury
Modifiers of mitochondrial biogenesis		
SIRT1 activators	Upregulation of SIRT1 transcription	Increasing PGC-1 α expression Antioxidant properties (see above) Reduced NF- κ B activation Electrophysiological modifications: Inhibition of I _{Na-L} , I _{Ca-L} Reduction of intracellular Ca ²⁺ transients
Rapamycin	Inhibition of mTOR signaling	Reduced ROS production Reduced cardiac hypertrophy Normalization of age-related Ca ²⁺ homeostasis disruption Increased SERCA expression Reduced RyR current amplitude Increased mitophagy

PGC-1 α , Peroxisome proliferator-activated receptor gamma coactivator 1- α ; ROS, reactive oxygen species; RyR, ryanodine receptor; SERCA, sarco-/endoplasmic reticulum Ca²⁺-ATPase; TPP, triphenylalkylphosphonium ion.

controlled trials collectively involving tens of thousands of patients found no significant positive effects of non-targeted antioxidants on cardiovascular health or overall mortality (205–207). This may be due to the types of antioxidants investigated by clinical studies. For example, vitamin E has been shown to have pro-oxidant effects (208). Endogenous non-targeted antioxidant enzymes such as superoxide dismutase and catalase which were used in experiments to support the use of antioxidant therapy are not feasible in a clinical setting due to their size, rapid degradation, and potential antigenicity (5).

The failure of non-targeted antioxidants in clinical studies coupled to experimental findings that the source of ROS in aging arises primarily from the mitochondria has motivated the development of mitochondria-targeted antioxidants.

Triphenylalkylphosphonium Ion (TPP⁺) Conjugated Antioxidants

The highly negative mitochondrial membrane potential (150–180 mV) has been utilized to target molecules to the mitochondria. Thus, coupling lipophilic antioxidants to strongly positive cations such as TPP⁺ increases accumulation

in the mitochondria by 100- to 1000-fold compared to the cytosol (5). Such antioxidants include coenzyme Q (MitoQ), plastoquinone (SkQ1), and piperidine nitroxide in combination with triphenylphosphonium chloride (MitoTEMPO) (209, 210). Experimental studies found that they significantly reduce ROS generation, oxidation of mitochondrial components such as cardiolipin, ROS-induced apoptosis and necrosis, and prolonged lifespan of the fungus *Podospira anserina*, the crustacean *Ceriodaphnia affinis*, *Drosophila*, and mice models (210–212). Additionally, SkQ1 inhibited development of age-related conditions including retinopathy and osteoporosis in mammalian models of those conditions (210). In a rat model of H₂O₂- and ischemia/reperfusion-induced cardiac arrhythmias, treatment with SkQ1 for 3 weeks abolished the steady heart arrhythmia (213). Furthermore, experiments in a guinea pig model of non-ischemic heart failure that recapitulates features of prolonged QT interval and high incidence of spontaneous arrhythmic SCD, MitoTEMPO normalized cellular ROS levels, avoided and reversed heart failure, and prevented SCD by decreasing dispersion of repolarization and ventricular arrhythmias (214). So far, clinical trials are yet to investigate the anti-arrhythmic effects of TPP⁺ conjugated antioxidants on human patients.

Limitations of TPP⁺ conjugated antioxidants include their reliance on the mitochondrial membrane potential gradient. This gradient is disrupted with mitochondrial dysfunction in aging, as well as a direct effect of the antioxidants at high concentrations hence limiting their uptake and effectiveness (5, 211, 212). Additionally, at higher micromolar concentrations, these molecules appear to show pro-oxidant rather than antioxidant effects (212). It is thus important to clarify the window between anti- and pro-oxidant concentrations before proceeding to clinical trials.

Szeto-Schiller (SS) Peptides

SS peptides are synthetic aromatic-cationic tetrapeptides that selectively target and concentrate in the inner mitochondrial membrane (215, 216). Hence, *in vitro* experiments have shown that SS peptides scavenge ROS including hydrogen peroxide, hydroxyl radical, and peroxynitrite (215, 217). As such, they prevent lipid peroxidation as well as Ca²⁺-mediated mitochondrial swelling or reperfusion injury by inhibiting mitochondrial permeability transition and cytochrome c release (215, 216, 218, 219). In mouse models of ANG II-induced cardiomyopathy and G α q-overexpression induced heart failure, SS peptide administration prevented mitochondrial dysfunction and ROS generation, downregulated pro-oxidative pathways, and reduced cardiac hypertrophy and fibrosis (220). Similarly, in a rat model of ischemia-reperfusion injury, SS peptides significantly reduced myocardial lipid peroxidation and infarct size as well as reducing the frequency and severity of cardiac arrhythmias (221).

A significant advantage of SS peptides over MitoQ and SkQ1, is that SS peptides do not depend on the mitochondrial membrane potential gradient for accumulation in the mitochondria as they have been shown to concentrate in dysfunctional depolarized mitochondria (5, 215). Additionally,

unlike most oligopeptides, SS peptides are water soluble and have good transcellular permeability (215, 222).

Targeting Mitochondrial Biogenesis SIRT1 Activators (Caloric Restriction Mimetics)

Caloric restriction (CR) has been identified as one of the most potent interventions to improve health and slow down aging (223). Though the beneficial effects of CR are likely multifactorial, the sirtuin family of NAD⁺-dependent histone deacetylases, of which the predominant mammalian isoform is SIRT1, appear to be responsible for a large number of those beneficial effects (224, 225). SIRT1 acts through multiple pathways to regulate inflammatory responses, cellular senescence and its associated secretory phenotype, telomere attrition, and DNA damage responses (161, 226). As such, aging and its related mitochondrial dysfunction and ROS production are associated with decreased SIRT1 expression and activity (227). Thus, expression of SIRT1 was induced in rats undergoing CR and in human cells exposed to serum from CR rats, and in turn SIRT1 deacetylated the DNA repair factor Ku70 and sequestered the proapoptotic factor Bax away from mitochondria (224, 227). In mice, gain-of-function mutation of SIRT1 improved endothelial function through activating endothelial NO synthase (eNOS), preventing ROS production, inhibiting NF- κ B signaling by deacetylating RelA/p65, and reducing the inflammatory response (228). Similarly, other experiments replicated the positive effects of SIRT1 activation including enhanced mitochondrial biogenesis by inducing eNOS expression (229). Therefore, it is expected that compounds capable of activating SIRT1 will recapitulate the protective anti-aging effects of caloric restriction and hence prolong life and improve cardiovascular health including reduced arrhythmic risk. Resveratrol is one such compound being investigated. Its presence in red wine is thought to account for the cardiovascular protective effects of red wine drinking particularly in southern France (230). Resveratrol induced similar transcription profiles as SIRT1 and CR and promoted the same protective effects in heart, skeletal muscle and brain tissue in mice where it also prolonged lifespan and prevented age-related cardiac dysfunction (231).

One of the main mechanisms through which SIRT1 acts is through stimulating PGC-1 α expression which acts as an important regulator of mitochondria bioenergetics (232, 233). In rats, resveratrol demonstrated significant antioxidant properties in cultured aortic segments and endothelial cells through reducing ROS production and damage by reducing H₂O₂ levels and H₂O₂-mediated apoptosis, preventing UV-induced DNA damage, as well as increasing expression of antioxidant enzymes glutathione peroxidase, catalase, and heme oxygenase-1 (230). Similar antioxidant effects were reported in experiments using guinea pigs (234). Furthermore, it inhibited NF- κ B activation and reduced vascular tissue inflammation (235). As such, it has been shown to block age-related cardiac hypertrophy and fibrosis in animal models (173, 236, 237). Interestingly, resveratrol has been suggested to normalize intracellular Ca²⁺ in a murine model of chronic diabetes through increasing SERCA2a expression (238, 239). Moreover, resveratrol exerts its antiarrhythmic effects on cardiac electrophysiology through

regulating a number of ionic currents including inhibition of I_{Na-L}, inhibition of I_{CaL} and reduction in the amplitude of intracellular Ca²⁺ transients, (232, 237, 240, 241). Intriguingly, resveratrol effects on repolarization currents appear more complex with studies finding contradictory changes, nonetheless, in all of those studies the change exerted antiarrhythmic effects (232, 233, 241–243). Additionally, resveratrol promotes the inotropic effect of sympathetic stimulation, without enhancing their proarrhythmic effects and hence evading sinoatrial tachycardia (244).

Therefore, in a rat model where ventricular arrhythmias are enhanced via ischemia-reperfusion, application of resveratrol significantly reduced the severity of ventricular arrhythmia and mortality rate (245, 246). Similarly, in a rabbit model of heart failure, inducibility of atrial fibrillation was markedly reduced by treatment with resveratrol (237). These antiarrhythmic properties have been demonstrated under a number of different experimental models (232, 233, 240, 243) confirming the potential of resveratrol to act as an effective cardioprotective antiarrhythmic agent. While significant clinical data regarding the protective effects of resveratrol, particularly its antiarrhythmic potential, are yet to be obtained, initial clinical trials focusing pharmacokinetics and metabolism of resveratrol have found it to be safe and reasonably well-tolerated at doses of up to 5 g/day (247).

Rapamycin and mTOR

In addition, mammalian target of rapamycin (mTOR) is an important component of nutrient signaling pathways implicated in the aging process (248). mTOR is a protein kinase that forms the core of two protein complexes, mTOR complex 1 and mTOR complex 2, which play an important role in aging through regulation of a variety of cellular pathways controlling cell growth and proliferation (249). Of those, complex 1 appears to be more important in cardiac aging accelerating ribosomal synthesis and cap-dependent translation through phosphorylation of p70S6K (S6K1) and 4E binding protein 1, respectively (5, 249). mTOR signaling is increased with age reflecting its role in the aging mechanism but is normalized with caloric restriction in mice (250).

Inhibition of mTOR signaling through rapamycin has been shown to prolong lifespan in numerous animal models including mice (251). In a murine model of load-induced cardiac hypertrophy via aortic constriction, rapamycin application suppressed S6K1 levels and prevented cardiac hypertrophy (252). Furthermore, application of rapamycin following established cardiac fibrosis improved ventricular function and reversed cardiac fibrosis (253, 254). Similar results were replicated clinically where patients who received rapamycin following cardiac transplant had reduced cardiac hypertrophy and improved cardiac function (255). Rapamycin has also been shown to normalize age-related disruption in ion homeostasis particularly of Ca²⁺. As such, rapamycin increased SERCA expression, and reduced RyR current amplitude, elevation in [Ca²⁺]_i and activation of downstream Ca²⁺ pathways such as mitogen-activated protein (MAP) kinases (253, 256, 257). Mitochondrial ROS production and pro-arrhythmic

disturbances in Ca^{2+} homeostasis are also caused by age-related decrease in mitochondrial autophagy (mitophagy) (258–260). Autophagy is negatively regulated by mTOR. Hence enhancing autophagy via Torin1 potent mTOR inhibitor in aged rabbit hearts reduced the rate of ROS production and restored both the depolarized mitochondrial membrane potential and defective Ca^{2+} handling (261). Therefore, rapamycin pharmacological inhibition of mTOR may offer feasible anti-aging and hence anti-arrhythmic therapy. However, the anti-arrhythmic effects are yet to be explored by laboratory and clinical studies.

CONCLUSION

Aging is a cardinal risk factor for arrhythmic incidence in the general population and in individuals with inherited channelopathies. Aging is closely related to mitochondrial dysfunction which promotes arrhythmogenesis whereby it increases arrhythmic triggers and substrates via modifying sodium ($\text{Na}_V1.5$) and potassium (K_V , sarK_{ATP}) ion channels, gap junctions, Ca^{2+} homeostasis ($\text{Ca}_V1.2$, SERCA, RyR), and

tissue fibrosis. Hence, stratification using “mitochondrial health” as a marker of arrhythmic risk such as through the utilization of metabolomics to analyze biopsy samples allows identification of vulnerable patients amenable to pharmacological therapy. As such, a number of exciting pharmacological therapies targeting mitochondrial dysfunction have been discussed including targeted antioxidants (TPP⁺-conjugated antioxidants and Szeto-Schiller peptides), SIRT1 activators (resveratrol), and mTOR inhibitors (rapamycin).

AUTHOR CONTRIBUTIONS

KS: conceptualization. KS and IF: writing—original draft preparation, writing—review, and editing. Both authors have read and agreed to the published version of the manuscript.

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The Link Between Sex Hormones and Susceptibility to Cardiac Arrhythmias: From Molecular Basis to Clinical Implications

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It is well-known that gender is an independent risk factor for some types of cardiac arrhythmias. For example, males have a greater prevalence of atrial fibrillation and the Brugada Syndrome. In contrast, females are at increased risk for the Long QT Syndrome. However, the underlying mechanisms of these gender differences have not been fully identified. Recently, there has been accumulating evidence indicating that sex hormones may have a significant impact on the cardiac rhythm. In this review, we describe in-depth the molecular interactions between sex hormones and the cardiac ion channels, as well as the clinical implications of these interactions on the cardiac conduction system, in order to understand the link between these hormones and the susceptibility to arrhythmias.

Keywords: arrhythmia, sex hormones, cardiomyopathy, channelopathy, testosterone, estrogen

INTRODUCTION

Cardiac arrhythmias encompass a wide spectrum of clinical presentations, ranging from benign extrasystoles on electrocardiogram (ECG) to arrhythmias that may pose a significant clinical threat, such as atrial fibrillation (AF), which is an important cause of stroke, and ventricular tachycardia or fibrillation (VT/VF) that can result in sudden cardiac death (SCD) (1). A significant proportion of SCD events occurs in patients without any known cardiac disease, and even in the presence of diagnosis of a cardiac disorder, current knowledge on prediction of arrhythmic risk is limited (2). It is well-known that gender is an independent risk factor for some types of cardiac arrhythmias. For example, males have a greater prevalence of AF and the Brugada Syndrome (3, 4). In contrast, females are at increased risk for Torsade de pointes (TdP), both in congenital and acquired Long QT Syndrome (LQTS) (5). Despite the fact that the incidence of a variety of cardiac arrhythmias differs between men and women, the underlying mechanisms of these gender differences have not been clearly identified.

The predominant factors determining gender differences are the sex hormones. These steroid hormones are known to exert their multiple physiological effects by binding to cytosolic or membrane receptors (6). One of the target organs of action of these hormones is the heart, and there is now accumulating evidence indicating that sex hormones may have a significant impact on the cardiac rhythm (7, 8). It was initially thought that sex hormones only had genomic actions,

such as exerting their effects through the regulation of transcriptional processes, hence influencing gene expression after nuclear translocation (9). However, recent literature has shown that their scope of action goes well-beyond that, and encompasses non-genomic actions, as well. In fact, these hormones can acutely regulate cardiac ion channels and affect their currents, and these actions are mediated by transcriptional processes, such as RNA and protein synthesis (6–8).

In this review, we aim to describe in-depth the molecular interactions between sex hormones and the cardiac ion channels, as well as the clinical implications of these interactions on the cardiac conduction system, in order to understand the link between these hormones and the susceptibility to arrhythmias.

TYPES AND FUNCTIONS OF SEX HORMONES

Sex hormones play a key role in reproduction and sexual development. Moreover, they are also involved in other processes, such as regulating cholesterol levels and determining inflammatory response. They are produced by the gonads (ovaries and testicles) and adrenal glands. Estrogen and progesterone are the two main female sex hormones. Estrogen promotes growth of uterine tissue and breasts, maintains libido and secondary sexual characteristics in females, whereas progesterone has a significant role in the female menstruation cycle and pregnancy. The main male sex hormone is testosterone, but females also produce a small amount of this hormone. Testosterone affects many organs and has a variety of functions, including spermatogenesis, muscle growth, maturation of genitalia and bone metabolism (Figure 1).

Fluctuations in Sex Hormone Levels

The levels of sex hormones change during life as part of physiological processes. For example, the female sex hormones increase during adolescence and drop during menopause. Moreover, cyclic hormonal changes occur during the reproductive cycle. In certain situations, fluctuations of sex hormone levels can lead to health issues such as infertility, change in sexual desire, hair loss, osteoporosis, etc.

Sex hormone fluctuations also occur in the short-term. For example, there is a substantial circadian variation in the circulating levels of testosterone. Normal values for testosterone in a healthy adult male range between 300 and 1,000 ng/dL (10). The highest level of testosterone is reached in the morning, and therefore, it is recommended to determine it between 8:00 and 10:00 am in order to achieve accurate (and comparable) measurements (11). In addition, there may also be a seasonal variability of testosterone measurements. However, this is a contradictive issue, since there is both literature supporting and rejecting this hypothesis (12). After the third decade of life, men start to have ~1–2% decrease of testosterone per year (13). Finally, the amount of active serum testosterone is heavily reliant on the concentrations of sex hormone-binding globulin (SHBG) and albumin, thus being subject to fluctuations in those proteins. Such changes of testosterone levels across the healthy population

pose challenges in determining its effect on cardiac physiology, as values measured have to be adjusted for multiple factors, in order to have an unbiased result.

Estradiol (E2), the most potent estrogen, has the function of regulating reproductive cycles in females (Table 1, Figure 2). Evaluating estradiol levels is challenging, given that it changes widely during the menstrual cycle. Estradiol increases prior to ovulation and falls during ovulation, and there is a gradual increase of estradiol and then decrease prior to its lowest point in early menses. Moreover, it is also subject to a circadian variability, which is further affected by the menstrual cycle in post-menopausal women, estrogen levels decrease and show fluctuations (14).

Sex Hormones and Exercise

Both testosterone and estrogen play an important role in the neuromuscular adaptation to exercise in males and females. This adaptation is mediated by the hypothalamic-pituitary-gonadal (HPG) axis, which is responsible for both acute and chronic responses to exercise (15). In males, testosterone is pivotal for athletic activity during adolescence and in adulthood. While it is well-known that exercise acutely increases testosterone levels in men, its impact in the long-term is much less clear (16). Multiple cross-sectional studies have identified decreased testosterone levels in endurance and resistance athletes, as compared to controls. Furthermore, a study by Grandys et al. (17) found that testosterone levels vary significantly even within the athlete group, depending on the training period. In fact, testosterone increases during low-intensity training periods in comparison to high-intensity training periods. Conversely, in a cross-sectional study by Fitzgerald et al. (18) which compared trained cyclists to recreational cyclists, the former group had higher levels of serum testosterone than the latter. The conflicting results could be due to the retrospective nature of the studies evaluating the impact of chronic exercise. On the other hand, the few prospective studies also showed contradictory results, most likely due to differences in the training period, the magnitude of training stimulus and the volume of training load employed. In females, the acute effects of exercise training on the HPG axis are less well-known. Nindl et al. (19) have shown that estradiol may increase acutely after exercise, whereas the long-term effects of exercise still need to be determined.

THE INTERACTION BETWEEN SEX HORMONES AND CARDIAC ION CHANNELS

The differences in cardiac electrical activity between males and females had been observed with the earliest ECG recordings a century ago. In the meantime, while the scientific community has gathered a thorough understanding of the ECG as the net sum of electrical current flow of ions crossing the cardiomyocyte cell membrane, the molecular basis underlying gender differences are still largely debated (20).

The transit of ions across the cell membrane is permitted by specific transmembrane proteins, i.e., ion channels. Any

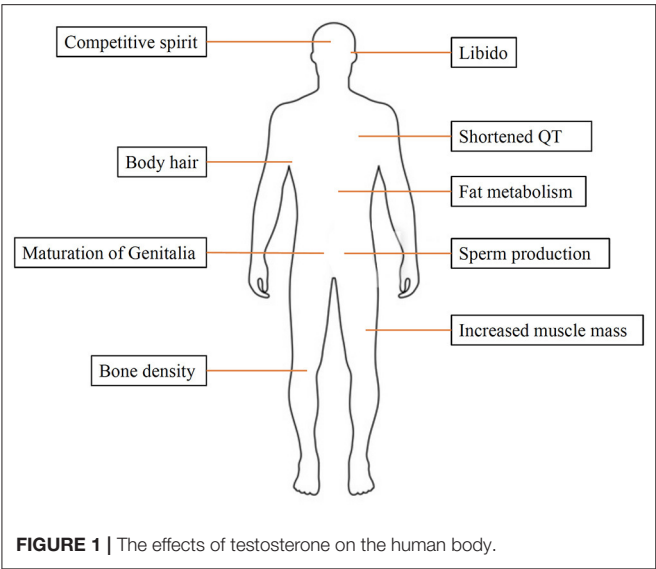


TABLE 1 | Sex hormones and their biological functions.

Sex hormones	Biological functions
Androgens	
Testosterone	Development of male genitalia, increase in muscle and bone mass, growth of body hair
Dihydrotestosterone	Catalyzed from testosterone, considerably more potent agonist of the androgen receptor
Androstenedione	Precursor of testosterone, endogenous pro-hormone, weak androgenic, and estrogenic activity
Estrogens	
Estrone (E1)	Weak estrogen, precursor of estradiol
Estradiol (E2)	Potent estrogenic hormone: regulation of the estrous and menstrual female reproductive cycle, development of female genitalia, bone growth and density, skin health, neuroprotective
Estriol (E3)	Weak estrogen, almost undetectable in non-pregnant women, high levels during pregnancy
Estetrol (E4)	Weak estrogen found only in pregnancy, physiological function unknown

change that affects the flow of ions through these channels will have an impact on the ECG (21). The voltage-gated channels are the major ion channel group in charge of cardiac electric activity. These possess a rapid response mechanism to changes in membrane voltage and interact with each other to produce the cellular action potential (AP). In the myocardium, the rapidly activating sodium channel is the principal driver for cellular depolarization, while L-type calcium current is responsible for the plateau phase and the delayed rectifier potassium current is mostly in charge of the repolarization. These give rise to the clinically measurable QRS complex, the magnitude and dispersion of which are strongly influenced by changes to the sodium current, and the QT interval, which is dependent on the calcium and potassium currents (22). These parameters are also widely influenced by cell-cell connections, which are mediated by

the connexin family of proteins at gap junctions, and therefore, ECG abnormalities often occur in diseases of the connexome (e.g., arrhythmogenic right ventricular cardiomyopathy). A decrease in cell-cell conductance typically leads to a longer QRS duration with or without terminal activation delay (23). Since the above-mentioned gender differences are not present at birth, but only appear at puberty, this has led to the hypothesis that they are dependent on sex hormone regulation (24).

Cardiac myocytes have a variety of receptors for sex hormones, specifically estrogen, progesterone and testosterone, whose activation can alter the electrical activity of the heart through modulation of ion channels. Although literature is scarce, fluctuating hormone levels can lead to changes in the behavior and expression of myocardial ion channels (Figure 3) (25).

The Effect of Sex Hormones on Ventricular Ion Channels

Ventricular Potassium Channels

Potassium channels play a pivotal role in the regulation of muscle excitability. Potassium conductance through potassium channels present on the plasma membrane enables the membrane potential to reach the equilibrium potential of potassium, leading to hyperpolarization or accelerated repolarization of action potential in muscle cells. This results in a controlled cell excitability, elicited by various signals such as depolarization of membrane potential, ligand-binding, and so on (26). Sex hormones can regulate these via modulation of gene expression (by binding to their unique nuclear receptor) or non-genomic pathways after binding to membrane receptors. The rapid non-genomic effects of sex hormones are exerted through the activation of specific signaling pathways, which are initiated from sex hormone receptors or binding proteins in the plasma membrane and cytosol. Furthermore, sex hormones may also directly bind to potassium channels or the auxiliary subunits to modulate the activities of potassium channel blockers and potassium channel openers.

Several different types of currents pass through the potassium channels, i.e., the transient outward current (I_{to}), the rapid-delayed rectifier current (I_{kr}), the slow delayed-rectifier current (I_{ks}), and the inward rectifier potassium current (I_{kl}). However, there are contradicting reports concerning the interaction of sex hormones and the potassium channels. Previous studies either showed no influence of sex hormones on I_{to} (27) or greater expression in males than in females (28, 29). Consequently, an increased expression of this current was demonstrated in ovariectomized mice, with a concurrent increase in their associated channel mRNA (30). This has been confirmed by the fact that in the high estradiol state (as is pregnancy), a reduction of I_{to} is observed (31).

Similarly to the I_{to} , the I_{kr} has also been shown to be either not influenced by gender (29), or less expressed in females (32). This was confirmed in an *in vitro* study upon acute administration of exogenous estradiol on cultured guinea pig cardiomyocytes with human ether-a-go-go-related gene (hERG) overexpression, which unmasked the blockage of the hERG channel and thus

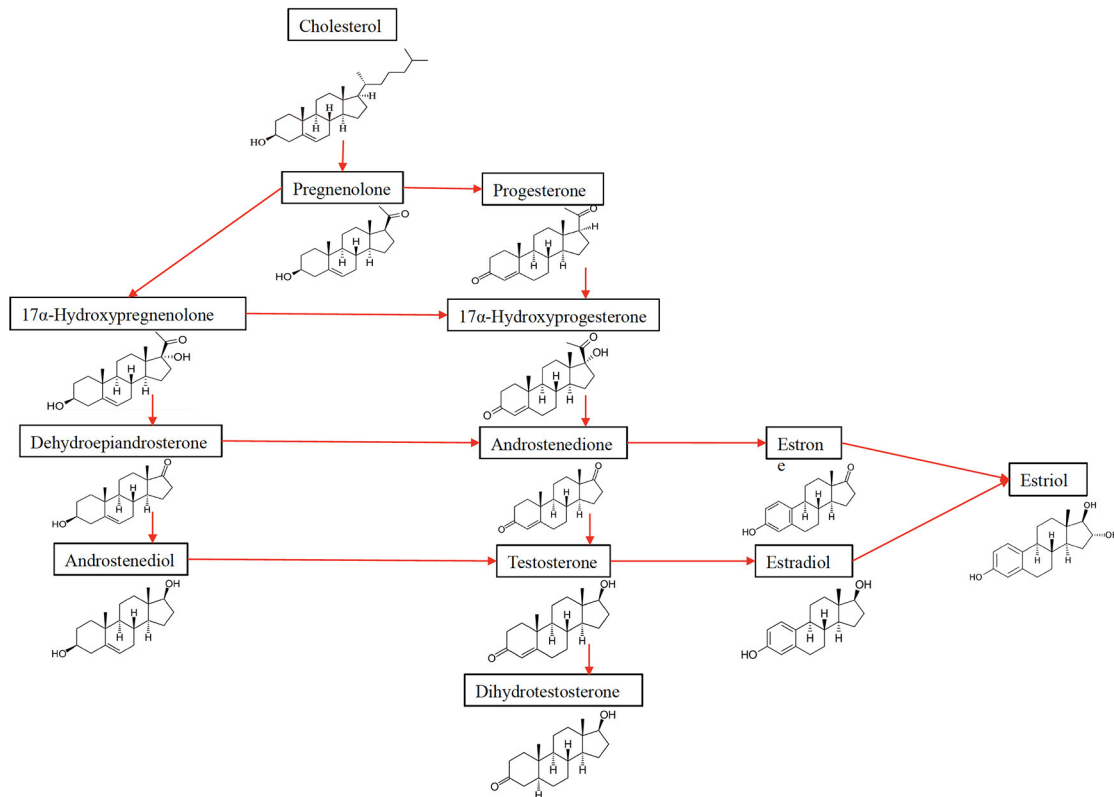


FIGURE 2 | The synthesis of sex hormones from their precursor cholesterol.

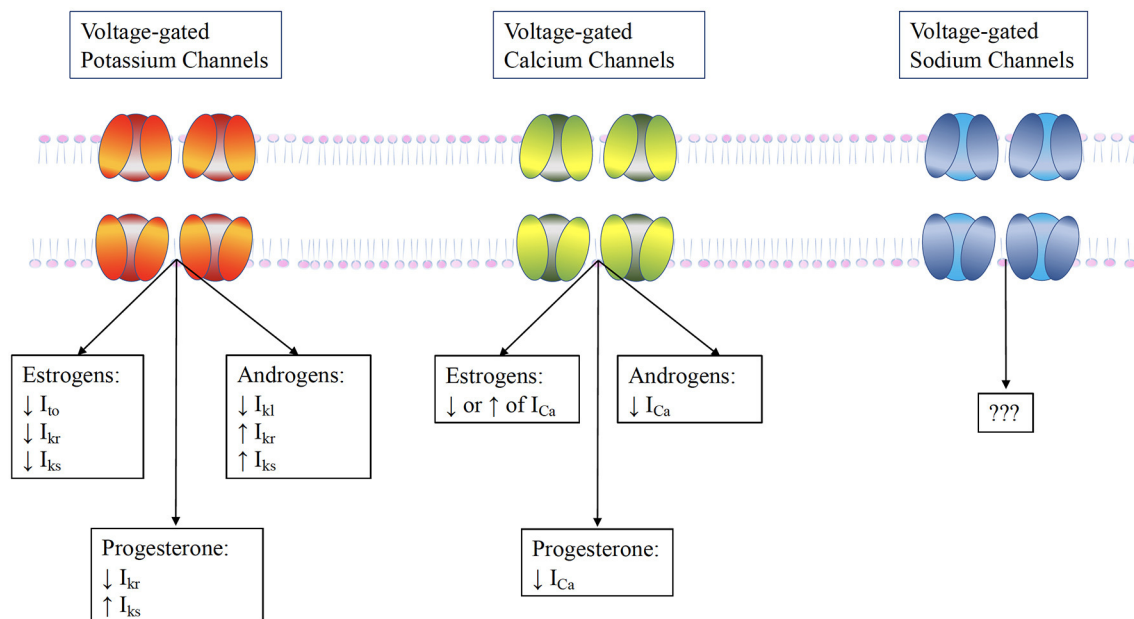


FIGURE 3 | The influence of sex hormones on ventricular ion channels. Testosterone increases repolarizing currents I_{kr} and I_{ks} and the I_{to} , while it acutely increases the L-type calcium current I_{CaL} . Estrogen has a more complex effect. It directly blocks the I_{kr} , but may also increase it by promoting HERG trafficking. Furthermore, it reduces the KCNE1, thereby reducing I_{ks} . These alterations result in prolongation of AP duration by estrogens, but its shortening by testosterone.

I_{kr} (33). In various animal models, the I_{ks} has been observed as either larger in males (34), larger in females or with no gender difference at all (29), depending on which region the cells were taken from. This is in line with increasing evidence suggesting that the differences exerted by sex hormones may not be uniform across the heart. As in the case of I_{kr} , this current has also been shown to be blocked upon administration of exogenous estradiol through blockage of its related channels (KCNQ1/KCNE1) (35). Interestingly, while this current is enhanced by progesterone, as shown in the *in vitro* patch clamping study by Nakamura et al. (36) the hERG channel trafficking is reduced with progesterone. Furthermore, it has been demonstrated in guinea pig ventricular myocytes through acute applications of physiological serum levels of testosterone that this increases the outward potassium currents (I_{kr} and I_{ks}) and the inward rectifier current (I_{kl}) (7). Thus, progesterone and testosterone shorten the ventricular APs, while estrogen lengthens the APs and may exert a pro-arrhythmic effect (8).

Ventricular Calcium Channels

The L-type Ca^{2+} channels are heteromultimeric proteins, consisting of multiple subunits. Similar to the mechanism with the above-mentioned potassium channels, signaling of gonadal steroids has traditionally been associated to a genomic action, i.e., the transcriptional control of target genes through binding of the nuclear receptors and ligands to the genomic consensus sequence in reproductive organs. However, several biological actions of gonadal steroids have recently been shown to be too rapid to be compatible with transcriptional mechanisms.

The evidence on interaction of sex hormones and the cardiac calcium channels is conflicting. In fact, there are reports of increased (29), decreased (32), or indifferent calcium currents (I_{Ca}) in females as compared to males (27). Similarly to what was observed for the cardiac potassium channels, there is evidence of regional heterogeneity in the different interactions between sex hormones and ion channels (37). In fact, in animal studies, gender differences were observed for the I_{Ca} in the apex, but not in the base of the left ventricle of rabbits (37). Moreover, an increase in L-type calcium channels was shown in the basal cardiomyocytes of rabbits by incubating these with physiological concentrations of estrogen. This effect, however, was not seen in the apical cardiomyocytes (38). Acute administration of progesterone was shown to decrease the I_{Ca} by 60% (8). Interestingly, ovariectomized mice had an increase in the expression of L-type calcium channels, which were reversed upon administration of exogenous estrogen (39). Testosterone also plays a part in the modulation of the voltage-gated calcium channels. It is a selective and potent inhibitor of L-type calcium channels of vascular smooth muscles (40). The likely reason may be the fact that it blocks the major α_1 subunit of the L-type channel, similarly to the effects of the commonly prescribed calcium channel antagonists.

Ventricular Sodium Channels

The influence of sex hormones on the cardiac sodium channels are yet unknown. Even though there is no reported association between sex hormones and the LQT3 (41), there is increasing

evidence linking sex hormones to the phenotypic expression of the Brugada Syndrome (42), despite the fact that both diseases stem from cardiac sodium channel mutations. The interesting link between the Brugada Syndrome and sex hormones is thoroughly discussed in the later section.

Ventricular Ryanodine Receptor

The contractile function of the heart is determined by intracellular calcium concentration, which is influenced by the expression and activity of calcium regulatory proteins. When the sarcolemma is depolarized, the voltage-gated L-type calcium channels are opened and the influx of calcium into cytosol increases. This influx induces a massive release of calcium from the sarcoplasmic reticulum via the ryanodine receptor (RyR). This results in a sudden increase in calcium, which then triggers cardiac contraction. Tsang et al. (43) demonstrated how testosterone increased contraction and relaxation velocities that were associated with increased calcium release and recovery through activities of the RyR receptor and sarcoendoplasmic reticulum calcium transport ATPase (SERCA2). This observation may explain the predominantly male phenotype in catecholaminergic polymorphic ventricular tachycardia (CPVT).

SEX HORMONES AND VENTRICULAR ARRHYTHMIAS

Sex hormones may alter the susceptibility to ventricular tachyarrhythmias in patients with underlying structural heart disease and in those with cardiac channelopathies.

Coronary Artery Disease

The higher incidence of coronary artery disease, and especially of SCD, in male patients but also in females following menopause, has highlighted the role of estrogen as a cardioprotective hormone. It was shown that estrogen has beneficial effects by improving cardiac function, preserving calcium homeostasis and inhibiting the mitochondrial apoptotic pathway (44). In the context of an ischemic insult to the heart, reperfusion that accompanies the opening of a blocked coronary artery may trigger arrhythmias and result in SCD. Estrogen was shown to decrease reperfusion arrhythmias in multiple animal studies. Savergnini et al. (45) demonstrated in young female rats that administration of estradiol was protective against this type of arrhythmia. This finding was also confirmed by Wang et al. (46) who showed that this action was mediated by the genomic action of estrogen, mainly the upregulation of the Estrogen Receptor β (Er β) activation.

The role of testosterone during cardiac ischemia and in the prevention of reperfusion arrhythmias is controversial. Preclinical studies showed that pretreatment with testosterone of rat hearts exposed to ischemia decreased arrhythmias, as effectively as it was the case after estradiol administration (47). Likewise, testosterone replacement was shown to exert cardioprotective effects in orchietomized rats (48). In another study in isolated rat hearts exposed to acute ischemia,

testosterone significantly reduced norepinephrine release and consequent arrhythmias (49).

Arrhythmogenic Cardiomyopathy

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited heart disease that is associated with life-threatening ventricular arrhythmias. It is one of the leading causes of SCD, especially in the young, athletic population (50). While risk stratification traditionally relied on measurements of structural dysfunction and electrophysiological indices (51), there have been recent attempts to identify circulating biomarkers for prediction of arrhythmic risk. Among these, sex hormones, and particularly testosterone, seem to play an important role in arrhythmogenesis. In the study by Akdis et al. (52) our group investigated the role of sex hormones and their association with major arrhythmic cardiac events (MACE) in patients with ARVC. We observed that elevated serum testosterone levels were independently associated with MACE in male ARVC patients, whereas estradiol levels were significantly lower in female patients with MACE. These findings have recently been validated in another study by Ren et al. (53) in a different ARVC cohort. The study showed that testosterone levels were a strong predictor of future adverse arrhythmic events in male patients with ARVC, independently from baseline systolic function. However, there was no association between the circulating levels of sex hormones and future heart failure events.

It is important to note that SCD may occur during early, clinically occult stage of ARVC, which still bears the pathological hallmarks of progressive cardiomyocyte loss and fibrofatty infiltration. Hence, there is an underlying electrophysiological substrate in ARVC that can facilitate reentry. This is in contrast with the mechanisms of arrhythmogenesis in patients with outflow tract ventricular tachyarrhythmias in the absence of structural heart disease. The study by Hu et al. (54) did not reveal any differences in circulating testosterone levels between diseased and control males with such idiopathic arrhythmias, while estradiol levels were indeed lower in diseased males. While the proarrhythmogenic effect of testosterone in ARVC may be partially related to increased apoptosis and lipogenesis, as shown in the study by Akdis et al. (52) arrhythmia susceptibility in patients with idiopathic outflow tract tachyarrhythmias may be due to estrogen deficiency and its consequences on the cardiac calcium and potassium channels, as discussed previously.

Takotsubo Syndrome

Takotsubo Syndrome is characterized by acute and mostly transient left ventricular dysfunction, which is often preceded by emotional or physical triggers. The patients have a substantial risk for MACE (55). The fact that this condition typically occurs in post-menopausal women (up to 90% of cases) has pointed out the role of estrogen deficiency as a causal factor, although pathophysiological mechanisms underlying the disease are still largely debated. The lack of estrogen together with an excess in catecholamines may not only underlie the pathophysiology of the disease itself, but also the risk of arrhythmic complications. This has been shown in a small study by El-Battrawy et al. (56) on hiPSC-CMs, which demonstrated that catecholamine excess

may increase reactive oxygen species (ROS) production, which in turn enhances the late sodium current (I_{Na}) and suppresses the I_{to} , causing prolongation of AP duration. Interestingly, when these hiPSC-CMs were treated with estradiol, this reduced their sensitivity to catecholamines by reducing adrenoreceptor expression, and thus, showed a protective effect of estrogen in the context of this disease. Further studies are needed to determine the exact pathophysiological and prognostic role of sex hormones in Takotsubo Syndrome.

Cardiac Channelopathies

Long QT Syndrome (LQTS)

This condition defines prolonged rate-corrected QT intervals (QTc) in patients who are at risk for ventricular tachyarrhythmias (typically TdP) and SCD (36). LQTS can be either congenital or acquired (as is the case for drug-induced LQTS). Female sex is known to be an independent risk factor, as females have 10–20 ms longer QTc intervals. However, the gender difference only manifests after puberty, suggesting that female sex hormones may play a role in the increased propensity for arrhythmias (36, 57, 58).

The QTc is most affected by alterations in phase 2 and 3 of the AP, which are dominated, respectively, by the L-type calcium current (upregulation lengthens the QTc) and the delayed rectifier potassium currents (I_{Kr} and I_{Ks} upregulation shortens the QTc). As mentioned previously, estrogen, progesterone and testosterone have varying effects on these currents, which could explain the gender differences (7). While the hypothesis that increased estrogen might be responsible for arrhythmogenesis in females has not been proven in humans yet, there are several clinical studies showing that QTc and arrhythmogenicity may increase in males with decreased testosterone. A recent study by Salem et al. (59) has reported that male hypogonadism secondary to androgen-deprivation therapies (ADTs) is associated with acquired LQTS and increased incidence of TdP. Furthermore, the same group has also studied hiPSCs from men and treated them either with ADTs and dihydrotestosterone, reporting that while the ADTs indeed prolonged the QT interval, this was acutely reversed upon dihydrotestosterone administration (60).

Brugada Syndrome

This genetically-determined disease is characterized by the appearance of a coved-type ST segment elevation in the right precordial ECG leads, and puts the patients at significant risk for SCD in the absence of an underlying structural heart disease (61). The most common genetic mutations found in affected patients (up to 25%) are loss-of-function mutations in the SCN5A gene, which encodes the α subunit of the cardiac sodium channel protein ($Nav_{1.5}$) (62). Despite the autosomal dominant inheritance of the disease, its incidence is significantly higher in males (8:1 in Western countries and 9:1 in Asia). Indeed, a study by Shimizu et al. (42) showed that males with the Brugada Syndrome had significantly higher testosterone levels as compared to their control counterparts, even after adjusting for age, exercise, stress, smoking habits, and medications. Furthermore, there are a few interesting case reports correlating

the Brugada ECG pattern to blood testosterone levels. Matsuo et al. (63) described two cases of males with an asymptomatic Brugada ECG pattern, which disappeared following surgical castration for prostate cancer. Another recently published case study by Sichrovsky et al. (64) has demonstrated the development of a previously unrecognized Brugada pattern and SCD in a genetic female living as a transgender male through the use of exogenous testosterone.

As previous literature has suggested, the accentuation of the epicardial AP notch and loss of its dome induced by a potassium channel opener, seems to be the cause of the typical Brugada-type ST segment elevation. This can result in phase II reentry and trigger ventricular tachycardia or ventricular fibrillation (65). Moreover, it matches the evidence of the sodium channel being the primary gene candidate for the Brugada Syndrome. Since either a decrease in the density or an acceleration of inactivation of the sodium channel would leave I_{to} unopposed during the early phases of the AP, the mechanism of the AP notch accentuation may indeed be due to a more prominent I_{to} , which is physiologically more expressed in males as compared to females. This has been proven in a dog model, showing that the I_{to} current density of the RV epicardium is significantly higher in males than in females, culminating in increased transmural dispersion of repolarization (4). This substrate facilitates the presence of the Brugada-type ECG pattern and occurrence of arrhythmias in males. Furthermore, as previously mentioned, testosterone may augment outward repolarizing currents (such as I_{kr} and I_{ks}), and thus, lead to loss of the AP dome, explaining the male predominance of the Brugada Syndrome. A recently published *in vitro* study by Yang et al. (66) examining the effects of estrogen and testosterone on the wild-type and the mutant $Nav_{1.5}$ has shown no influence of these hormones on either channel, but this study had the limitation of lacking an *in vivo* validation.

Catecholaminergic Polymorphic Ventricular Tachycardia

CPVT is an inherited arrhythmic syndrome, which affects 1 in 10,000 individuals. It is characterized by potentially lethal ventricular arrhythmias, which are often biventricular or polymorphic in origin, and which mostly appear following an adrenergic challenge precipitated by emotional or physical stress (67). CPVT arises from disrupted intracellular calcium homeostasis and is most often associated with gene abnormalities involving the RyR2. The resulting dysregulated RyR2-mediated leak of sarcoplasmic reticular calcium elevates cytosolic calcium, increasing electrogenic sodium/calcium exchanger and/or calcium-activated chloride transient inward currents. The consequently occurring delayed afterdepolarizations may trigger arrhythmic events. Interestingly, the majority of phenotypes related to RyR2 mutations show a higher mortality in males as compared to females. The molecular basis for this entity has been explored in a recent study by Saadeh et al. (68) in a murine model of CPVT (homozygotic RyR2^{S/S}), in which they investigated the impact of gender on the expression levels of molecular determinants of calcium homeostasis and conduction velocity. While the authors have found no difference in the expression levels of calcium homeostasis proteins, they showed a decreased

Cx43 expression, which correlated with slowed conduction velocity in female mice, but not in males.

HORMONE REPLACEMENT THERAPY AND ARRHYTHMIAS

Male hypogonadism, which may arise from multiple etiologies including androgen-deprivation therapy (ADT), has been reported as a risk factor for acquired LQTS and the occurrence of TdP. This has led to multiple pharmacovigilance studies assessing the link between hormone replacement therapy (HRT) and the incidence of ventricular arrhythmias. Interestingly, this has helped to shed some light on the link between arrhythmias and sex hormones, supporting the hypothesis that hypogonadism is a correctable and identifiable risk factor for TdP, especially in men. This has clinical implications, for example while considering ADT, which is the cornerstone of the treatment of prostate cancer.

Ventricular Arrhythmias

Male hypogonadism is a condition in which clinical symptoms occur due to testosterone deficiency (69). As discussed previously, low testosterone values may be associated with a higher risk of ventricular arrhythmias and SCD due to a lengthening of the myocardial repolarization phase. In fact, the QTc values of males after puberty are significantly shorter and aging men with decreasing testosterone levels seem to have a gradual increase in QTc. Some types of male hypogonadism can be treated with testosterone replacement therapy (TRT). The data available on the effects of TRT on cardiovascular risks are contradicting. The RHYME study, which investigated hypogonadal men receiving TRT, did not find any increased cardiovascular risk (70). The same results were shown in a systematic review by Corona et al. (71) which reported the absence of a causal role between TRT and cardiovascular events. Interestingly, a study by Muensterman et al. (58) reported that in older men, the use of transdermal testosterone combined with oral progesterone attenuates drug-induced QTc lengthening. This shows that there may be a probable protective role of both testosterone and progesterone on arrhythmia occurrence due to prolonged QTc.

Concerning hormone replacement in females, there are several studies in post-menopausal women showing that estrogen replacement therapy (ERT) prolongs the QTc interval. Indeed, as mentioned above, estrogen has a lengthening effect on the myocardial repolarization phase. This effect is not evident in female children, but only manifests itself after adolescence, and significantly decreases after menopause (72). A study on hormone replacement for 1 year confirmed that the use of ERT increases the QTc interval (73). Interestingly, this effect was not seen in combined estrogen-progestin replacement therapies, strengthening the theory that progesterone most likely has a similar effect on the QTc interval as testosterone. It is important to note that a study by Saba et al. (74) did not find any significant difference in QTc interval between premenopausal, post-menopausal and post-menopausal women treated with hormone replacement. A major limitation of this study was

that it was not clear whether hormone replacement consisted of estrogen alone or combined with progesterone in the study population (74).

CONCLUSIONS

Gender is known to be an independent risk factor for some types of cardiac arrhythmias. However, the link between the sex hormones and susceptibility to arrhythmias is still a matter of debate. Nonetheless, despite conflicting results, these hormones may influence arrhythmia occurrence both in the presence or absence of underlying structural heart disease. Further studies, which validate or contradict the already present literature, will be of invaluable importance to fully understand the pathophysiological mechanisms that lie at the basis of arrhythmogenesis.

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AUTHOR CONTRIBUTIONS

SC manuscript drafting and data collection. AS manuscript drafting and review. AG and DA data collection and manuscript review. CB funding recruitment and manuscript review. FD coordinator, manuscript structuring, and manuscript review. All authors contributed to the article and approved the submitted version.

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The Key Role of Uric Acid in Oxidative Stress, Inflammation, Fibrosis, Apoptosis, and Immunity in the Pathogenesis of Atrial Fibrillation

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Atrial fibrillation (AF) is a highly prevalent cardiac arrhythmia that leads to numerous adverse outcomes including stroke, heart failure, and death. Hyperuricemia is an important risk factor that contributes to atrium injury and AF, but the underlying molecular mechanism remains to be elucidated. In this review, we discussed the scientific evidence for clarifying the role of hyperuricemia in the pathogenesis of AF. Experimental and Clinical evidence endorse hyperuricemia as an independent risk factor for the incidence of AF. Various *in vivo* and *in vitro* investigations showed that hyperuricemia might play a critical role in the pathogenesis of AF at different UA concentrations through the activation of oxidative stress, inflammation, fibrosis, apoptosis, and immunity.

Keywords: uric acid, atrial fibrillation, mechanisms, oxidative stress, inflammation

INTRODUCTION

Atrial fibrillation (AF) is considered to be the most frequent cardiac arrhythmia and its prevalence is increasing substantially. In 2016, 46.3 million individuals had prevalent AF/atrial flutter globally, and the prevalence of AF has been estimated between 2 and 4% in adults (1). Also, the prevalence of AF is expected to rise more than double in the next three decades, largely owing to the extended life expectancy of the general population, intensifying search for undiagnosed AF (2), and longer survival with chronic conditions (3). AF is associated with a 5-fold risk for stroke and is estimated to cause 15% of all strokes (4), and among various cardiac arrhythmia, AF is receiving significant attention for its contribution to cardiac mortality and morbidity (5).

Chronic diseases such as rheumatic heart disease, hypertension, hyperthyroidism, chronic kidney disease, and diabetes mellitus have all been regarded as risk factors for AF (6, 7). Although the pathophysiology underlying AF remains to be fully elucidated, inflammation and oxidative stress are partially known for their involvement in the pathogenesis of AF (8). Recently, increased focus has been given to the possible mechanism by which hyperuricemia causes AF. Similarly, the link between hyperuricemia and other conditions such as hypertension, metabolic syndrome, diabetes mellitus, and chronic kidney disease has been reported (9–11). Uric acid [UA; 7,9-dihydro-1H-purine-2,6,8(3H)-trione; C₅H₄N₄O₃; molecular weight of 168.11 Da] is a heterocyclic organic compound and an end product of purine metabolism in humans. UA acts as an antioxidant and pro-oxidant at its normal and high concentration, respectively (12). Difficulties in determining whether UA acts as a risk marker or a risk factor for AF remained debatable due to the frequent association and intricate relationship with other cardiovascular risk factors. Despite such controversy, the interest in UA has recently resurrected.

A recent review on the role of UA in cardiovascular diseases (CVD) has focused on summarizing the association between uric acid and various cardiovascular diseases, mainly from experimental evidence (13). Also, the review was shallow in the area of AF despite its well-organized content on the relationship between UA and CVD. Bearing in mind the significance of bridging the experimental findings with clinical evidence, it is undoubtedly important to summarize the association of UA with AF based on both experimental and clinical evidence. Therefore, this review will discuss the potential mechanism on how UA involves in AF pathophysiology. In particular, our review will summarize the effects of fibrosis, apoptosis, and immunity on the progress of AF and how elevated UA associated with hypertension and metabolic syndrome aggravates the risk of AF.

THE EXPERIMENTAL EVIDENCE IN AF

Bearing in mind that the extensive overlap exists between comorbidities and risk factors of hyperuricemia and arrhythmias, multivariable analyses of epidemiologically collected data cannot substitute proof generated from basic and clinical studies. As such, there is a need for further basic research to establish a causal relationship between UA and AF and to identify the mechanism by which UA is involved in AF pathology. Elevated UA can cause arrhythmia either directly or indirectly, depending on the availability of other risk factors. In this review, we have summarized *in vitro* and *in vivo* studies regarding UA acting on several cellular signaling pathways in **Table 1**. The summary of the elevated UA induced cardiac remodeling related to electrophysiological and structural alterations via various mechanisms, including oxidative stress, inflammation, fibrosis, apoptosis, and immunity is described in **Figure 1**.

UA Participates in the Progress of Hypertension-Induced AF

Clinically, hypertension is an independent risk factor for AF. In the past, it was well-established that UA and nitrate concentrations were positively associated with elevated blood pressure (27). Some researchers also demonstrated that UA, the most abundant antioxidant in plasma, reacts directly with nitric oxide (NO) in a rapidly irreversible reaction resulting in the formation of 6-aminouracil and depletion of NO (28). Also, elevated UA was found to inhibit the production of NO in bovine aortic endothelial cells caused by the vascular endothelial growth factor (27). This evidence proves that hyperuricemia-induced vascular insufficiency can be achieved by reducing NO production. Besides, the angiotensin system is also believed to involve in UA-induced NO production reduction. According to earlier evidence, UA activates the renin-angiotensin system and inhibits NO production by downregulating NOS1 expression.

UA can also indirectly affect NO production through classical inflammatory pathways. Different concentration of UA *in vitro* has been found to involve I κ B α phosphorylation via NF- κ B activated inflammatory signaling pathway. This leads to the up-regulation of inducible NO synthase (iNOS) expression and

excessive NO production, which subsequently contribute to the injury of cells (17).

Elevated blood pressure is a known risk factor for AF, and hypertension often coexists with AF. Whereas, hyperuricemia, as a risk factor of AF, can directly trigger the occurrence of AF or indirectly contribute to AF through a hypertension-induced mechanism. Mazzali et al. used oxonic acid (OA) to create a mild hyper-UA model *in vivo*. After a low-salt diet in the rats, they found that the diameter of the arterioles of the mice with hyperuricemia was shortened and the blood pressure increased significantly (22). What's more, vascular hypertension can cause an increase in cardiac preload, resulting in left atrial structural remodeling (26). Studies have shown that after entering into cells through an organic anion transport, UA stimulates the proliferation of vascular smooth muscle cells. The changes in the vascular muscle cells may increase the thickness of the vascular wall, which could further result in cardiac afterload, and long-term atrial structural remodeling (29, 30), which could be the potential mechanism of AF.

UA Participates in the Progress of Metabolic Syndrome-Induced AF

Experimental studies proposed that UA may have a causal role in metabolic syndrome (MetS) and obesity (22, 31). Earlier evidence pointed out that UA enters the cell through UA transporters, and intracellular UA increases the activity of xanthine oxidase (XO) and NADPH oxidase (NOX) (18). As a result, these activities promote the formation of superoxide. These common UA transporters include URATv1, ABCG2, MRP4, and MCT9 (16). A piece of evidence revealed, soluble UA provokes an increase in NOX activity in differentiated 3T3-L1 adipocytes by promoting the action of URATv1. The NOX activity *per se* is a cytoplasmic enzyme consisting of at least one catalytic transmembrane-spanning NOX subunit, which produces ROS by transferring electrons from NADPH to molecular oxygen. In addition, the reduction of bioavailability can result in the down-regulation of NO and an increase in protein nitrosylation and lipid oxidation (14, 19). Consequently, the formation of downstream superoxide-dependent ROS is increased, which leads to the up-regulation of monocyte chemotactic protein (MCP-1). These pathophysiological alterations can eventually lead to obesity-related low-grade inflammation, metabolic syndrome, and cardiovascular diseases (32).

The Role of Oxidative Stress on the Progress of Elevated UA-Induced AF

A substantial body of evidence suggests that oxidative stress plays a key role in the pathophysiology of AF. However, the molecular pathways of this pathologic process are complex. Therefore, oxidative stress and its modulation in AF require the development of strategies that target specific sources of ROS implicated in atrial remodeling (33). XO is deemed to be a key enzyme in UA metabolism, which is also a critical source of reactive oxygen species (ROS), free radicals responsible for oxidative damage (34) in cardiovascular diseases (35). A study that involved a histochemical staining technique based on the

TABLE 1 | *In vitro* and *in vivo* studies regarding uric acid acting on several cellular signaling pathways in different hyperuricemia models.

The types the cells	Concentration	Acting duration	Pathway	Animal model	References
Human proximal tubular cell	4,8,16 mg/dl	24, 48 and 72 h	MAPK pathway	No	(14)
Cardiomyocyte	0–15 mg/dl	12, 24, 48 and 72 h	ERK/P38	Yes ^a	(15)
Renal proximal tubule cell	500 μ M	8 h	PKC, MAPK, cPLA2, and NF- κ B	No	(15)
Human umbilical vein endothelial cells	10, 50, and 100 g/ml	60 min	MEK/Erk pathway	No	(16)
β -cell	5 mg/dL	24 h	NF- κ B-iNOS-NO signaling axis.	Yes ^b	(17)
Mouse atrial myocytes	7 mg/dl	24 h	ERK pathway	No	(18)
Pre-adipocyte 3T3-L1 cells	15 mg/dl	5–30 min	p38 and ERK1/2 MAP kinases pathway	No	(19)
Cardiomyocytes	0, 5, 10, and 15 mg/dl	30 min, 1, 8, 16 and 24 h	IRS-PI3K-Akt signaling	No	(20)
Rat cardiac fibroblasts	3–300 μ M	24 h	ERK	No	(21)
Interstitial macrophages	3% UA	7 weeks to feed	RAS-NOS1	Yes ^c	(22)
Human vascular smooth muscle cells	6 to 12 mg/dl	1 to 48 h	p38	No	(23)
Vascular smooth muscle cells	2.5 to 10 mg/dL	72 h	MAPK signaling molecules ERK p44/42 and p38 NF- κ B	Yes ^d	(24)
Human mesangial cells	8–50 mg/dl	24 h	COX-2 expression and PGE2 synthesis	No	(25)
Pulmonary artery endothelial cells.	2.5–15 mg/dl	24 h	L-arginine-eNOS pathway	No	(26)

^aIntraperitoneal injection of potassium oxonate (300 mg/kg) and intragastric administration of hypoxanthine (500 mg/kg) for 1–2 h to create acute hyperuricemia.

^bThe mouse hyperuricemia model was generated by daily intraperitoneal injection of uric acid (250 mg/kg, Sigma) for 4 weeks.

^cMild hyperuricemia was induced in rats by providing a uricase inhibitor- oxonic acid (OA) and marked hyperuricemia were fed with 2% OA and 3% UA in the diet.

^dOA feeding for rats.

reduction of nitro blue tetrazolium to formazan by superoxide radical also revealed the presence of XO activity in human hearts (36). Moreover, an analysis of the correlation between maximal oxygen and UA level in patients with chronic heart failure reflects the impairment of oxidative metabolism (37). Autonomic nervous system activation can induce significant and heterogeneous changes of atrial electrophysiology and induce atrial tachyarrhythmias, including atrial tachycardia and AF (38). Recently, some researchers showed that a continuous 4 weeks inhibition of XO in infarcted rats down-regulated sympathetic innervation (39). This suggests that UA involves in sympathetic nerve activity via sympathetic innervation probably through a superoxide-dependent pathway, which eventually contributes to arrhythmia.

Cell experiments have been conducted to reveal the effect of UA on cardiac remodeling by stimulating the vascular Renin-Angiotensin System (RAS) (40). The study demonstrated that UA stimulates vascular smooth muscle cell proliferation and oxidative stress via the vascular renin-angiotensin system. Landmesser et al. have proved that angiotensin II induces the increased activity of NOX and XO, and eventually causes oxidative damage (41). An experimental test by Corry et al. also found that the mRNA and intracellular protein of angiotensin II were upgraded after 48 h of UA stimulation of vascular smooth muscle cells, and this effect was inhibited after the use of losartan and captopril (40). Moreover, increased oxidative stress levels that result in the upregulation of hydrogen peroxide and 8-isoprostatin was slowed down by losartan, captopril, and PD

98059 (a mitogen-activated protein (MAP) kinase inhibitor) treatment, suggesting UA causes vascular dysfunction through the angiotensin system.

Note, elevated UA levels cannot only increase the risk of myocardial oxidative damage through activating RAS but also lead to cellular damage by activating other pathways. For instance, a shred of evidence has shown that high UA can promote the up-regulation of NOX4 expression in renal proximal tubule cells and result in an increase in ROS production via activating P38 and ERK1/2 phosphorylation. Such ROS production through activation of P38 and ERK1/2 phosphorylation can further inhibit PI3K and Akt activation, and unbalance Bax/Bcl-2 equilibrium, which could eventually contribute to increased apoptosis and decreased cell activity (42).

Hyperuricemia can also cause oxidative damage and inhibit cardiomyocyte activity. Similar results were obtained *in vivo*, consistent with those obtained *in vitro*. The phosphorylation of ERK and P38 was up-regulated in mice with acute hyperuricemia model (15). Another experimental study endorsed hyperuricemia-induced oxidative stress in cardiomyocytes to further progress to myocardial structural remodeling (43).

Plasma urate level is directly regulated by a voltage-driven urate efflux transporter (URATv1) in humans (44). Recently, researchers have found that UA in the blood can promote the formation of ROS in atrial myocytes through UA transporters. In their study, they claimed that ROS activates ERK pathways and

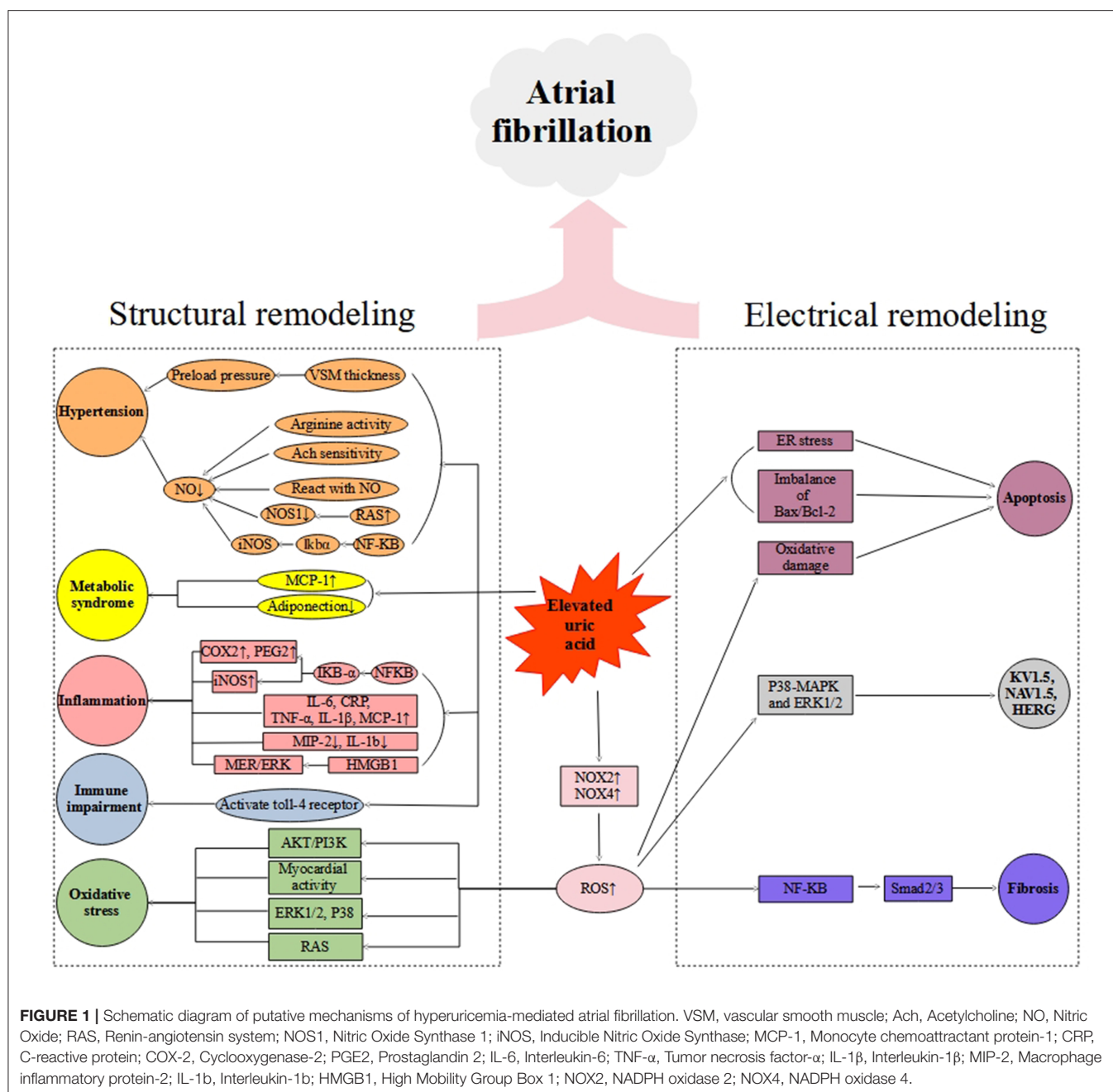


FIGURE 1 | Schematic diagram of putative mechanisms of hyperuricemia-mediated atrial fibrillation. VSM, vascular smooth muscle; Ach, Acetylcholine; NO, Nitric Oxide; RAS, Renin-angiotensin system; NOS1, Nitric Oxide Synthase 1; iNOS, Inducible Nitric Oxide Synthase; MCP-1, Monocyte chemoattractant protein-1; CRP, C-reactive protein; COX-2, Cyclooxygenase-2; PGE2, Prostaglandin 2; IL-6, Interleukin-6; TNF-α, Tumor necrosis factor-α; IL-1β, Interleukin-1β; MIP-2, Macrophage inflammatory protein-2; IL-1b, Interleukin-1b; HMGB1, High Mobility Group Box 1; NOX2, NADPH oxidase 2; NOX4, NADPH oxidase 4.

regulates the mRNA and protein expression of KV1.5, Nav1.5, and HERG channels. These researchers also demonstrated that hyperuricemia induces atrial electrical remodeling by activating URATv1. And after using URATV1 inhibitors, the expression of related sodium and potassium channel proteins and mRNA decreased. At the same time, this electrical remodeling effect was also inhibited by NOX inhibitors, apocynin, and antioxidant N-acetylcysteine (18). A recent experimental study examined the relationship between oxidative stress and atrial electrical and structural remodeling, and the beneficial effects of XO inhibitor allopurinol in alloxan-induced diabetes mellitus rabbits.

The study found that allopurinol attenuated atrial structural and electrical remodeling and suppresses AF vulnerability. These protective effects of allopurinol were highly associated with reductions in ROS formation and atrial fibrosis-related factors and abnormal calcium homeostasis (45). Allopurinol can improve atrial electrical remodeling by inhibiting CaMKII activity and protein expression of Na⁺/Ca²⁺ exchanger in diabetic rats (46). In aggregate, these findings indicate that oxidative stress is involved in atrial electrical remodeling caused by hyperuricemia, and XO inhibition can reduce oxidative stress and ameliorate atrial electrical remodeling.

The Role of Inflammation on the Progress of Elevated UA Induced-AF

Systemic inflammation, in particular, has been connected to endocardial inflammation of the endothelium, as well as atrial remodeling (47). Experimental animal models and human studies have verified that several major signaling pathways, including the renin-angiotensin system and inflammation, are involved in AF (48). Earlier evidence documented that hyperuricemia is responsible, at least in part, for the increased cytokine production. For example, Netea et al. explored the role of hyperuricemia in increased cytokine production after lipopolysaccharide challenge in neutropenic mice, and hyperuricemia induced by repeated administrations of uric acid in normal mice led to an increased TNF production after lipopolysaccharide (49). In general, an increase in cytokine is an indicator of systemic inflammation. In 2016, Chen et al. found that a combination of Na⁺ and UA can trigger an overproduction of TNF, IL-1, IL-6, and KC (50). In addition, some cytokine was reported to participate in the process of electrical and structural remodeling of the heart, such as TNF α (51), and IL-1 β (52).

In response to oxidative stress, activation of the inflammation signaling pathway NF-KB is reported to have direct effects on ion channel promoter regions, transcription factor expression levels, or mRNA splicing, which are responsible for the occurrence of AF. Acting on cultured tubular epithelial cells through the UA transporters, UA activates NF-KB signaling pathways and induces the expression of inflammatory factors and chemokines, including TNF- α , MCP-1, and RANTES (53). Moreover, UA activates the transcription factors nuclear factor-kappaB and activator protein-1, as well as the MAPK signaling molecules ERK p44/42 and p38, and increased cyclooxygenase-2 (COX-2) mRNA expression (24). Notably, UA-induced MCP-1 expression at 24 h was suppressed following the inhibition of p38, ERK 44/42, or COX-2, implicating these pathways in response to UA. Also, the ability of both diphenyleneonium (antioxidants) and n-acetyl-cysteine to obstruct UA-induced MCP-1 production suggested the involvement of intracellular redox pathways. Therefore, it is worthy to conclude that UA regulates critical proinflammatory pathways.

The effects of UA on human umbilical vein endothelial cells (HUVEC) and human vascular smooth muscle cells have been reported to produce a certain degree of vascular inflammation and vascular remodeling, mainly in the up-regulation of the expression of C-reactive protein, one of the independent risk factors for cardiovascular diseases and an important marker of inflammation (54). Another study showed that soluble UA, at physiologic concentrations, has profound effects on human vascular cells. The study reported that UA alters the proliferation/migration and NO release of human vascular cells, mediated by the expression of CRP (23). This suggests UA induced inflammation cause vascular dysfunction.

When UA stimulates endothelial cells, it induces the acetylation of an intracellular high-mobility group box-1 protein (HMGB1) through calcium mobilization and MERK/ERK pathway. A study that involved treatment of HUVEC with UA resulted in increased HMGB1 mRNA expression and acetylation of nuclear HMGB1 (55). UA after ischemia-reperfusion injury

mediates the acetylation and release of HMGB1 from endothelial cells by the MEK/Erk pathway, calcium mobilization, and activation of Toll-like receptor-4. Once released, HMGB1 *per se* promotes its cellular release and acts as an autocrine and paracrine to activate both proinflammatory and pro-reparative mediators (16). Besides, UA has the ability to stimulate pro-inflammatory effect in vascular smooth muscle cells to produce MCP-1 at transcriptional levels and protein expressions. Also, UA could cause necrosis of human mesangial cells at an ecological concentration, but UA could increase significantly at 8 mg/dl concentration and then increase the expression of COX-2 and PGE-2 to promote inflammation (25).

The Role of Fibrosis on the Process of Elevated UA-Induced AF

Atrial fibrosis produces heterogeneous pathways of slow conduction and atrial dilatation. In male adult Sprague Dawley rats, abnormal morphology of atrial myocytes, apoptosis, and atrial fibrosis were observed in hyperuricemic rats compared to the control. The study demonstrated that apoptosis and fibrosis of atria were partly mediated by B-cell lymphoma 2-extra-large (Bax), caspase-3, α -smooth muscle actin, and TGF- β 1. Also, the study reported that uric acid significantly induced primary rat cardiomyocyte apoptosis and fibrosis *in vitro* and AF induced by hyperuricemic rats occurred primarily via induction of atrial remodeling (56). Also, another study supports that UA can induce cell proliferation and endothelin-1 gene expression in rats with myocardial fibroblasts. Notably, the researchers were able to reverse the myocardial fibroblasts by increasing NADPH oxidase activity, ROS generation, ERK phosphorylation, and activator protein-1(AP-1)-mediated replacement activity (21). Thus, UA plays an important role in the pathogenesis of cardiac fibroblasts. Other researchers who were used the OA to create a mild hyper UA model *in vivo*, after a low-salt diet in the mice, found the shortened diameter of the arterioles in the hyperuricemia group and a significant increase in blood pressure (57). Likewise, some researchers reported an increase in cardiac afterload, interstitial fibrosis, and collagen deposition in the hyperuricemia mice compared with those in the control group (58). Importantly, another study draws a similar conclusion in mice fed a Western diet. In assessing the role of Western diet-induced increases in uric acid, the researchers found that increased cardiomyocyte hypertrophy, interstitial fibrosis, myocardial oxidative stress, and impaired diastolic relaxation. Further, the Western diet enhanced the profibrotic transforming growth factor- β 1/Smad2/3 signaling pathway, activation of the S6 kinase-1 growth pathway, and macrophage proinflammatory polarization. Importantly, all these Western diet-induced pathophysiological alterations were improved with allopurinol treatment (59). These results suggest increased production of UA promotes cardiomyocyte hypertrophy, oxidative stress, and inflammation that result in myocardial fibrosis and associated impaired diastolic relaxation.

The Role of Apoptosis and Immunity on the Progress of the AF

A significant association has been reported between UA and cell apoptosis. Of note, Bcl2 family proteins are key regulators

of apoptosis cell death. An experimental study suggested a high concentration of UA downregulates B-cell lymphoma 2 (Bcl-2) expression in pancreatic β -cells (17). This could substantially lead to the imbalance of Bax/Bcl-2. In another recent experimental study, Yan et al. demonstrated, uric acid induces cardiomyocyte apoptosis via activation of calpain-1 and endoplasmic reticulum stress (ERS) (60). The study extended that Calpain-1 expression was significantly increased after oxonic acid (OA) gavage administration for 16 weeks and p-PERK, GRP78, and CHOP expression was increased in H9c2 cardiomyocytes, suggesting hyperuricemia induced ERS activation. Another study demonstrated that elevated UA promoted ROS-induced tubular cell apoptosis by upregulating Nox4 expression in HK-2 cells that were used as a human proximal tubular cell model. The results of the study showed that treatment with UA reduced HK-2 cell viability and enhanced apoptosis in a dose-dependent manner. This was consistent with Nox4 upregulation as well as ROS overproduction, which resulted in Bax/Bcl-2 imbalance in HK-2 cells. Interestingly, inhibition of Nox4 with DPI prevented UA-induced cell apoptosis (14). Therefore, UA may involve in atrial fibrosis induced AF.

Immune cells may play a vital role in the immunological pathogenesis of AF (61), and UA has been identified as one of the endogenous adjuvants that can augment immune responses to particulate antigens (62). UA alerts the immune system to dying cells by stimulating dendritic cell maturation and activation (63). The study demonstrated that UA increases the expression of dendritic cell maturation markers, including CD80 and CD86. Also, Webb et al. demonstrated that UA can directly activate T-cells in the absence of antigen presentation (64). UA release leads to antigen-independent T-cell stimulation and provides an adjuvant effect for autoantigens released from apoptotic cells. It is reported, UA could lower the threshold for T-cell activation and potentially facilitate the break of peripheral T-cell tolerance (64). When UA stimulates endothelial cells, it activates toll receptor four, which eventually leads to increased expression and secretion of angiotensin II and stimulation of NF- κ B inflammatory channels (16).

CLINICAL EVIDENCE: HYPERURICEMIA AND ATRIAL FIBRILLATION

Epidemiological evidence suggests that the prevalence of hyperuricemia and AF are on the rise in many corners of the world. According to a community-based study that involved the elderly population, high UA population (SUA > 416 μ mol/L in men and >357 μ mol/L in women) had a higher risk of AF (OR: 2.080, 95% CI: 1.103–4.202; $P < 0.001$) (65). A single centered observational study by Mantovani et al. also concluded that hyperuricemia is independently associated with increased prevalence of AF [odds ratio (OR): 3.41, 95% confidence interval (CI): 2.19–5.32; $p < 0.001$] in patients with type 2 diabetes after adjusted for multiple confounding factors (66). Also, a cohort study from Taiwan reported similar findings (67). Over a mean follow-up of 6.3 years, Chao et al. reported that individuals with a history of one or more episodes of gouty arthritis had a higher

risk of AF (2.1 vs. 1.7% in controls, $P < 0.001$), even after adjustment for age and gender (67). Another study also showed a positive and independent association between UA and AF in coronary heart disease (CHD) events complicated with chronic comorbidity (68). This evidence from the epidemiological studies may explain the tremendous influence of the chronic disease comorbidity in the association between hyperuricemia and AF. **Table 2** summarizes related studies regarding the elevated UA and risk of AF.

Different treatment modalities have shown different results. A cohort study tested the use of allopurinol and the risk of atrial fibrillation in the elderly in 8,569 beneficiaries using Medicare data. After adjustment for age, sex, race, Charlson–Romano comorbidity index, and use of statins, diuretics, ACE inhibitors, and β -blockers, the use of allopurinol for 6 months or more was associated with a reduced risk of incident AF in the elderly (HR: 0.83; 95% CI: 0.74–0.93) (75). On the other hand, a meta-analysis of two retrospectives and two prospective cohort studies showed that elevated UA was not associated with an increased risk of AF recurrence after catheter ablation (76). However, the meta-analysis was criticized for large heterogeneity regarding AF type, ablation technique, and follow-up duration. Also, the meta-analysis included a small number of studies. In summary, clinical and epidemiological evidence showed that gender, aging of the population, CVD comorbidity, and allopurinol drugs seem to affect the prevalence of AF.

Colchicine, an anti-inflammatory drug that is used for a wide range of inflammatory diseases also associated with decreasing AF risk. A meta-analysis that included 17 prospective controlled randomized studies with 2082 patients that received colchicine and 1982 controls with an average follow-up duration of 12 months reported that treatment with colchicine reduced the recurrence of atrial fibrillation significantly in patients after cardiac surgery or pulmonary vein isolation (OR: 0.54, 95% CI: 0.41–0.7; $P = 0.001$) (77). However, a double-blind, placebo-controlled, randomized clinical trial among 360 patients undergoing cardiac surgery indicated that perioperative use of colchicine compared with placebo reduced the incidence of the postpericardiotomy syndrome but not of postoperative AF or postoperative pericardial/pleural effusion (78). Further RCTs are required to determine if AF events are lowered with colchicine.

Relationship Between Uric Acid Level and Atrial Fibrillation

Several studies have demonstrated the association between hyperuricemia and the development of AF. These studies that show a clear association between hyperuricemia and AF raise the question: how much elevation in UA concentration will significantly increase the risk of AF? Kuwabara et al. found that UA concentration was significantly higher in patients with AF than in non-AF (OR: 2.75; 95% CI: 1.22–1.50; $P < 0.05$) after adjusting for traditional risk factors such as hypertension, diabetes, nephropathy, and lipid metabolism disorders (70). And the incidence of AF increases when UA levels reach a certain limit. For instance, the first dose-response meta-analysis regarding the relationship between elevated UA concentration

TABLE 2 | The impact of hyperuricemia on the risk of atrial fibrillation.

References	Study design	Research population	Number of patients with AF	Number of population	Main finding
Sun et al. (69)	Cross-sectional	People from rural regions	139	11,956	Hyperuricemia is closely related to the increased prevalence of AF with the OR of 1.94
Mantovani et al. (66)	Cross-sectional	T2DM inpatients	91	867	T2DM patients with hyperuricemia had a greater likelihood to have AF than patients with normal uric acid level (OR: 3.41)
Kuwabara et al. (70)	Randomized control	General population	291	90,143	AF groups (OR: 1.35) have an significantly higher SUA level and hyperuricemia (OR: 1.73) was a significantly independent competing risk factor for AF
Liu et al. (61)	Cross-sectional	General population	55	1,056	Hyperuricemia (OR: 1.69) was an independent predictor for left atrial thrombus/spontaneous echo contrast in non-valvular AF patients
Lin et al. (71)	Cross-sectional	Residents of community	144	11,488	Elevated SUA level (OR: 2.19) is independently with the increased risk of AF
Zhang et al. (11)	Cohort	Individuals	6,831	527,908	The highest (HR:1.9) and the intermediate level (1.36) of UA significantly increase the risk of AF after adjusted for traditional risk factors
Li et al. (10)	Cohort	General population	871	123,238	High SUA level (HR:1.91) and the augment in SUA increases the occurrence of AF
Kawasoe et al. (72)	Cohort	General population	647	111,566	High level of baseline SUA (HR:1.74) was closely related with a higher incidence of AF in women.
Hong et al. (73)	Mendelian Random	Inpatients	633	4,166	Hyperuricemia gene rs1165196 (OR: 0.21) was causally associated with AF
Zhang et al. (74)	Meta-analysis	General population	N/A	426,159	Hyperuricemia (RR: 1.49) was significantly associated with increased risk of AF

T2DM, Type 2 diabetes; AF, Atrial fibrillation; SUA, serum uric acid; CHD, Coronary heart disease; OR, odds ratio; HR, hazard ratio; RR, relative risk.

and the incidence of AF reported that both the uppermost [Relative risk (RR): 1.9, 95% CI: 1.64–2.23; $I^2 = 0\%$] and medial (RR: 1.36, 95% CI: 1.16–1.59; $I^2 = 36\%$) level of serum UA were associated with increased risks of AF in comparison to patients with the lowest level of serum UA (79). The Kailuan cohort study has also reported similar findings. After repeated measurements of UA concentrations, patients with high levels of both serum UA had a substantially higher risk of AF. Most importantly, the study has denoted that the first rise in UA concentration is sufficient to increase the risk of AF in both men and women (male UA baseline level was >6.5 mg/dL, and female UA baseline level was >4.9 mg/dL) (10). This evidence is sufficient that hyperuricemia is associated with an increased risk of AF in patients with or without other chronic conditions.

Previous studies reported a positive association between UA levels and left atrial diameter in patients with hypertension. A study that included 451 hypertensive patients demonstrated an independent association between increased serum UA levels and AF (80). Also, a single centered retrospective data from Northeast China showed that SUA levels and left atrial diameter (LAD) were associated with AF in patients with hypertension, and the risk of AF associated with LAD increases among those with hyperuricemia (Hidru et al.). It should be noted that enlarged left atrial diameter is a conventional marker of atrial structural remodeling (81). Therefore, UA may participate in the pathophysiology of AF in patients with hypertension.

UA is an independent risk factor for MetS (82) and patients with MetS are considered to be at a higher risk of developing

AF (83). A prospective, observational study that enrolled 843 AF patients (mean age, 62.5 ± 12.1 years, 38.6% female) without overt coronary artery disease reported a significant risk of major adverse cardiovascular events, including myocardial infarction, coronary revascularization, and cardiac death (84). The study suggested that the prevention and treatment of MetS may reduce the burden of non-thromboembolic complications in AF. Since AF often associates with diabetes, hypertension, and obesity, it is possible that convergence of multiple risk factors could potentiate AF risk. As such, understanding the link among MetS, UA, AF, and non-thromboembolic MACE is imperative for investigating the possible mechanism and devising effective preventive strategies.

The Effect of Gender in the Link Between Uric Acid and Atrial Fibrillation

The reports on the effect of hyperuricemia on AF incidence have tremendous discrepancies. Epidemiological evidence suggested that hyperuricemia was more prevalent in males than females (7.9 vs. 4.9%) (85). Similarly, a cross-sectional study in three rural regions of China revealed a positive relationship between hyperuricemia and AF in men, but not in women (86). In contrast, a recent large cross-sectional study that includes urban and rural residents revealed a significant association between high UA levels and the prevalence of AF, especially in females (10). Also, a survey of healthy adults found that hyperuricemia is broadly correlated to the occurrence of cardiovascular diseases in females (87). And earlier in 2012, a Japanese study of 7,155

patients reported that hyperuricemia significantly increased the prevalence of crude AF, but after adjusting for all cardiovascular risk factors, such independent correlations were only confirmed in women (OR: 1.888, 95% CI: 1.278–2.790; $P < 0.05$) but not in men (OR: 1.176, 95% CI: 0.935–1.478; $P > 0.05$) (88). Similarly, prospective follow-up studies confirmed that women with high UA are more likely to develop AF (89), although the number of women with high UA is much lower than that of men (90). Recently, a large prospective study of 15,737 participants (52% women), tested the established and novel risk factors for atrial fibrillation in women compared with men, over 20 years follow-up. Women showed a stronger relationship between UA and AF (91).

The Causal Relationship Between UA and AF

Ample evidence is available from a large number of clinical studies to reach a consensus that hyperuricemia is an independent risk factor for AF. However, whether UA causes AF required explicit evidence. A human study has confirmed the association between UA and concentration levels of cytokine including C-reactive protein (CRP), IL-6, and TNF- α and IL-1 β , especially in women (26). This evidence explains that UA induced inflammation involve pathophysiological alteration either directly or indirectly. A previous Mendelian randomization analysis indicated that a genetic causal relation between elevated UA level and adverse cardiovascular outcomes, such as sudden cardiac death (92). Hong et al. designed a genomic study on the susceptibility of AF associated with UA using 9 selected single nucleotide polymorphism (SNPs) and found that the SNP rs1165196 on SLC17A1 (F-statistics = 208.34, 0.18 mg/mL per allele change; $P < 0.001$) and weighted genetic risk score (wGRS) (F-statistics = 222.26, 0.20 mg/mL per 1 SD change; $P < 0.001$) were significantly associated with increased

UA levels. The mendelian randomized analysis was causally associated with rs1165196 (OR: 0.21, 95% CI: 0.06–0.75; $P = 0.017$), but not with wGRS (OR: 1.07, 95% CI: 0.57–2.01; $P = 0.832$) (73), confirming that the UA was independently associated with the AF risk.

CONCLUSION

Existing studies strongly suggest that hyperuricemia is independently associated with the increasing incidence of AF. Epidemiological and clinical studies highlight a close association between various conditions including hypertension, metabolic syndrome, DM, and other CVD comorbidities and increased risk of AF. Therefore, the mechanistic links between UA and AF are complex with several underlying diseases and conditions. Experimental and clinical data indicate that UA is implicated in the pathophysiology of AF via activation of inflammation, oxidative stress, and fibrosis induced atrial remodeling. Briefly, atrial remodeling involves electrophysiological and structural abnormalities that promote the development of UA induced AF. Also, UA induced AF activates apoptosis and immune system. There is still a need for further investigation to obtain a more comprehensive understanding of the role of UA in the pathophysiology of AF.

AUTHOR CONTRIBUTIONS

YD and FL were contributed to literature researches, data collection, and were involved in the draft of the manuscript. XY and YX were contributed to the coordination and designing of the review and writing of the final draft of the manuscript. All authors have read and approved the final manuscript.

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Theoretical Models and Computational Analysis of Action Potential Dispersion for Cardiac Arrhythmia Risk Stratification

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Reentrant cardiac arrhythmias such as atrial fibrillation (AF) and ventricular fibrillation (VF) are common cardiac arrhythmias that account for substantial morbidity and mortality throughout the world. However, the mechanisms and optimal ablation treatment strategies for such arrhythmias are still unclear. Using 2D optical mapping of a mouse model with AF and VF, we have identified regional heterogeneity of the action potential duration (APD) in the atria and ventricles of the heart as key drivers for the initiation and persistence of reentry. The purpose of this paper is to discuss theoretical patterns of dispersion, demonstrate patterns of dispersion seen in our mouse model and discuss the computational analysis of APD dispersion patterns. These analyses and discussions may lead to better understanding of dispersion patterns in patients with these arrhythmias, as well as help comprehend whether and how reducing dispersion can lead to arrhythmia risk stratification and treatment strategies for arrhythmias.

Keywords: action potential duration, optical mapping of calcium and action potentials, action potential dispersion, cardiac arrhythmia, heart

INTRODUCTION

Complex reentrant cardiac arrhythmias such as atrial fibrillation (AF), and ventricular fibrillation (VF) are prevalent clinical cardiac diseases affecting millions of people around the world. AF may result in clot formation in the atria of the heart leading to stroke, and when uncontrolled, may lead to heart failure. AF has also been found to be an independent risk factor for cognitive decline and dementia (1). The mechanisms of reentry causing AF are not clear and the treatment options such as pharmaceutical therapy and ablation therapy are only met with moderate success (2, 3).

It is thought that the mechanism of AF reentry in the atria of the heart also occurs in the ventricles, deteriorating into VF (4). VF accounts for >700,000 sudden cardiac deaths in the US and Europe (5). The risk of VF increases in patients after myocardial infarction, but can also occur in patients with genetic abnormalities such as mutations in sodium and calcium channels. This suggests that anatomical and electrophysiological alterations due to genetic or physiological causes may cause a predisposition to reentry (4, 6, 7).

Reentry refers to the persisting activation sequence of a wavefront that repeatedly propagates around an anatomic or functional region, referred to as a core (5). The substrate requirements

for reentry include: (i) slow conduction in one limb allowing recovery of excitation just in time for re-excitation by the depolarizing wavefront; (ii) unidirectional conduction block; and (iii) inhomogeneous tissue causing conduction abnormalities, which may be due to an anatomical obstacle, or differences in conduction velocity due to altered electrophysiological properties in the myocardial tissue. Allesie et al. have shown that dispersion of refractory period of 11–16 milliseconds (ms) was sufficient to allow reentry around a line of conduction block of 5 millimeter (mm) after premature stimulation, also known as “short excitable gap reentry” (8, 9).

Increased temporal dispersion of excitation recovery in the heart has previously been established to enhance the development of complex reentry, namely fibrillation and nonuniform recovery of excitability. This further emphasizes the role of nonuniformity of excitability and conduction velocity during the relative refractory period in the initiation of turbulent impulse propagation (9). Dispersion of repolarization and recovery of excitability are also influenced by heart rate and the autonomic nervous system (10). Regional differences in refractoriness or heterogeneity in the passive electrical coupling of the myocardial cells may cause local areas of conduction block and irregular propagation of early premature impulses (9). Areas of longer refractory period may coincide with sites of unidirectional conduction block and cause intramyocardial reentry, e.g., between the endocardium and epicardium (11).

The refractory period is the interval from depolarization to the recovery of excitability (8, 12). Action potential duration (APD) is the time interval from the onset of phase 0 to the recovery of the membrane potential to the resting level. APD can be measured at 50% and up to 90% of repolarization. Over the years, the use of dispersion and heterogeneity has been translated to clinical indicators of repolarization abnormalities, such as QT interval dispersion on the 12-lead electrocardiogram or electrogram dispersion (13, 14). Repolarization time and refractory period have also been measured using a single cell microelectrode, monophasic action potentials (MAPs) (15), and extra stimulus techniques, during electrophysiology study and optical mapping (8).

In this paper, we will present APD dispersion data from a mouse model of atrial and ventricular fibrillation. This double transgenic (TG) mouse model of tetracycline inducible, cardiac-specific overexpression of F1759A SCN5A, initially created to study long QT3, spontaneously develops sustained atrial and ventricular fibrillation, secondary to late persistent sodium current (16). Dispersion will be quantified here by comparing the differences in APD within a tissue, region or area. We will measure the standard deviation (std) of APD across neighboring pixels within a selected area. A low value of dispersion suggests that the data are closely clustered and the tissue is relatively electrophysiologically homogeneous, whereas, a high value of dispersion indicates that there is a clear discrepancy between neighboring data points and an increased potential for reentry.

Theoretical Models of APD Dispersion

We propose in this paper several patterns of APD dispersion that may be theoretically observed (Figure 1).

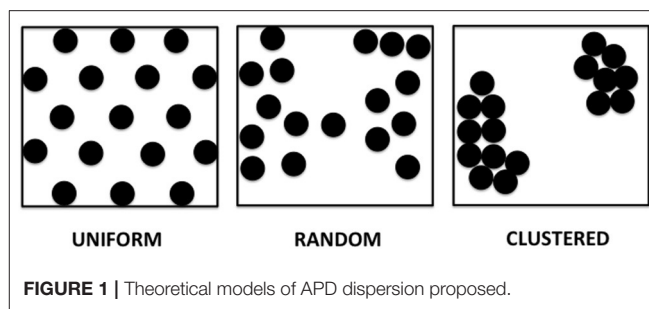


FIGURE 1 | Theoretical models of APD dispersion proposed.

- **Uniform dispersion:** areas of dispersion that are evenly spaced over a spatial region of the tissue. This pattern can be observed in genetically modified expression systems, where selected areas have higher dispersion, such as in monolayers with adenovirus inoculation allowing for channelrhodopsin light activation.
- **Random dispersion:** areas of dispersion that have unpredictable distribution. A given area of dispersion is equally likely to occur at any location. There is no cell specific regulation causing dispersion evident in that area, which can occur with either relatively low sodium channel expression or little fibrosis or scar. This can even be present for example, in young mice with a structurally normal heart and little to no fibrosis.
- **Clustered dispersion:** areas of dispersion that are clumped into groups, causing a patchy distribution or islands of areas of dispersion, especially near the anatomic borders, for example, post-myocardial infarction with dispersion along border zones.

MATERIALS AND METHODS

Transgenic Mice

The TG mouse line, F1759A-Nay1.5, was generated as previously described (16). Both male and female genders were included, between 3 and 12 months of age. All animal experiments were performed according to NIH guidelines. The Institutional Animal Care and Use Committee at Columbia University approved all animal experiments. Non transgenic littermates were used as controls.

Optical Mapping Data Acquisition and Processing

Optical mapping of the Langendorff perfused mice hearts was performed (16) using a complementary metal-oxide-semiconductor (CMOS) camera (MICAM Ultima, SciMedia). Movies acquisitions were performed at 1,000 f/s for 4–5 s, with 100×100 pixel resolution (0.095 mm per pixel). Image processing was performed using a custom software, PV-WAVE (Precision Visuals - Workstation Analysis and Visualization Environment, Visual Numerics, Inc) (17). Dominant frequency (DF) and phase maps were obtained in AF or ventricular tachycardia (VT)/VF, while APD and conduction velocity maps

were obtained with atrial or ventricular pacing at 10-Hz. Pacing-induced AF and VT/VF were assessed by 3 attempts of burst pacing at twice the excitation threshold of the left atrium and left ventricle, respectively (20 Hz, amplitude 0.5–2.0 mA, 5 ms). Two wires in the bath acted as single vector ECG monitoring for the Langendorff perfused hearts. Average APD, maximum APD, and APD dispersion [std(APD)], were calculated for the whole atrium and ventricle, and compared between the regions of highest singularity point density (SPD) to a neighboring 10×10 pixel area (17). High APD gradients were defined as regions where the difference between a long APD and a neighboring short APD within a 10×10 pixel area was greatest. Dispersion was calculated using a customized processing code in MATLAB.

Statistical Analysis

Group data are presented as mean \pm standard error of the mean (SEM). Statistical comparisons between the groups were tested using a one sample *t*-test. Values of $P < 0.05$ were considered statistically significant. All statistical analyses were performed using Prism 6.0 (San Diego, CA, 2018).

RESULTS

We performed 2D epicardial surface voltage optical mapping on the atria and ventricles of Langendorff perfused TG mice (Figures 2, 3). Surprisingly, we observed islands or regions of APD dispersion patterns most consistent with the clumped phenotype in most of the hearts. We also analyzed the percentage of atrial and ventricular tissue imaged that exhibited these islands/regions by calculating the yellow (APD dispersion > 10 ms) to green area (APD dispersion < 10 ms) ratio on the binarized dispersion maps (Figures 2, 3, right column). In other terms, we measured the percentage of mapped tissue exhibiting APD dispersion > 10 ms (Figures 4, 5). We have previously shown that areas of reentry were most common in regions with APD dispersion > 10 ms (18). Hence, we used this cut-off to quantify the percentage of tissue with dispersion > 10 ms necessary for AF and VT/VF arrhythmogenesis, respectively.

Representative images from 3 TG AF mice mapped after hyperkalemic induced sinus conversion and 10 Hz pacing are displayed in Figure 2. These three reconstructions exhibit the clustered APD dispersion pattern, namely patchy islands of increased dispersion, especially near the atrial edges. This was found to be the predominant pattern of distribution in the left atrium for the 22 TG mice that were analyzed. The optical maps of the TG mice were compared to control mice in normal sinus rhythm without late persistent Na^+ current, in which we had previously shown homogenous atrial APD maps (16). We observed in the three rows of representative images (Figures 2A–C) that there was heterogeneity of the APD maps, which did not appear to relate to anatomical structure, and the dispersion maps took the form of serpiginous patterns within the atria. We found that the islands/regions of APD dispersion might be singular, or that there might be an archipelago of regions within the atria. The percentage of areas with dispersion > 10 ms over the total epicardial area of the atrial tissue imaged

by optical mapping ranged from ~ 2 to $\sim 22\%$ (Figure 4). The average area of TG atrial tissue with dispersion > 10 ms was $10.24 \pm 1.15\%$, while the control mice group with no AF was significantly different ($p < 0.001$, Student's *t*-test) and did not exhibit any dispersion, as seen on Figure 4.

As for the ventricular optical mapping results, serial representative images from three mice with spontaneous and sustained VT/VF that were captured in normal sinus rhythm are displayed in Figure 3. These three representative dispersion maps show clustered areas with mostly serpiginous patterns of APD dispersion. This was the predominant pattern of distribution in the ventricles of the five F1759A-dTG mice that were analyzed. The optical maps of the TG mice were compared to control mice in normal sinus rhythm without late persistent Na^+ current, that had completely homogenous ventricular APD maps (16). The three rows of representative images (Figures 3A–C) show that there was heterogeneity of the ventricular APD maps. Just as in the atria, these areas of dispersion were inconsistent from mouse-to-mouse and did not appear to be correlated with anatomical ventricular structures, as the APD dispersion patterns were inconsistent from mouse to mouse. We found that the islands/regions of APD dispersion in the ventricle were similar to the patterns observed in atria. There were evident serpiginous islands/regions, consistent with the clustered phenotype. The percentage of areas with dispersion > 10 ms over the total epicardial area of the ventricle imaged by optical mapping ranged from ~ 8 to $\sim 16\%$ of the two-dimensional area (Figure 5). The average area of TG ventricular tissue with dispersion > 10 ms was $12.9 \pm 1.4\%$, while the control mice that exhibited no VT/VF were significantly different ($p < 0.001$, Student's *t*-test) and did not show any dispersion (Figure 5).

DISCUSSION

There have been studies on dispersion as the substrate for persistence of arrhythmias like AF and VT/VF. Tissue spatial dispersion differences may be due to inherent alterations in cellular electrophysiological properties between cells. It may also be due to the presence of collagenous connective tissue septa, which may separate and insulate different myocardial muscle bundles to create a nidus for local conduction inhomogeneity (9, 19). Nevertheless, the methods to measure and quantify dispersion vary. Numerous methods, including measuring spatiotemporal electrogram dispersion, and the use of 12-lead ECG to determine the QT interval dispersion, have limited efficacy and correlate poorly with basic cellular electrophysiological data. Intracellular microelectrode recordings can be used to measure APD but has limited application, since it can be technically challenging to maintain stable cell membrane punctures. In addition, isolation of cardiac myocytes from ventricular tissue after enzymatic digestion can lead to various ventricular regional cardiomyocytes being measured at the same time (8). Other noninvasive clinical measurements of dispersion, such as 12-lead ECG QT dispersion, measure ventricular repolarization time and have been used for arrhythmia risk stratification. However, previous research

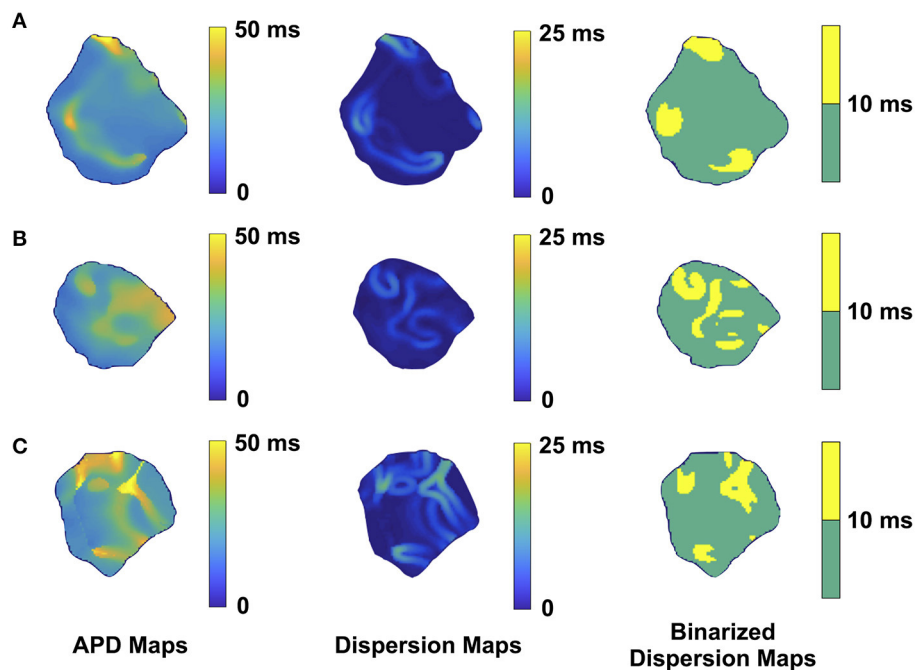


FIGURE 2 | Representative optical mapping images in the atria of three TG mice (A–C) with AF. The column on the left shows the APD maps of the epicardial surface of the atria captured during optical mapping. The middle column displays the corresponding APD dispersion maps. The column on the right demonstrates the binary APD dispersion patterns obtained when applying a threshold for areas with APD differences >10 ms in the atria. The mice were mapped after hyperkalemic induced sinus conversion and 10Hz pacing.

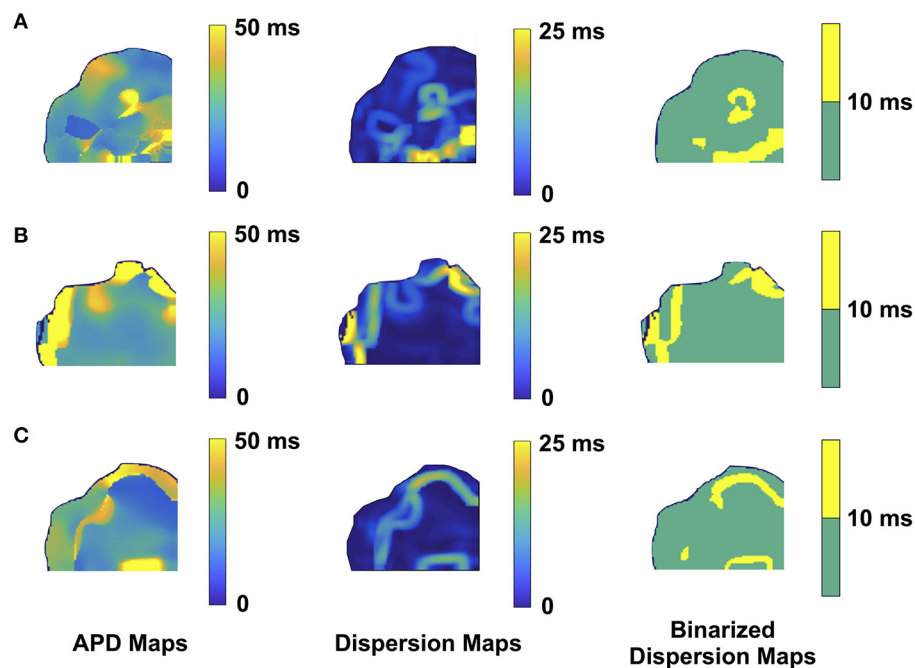


FIGURE 3 | Representative optical mapping images in the ventricles of three TG mice (A–C) with VT/VF. The column on the left shows the APD maps of the epicardial surface of the ventricle captured upon optical mapping. The middle column displays the corresponding APD dispersion maps. The column on the right demonstrates the binary APD dispersion patterns obtained when applying a threshold for areas with APD differences >10 ms in the ventricle. The three mice with spontaneous and sustained VT/VF were captured in normal sinus rhythm.

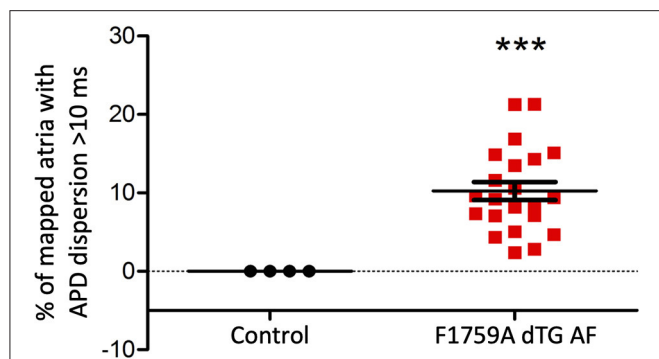


FIGURE 4 | Analysis of the APD maps showing the percentage of areas with dispersion > 10 ms over the total epicardial area of the left atria imaged by optical mapping, in TG mice with AF. One sample *t*-test $p < 0.05$, $n = 4$ control and 22 TG mice. The average area of TG atrial tissue with dispersion > 10 ms was $10.24 \pm 1.15\%$, while the control mice group with no AF was significantly different ($***p < 0.001$, Student's *t*-test) and did not exhibit any dispersion.

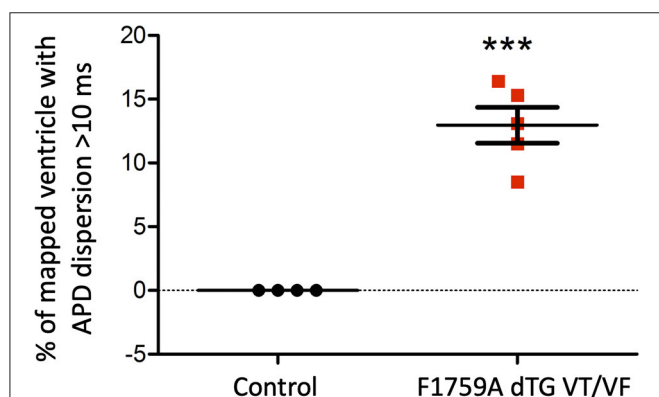


FIGURE 5 | Analysis of the APD maps showing the percentage of areas with dispersion > 10 ms over the total epicardial area of the ventricle imaged by optical mapping, in TG mice with spontaneous VT/VF. One sample *t*-test $p < 0.05$, $n = 4$ control and 5 TG mice. The average area of TG ventricular tissue with dispersion > 10 ms was $12.9 \pm 1.4\%$, while the control mice that exhibited no VT/VF were significantly different ($***p < 0.001$, Student's *t*-test) and did not show any dispersion.

studies have shown their limited ability to correlate dispersion with MAPs, as well as epicardial activation-recovery (8, 20).

To address these limitations, we used optical mapping of epicardial tissue recordings and applied it to the atria and ventricles of F1759A SCN5A TG mice in this study. This method relies on voltage sensitive dye fluorescence, captured by CMOS cameras, to obtain thousands of simultaneous recordings in both the atria and ventricles. The technique was used to study spatial APD dispersion in our TG mouse model with heterogenous cardiomyocyte increased late Na^+ current, which allowed spontaneous and sustained AF and VT/VF (16). We presented theoretical models for APD dispersion, methods for analysis and calculation of APD dispersion, and showed that APD dispersion in clustered patterns is the predominant configuration for spontaneous occurrence and persistence of

AF and VF in this murine model. We suggest herein that the presence of and quantification of APD dispersion of a tissue or organ may be a method to risk-stratify its propensity to propagate reentrant arrhythmia.

In the clinical electrophysiology laboratory, there are several methods for measuring tissue electrophysiological properties and refractoriness. Specific action potential tissue recordings can be obtained using a MAP catheter. However, MAP catheter electrograms may be distorted due to tissue movement, and maintaining tissue stability can be technically difficult. Electrophysiological measurement of effective refractory period can also be measured by transmitting regular trains of pacing stimuli followed by premature stimuli at progressively shorter coupling intervals. These measurements are limited to the tissue surface in contact with the catheter at epicardial or endocardial sites. However, consecutive measurements are required to estimate spatial dispersion (8, 15). Recently, a splined, multi-electrode catheter was used to record electrograms in the human atria in order to visualize electrogram dispersion sites, and simultaneously target these areas for ablation (13).

Reentry as the Mechanism for Cardiac Arrhythmogenesis in the Atria and Ventricles

Atrial Dispersion

APD heterogeneity in the atria of the heart may be due to many factors, including age, gender, and pressure or volume overload, leading to increased fibrosis (21). Increased atrial fibrosis in patients with AF has been thought to be a nidus for reentry. Previous studies have suggested that fibrosis, no matter whether it is focal or diffuse in the atrial tissue, may act as a substrate for AF (22). 2D computational simulations suggest that fibrosis can lengthen APD, increase APD dispersion, and increase the likelihood of reentry (22, 23). Simulated fibrosis at small scales was associated with increased vulnerability to sustained reentry relative to fibrosis at larger scales. Other predisposition factors to AF include among other things altered ion channels expression, such as heterogeneous distribution of late sodium current (24). Besides, vagal stimulation shortens the atrial effective refractory period and APD, thus enhancing APD dispersion (25). Atrial dilation and remodeling may also be associated with an increased risk of AF inducibility (26, 27).

Ventricular Dispersion

The ventricular myocardium is thicker than the atrial myocardium and is more electrically heterogeneous, as it is comprised of at least three electrophysiologically and functionally distinct cell types: epicardial, M, and endocardial cells. These three principal ventricular myocardial cell types differ with respect to phase 1 and phase 3 repolarization characteristics (28). These differences in the three ventricular layers account for the transmural dispersion of repolarization, thus providing a substrate for the development of ventricular reentrant arrhythmias such as VT and VF. Ventricular arrhythmias, including VT/VF, can occur due to acute ischemia or infarction. They can also be seen in hearts with extensive

cardiac remodeling, which may be caused by myocardial infarction, or by increased volume or pressure overload (14). Myocardial ischemia due to coronary artery disease has been associated with alterations in action potential duration. Downstream effects of ischemia, simulated by carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP)-induced oxidative phosphorylation, cause an initial prolongation and then a subsequent reduction in APD. Action potential prolongation can be reduced by blocking the inward calcium current (I_{Ca}), inward potassium current (I_{K1}) and transient outward potassium current (I_{to}). Alterations in I_{Ca} , I_{to} and I_{K1} can be modulated by intracellular decreased ATP and pH or decreased calcium, all of which are metabolic fluctuations observed in ischemia (29).

Our TG F1759A SCN5A mouse model had episodes of spontaneous and sustained VT/VF, likely due to increased persistent late Na^+ current, with modest reduction in ejection fraction. This model is not representative of all possible models of VT/VF as there are models of ventricular arrhythmias due to structural heart disease such as myocardial infarction, ischemia or other ion channel mutations. However, we used this model herein to understand the possible APD dispersion pattern for reentry in VT/VF.

Summary of Findings

Although three theoretical APD dispersion patterns were possible: uniform, random and clustered, the clustered pattern was the one overwhelmingly present in this mouse model. Underlying pathophysiological mechanisms that may cause clustered dispersion patterns rather than uniform or random in the atria and ventricles include fibrosis, genetic abnormalities or possibly infiltrative heart disease.

When we analyzed our optical mapping images for dispersion > 10 ms, APD dispersion patterns in both the atria and ventricles most often had one or more islands or regions within the tissue in either an ovoid or serpiginous shape. When measuring the percentage of areas with APD dispersion > 10 ms in the atria and ventricles, we found that commonly 10% of the overall tissue fell within the dispersion > 10 ms category.

Additionally, we found that the clustered patterns were likely not due to fibrosis, since histologically, there was only a small increase in fibrosis in AF TG mice compared to controls (16, 18). Our mouse model had only modest increase in fibrosis, but we acknowledge it may not be representative of all possible AF models. However, we used this model as a first step in understanding the possible APD dispersion patterns necessary for inducing reentry in AF.

Lastly, translation of our murine model findings to humans is critical. Optical mapping which uses blebbistatin, a calcium uncoupler, cannot be performed in patients *in vivo*. However, correlative findings may be obtained using MAPs and 3D electroanatomical mapping to locate and quantify areas of heterogeneous conduction. Initial *in vivo* comparison between the mouse model and 5 patients undergoing AF ablation was previously performed by our group (18). Electroanatomical voltage maps and MAP recordings in sinus rhythm for these patients successfully found APD heterogeneity, similarly to APD

dispersion in mice. In the advent of new technologies and in the era of high-density mapping, we envision the development of new high density catheters allowing measurement of APDs at high resolution at several myocardial tissue locations simultaneously. This information may be used to further stratify patients at risk in addition to other standard clinical diagnostic tests.

Limitations and Future Work

A major limitation of standard 2D optical mapping is its inability to view the structure in three dimensions. Hence, the appearance of clustered patterns near the edge of the tissue border might actually be due to the abrupt curvature of the tissue that could not be appreciated in two-dimensional imaging. Our group has since then developed a three-dimensional panoramic optical imaging set-up, currently being investigated in other mouse models, that could help overcome this limitation in future studies.

Moreover, we are developing a 3D visualization tool to co-register multiple imaging modalities for improved computational modeling and risk stratification of arrhythmias. On top of tissue electrophysiological information from panoramic optical imaging such as activation mapping and APD measurements, we have integrated and overlayed the data and structural information extracted automatically from anatomical imaging techniques such as computed tomography (CT) or magnetic resonance imaging (MRI) with deep learning algorithms. The purpose of this integration would be to study the effects of structural changes such as fibrosis and hypertrophy on arrhythmogenesis. These measurements can be derived through myocardial wall thickness measurements from the reconstructed CT, while MRI scans can give us access to complementary information like fibrosis and myocardial fiber orientation. Merging the spatial information from different imaging modalities may allow for a more in-depth three-dimensional characterization of the arrhythmogenic substrate. Visualizing how anatomical markers correlate with arrhythmia mechanisms and translating this information clinically would result in improved understanding of disease progression. Consequently, interactions and inter-dependence of functional and structural measurements through the fusion of multi-imaging modalities may lead to better arrhythmia risk stratification performance.

Finally, our study is limited by the usage of a single mouse model but it is an *in vivo* model with known spontaneous and sustained atrial and ventricular arrhythmias, and exhibits structural and functional changes similar to what is observed in human patients with these clinical arrhythmias. Future investigation will include additional pacing experiments in this murine model to confirm that tissues with areas of high APD dispersion have a high likelihood of inducible reentrant arrhythmias. Further studies evaluating the arrhythmia risk stratification performance of the APD dispersion computational analysis approach presented herein may provide further insight into how to study, prevent and treat patients.

CONCLUSION

Analysis and discussion of APD dispersion patterns, which may be a leading cause of cardiac arrhythmogenesis, may be helpful to improve computational modeling and justify further studies to characterize and quantify APD dispersion for risk stratification of atrial and ventricular arrhythmias. Further comprehension of dispersion patterns may lead to better understanding of whether and how reduction of APD dispersion may lead to new treatment strategies for patients with arrhythmias.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by The Institutional Animal Care and Use Committee at Columbia University.

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AUTHOR CONTRIBUTIONS

UA and EW designed the study, performed experiments, and collected and analyzed the data. UA, LM, JK, SL, and EW wrote the manuscript. UA and LM were involved in generating the figures. All authors contributed to the article and approved the submitted version.

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Automatic Detection for Multi-Labeled Cardiac Arrhythmia Based on Frame Blocking Preprocessing and Residual Networks

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Introduction: Electrocardiograms (ECG) provide information about the electrical activity of the heart, which is useful for diagnosing abnormal cardiac functions such as arrhythmias. Recently, several algorithms based on advanced structures of neural networks have been proposed for auto-detecting cardiac arrhythmias, but their performance still needs to be further improved. This study aimed to develop an auto-detection algorithm, which extracts valid features from 12-lead ECG for classifying multiple types of cardiac states.

Method: The proposed algorithm consists of the following components: (i) a preprocessing component that utilizes the frame blocking method to split an ECG recording into frames with a uniform length for all considered ECG recordings; and (ii) a binary classifier based on ResNet, which is combined with the attention-based bidirectional long-short term memory model.

Result: The developed algorithm was trained and tested on ECG data of nine types of cardiac states, fulfilling a task of multi-label classification. It achieved an averaged F1-score and area under the curve at 0.908 and 0.974, respectively.

Conclusion: The frame blocking and bidirectional long-short term memory model represented an improved algorithm compared with others in the literature for auto-detecting and classifying multi-types of cardiac abnormalities.

Keywords: electrocardiogram, cardiac arrhythmia, residual neural network, attention-based bidirectional, long short-term memory, frame blocking, auto-detection algorithm

INTRODUCTION

Cardiac arrhythmias refer to irregular heart rhythms, representing abnormal cardiac electrical activities associated with abnormal initiation and conduction of excitation waves in the heart (1). Cardiovascular diseases in association with cardiac arrhythmias can cause heart failure, stroke, or sudden cardiac death (2). Early detection and risk stratification of cardiac arrhythmias are

crucial for averting severe cardiac consequences. With their ability to represent useful information regarding the electrical activity of the heart, electrocardiograms (ECG) measured via electrodes placed on the body surface played an important role in diagnosing cardiac abnormalities (3). Recently, artificial intelligence-based algorithms (4, 5) have shown promises in screening abnormal features of ECG to achieve an automatic diagnosis of cardiac arrhythmias with high accuracy but less labor demand.

In previous studies, several auto-detection algorithms have been developed (6, 7). These algorithms focus on extracting physiological features of ECGs, such as heart rate variation (calculated from the time interval between two consecutive R peaks), the width of the QRS complex, and QT intervals. However, these algorithms do have limitations for practical application, as ECG features were merely extracted from RR or QT intervals, providing insufficient information for multiple types of cardiac event classification. To extract sufficient features automatically and achieve high classification accuracy, recent advancements in deep neural network (8) helped to develop several improved auto-detection algorithms (5, 9, 10) for ECG analysis and classification. These studies illustrated that the deep-learning-based algorithms have the advantages of extracting and processing ECG features automatically.

However, the algorithms discussed earlier are mainly focused on processing single-lead ECG rather than the 12-lead ECG, which is commonly used in the clinical setting for providing more diagnostic information than a single-lead ECG on cardiac excitations (11). Also, it is still a challenge to auto-detect multi-types of cardiac diseases based on 12-lead ECG due to (i) similar morphological features of ECG among different types of diseases, such as between atrial fibrillation (AF) and premature atrial contraction (12); (ii) imbalanced ECG data for various heart diseases in some training datasets, which may result in excessive bias or over-fitting of the neural network for diagnosis; (iii) unequal recording length of clinical ECG recordings, which may result in loss of some essential signals in the process of preprocessing for training the neural network.

Therefore, this study aims to develop a novel method for preprocessing raw ECGs and design an appropriate neural network for classifying 12-lead ECG data with multi-labeling and varied lengths.

METHODOLOGY

The proposed algorithm for classifying 12-lead ECG with multi-labeling consists of components of data denoising, framing blocking, and dataset balance for data preprocessing and a neural network structure based on ResNet in combination with attention-based bidirectional long short-term memory (BiLSTM). The general structure of the proposed algorithm is shown in **Figure 1**.

Dataset Description

China Physiological Signal Challenge in 2018

The China Physiological Signal Challenge (CPSC) 2018 dataset consists of 6,877 (females: 3,178; males: 3,699) recordings of

12-lead ECG data collected from 11 hospitals. Each recording is saved as a MAT file with a hea file presenting labels and relevant information of the ECG recording at the end of the file. The ECG recordings are sampled at 500 Hz with different recording lengths, ranging from 6 to 60 s. The dataset contains ECG recordings for nine types of cardiac states, including AF, intrinsic paroxysmal atrioventricular block, left bundle branch block (LBBB), normal heartbeat (Normal), premature atrial contraction (PAC), premature ventricular contraction (PVC), right bundle branch block (RBBB), ST-segment depression (STD), and ST-segment elevation (STE). To illustrate the morphological variation of the ECG among different cardiac states, the visualization of ECG lead II waveforms for nine types of cardiac states and a multi-labeled ECG recording can be found in **Supplementary Figures 1, 2**, respectively. Among the 6,877 recordings, 476 of them have two or three different labels. **Table 1** lists the numbers and distribution of eight-type cardiac arrhythmias in the 476 multi-labeled recordings of the CPSC 2018 dataset.

China Physiological Signal Challenge in 2020

An independent dataset, the CPSC 2020 dataset, is also used for testing the robustness of the proposed model. The dataset from CPSC 2020 contains two subsets of annotated recordings, one with 6,877 (males: 3,699; females: 3,178) recordings and the other with 3,453 (males: 3,453; females: 1,610) recordings of 12-lead ECG data, each of which was collected by a sampling frequency of 500 Hz. Furthermore, the dataset from CPSC 2020 contains public and unused datasets from the CPSC 2018 dataset for seven common types of cardiac states, details of which are listed in **Table 2** for the total number and distribution of cardiac abnormality in the CPSC 2020 dataset. Except for normal heart rhythm, the numbers and distribution of six types of abnormalities in multi-labeled recordings of the CPSC 2020 dataset can be found in **Supplementary Table 1**. In the experimental process, the total recordings for seven common types of cardiac states in CPSC 2020 were used for robustness testing.

PTB XL

To demonstrate the universality and robustness of the proposed algorithm, the cross-validation of the algorithm was processed on the PTB XL dataset. The PTB XL dataset comprises 21,837 clinical 12-lead ECG records from 18,885 patients (males: 9,820; females: 9,064) of 10-s length. As a multi-labeled dataset, the ECG records were annotated by two cardiologists based on the Standard Communication Protocol for Computer-Assisted Electrocardiography standard (14). **Table 3** illustrates the distribution of diagnosis, where the diagnostic labels are aggregated into superclasses.

Preprocessing

Noise Processing

Most ECG signals have a frequency range between 0.1 and 35 Hz and are non-stationary in the low-frequency range (15). Noises normally contaminate them from sources of power-line interference, muscle movement, and baseline wander, which blur

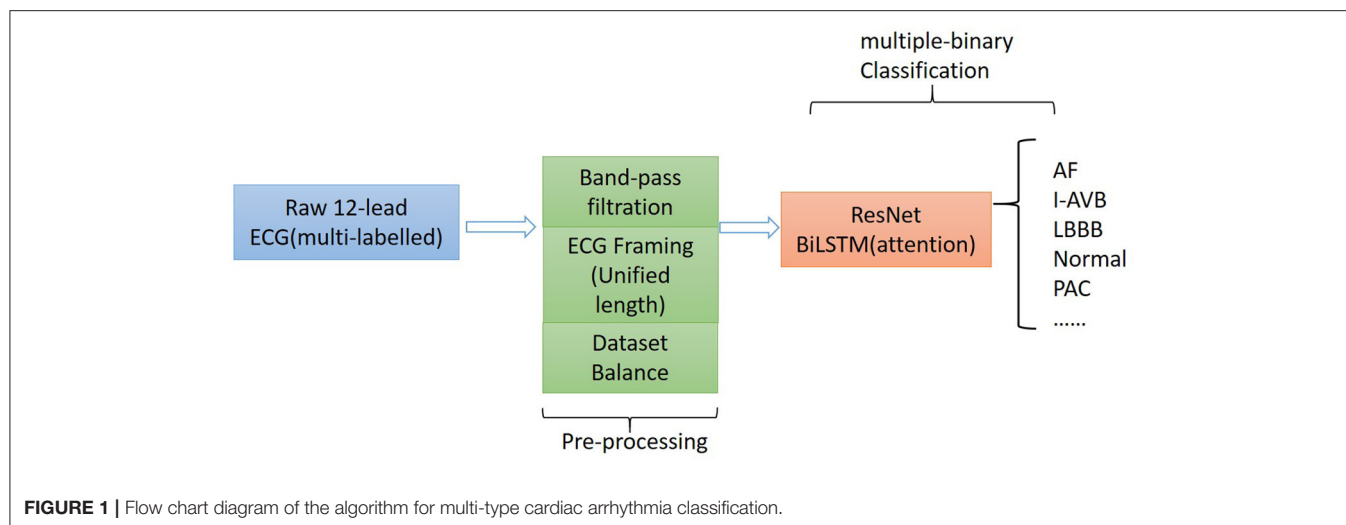


TABLE 1 | Numbers and distribution of ECG recordings with multiple labels (13) for eight different types of abnormalities in CPSC 2018.

	AF	I-AVB	LBBB	RBBB	PAC	PVC	STD	STE
AF	0	0	29	172	4	8	33	2
I-AVB		0	8	10	3	5	6	4
LBBB			0	0	10	6	3	4
RBBB				0	55	51	20	19
PAC					2	3	6	5
PVC						0	18	2
STD							0	2
STE								0

TABLE 2 | Recording numbers and distribution of seven types of abnormalities in CPSC 2020.

Abnormalities	CPCS 2020	CPCS 2020	Total
	Training	Training	
	set1	set2	
AF	1,221	153	1,374
I-AVB	722	106	828
LBBB	236	38	274
Normal	918	4	922
RBBB	1,857	1	1,859
PAC	616	73	689
PVC	0	188	188

TABLE 3 | Recording numbers of distribution of five types of diagnostic labels in PTB XL.

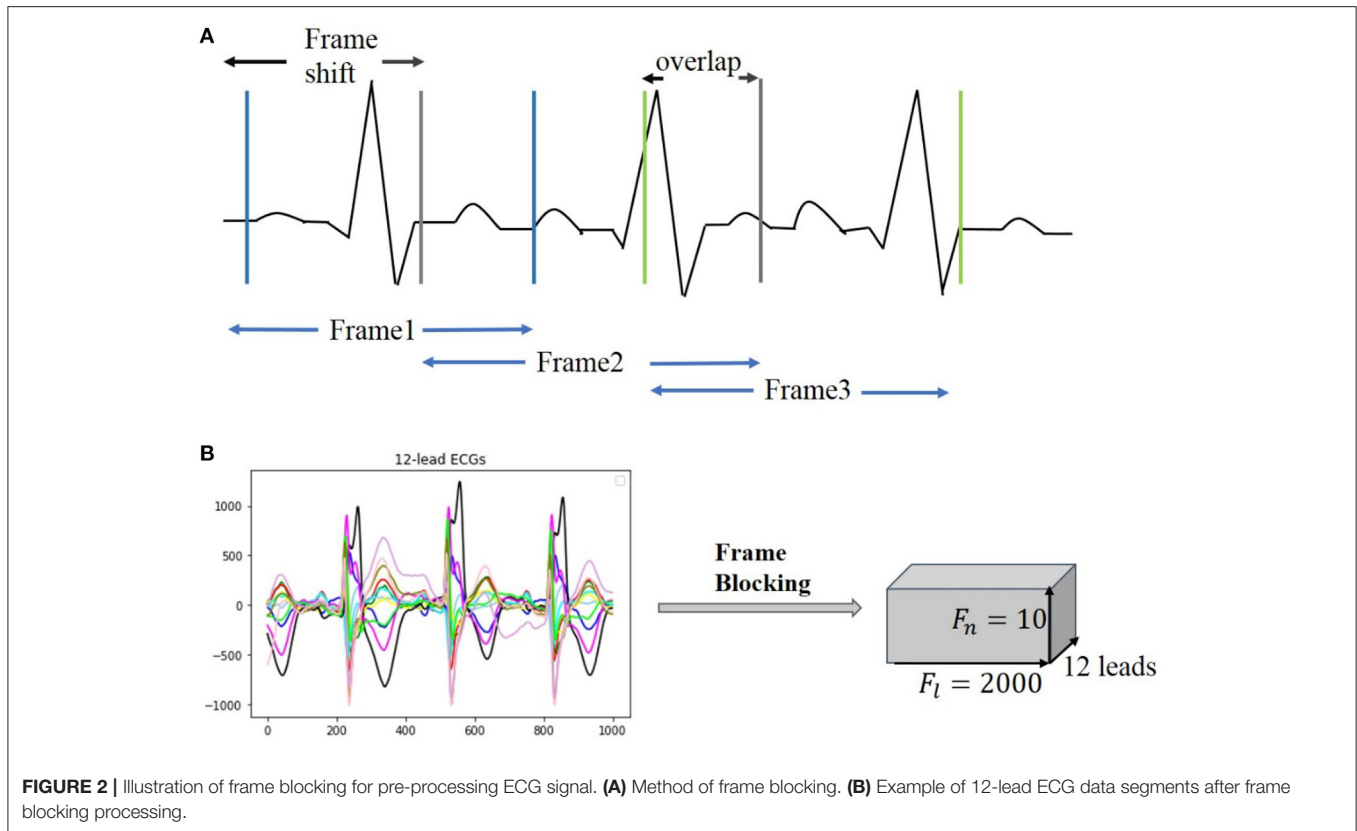
Superclass	Description	Record_Num
NORM	Normal ECG	9,528
MI	Myocardial Infarction	5,486
STTC	ST/T Change	5,250
CD	Conduction Disturbance	4,907
HYP	Hypertrophy	2,655

the features of the ECG signals for classification. For minimizing possible effects of noise on model classification, raw ECG data in the two databases were denoised by using an eight-order Butterworth lowpass (35 Hz) filter for eliminating noise and removing baseline wander.

Frame Blocking

Clinical ECG data are normally collected with non-uniform duration, ranging from 10 s to 24 h, causing difficulties for

training and testing neural networks. For unifying the length of each of the ECG recordings, a frame blocking method adapted from speech recognition (16) is utilized in the present study. In speech recognition, frame blocking is used to segment speech signals into short frames with overlapping, enabling a smooth transition between adjacent frames that maintains the continuity of the signal. As there is a similarity between speech signals and ECG time series (17, 18), the frame blocking method can be implemented in ECG data for unifying their recording length. **Figure 2A** illustrates the implementation of the frame blocking method on the cardiac signal. In the figure, F_s , the frameshift, denotes the time lag of the frame (from the starting time of the ECG recording), and f_o denotes the overlapping part between



adjacent frames. Thus, the length of each frame, F_l , can be expressed as:

$$F_l = F_s + f_o \quad (1)$$

For a raw ECG recording with a total length of S_l , given the number of frames F_n and frame length F_l , then the framing equation can be represented as:

$$F_s = (S_l - F_l) / (F_n - 1) \quad (2)$$

The length of each ECG recording in the CPSC 2018 dataset is variable, of which 6,634 recordings have their length shorter than 40 s (i.e., ~20,000 sampling data points). To retain the available ECG signals for each record as much as possible, we set F_l and F_n as a constant of 2,000 (sampling points) and 10, respectively, but F_s variable for fitting the required length and number of frames. **Figure 2B** illustrates an example of a 12-lead ECG recording processed by the frame blocking, with each ECG recording can be transformed into a frame-block with a uniform size [i.e., $(F_n, F_l, \text{lead_num})$]. As such, the frame blocking acted on each lead of the signals and divided them into 10 frames with a frame length of 2,000 sampling points.

Dataset Balance

In the present study, the multi-labeled dataset was converted into multiple types of sub-dataset classes, each of which represented one of the multiple types of cardiac states. The length of the ECG data for each type of cardiac abnormalities is imbalanced,

leading to over-fitting and weak generalization of the proposed neural network. To address this problem, a random under-sampling method (19) is used. For training and testing each binary-classifier, data samples are selected randomly from the dataset until a 2:1 ratio of samples in the majority class to the minority class is obtained.

Construction of the Model

Residual Convolution Neural Network

Residual convolutional neural network (CNN) (20) has shown excellent performance on image recognition for addressing the degradation problem of a deeper neural network, and it is believed to be useful for analyzing time-series signals, such as ECG. Here, we implemented one-dimension residual CNN with 13 layers based on the structure of ResNet. As shown in **Figure 3** for the general structure of the network, both dense blocks 1 and 2 belong to the residual block, and the shortcut connection simplifies the optimization of the deep neural network.

Attention-Based Bidirectional Long Short-Term Memory

In the proposed model, the residual blocks primarily focus on extracting features from ECG signals, and the attention-based BiLSTM structure focuses on learning and analyzing the feature map produced by the residual blocks. The bidirectional structure provides contextual information in the forward and backward directions for the output layer, providing more prediction information (21); thus, in this study, a BiLSTM (17)

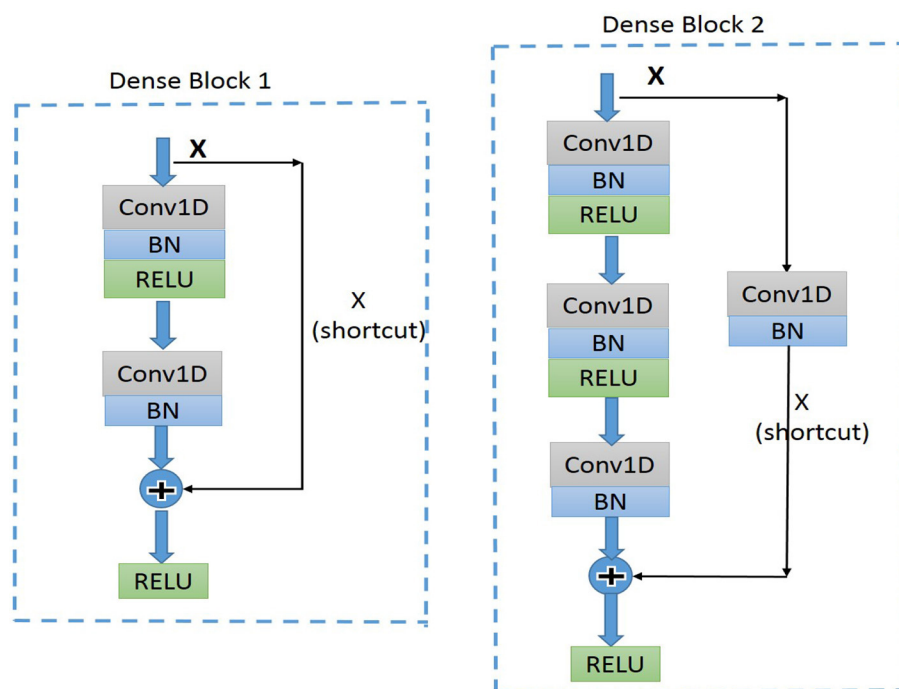


FIGURE 3 | Diagram of the structure of dense block1 and dense block2. BN, batch normalization; ReLU, rectified linear units; Conv1D, one-dimension convolutional layer.

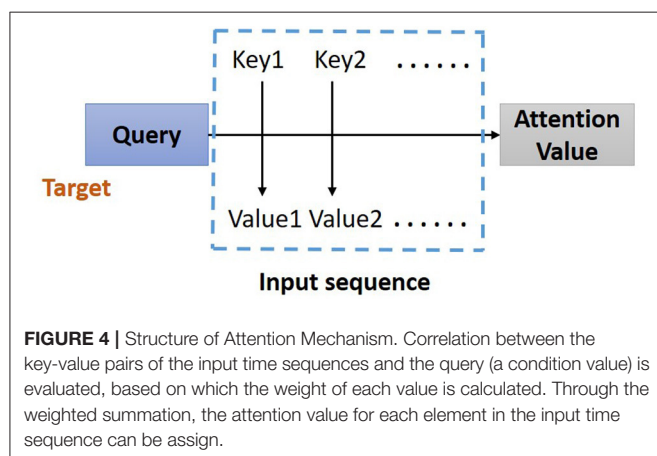


FIGURE 4 | Structure of Attention Mechanism. Correlation between the key-value pairs of the input time sequences and the query (a condition value) is evaluated, based on which the weight of each value is calculated. Through the weighted summation, the attention value for each element in the input time sequence can be assigned.

is used to catch some essential information from a long-distance correlation of the ECG data. The proposed model implements the Attention Mechanism (Figure 4) to allocate different attention values to each input query, which assists BiLSTM to precisely identify valid information and reduce the loss of key features. The attention-based BiLSTM can focus on the essential part of the input, meanwhile, it catches global, and local connection precisely because of the weight and attention allocation for the input time sequences.

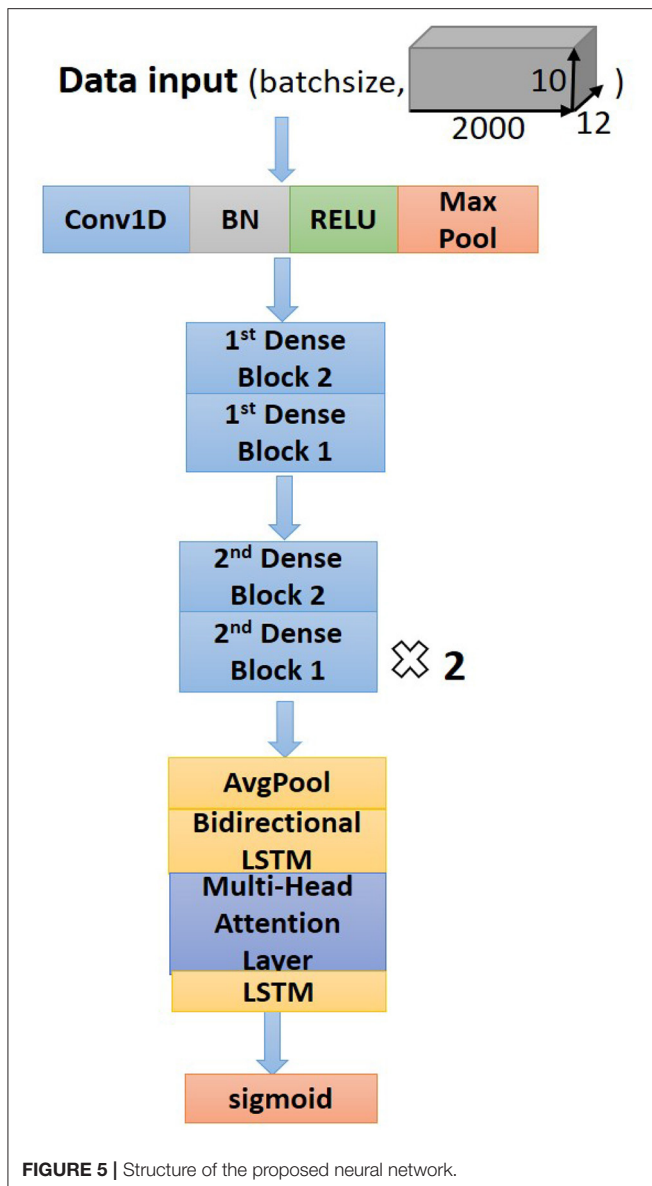
Structure of the Proposed Network

The dense block 1 shown in Figure 3 is a standard residual block in ResNet. It consists of two one-dimension convolutional layers

(Conv1Ds), two Batch Normalization (BN) (22), and rectified linear units (ReLU) (23) for the activation function layers, as well as a shortcut connection that transmits the input to output directly before applying the second ReLU nonlinearity. As for the structure of dense block 2, a Conv1D layer and BN are added in a shortcut for adjusting channels or stride to fit the desired shape of output. The overall structure of the proposed model is shown in Figure 5.

Following the Conv1Ds are the BN and ReLU layers, which help to simplify the parameter adjustment, improve the learning speed, and address the vanishing gradient problem of the model. Then a 1D max-pooling layer is used to down-sample the feature map by computing and extracting maximums of every three values in the feature map matrix, thus retaining the most valuable features and avoiding unnecessary memory usage during the training process. After the max-pooling layer, dense block 2 is connected to dense block 1 to fulfill a complete residual CNN. Before processing the attention-based BiLSTM for feature analysis, the global average pooling layer (24) is used to process the regularization of the global structure of the network, preventing it from overfitting.

Supplementary Table 3 lists a set of optimal parameters for each layer and the residual blocks. Among different residual blocks in different positions (first or second), convolution kernels have different sizes and numbers. For classification, sigmoid activation with binary cross-entropy (25) is used to convert the output sequence from the last LSTM layer into a probability for a specific label, based on which classification is determined with a given threshold.



Experimentation Details and Evaluation Matrix

The proposed model is initially trained and implemented using the CPSC 2018 datasets and run on Tesla T4 GPU with Keras frameworks (26). As described in the section of dataset balance, the positive and negative samples of each cardiac abnormality with the ratio of 1:2 are randomly selected and combined as input datasets for the model. For each binary classifier, the input data were divided into three subsets: 64% for training, 16% for validation, and 20% for testing. The 5-fold cross-validation was also implemented for training and validation. The test dataset was used purely for evaluating the performance of the model and was not involved in training and validation of the proposed model.

The classification performance can be comprehensively evaluated by precision, Recall, F score, receiver operator

characteristic (ROC) curve, and area under the curve (AUC). These evaluated measures are calculated by the following equations:

$$Precision_i = \frac{TP_i}{TP_i + FP_i}$$

$$Recall_i = \frac{TP_i}{TP_i + FN_i}$$

$$F1score_i = \frac{2 \times (Precision_i \times Recall_i)}{Recall_i + Precision_i}$$

In these equations, i denotes each of the types of cardiac arrhythmias. TP_i and TN_i represent the number of correctly predicted positive and negative samples, respectively. On the other hand, FP and FN are the values of false prediction for positive and negative samples separately. The ROC curve measures the performance of the model *via* plotting the trade-off between sensitivity and specificity, and the AUC is the value of the area under the ROC curve. A ROC curve is closed to the top-left corner and has the AUC close to 1 indicates the good performance of the classification model.

RESULT

Adjustment of Hyperparameter

The process and outcome of tuning of hyperparameters can be found in the **Supplementary Material**.

Comparison of Model Performance to Different Model Structures

To compare the performance of the proposed model to others, results obtained here were compared with those obtained from multiple models with different network structures, which included (i) the plain CNN with attention-based BiLSTM; (ii) Plain CNN + LSTM; and (iii) Challenge-best deep neural network model.

i) Plain CNN + attention based BiLSTM

Supplementary Table 5 lists the architecture of plain CNNs and attention-based BiLSTM. Except for the structure of shortcut, the convolutional layers, batch normalization layers, and ReLU layers of this model are similar to those of the proposed model. Multiple dropout layers were added to this structure, which could reduce the complexity of coadaptation between hidden neurons and improve the robustness of the neural network (27).

ii) Plain CNN + LSTM

Similar to the plain CNN + attention-based BiLSTM model, the structure of the plain CNN + LSTM model contains plain CNNs without shortcut. Moreover, the attention-based BiLSTM is replaced by LSTM layers with a simpler structure for feature analysis.

iii) Challenge-best deep neural network model

Supplementary Table 6 depicts the structure of the first prize model (13) for the automatic diagnosis of cardiac abnormalities in the CPSC 2018 dataset. The model consists of five CNN blocks

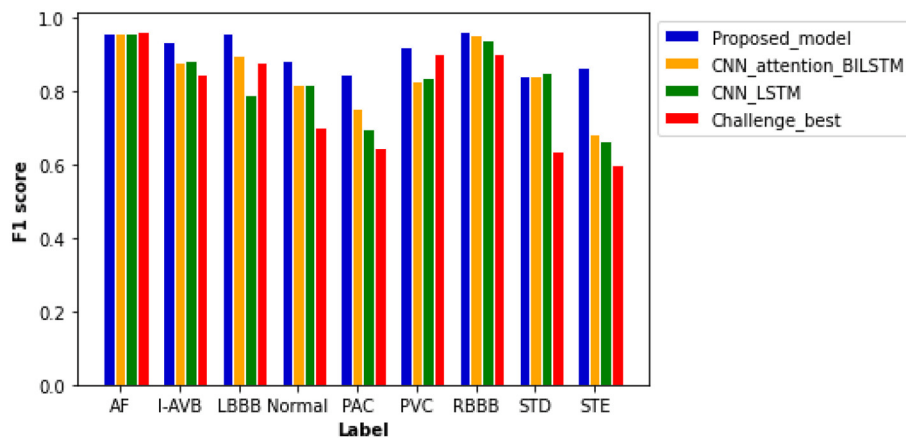


FIGURE 6 | Comparison of F1 scores between different models based on the same test samples. F1 scores of the proposed model show the best performance of the model as compared with others with values of 0.959 for AF, 0.937 for an intrinsic paroxysmal atrioventricular block (I-AVB), 0.958 for LBBB, 0.885 for Normal, 0.848 for PAC, 0.920 for PVC, 0.965 for RBBB, 0.841 for STD, and 0.868 for STE. Specific values of F1 scores of the other three models are shown in **Supplementary Table 7**.

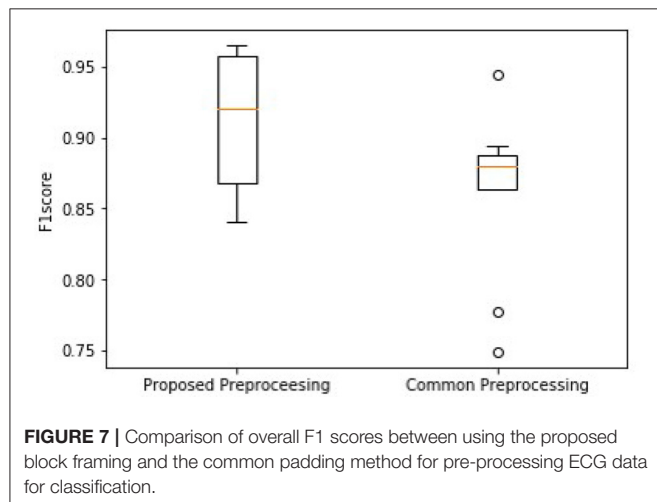


FIGURE 7 | Comparison of overall F1 scores between using the proposed block framing and the common padding method for pre-processing ECG data for classification.

and attention-based bidirectional GRU. Each block includes two convolutional layers, with one pooling layer appended for reducing the over-fitting and the amount of computation. To achieve optimal performance of classification, the bidirectional GRU layer followed by an attention layer is connected to the last convolutional block. Moreover, the hyperparameters of the challenge-best model have been modified based on our proposed model, enabling a direct comparison.

Figure 6 plots computed F1 scores achieved by the proposed model, which are compared with results from other comparable models using the same dataset. As shown in the figure, the F scores of six labels in the proposed model are notably higher than others. The proposed model achieved the highest F score of 0.965 for the RBBB case, followed by 0.959 and 0.958 for AF and LBBB, respectively. The probability results illustrated by the confusion matrix (**Supplementary Figure 3**) demonstrated

a low probability of misclassification by our proposed model; especially, the probability of false positive and false negative for AF, LBBB, PVC, and RBBB is closed to zero.

The plain CNN with attention-based BiLSTM ranks second with an average F score of 0.846. The computed F scores from the model for PAC, PVC, and STE are much smaller than those of the proposed model. Thus, the replacement of residual networks reduced the performance of the model. The performance of plain CNN with the LSTM model is not optimal for each type of cardiac abnormalities, especially for the cases of LBBB, PAC, and STE, for which F score is < 0.800. Although the Challenge-best model achieved the highest F score for the AF case, its performance for other abnormalities is relatively poor. Over-fitting occurred when the Challenge-best model was implemented for the data input of PAC, STD, and STE, leading to undesired F scores. Though the architecture and hyperparameters of the Challenge-best model are similar to the model shown in **Supplementary Table 5**, the computed average F score of the challenge-best model is much lower as compared with the presented model.

Supplementary Figure 4 shows the computed ROC curve from different models for each type of the nine cardiac states. Comparing with other models, the ROC curve of the proposed model is closer to the top left corner, with an averaged AUC at 0.974, suggesting out-performance to the other models.

Performance on Different Preprocessing

To illustrate the advantage of the frame blocking for pretreatment of the data, the performance of the proposed model was compared with that using a common preprocessing method (28–30), which uses direct cutting and zero-padding protocol to unify the length of ECG signals. As for a fixed length of 40-s ECG data (i.e., 20,000 sampling data points), the common method can either truncate the exceeding signal samples when the length of original records exceeds 40 s or pads zeros to the data when the length is < 40 s.

As shown in **Figure 7**, the higher median and minimum of the common method illustrate an improved model performance of the proposed frame blocking method. Moreover, the distribution of F1 scores by the common method is discrete, reflecting the instability of the performance of the classification model. To further evaluate the significant difference of this observation, the Wilcoxon signed-rank test is done on the two paired of F1 scores. The p -value is 0.028 (<0.05), revealing the difference between F1 scores produced by two pretreatments is significant.

Robustness Testing

Being tested on the CPSC 2020 dataset, the proposed model shows F1 scores significantly higher than those of the Challenge-best model (13) for all seven types of cardiac arrhythmias (**Figure 8**). The computed ROC curve and AUC (shown in **Supplementary Figure 5**) also demonstrate the better performance of the proposed model (with an averaged AUC of 0.951) than the challenge-best model (13). It is interesting to note that the Challenge-best model is much harder to converge on the CPSC 2020 than those of CPSC 2018. Also, the performance of the challenge-best model varies dramatically for different types of cardiac abnormalities with the use of the CPSC 2020 dataset as indicated by low values of F1 score for LBBB, Normal, PAC, and PVC conditions.

Cross-Validation

Besides the CPSC datasets, the PTB XL dataset was adapted for cross-validation of the proposed novel algorithm for preprocessing and classification. As shown in **Figure 9**, the F1 scores of four diagnosis labels are higher than 0.800, achieving an average F1 score of 0.838 for all diagnosis labels in that dataset. The computed ROC curve and AUC (shown in **Supplementary Figure 6**) also illustrated a satisfying performance of the proposed algorithm on an external dataset with an average AUC of 0.950.

DISCUSSION

The novelty and major contributions of the present study are the following: (i) we proposed a preprocessing algorithm of frame blocking adapted from speech recognition, which decomposes ECG signals into overlapped frames. The proposed frame blocking method minimizes the loss of valid signals while maintaining the continuity of ECG signals in the process of unifying the length of variant ECG recordings; (ii) we developed a neural network based on the residual networks (31) with attention-based BiLSTM. As compared with the previous algorithms mentioned earlier for ECG detection, the presented network can extract and analyze ECG features automatically, thereby improving the model performance. It also alleviates the vanishing and exploding gradient problem as seen in deep neural networks, and (iii) by training and testing the model using three independent datasets of 12-lead ECG signals provided in CPSC (32) and PTB XL (33), the proposed algorithm demonstrates superiority and robustness in classifying 12-lead ECGs with multi-labeling.

In recent years, numerous automatic detection methods for ECG analysis and classification have been developed. These methods are mainly based on and tested using the open-source MIT-BIH database (34), which are mainly single lead ECGs with single labeling. Thus, the general applicability of these algorithms for automatic stratifying multi-leads ECG and multiple types of arrhythmias is unclear. In this study, we developed a new algorithm based on frame blocking and the structure of ResNet, in combination with attention-based BiLSTM. Initially, the novel algorithm was trained and evaluated on the datasets of CPSC for classifying 12-lead ECG for nine types of arrhythmia labeling. By comparing the performance of other model structures (**Figure 6**), the superiority of the proposed model was confirmed. Comparing with the common preprocessing method (**Figure 7**), the frame blocking method reduces the number of zeroes padded at the end of the signal recording, enhancing the valid part of ECGs, as well as the autocorrelation of ECG records. Thus, the proposed preprocessing method is more conducive to feature extraction for further classification.

The proposed algorithm demonstrated its robustness and clinical value via robustness testing and cross-validation. Through the robustness testing, the proposed algorithm shows a consistent performance on the two datasets and various types of abnormalities, illustrating the robustness of the proposed algorithm and hyperparameters. Considering the cross-validation, both the frame blocking method and classification model are also applicable to the PTB XL dataset with a vast number of clinical records.

Regarding the model structure, the proposed model adopts a similar neural network structure as the Challenge-best model. Both are based on a bidirectional recurrent neural network with the attention mechanism, but the proposed model used residual networks to avoid gradient explosion and vanishing. The strength of ResNet has also been demonstrated by several studies (31, 35). In their studies, He et al. (35) showed the deep residual networks achieved an overall F1 score of 0.806. Rajpurkar et al. (31) utilized a 34-layer residual neural network to classify 65,000 multi-lead ECG records with 14 classes of cardiac disease and achieved an average accuracy and F1 score of 0.800 and 0.776, respectively. Due to the differences between the original dataset and preprocessing method, the crosswise comparison of classification models is not persuasive. The studies mentioned earlier processed the classification *via* complex network structures and a large amount of annotated data. Although the deeper neural networks with sufficient training data contributed high classification accuracy, the computation of the model also increased and required expensive hardware support. Our model adapted a similar structure as the studies mentioned earlier but simplified the network structure, raising the computational efficiency of training and the probability of clinical practice.

As for the challenge-best model proposed by Chen et al. (13), its whole structure contains 10 plain convolutional layers and 5 pooling layers. The use of unnecessary multiple layers in the CNN layers may reduce the model performance on a small and unbalanced dataset due to over-fitting, causing difficulties in parameter tuning. Thus, the occurring of the

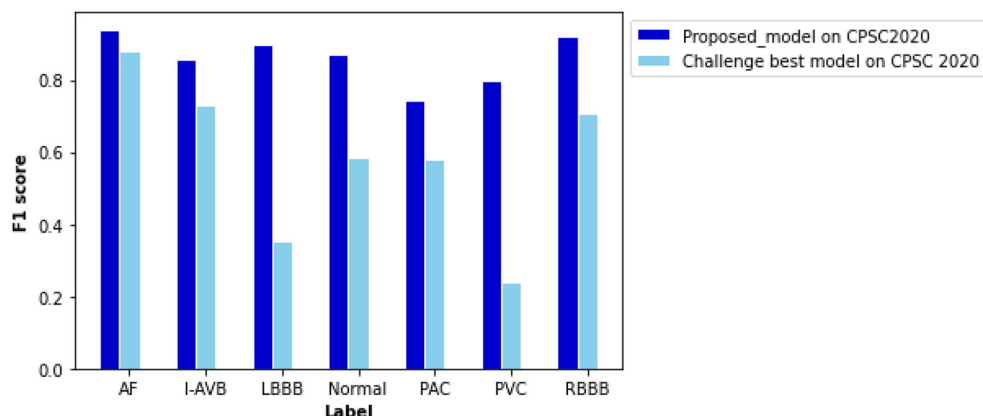


FIGURE 8 | Comparison of performance between the proposed model and the Challenge-best model tested on the CPSC 2020 dataset for various types of arrhythmias. F scores of the proposed model are 0.940 for AF, 0.856 for intrinsic paroxysmal atrioventricular block (I-AVB), 0.898 for LBBB, 0.870 for Normal, 0.743 for PAC, 0.798 for PVC, 0.922 for RBBB, 0.841 for STD, and 0.868 for STE. Comparison of the F1 score between them is listed in **Supplementary Table 8**.

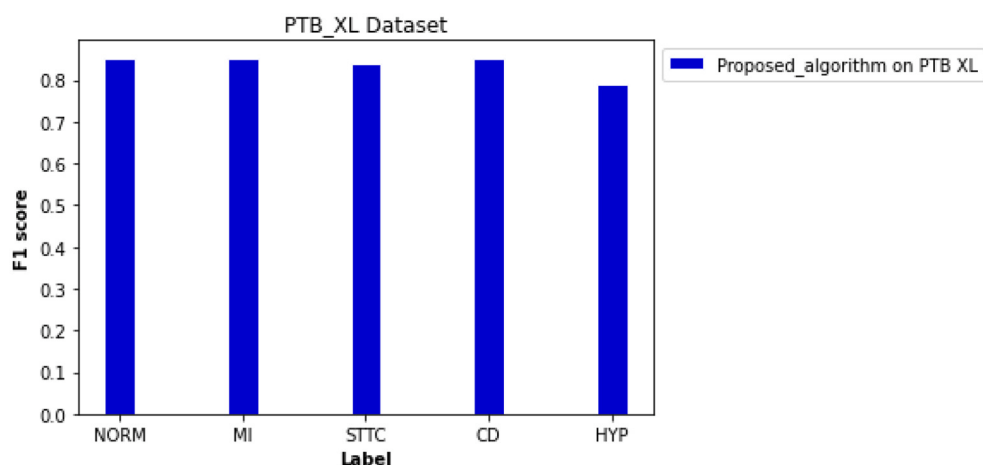


FIGURE 9 | Performance of the proposed algorithm on PTB XL dataset for five diagnosis labels. F scores of each label are 0.853 for NORM, 0.852 for MI, 0.842 for STTC, 0.853 for CD, and 0.791 for HYP.

internal covariate shift slows down the training process when the input distribution changes, impairing the convergence ability of the model. Different preprocessing methods may also affect the performance of the model. We have looked at this issue. Compared with the commonly used method, the frame blocking method used in this study demonstrated its advantage in retaining maximum valid cardiac signal, which contributed to signal enhancement. Therefore, it proved to be a feasible preprocessing method to help the model extract more available features that are useful for model classification.

As for algorithms for multi-label classification (36), they can fall into problem transformation and algorithm adaption. With the development of neural networks, more studies (12, 31, 35, 37) designed an adaptive algorithm for multi-label classification. However, algorithm adaption has a high demand for sufficient training data and effective parameter adjustment to reduce misdiagnosis for multi-labeled ECG. Additionally,

algorithm adaptation requires a complex model with proper parameters, increasing training cost and difficulties in data interpretation. In this study, each abnormality is considered as an independent binary problem, improving the interpretation of the features extracted. Although the binary relevance method cannot provide information about label correlation and interdependence directly, it still demonstrated some advantages for multi-label classifying performance and efficiency.

Regarding several recent studies (38–40), the risk stratification is in high demand to prevent sudden death or stroke caused by cardiac diseases. Inspired by the present algorithm, the risk prediction of cardiac diseases can be automated based on the clinical data collected from the ECG or electronic heart records. The shortcut connection in the residual network saved the computing time of the model and accelerated the convergence of the model, which is friendly to the clinical research setting. Thus, the model has the potential to automatically identify the

patients at a high risk of cardiac diseases, process early clinical interventions and therapy. Furthermore, the application of a warning system of cardiac arrhythmias can be implemented based on the risk stratification and auto-detected algorithm. The ECG and electronic heart records can be stored and processed via cloud infrastructure and the internet, realizing the real-time monitoring system for cardiac arrhythmias and improving the early warning for the patients suffered from cardiac diseases.

RELATED WORKS

Previous works into ECG auto-detection are mainly focused on manual feature extraction via the analysis in the time domain, frequency domain, and ECG morphology. After feature extraction, machine learning methods, such as Support Vector Machine (41) and linear discrimination analysis (42), are usually used for classifications. Compared with the algorithms mentioned earlier, ECG auto-detection based on deep neural networks focuses more on automatic feature extraction from ECG signals.

Hannun et al. (10) developed a deep CNN model for auto-detection of 12 classes of cardiac rhythms, achieving an averaged F1 score of 0.837. Besides, models based on LSTM have also been developed for processing ECG data with varied recording lengths and long-term time dependence to avoid the loss of valid features (43). For multiple label classification, the combined use of different neural networks demonstrates a better performance than the network structure purely based on the convolution layer. For example, the algorithm of multi-information fusion neural networks (44) consisting of BiLSTM and CNN has the advantages of simultaneously extracting the morphological features and temporal features, yielding an accuracy of 99.56%. Moreover, a similar BiLSTM–CNN model has been introduced to process data with long-term correlation, which could sufficiently extract features (45) to achieve high sensitivity and specificity of 98.98 and 96.95%, respectively.

The ECG auto-diagnosis algorithms discussed earlier demonstrated the advantages of deep learning algorithms in classification accuracy but were less focused on processing the 12-lead ECG with multiple diagnosis labels. Thus, it is in demand to develop an effective and auto-diagnostic algorithm to classify 12-lead ECG data for multiple cardiac arrhythmias.

LIMITATION OF STUDY

There are a few potential limitations in this study. Firstly, random under-sampling was used to address the imbalanced datasets of MIT-BIH (34), PTB XL (33), and CPSCs 2018 (32) and 2020. However, some potentially important and information-rich data might be discarded from the majority class, causing difficulties in fitting the decision boundary between majority and minority samples (19). Although the proposed model demonstrated good performance on two CPSC datasets (2018 and 2020) and PTB XL for 9, 7, or 5 different rhythmic abnormalities, it still needs

to be further tested and improved by using other ECG datasets with more types of rhythmic abnormalities. However, as the types of rhythmic abnormalities increase, it would be expected that the required training time and GPU memory usage will be substantially increased.

In addition, the proposed neural network algorithm is heavily dependent on a large amount of annotated training data, which is labor expensive. For some rare types of cardiac abnormalities, it is difficult to collect such a large ECG dataset with annotation. In following-up works, it warrants to study further how algorithm adaption method (46) and other neural network architectures (47–49) help to deal with multi-labeled data directly and reduce time-demand for training. Moreover, unsupervised and semi-supervised learning can also be tested for addressing the lack of enough annotations.

CONCLUSION

This study proposed a new framing preprocessing method that can minimize the loss of ECG signals to enhance the features of signals. The proposed algorithm can diagnose multiple types of cardiac arrhythmias with promising accuracy, clinical value, and robustness, which may be potentially useful in assisting risk stratification, clinical diagnosis, and real-time ECG monitoring. Furthermore, we have shown that the residual neural network helps to extract deep features while saving computing time *via* processing the convolutional layers in parallel. For feature analysis, the attention-based BiLSTM demonstrated its advantage in addressing problems of long-distance dependency, allowing focus on the most significant features based on the assigned attention values.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

HZ conceived the study. ZL designed the model and accomplished experiments. ZL and HZ wrote the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcvm.2021.616585/full#supplementary-material>

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Hypertension and Atrial Fibrillation: A Study on Epidemiology and Mendelian Randomization Causality

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Introduction: Hypertension (HT) and atrial fibrillation (AF) often coexist. However, the causality between these two conditions remains to be determined.

Methods: We used individual participant data from the Atherosclerosis Risk in Communities (ARIC) prospective cohort with 9,474 participants. HT was ascertained at visit 1 (1987–1989), and incident AF was identified by ECGs conducted during study examinations at each visit, hospital discharge codes, and death certificates. We used the Kaplan–Meier estimate to compute the cumulative incidence of AF by the HT subgroup. Then we used Cox hazard regression model to assess the association between HT and incident AF. The causality between genetically determined HT and AF was analyzed by the two-sample Mendelian randomization (MR) based on publicly summarized genome-wide association studies (GWASs) data.

Results: A total of 1,414 cases (14.9%) of AF were identified during the follow-up period (median 24.1 years). After adjusting for all covariates, the hazard ratio between the participants with HT and incident AF was 1.50 [95% confidence interval (CI) 1.29–1.73]. In the HT → AF MR analysis, we detected a causal correlation between HT and AF (OR: 1.90, 95% CI 1.18–3.04, $P = 0.01$) with no evidence of heterogeneity from single-nucleotide polymorphisms. Besides, the genetically determined SBP and DBP (10 mmHg) were consistently associated with a higher risk of AF.

Conclusions: In the ARIC study, the incident AF increased by 50% in patients with HT. In the MR analysis, our results supported causal inference between HT and AF.

Keywords: hypertension, atrial fibrillation, mendelian randomization, genome-wide association study, causality

A KEY MESSAGES BOX

In epidemiological studies, the incidence of atrial fibrillation (AF) increased by 50% in patients with hypertension (HT). HT might be a genetic cause of AF.

INTRODUCTION

Hypertension (HT) and atrial fibrillation (AF) are two important public health priorities. Their prevalence is increasing worldwide, and the two conditions often coexist in the same patients (1).

Both conditions are associated with aging. Additionally, HT is also related to other cardiovascular comorbidities that increase the risk of AF, including coronary heart disease (CHD), heart failure (HF), metabolic syndrome, chronic kidney disease, and sleep apnea (2). The epidemiological association between HT and AF was established in many previous studies (3–5). In patients with established AF, their HT morbidity was reportedly much higher than that of non-AF (3). Moreover, following HF, aging, and valvular heart disease, HT portends an excess risk of AF by 50% in men and 40% in women (4). Considering the high prevalence of HT in the population, it accounts for more cases of AF than other risk factors (6, 7).

Despite the well-established epidemiological association between HT and AF, these preliminary observational data were limited for causal inference due to the potential bias introduced by confounding factors and reverse causality (3, 7). Hence, understanding the causal relation between HT and AF has important public health significance for disease prevention and complication management. Mendelian randomization (MR) is a robust genetic methodology used to identify causal risk factors for diseases (8). It relies on three main assumptions, which are shown in **Supplementary Figure 1** (9). Subject to a genetic variant satisfying the instrumental variable assumptions, an association between the variant and outcome implied a causal effect of the exposure on the outcome. In this study, our goal was to describe the association between HT and AF in a considerable prospective cohort Atherosclerosis Risk in Communities (ARIC) study. We also conducted a two-sample MR analysis for the causal relationship between HT and AF and systolic blood pressure (SBP), diastolic blood pressure (DBP), and AF.

METHODS

Ethics

The studies involving human participants were reviewed and approved by the ethics committee of Guangdong Pharmaceutical University. The participants provided written informed consent to participate in this study.

Study Population

The ARIC study design was previously described (10). A total of 15,792 participants, aged 45 to 64 years, were recruited between 1987 and 1989 (visit 1). Later on, there were four subsequent study visits in 1990–1992 (visit 2), 1993–1995 (visit 3), 1996–1998 (visit 4), and 2011–2013 (visit 5). We excluded participants with prevalent AF or missing follow-up data, HT data, and other covariates. A total of 9,474 participants were eventually included in our analysis.

HT and AF Assessment

HT was ascertained at visit 1 (a measured SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg). Three blood pressure measurements were obtained from seated participants with a 5-min rest period. The average of the second and third measurements was recorded.

Incident AF was identified by the following three methods, (1) electrocardiograms (ECGs), (2) hospital discharge codes, and (3) death certificates. Twelve-lead ECGs were conducted

with participants lying in a supine position at each visit. ECGs were automatically coded as a cardiologist confirmed AF. ECG data were transmitted electronically to a reading center (EpiCare, Wake Forest University, Winston-Salem, NC, USA), reviewed, and analyzed using the GE Marquette 12-SL program (GE Marquette, Milwaukee, WI, USA). Trained abstractors collected information from all participant hospitalizations using the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) codes for diagnoses. AF was ascertained if the ICD-9-CM code 427.31 (AF) or 427.32 (atrial flutter) was present in any hospitalization. AF associated with open cardiac surgery was excluded. AF was also defined if ICD-9-CM codes 427.31 or 427.32 were listed as a cause of death (11).

Measurement of Other Covariates

All of the covariates such as race, gender, and age were assessed at visit 1. The educational level was self-reported. Physical activity was accessed using the validated Baecke questionnaire. Height and weight were measured with the participants wearing light clothes. Body mass index (BMI) was calculated as weight (in kilograms) divided by squared height (in meters). Diabetes was defined as fasting blood glucose ≥ 126 mg/dl, non-fasting blood glucose ≥ 200 mg/dl, use of antidiabetic medicine, or self-reported physician diagnosis of diabetes. Total cholesterol, high-density lipoprotein cholesterol (HDL-c), and triglycerides (TG) were measured using standardized enzymatic assays, and low-density lipoprotein (LDL-c) was calculated based on the Friedewald formula. Creatine was measured using a modified kinetic Jaffe method (10). Stroke, CHD, and HF were defined as previously described (12–14).

Summary of Genome-Wide Association Studies (GWAS) Data

Data included in this MR study were the GWAS summary statistics datasets from the Neale Lab consortium for HT, SBP, and DBP, and the Ben Elsworth consortium for AF. Details of the studies and datasets used for the analyses are presented in **Table 1**.

Data Extraction and Harmonization

We requested the following SNP genotype quality metrics from disease and risk factor studies: strong evidence of between-study heterogeneity in the SNP-trait association ($P \leq 0.001$), Hardy-Weinberg disequilibrium ($P \leq 0.001$), or imputation quality metric (info or r^2) ≤ 0.90 . We harmonized the summary data for diseases and risk factors so that the allele effect reflected the alleles associated with exposure. When SNPs were palindromic, A/T or G/C, we used information on the allele frequency to resolve strand ambiguity. We excluded SNP-trait associations from the GWAS catalog if they missed a P -value, beta, or an SE for the beta. The included SNPs are shown in **Supplementary Tables 1–3**.

Mendelian Randomization Analysis for Genetic Causality Assessment

Since MR for the SNP exposure effects and SNP outcome effects were obtained from separate studies, it was possible to estimate the causal influence of the exposure on the outcome (9). Our MR

TABLE 1 | Details of studies and datasets used for the MR analyses.

Exposure/ outcome	Participants	Sample size	Data source	First author	Consortium	Year	Units
HT	European, males, and females	337,199	http://www.nealelab.is/blog/2017/9/11/details-and-considerations-of-the-uk-biobank-gwas	Neale	Neale Lab	2017	NA
SBP	European, males, and females	317,754	http://www.nealelab.is/blog/2017/9/11/details-and-considerations-of-the-uk-biobank-gwas	Neale	Neale Lab	2017	10 mmHg
DBP	European, males, and females	317,756	http://www.nealelab.is/blog/2017/9/11/details-and-considerations-of-the-uk-biobank-gwas	Neale	Neale Lab	2017	10 mmHg
AF	European, males, and females	463,010	41202#148: Output from GWAS pipeline using Pheasant derived variables from UKBiobank	Ben Elsworth	MRC-IEU	2018	NA

MR, Mendelian randomization; HT, Hypertension; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; AF, Atrial fibrillation; NA, not available.

study was conducted on the MR-Base platform online (<http://www.mrbase.org>). We conducted two-sample MR approaches for the genetic causality assessment (HT→ AF, SBP→ AF, and DBP→ AF) using publicly available summarized data from the GWAS (15).

Statistical Analysis

For the ARIC study, baseline characteristics between the HT subgroups were compared using one-way ANOVA, the χ^2 -test, and the Kruskal–Wallis test as appropriate. The Kaplan–Meier estimate was used to compute the cumulative incidence of AF by the HT subgroups, and differences in estimates were compared using the log-rank procedure. Cox hazard regression models were used to assess the association between HT and incident AF. The follow-up time was defined as the time from baseline (visit 1) to outcome occurrence, loss to follow-up, death, or December 31, 2014, whichever occurred first. Pre-specified subgroup analyses were performed by gender, age, race, smoking, drinking, BMI, creatine, LDL-c, TG, and potential interactions with HT. We also conducted sensitivity analyses, excluding participants with HF, CHD, and diabetes. Cox hazard regression models were also used to assess the association between SBP or DBP and AF separately as continuous variables. We also used a restricted cubic spline with three knots to explore the potential non-linear trends for SBP and DBP hazard ratios, respectively.

For the MR analysis, the strength of the instrumental variables was assessed using the F statistic. Causality between genetically determined HT, SBP, DB, and AF was estimated. Using the HT→ AF MR analysis as an example, each SNP's association with AF was weighted by its association with HT, and estimates were combined using an inverse variance weighted (IVW) method (16). Several sensitivity analyses were carried out, including (1) the weighted median method, (2) the weighted mode method, (3) MR-Egger regression, (4) funnel plots, and (5) leave-one-out analysis.

All of the statistical tests were two-sided. The statistical test for the MR analyses was considered statistically significant at $P < 0.05$. All of the analyses were conducted using Stata (version 14.2, StataCorp LP, College Station, TX, USA) and R (version 3.2.5, R Foundation for Statistical Computing, Vienna, Austria) (16).

RESULTS

Hypertension, Systolic Blood Pressure, Diastolic Blood Pressure, and Incident Atrial Fibrillation

The baseline characteristics are shown in **Supplementary Table 4**. Of the 9,474 participants, 1,190 had HT. Cases, 1,414 (14.9%), of AF occurred during a median 24.1 follow-up years. Unadjusted cumulative curves for incident AF are demonstrated in **Supplementary Figure 2**. Restricted cubic spline showed an increasing linear risk for SBP and a potential U-shaped risk tendency for DBP (**Supplementary Figures 3, 4**). In the adjusted model, the participants with HT were associated with a 50% increased rate of incident AF [hazard ratio, 1.50; 95% confidence interval (CI), 1.29–1.73] (**Supplementary Table 5**). The hazard ratios of SBP and DBP for incident AF were 1.17 (95% CI, 1.12–1.22) and 0.90 (95% CI, 0.84, 0.97), respectively, after adjusting for each other in the final models (**Supplementary Table 5**). The results were similar when stratified by sex, race, smoking, drinking, BMI, creatine, LDL-c, and TG in the subgroup analyses (all $P_{\text{interaction}} > 0.05$, **Supplementary Figure 5**). After excluding the participants with HF, CHD, or diabetes, the association between HT and incident AF persisted (**Supplementary Figure 5**). To summarize, the HT participants were associated with an increased AF incident rate.

Causal Associations Between Genetically Determined Hypertension, Systolic Blood Pressure, Diastolic Blood Pressure, and Atrial Fibrillation

The 3 HT-associated SNPs explained 1.04% of the variance in the AF levels, and the mean F statistic was 68. In the HT→ AF MR analysis using the conventional method (inverse variance weighted, IVW), we detected a causal relationship between HT and AF [odds ratio (OR): 1.90, 95% CI: 1.18–3.04, $P = 0.01$] with no evidence of heterogeneity between estimates from individual SNPs [$P_{\text{heterogeneity}} = 0.42$ (MR-Egger) and $P_{\text{heterogeneity}} = 0.72$ (IVW)] and the pleiotropy effect ($P_{\text{pleiotropy}} = 0.97$) (**Supplementary Table 6, Figures 1A,B**).

In the SBP→ AF and DBP→ AF MR analyses using the IVW method, our MR analyses showed that the genetically

determined SBP and DBP were consistently associated with a higher risk of AF (SBP→ AF, OR: 1.03, 95% CI: 1.01–1.05, $P = 0.01$; DBP→ AF, OR: 1.02, 95% CI: 1.00–1.04, $P = 0.03$) with no evidence of heterogeneity between estimates from individual SNPs or the pleiotropy effect [SBP→ AF, $P_{\text{heterogeneity}} = 0.88$ (MR-Egger), $P_{\text{heterogeneity}} = 0.88$ (IVW), and $P_{\text{pleiotropy}} = 0.46$; DBP→ AF, $P_{\text{heterogeneity}} = 0.72$ (MR-Egger), $P_{\text{heterogeneity}} = 0.73$ (IVW), and $P_{\text{pleiotropy}} = 0.50$) (Supplementary Table 6, Supplementary Figures 6A,B, 7A,B). The results were the same as in the weighted median and weighted mode methods in the SBP→ AF analysis (all $P < 0.05$). In the DBP→ AF analysis, the results were similar in the weighted mode method ($P = 0.02$) (Supplementary Table 6).

Furthermore, in the leave-one-out analysis, we found that no single instrument was strongly driving the overall effect of HT→ AF (Figure 2A). There was no funnel plot asymmetry (Figure 2B). Both the leave-one-out analysis and funnel plots further suggested that no SNPs exhibited horizontal pleiotropy. The horizontal pleiotropy results were similar in the SBP/DBP→ AF analysis (Supplementary Figures 8A,B, 9A,B).

DISCUSSION

In a large-scale ARIC cohort, we demonstrated that the participants with HT were associated with a 50% increase in incident AF. In the MR analysis, our results supported causal inference between HT and AF. These findings highlight the importance of optimal blood pressure control in the HT population to prevent AF. Therapeutics targeting HT treatment are likely to prevent AF effectively.

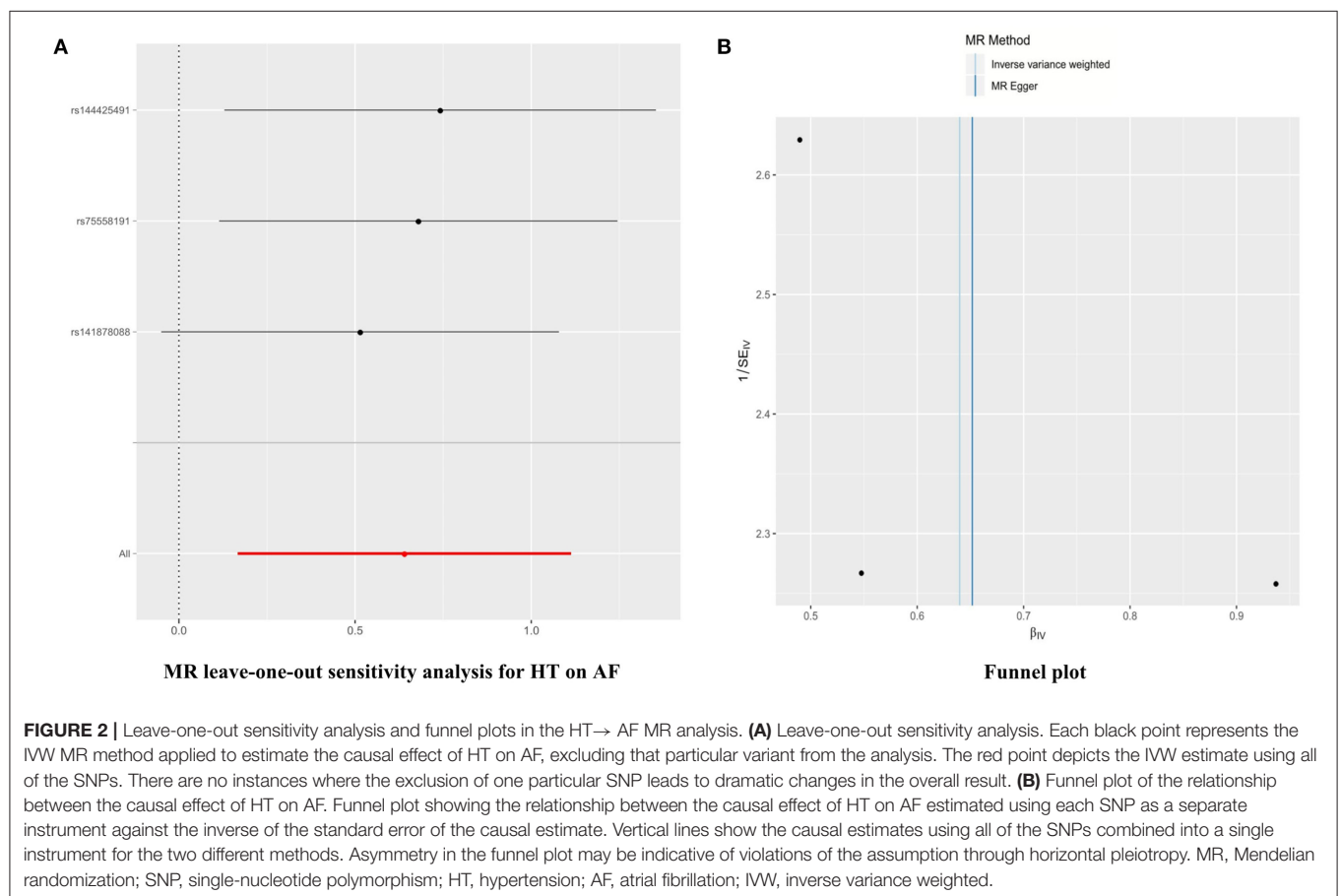
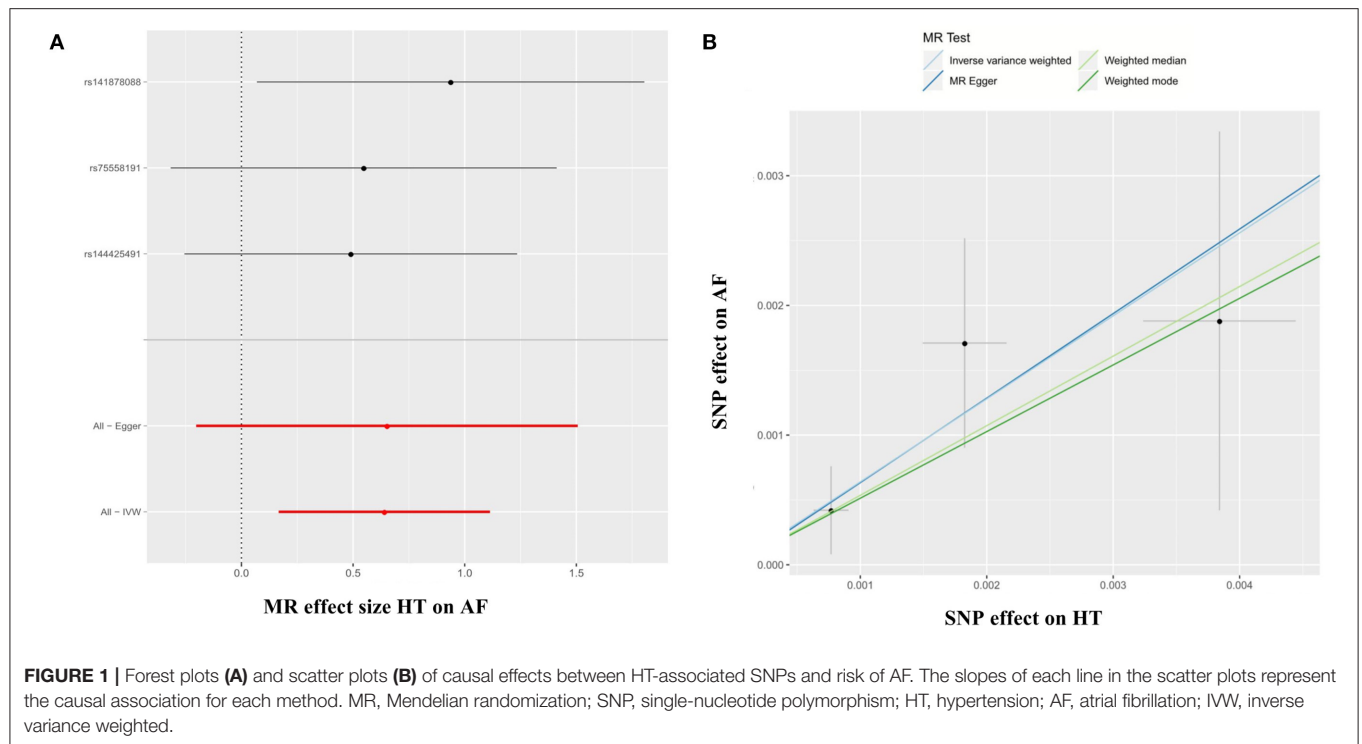
Previous epidemiological studies revealed that HT was an established risk factor for new-onset AF (4, 17, 18). However, these findings were limited in demonstrating a causal role for HT in AF development due to the potential of residual confounding and reverse causation (19). Previously, also in the ARIC study, it was reported that overall, 56.5% of AF cases could be explained by having ≥ 1 elevated risk factors, of which elevated blood pressure was the most important contributor (7). In our study, after adjusting for all of the covariates, the results indicated that the HT participants were associated with a 50% increased rate of incident AF. It was consistent with the previous study demonstrating the relationship between HT and AF (7). However, in our study, we had a much longer follow-up time (a median 24.1 years), while the mean follow-up time is 17.1 years in the previous survey. Moreover, our aim was different, resulting in different patient classification. In our study, our goal was to describe the association between HT and AF in the ARIC study, so patients were divided into two groups according to whether they had HT or not in visit 1. In the previous ARIC study, individuals were classified into three groups (optimal, borderline, and elevated level), according to the established AF risk factors (high blood pressure, elevated body mass index, diabetes, cigarette smoking, and prior cardiac disease) (7). Furthermore, in our study, the hazard ratios of SBP and DBP for incident AF were 1.17 (95% CI, 1.12–1.22) and 0.90

(95% CI, 0.84, 0.97), respectively, after adjusting for each other in the final models (Supplementary Table 5). We speculated that the effect of HT on AF was primarily through SBP instead of DBP.

Nowadays, the most common sustained cardiac arrhythmia is AF. It is associated with substantial healthcare use, stroke, and mortality. Significant strides have been made in stroke prevention and rhythm control strategies, yet reducing the incidence of AF has been slowed by increasing the incidence and prevalence of AF risk factors, including obesity, physical inactivity, sleep apnea, diabetes mellitus, HT, and other modifiable lifestyle-related factors (20). Since HT is the most important modifiable risk factor for AF, and abundant previous evidence supported the association between HT and AF, we were eager to know whether HT served as an etiology for AF. So we conducted the MR study to test the causality between HT and AF. Our results provided evidence supporting a causal association between genetically determined HT and AF [odds ratio (OR): 1.90, 95% CI: 1.18–3.04, $P = 0.01$]. Two prior studies are looking at blood pressure genetics and AF (5, 21). The first one demonstrated that SBP and DBP mediated ischemic stroke risk, in part, through AF (21). The second one found that blood pressure was associated with increased risk of AF, and blood pressure reduction with calcium channel blockade or beta-blockade could reduce the risk of AF in another consortium, which was different from ours (5).

Several pathophysiologic mechanisms could explain the relationship between HT and AF (22–24). HT animal models elaborated that high blood pressure led to left atrial scarring and inflammation (22, 23), and then, it would create altered patterns of conduction and functional slowing, resulting in AF development and perpetuation (23, 24). Moreover, HT-induced neurohormonal activation and autonomic dysfunction could also contribute to the pathogenesis of AF (25). The last but not the least, HT and AF might share the same pathogenic factors. For example, a recent MR study reported that higher BMI and a particularly fat mass index were associated with an increased risk of both HT and AF (26).

It was noted that in clinical practice, a retrospective real-world cohort analysis revealed that earlier and stricter 24-h blood pressure control reduced the occurrence of new-onset non-valvular AF (27). It was noted that HT might serve as a pathogeny for AF. Yet the current HT guidelines, including the recently released US guidelines (28), did not recommend more aggressive blood pressure targets for AF prevention. Based on epidemiological evidence, this suggestion might sound quite reasonable. The prevalence of HT increased and was currently ~20 to 50% in the adult population worldwide (29, 30). Our MR study revealed a causal association between HT and AF. So we believed that HT was still the most important potentially modifiable risk factor responsible for the increasing burden of AF. Although there was a genetic relationship between HT and AF, this relationship could not be explained by genetics alone. Many additional factors were relevant, including obesity, HF, sleep apnea, and so on. It should be further noted that genetics could not be impacted; other



modifiable risk factors should be targeted to achieve better AF prevention.

LIMITATIONS AND STRENGTHS

There are several limitations in our study. First, the ascertainment of AF is not perfect in the ARIC study. It is identified by ECGs performed during study exams, hospital discharge codes, and death certificates. So, it may miss the paroxysmal AF, resulting in a lower AF incidence. Second, technically speaking, it is better to adjust all the confounders, which may affect the AF incidence, such as rheumatic heart disease, chronic obstructive pulmonary disease, etc. Yet, we can only adjust as many confounders as we can get in the real world. Third, we suggest providing more omics information on the SNPs used in the analysis to see if these SNPs break the hypothesis of horizontal pleiotropy. The last but not the least, GWAS data in our study mainly relates to European ethnic individuals; therefore, the analysis should be repeated in other populations before being generalized across ethnic groups. In spite of this, based on the epidemiology data in ARIC study and Mendelian randomization causality research, we believe the causality to HT and AF.

CONCLUSIONS

Participants with HT were associated with a 50% increased rate of incident AF. HT might be a genetic cause of AF.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Guangdong Pharmaceutical University. The participants provided written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

L-ZL: design of the study. X-YW: analysis the data. S-ZZ: interpretation of the data. W-DL: drafting of the article. X-DZ: conception and design of the study.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcvm.2021.644405/full#supplementary-material>

Supplementary Figure 1 | Diagram of the instrumental variable assumptions for Mendelian randomization. The three assumptions are: (1) the genetic variant must be robustly associated with the exposure; (2) the genetic variant should not be related to confounders of the exposure-outcome association; and (3) the genetic variant must influence the outcome through the exposure only and not through any direct or alternative pathways. The dashed lines represent pathways that violate the assumptions. AF, atrial fibrillation.

Supplementary Figure 2 | Unadjusted cumulative curves for incident AF. During a median follow-up of 24.1 years, 1,414 cases (14.9%) of AF occurred. Unadjusted cumulative curves for incident AF are shown. AF, atrial fibrillation.

Supplementary Figure 3 | Hazard ratio of SBP for AF. SBP, systolic blood pressure; AF, atrial fibrillation.

Supplementary Figure 4 | Hazard ratio of DBP for AF. DBP, diastolic blood pressure; AF, atrial fibrillation.

Supplementary Figure 5 | Subgroup and sensitivity analyses of the association between HT and incident AF. Pre-specified subgroups by sex, age, race, smoking, drinking, BMI, creatine, LDL-c, and TG were analyzed. Sensitivity analyses were conducted by excluding participants with prevalent HF, CHD, and diabetes. HT, hypertension; AF, atrial fibrillation; BMI, body mass index; LDL-c, low-density lipoprotein cholesterol; TG, triglycerides; HF, heart failure; CHD, coronary heart disease.

Supplementary Figure 6 | Forest plots and scatter plots of causal effects between SDP-associated SNPs and risk of AF. The slopes of each line in the scatter plot represent the causal association for each method. SBP, systolic blood pressure; AF, atrial fibrillation; SNP, single-nucleotide polymorphism; MR, Mendelian randomization; IVW, inverse variance weighted.

Supplementary Figure 7 | Forest plots and scatter plots of causal effects between DBP-associated SNPs and risk of AF. The slopes of each line in the scatter plot represent the causal association for each method. DBP, diastolic blood pressure; AF, atrial fibrillation; SNP, single-nucleotide polymorphism; MR, Mendelian randomization; IVW, inverse variance weighted.

Supplementary Figure 8 | Leave-one-out sensitivity analysis and funnel plot in the SBP → AF MR analysis. **(A)** Leave-one-out sensitivity analysis. Each black point represents the IVW MR method applied to estimate the causal effect of SBP on AF, excluding that particular variant from the study. The red point depicts the IVW estimate using all of the SNPs. There are no instances where the exclusion of one particular SNP leads to dramatic changes in the overall result. **(B)** Funnel plot of the relationship between the causal effect of SBP on AF. Funnel plot showing the relationship between the causal effect of SBP on AF estimated using each SNP as a separate instrument against the inverse of the standard error of the causal estimate. Vertical lines show the causal estimates using all SNPs combined into a single instrument for the two different methods. Asymmetry in the funnel plot may be indicative of violations of the assumption through horizontal pleiotropy. SBP, systolic blood pressure; AF, atrial fibrillation; SNP, single-nucleotide polymorphism; MR, Mendelian randomization; IVW, inverse variance weighted.

Supplementary Figure 9 | Leave-one-out sensitivity analysis and funnel plot in the DBP → AF MR analysis. **(A)** Leave-one-out sensitivity analysis. Each black point represents the IVW MR method applied to estimate the causal effect of DBP on AF, excluding that particular variant from the study. The red point depicts the IVW estimate using all of the SNPs. There are no instances where the exclusion of one particular SNP leads to dramatic changes in the overall result. **(B)** Funnel plot of the relationship between the causal effect of DBP on AF. Funnel plot showing the relationship between the causal effect of DBP on AF estimated using each SNP as a separate instrument against the inverse of the standard error of the causal estimate. Vertical lines show the causal estimates using all of the SNPs combined into a single instrument for the two different methods. Asymmetry in the

funnel plot may be indicative of violations of the assumption through horizontal pleiotropy. DBP, diastolic blood pressure; AF, atrial fibrillation; SNP, single-nucleotide polymorphism; MR, Mendelian randomization; IVW, inverse variance weighted.

Supplementary Table 1 | Characteristics of the SNPs associated with HT and AF.

Supplementary Table 2 | Characteristics of the SNPs associated with SBP and AF.

Supplementary Table 3 | Characteristics of the SNPs associated with DBP and AF.

Supplementary Table 4 | Baseline characteristic of study populations by hypertension.

Supplementary Table 5 | Hazard ratio of HT for AF.

Supplementary Table 6 | Causal associations between genetically determined HT, SBP, DBP and AF.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Evaluating the Use of Genetics in Brugada Syndrome Risk Stratification

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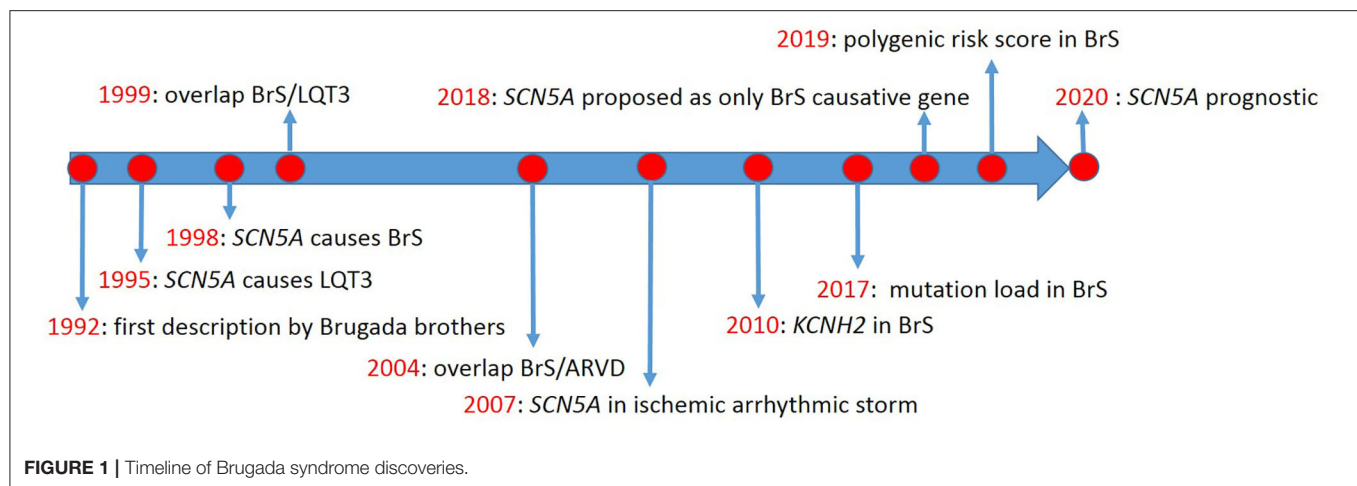
The evolution of the current dogma surrounding Brugada syndrome (BrS) has led to a significant debate about the real usefulness of genetic testing in this syndrome. Since BrS is defined by a particular electrocardiogram (ECG) pattern, after ruling out certain possible causes, this disease has come to be defined more for what it is *not* than for what it *is*. Extensive research is required to understand the effects of specific individual variants, including modifiers, rather than necessarily grouping together, for example, “all *SCN5A* variants” when trying to determine genotype-phenotype relationships, because not all variants within a particular gene act similarly. Genetic testing, including whole exome or whole genome testing, and family segregation analysis should always be performed when possible, as this is necessary to advance our understanding of the genetics of this condition. All considered, BrS should no longer be considered a pure autosomal dominant disorder, but an oligogenic condition. Less common patterns of inheritance, such as recessive, X-linked, or mitochondrial may exist. Genetic testing, in our opinion, should not be used for diagnostic purposes. However, variants in *SCN5A* can have a prognostic value. Patients should be diagnosed and treated per the current guidelines, after an arrhythmologic examination, based on the presence of the specific BrS ECG pattern. The genotype characterization should come in a second stage, particularly in order to guide the familial diagnostic work-up. In families in which an *SCN5A* pathogenic variant is found, genetic testing could possibly contribute to the prognostic risk stratification.

Keywords: Brugada syndrome, sudden cardiac death, genetic testing, mutation, variant, *SCN5A*, sodium channel, arrhythmia

INTRODUCTION

The first description of Brugada syndrome (BrS) included eight unrelated patients with recurrent aborted sudden cardiac death due to ventricular fibrillation (VF) (1), in whom basal ECG showed persistent ST-segment elevation in precordial leads V1 to V2-V3. However, the genetic background was not discussed. Thus, no genotype-phenotype relationship was established. Meanwhile, Gellens and coworkers characterized *SCN5A* for the first time (2). Later, *SCN5A* was described in two unrelated families with long QT syndrome (LQTS) type-3 (LQT3) (3) (timeline, **Figure 1**).

BrS was first considered a form of idiopathic VF, resulting from abnormal electrophysiologic activity in right ventricular epicardium (4). It was described to lie on the same spectrum of cardiac



electrophysiologic pathology as LQT3, caused by the same variant in *SCN5A* (5). Today, BrS is considered a Mendelian disorder inherited in an autosomal dominant fashion, even if alternative mechanisms of inheritance have been recently proposed (6). In BrS patients, variants in *SCN5A* are found more commonly than in any other gene (7) but confirm the clinical diagnosis in only a minority of cases (8). Many other genes have been proposed to cause BrS, but their roles are hotly debated (9, 10), with some groups suggesting that only *SCN5A* should be used in BrS genetic testing (9). However, variants in *SCN5A* have long been known to not necessarily segregate with BrS (4, 11). Recently, patients harboring *SCN5A* variants were demonstrated to have a worse prognosis (12).

These challenges have resulted in two important consequences: an overestimation of *SCN5A* diagnostic value and a contemporary underestimation of the clinical significance of genes different from *SCN5A*. All considered, the goals herein are to reevaluate the clinical significance of genetic data found in patients with BrS and to provide new insights about the complex genetics of BrS.

Clinical Definition of BrS

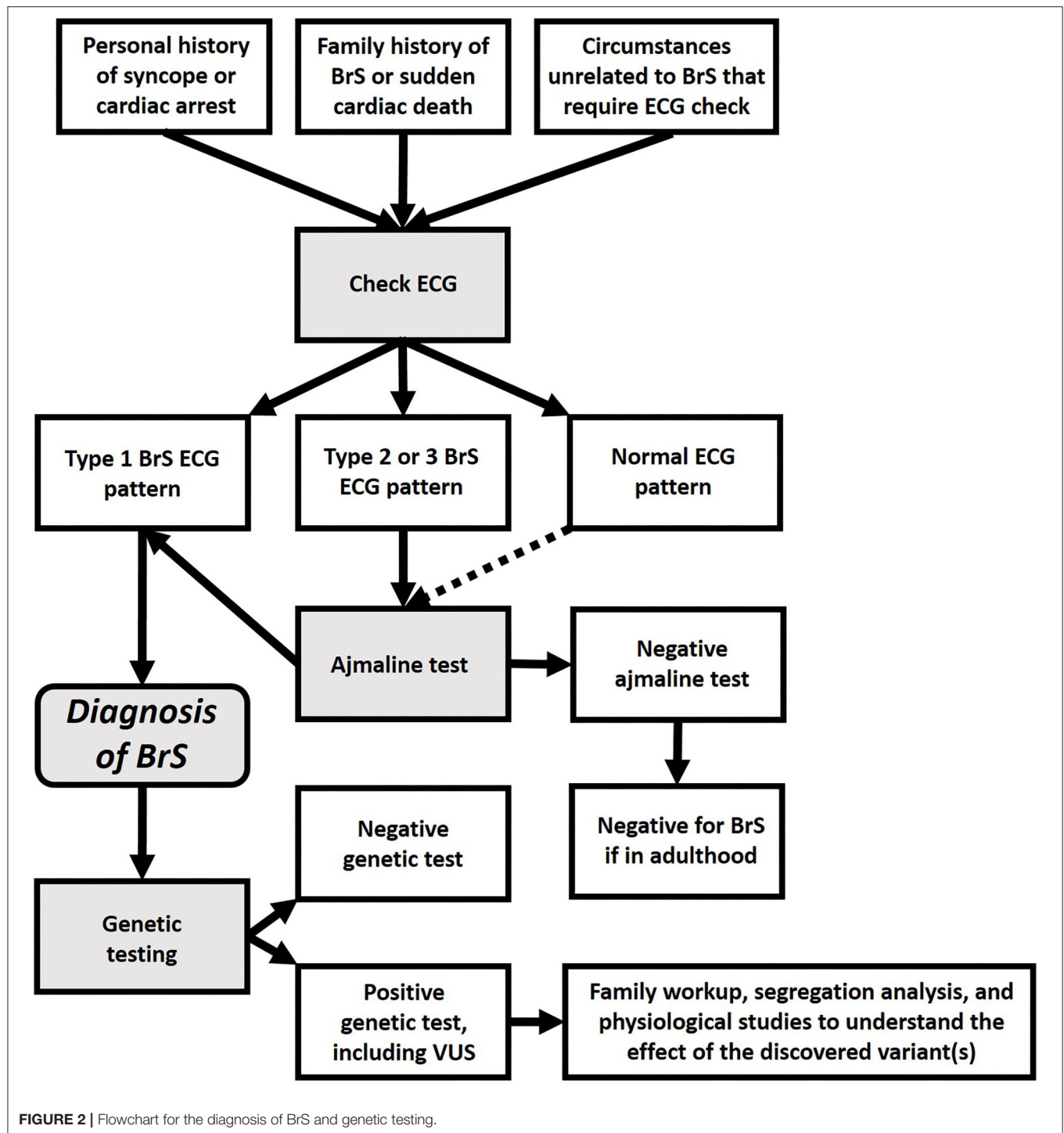
The difficulty in understanding BrS genetics may lie in the definition of BrS, based on the electrocardiogram (ECG), specifically the type-1 BrS pattern, an ST-segment elevation with coved morphology, ≥ 2 mm, often associated with a sharp transition from elevated ST-segment to negative T-wave, among right precordial leads V1-V2, positioned in the 2nd, 3rd, or 4th intercostal space (13). This type-1 BrS pattern can occur either spontaneously or be unmasked with intravenous administration of Class 1c antiarrhythmic drugs, such as ajmaline or flecainide (13). Recently, it was hypothesized that BrS might actually be a heterogeneous disease with a common ECG phenotype (14). While this phenotype has been commonly attributed to loss-of-function of the $\text{Na}_v1.5$ cardiac sodium channel, such phenotype could result from a number of molecular origins, not only *SCN5A* variants, but also alterations in proteins that modify the channel, or even environmental influences. Regarding the environmental

influences, “true BrS” is diagnosed by ruling out such causes as electrolyte disturbances or myocardial ischemia. BrS patterns in these cases are said to be “BrS phenocopies” (15, 16). We disagree with the definition of “phenocopy,” because it is based upon what BrS is *not* rather than providing a clear picture of what BrS is. This is especially concerning since environmental influences can have a pivotal role in BrS (17). Perhaps a better view would be to consider the “BrS pattern” as a warning of risk for sudden cardiac death, regardless of the underlying cause (18). We are aware that this concept challenges the autosomal dominant model of BrS, largely based on the accepted etiologic role of *SCN5A*.

BrS has also been attributed to an increase in potassium current (19, 20). Furthermore, several studies have suggested BrS may be similar to a cardiomyopathy (21–26). Thus, it is likely that the ECG pattern used to define “BrS” is actually a common clinical manifestation, resulting from a multitude of different molecular causes. Further development of this concept may lead to a new paradigm for BrS, which may be considered not only as a Mendelian disorder, but as a complex condition, which might be caused by a huge variety of genetic variants, interacting with environmental factors (14, 27). In any case, since our current understanding of BrS genetics is still elementary, today BrS should be diagnosed by the type-1 ECG pattern (see **Figure 2**), not by genetic findings, especially additional findings during screening for other diseases.

Genotype-Phenotype Relationships

Genotype-phenotype relationships are difficult to establish in BrS patients, because the clinical manifestations can be very subtle, and because the differential diagnosis can be extremely complex (28). Additionally, *SCN5A* variants have since been associated with a variety of pathologies (29, 30). Other works (31, 32) demonstrated both rare mutations and common variants in *SCN5A* can be considered phenotype modulators in myocardial infarction (33), arrhythmic storm (34), epilepsy (35), and even colon (36) and breast cancer (37). Thus, although *SCN5A* is



the only undisputed gene in which mutations are thought to cause BrS, genetic testing alone is insufficient to diagnose BrS, as mutations in this gene could result in a number of different phenotypes. Instead, BrS must be diagnosed only in the presence of a diagnostic type 1 BrS ECG pattern (spontaneous or drug-induced), not due to secondary causes, such as electrolyte disturbances or myocardial ischemia.

Other Candidate Genes

A recent study (9) concluded that only the *SCN5A* gene should be analyzed in BrS patients. We agree that mutations in *SCN5A* could be the cause of BrS in some patients. However, the study did not address what should be done in the majority of BrS patients, who test negative for any *SCN5A* mutations, nor provide clarity of the disease mechanism in those patients negative

for *SCN5A* mutations, especially regarding the role of copy number variations and mitochondrial DNA. London expressed his disagreement, arguing that eliminating other genes from testing panels could stifle scientific advancement (10). Wilde and Gollob (38), however, countered by arguing that undue harm from incorrect interpretation could result in a life-changing diagnosis, require intervention, create life-long anxiety, and impact asymptomatic family members. We believe that suspected candidate genes should be tested and studied so that we can better understand their effects. However, all suspected cases should be confirmed by the presence of the BrS pattern, including patients found to have mutations in *SCN5A*, as single mutations in this gene are responsible for a variety of phenotypes, not only BrS (39, 40), and may not even cause BrS on their own (41).

Many other genes such as *SCN10A* (42, 43), *SCN4A* (44), *SCN1B* (45), *KCNH2*(46), *RANGRF* (47), *PKP2*(48), *TPM1*(49), and several calcium channels genes (50–53) have been described in patients clinically affected by BrS. Whole exome sequencing with a high coverage was performed in a family with both hypertrophic cardiomyopathy and type-1 BrS, apparently caused by the same heterozygous *TPM1* mutation (49). Thus, several candidate genes exist and should be further studied. Physiologic studies should follow the discovery of candidate mutations in the clinic, as abnormal effects in the physiology laboratory can provide useful insights to understanding particular new mutations.

Modes of Inheritance

In spite of recent developments in the field of genetics, BrS is often still considered a monogenic Mendelian disease (54) inherited in an autosomal dominant fashion with incomplete penetrance (55–58). This is mainly due to the description of BrS in a family in which the genetics were consistent with this kind of transmission (59), making *SCN5A* the only accepted BrS gene (9). Another reason why *SCN5A* is so “popular” is because the segregation of variants in this gene show incomplete penetrance and marked variability in a significant percentage of patients (60). However, increasing evidence suggests that BrS in some patients might be actually caused by a digenic inheritance (61) or a combined effect of multiple variants (62), including polymorphisms (63). In this subset of patients, it is difficult to identify the real molecular cause of BrS, making it difficult to understand, using only genetic testing, which family members have inherited the syndrome and which have not. Additionally, since BrS may be due to a combined effect of multiple variants, the severity can often be different between family members (40). Furthermore, there might be other cases in which some family members have the syndrome but others do not, despite sharing certain variants, because of differences in modifier genes.

Although autosomal dominant inheritance with incomplete penetrance is the most commonly accepted mode of transmission of BrS, other forms of transmission have been suggested, such as recessive (64) and X-linked (19, 20). It is also possible that yet-undiscovered somatic mutations could have an effect on the heart. Furthermore, an autosomal dominant inheritance pattern could imply that the disease is Mendelian in nature, caused by a single mutation in a single gene. However, several studies have demonstrated an oligogenic mode of inheritance (7).

Therefore, likely, in some families, a particular variant causes BrS in a Mendelian fashion, while in other families, the pattern of inheritance is more complicated to understand, because the disease is caused by a combination of factors, resulting in different phenotypes even between family members (65). Tadros et al. calculating polygenic risk scores (PRSs) for PR interval, QRS duration, and BrS, reported that 44 common variants associated with PR, and 26 common variants associated with QRS, in the general population, were associated with ajmaline-induced PR and QRS prolongation, respectively. Also, a 3-single-nucleotide-polymorphism PRS derived from a case-control BrS GWAS was independently associated with ajmaline-induced type-1 BrS ECG (66). This demonstrates the importance of polymorphisms that might predispose to arrhythmias and create a pathological effect, especially in the presence of other variants in the same patient.

Overlap Syndromes

Since variants in *SCN5A* can be found in several cardiogenetic disorders, it is not surprising to observe an overlap between BrS and other pathologies. For example, BrS can be diagnosed in the proband while LQTS, epilepsy, febrile seizures, or complete bundle branch block can be present in the family members (67–70).

Overlap between arrhythmogenic right ventricular (RV) dysplasia/cardiomyopathy (ARVD/C) and BrS has been described by many groups (71), the mechanism of which may involve cell-cell junctions (24). Both ARVC and BrS can originate from mutations in the connexome, and the phenotype that emerges depends on the type of connexome mutation (72, 73). *PKP2* may be an important gene in this regard, as mutations in *PKP2* can result in loss of desmosomal integrity, cause sodium current deficit, and be found in patients with BrS (74, 75). The presence of ARVC in BrS patients has been associated with higher arrhythmic risk (76). The genetics of families with overlap syndromes should be carefully considered, as these genetic causes may be different than other families in which BrS is the only phenotype observed. This is yet another example of the need for personalized medicine and to consider the genetics of BrS on a family-by-family basis.

Mitochondrial Considerations

Many recent studies have related cardiac arrhythmias, and particularly BrS, to mitochondrial function, or the effect of mitochondrial products on the sodium channel. Heart arrhythmias can originate from pathophysiology of the mitochondria, which produce adenosine triphosphate, a compound required for normal ion channel function (77). Aiba et al. described a family with BrS and the *SCN5A* mutation R526H, which is a PKA consensus phosphorylation site and associated with reduced basal I_{Na} due to the inability of PKA to act on the sodium channel to increase the sodium current (78). A mutation in the GPD1-L protein reduces I_{Na} by raising intracellular NADH levels and inducing reactive oxygen species (ROS) (79). This process of ROS production, its release from mitochondria, and thus its detrimental effect on the sodium current can be reversed in several ways, namely by NAD⁺, inhibition of mitochondrial electron transport, a mitochondrial targeted antioxidant, and an inner membrane

anion channel modulator (80). A specific mitochondrial DNA (mtDNA) allelic combination and a high number of mtDNA single nucleotide polymorphisms (SNPs) have been reported in association with more severe cases of BrS, suggesting that these are important cofactors in the expression of the clinical phenotype (81, 82). Tafti et al. suggested that BrS may be caused by mutations in mitochondrial transfer RNA (tRNA) genes, leading to deficiencies in the translational process of critical proteins of the respiratory chain (83). Reports have demonstrated that tRNAMet, tRNAIle, tRNATrp and tRNAGln genes are hot spots for cardiovascular diseases (83, 84). Thus, mitochondrial function, or malfunction, contributes to sodium channel function and to cardiac rhythm.

Risk Stratification

Risk stratification in BrS has previously relied on clinical scores (85), including familial history of sudden cardiac death, personal history of syncope, aborted cardiac arrest, spontaneous type-1 BrS pattern, or male gender. It was also reported that proband status, inducibility toward ventricular arrhythmias (86), arrhythmogenic substrate area, and late potentials (87) were predictors of higher risk. Our group recently proposed the *SCN5A* genetic status as a prognostic factor for BrS patients (12, 88). In particular, *SCN5A* mutation carriers exhibited more pronounced epicardial electrical abnormalities and a more aggressive clinical presentation. In at least a subgroup of patients, the mutated *SCN5A* gene acts more like a phenotype modulator than a real Mendelian dominant cause of the displayed phenotype, possibly calling into question the autosomal dominant inheritance of BrS. This is true also for variants of “unknown significance” (VUS), which are generally treated as “benign.” However, in our experience, several of these VUS are later reclassified as pathogenic. We believe that, in time, many other VUS, especially in the *SCN5A* gene, will be determined to be pathogenic, considering also that the oligogenic model is likely to be accepted in the near future.

DISCUSSION

The genetics of BrS have likely remained elusive because of how the disease has been considered only an autosomal dominant Mendelian disorder. However, when BrS is considered an oligogenic disorder, it may be possible to use genetics to predict the BrS phenotype. Besides direct modifications in the *Nav1.5* protein, its function can be altered by many regulatory proteins like Hey2, Mog1, Gpd1-L, and others. According to us, studying the genes encoding those proteins is very important for the clinical management of BrS patients. Additionally, environmental factors might influence channel function through post-translational modifications. Even in families where *SCN5A* variants have been found, segregation analysis is not always consistent with autosomal dominant inheritance, demanding caution be used when interpreting genetic test results. Currently, it is necessary that all suspected cases of BrS are confirmed with ECG, using, when necessary, drug challenge to elicit the type-1 pattern. In other words, genetic testing alone should not be used for diagnostic purposes at this time, but rather, the

patients should each fulfill the diagnostic criteria for BrS at an arrhythmologic examination, as per the current guidelines (89). However, in families in which a *SCN5A* pathogenic variant is found, genetic testing could possibly contribute to the prognostic risk stratification.

Ideally, whole exome or whole genome testing should be performed to both confirm candidate genes and identify new ones. Collecting family segregation is mandatory to understand whether a particular variant might be clinically relevant. Ideally, such data should then be deposited into international databases. The specific effects of distinct variants should be studied, rather than necessarily grouping together, for example, “all *SCN5A* variants” when trying to determine genotype-phenotype relationships, because not all variants within a particular gene act similarly.

Identifying variants involved in oligogenic cases of BrS is extremely complicated. For this, the effect of polymorphisms, which, on their own, are considered benign, should be considered, as they may act as modifiers in the presence of other variants. For example, two variants in a particular gene may exist, which, individually, result in a benign phenotype, as neither variant, on their own, significantly modifies the ultimate function of the resulting protein. However, if those two (or three, or more) variants occur together in the same person, together they could ultimately impair the function of the protein, altering the clinical picture. This “mutational load” is an important concept in BrS, explaining why the genetics of this disease have been so difficult to elucidate. However, to understand the effect of mutational load, or compound heterozygosity (i.e., two or more heterogeneous recessive alleles at a particular locus), extensive research studies should be performed, also identifying other genes responsible for BrS, besides *SCN5A*. Only then it will be possible to study these concepts of oligogenic inheritance in the majority of patients. Probably, whole genome or whole exome studies would be useful in determining the genes involved, along with family segregation analysis.

Finally, non-genomic DNA considerations should be mentioned, as post-translational modifications of the sodium channel could affect its function without any variants in the *SCN5A* gene. Studies should be expanded to better understand any possible role for mitochondrial involvement, including the analysis of mitochondrial genes, their products, and their functional effects on the cells. Environmental factors should also be studied, including anything to which families may be exposed, resulting in post-translational effects, especially when probands test negative for variants in all BrS candidate genes. Environmental factors could be mistaken as a genetic condition when several family members living in the same environment are affected.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

MM and EM drafted the paper. EL and CP provided revisions and useful feedback. CP secured funding for the project. All authors approved the final version of the manuscript.

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Prenatal Management Strategy for Immune-Associated Congenital Heart Block in Fetuses

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Fetal congenital heart block (CHB) is the most commonly observed type of fetal bradycardia, and is potentially life-threatening. More than 50% of cases of bradycardia are associated with maternal autoimmunity, and these are collectively termed immune-associated bradycardia. Several methods have been used to achieve reliable prenatal diagnoses of CHB. Emerging data and opinions on pathogenesis, prenatal diagnosis, fetal intervention, and the prognosis of fetal immune-associated CHB provide clues for generating a practical protocol for clinical management. The prognosis of fetal immune-associated bradycardia is based on the severity of heart blocks. Morbidity and mortality can occur in severe cases, thus hierarchical management is essential in such cases. In this review, we mainly focus on optimal strategies pertaining to autoimmune antibodies related to CHB, although the approaches for managing autoimmune-mediated CHB are still controversial, particularly with regard to whether fetuses benefit from transplacental medication administration. To date there is still no accessible clinical strategy for autoimmune-mediated CHB. This review first discusses integrated prenatal management strategies for the condition. It then provides some advice for clinicians involved in management of fetal cardiovascular disorder.

Keywords: fetal immune-associated heart block, prenatal diagnosis, prenatal management, transplacental drug administration, outcome

HIGHLIGHTS

- This review first summarizes approaches for managing autoimmune-mediated congenital heart block based on the advantages and disadvantages of medications administration.
- Previous studies provide evidence that supports positive treatment for first-degree and second-degree atrioventricular blocks.
- Dexamethasone and hydroxychloroquine have not demonstrated any advantages with respect to reversing first-degree fetal atrioventricular block, but it is still recommended to administer medication for first-degree atrioventricular block due to therapeutic effects of dexamethasone and hydroxychloroquine to prevent progression of heart block.
- Transplacental administration of dexamethasone, intravenous immunoglobulin, and hydroxychloroquine is not considered viable for reversing third-degree atrioventricular blocks or benefiting fetal prognosis.

- Treatment to maintain heart function via digoxin and a β -sympathomimetic benefit delivery outcomes, but some patients born under such circumstances require pacemaker implantation soon after birth.
- Anti-Ro/SSA and La/SSB antibodies contribute to the most common causes of fetal immune congenital heart block.

INTRODUCTION

Congenital heart block (CHB) is one of the most commonly observed types of fetal bradycardia, and it entails potentially life-threatening problems for the fetus, and can result in fetal developmental delay and even intrauterine death (1). The general fetal mortality rate associated with CHB is 19%, and in ~70% of cases a pacemaker is implanted after birth. CHB is usually diagnosed between 18 and 24 gestational weeks (GWs) during pregnancy via fetal echocardiography techniques, but in some cases it has reportedly been identified at 16 GWs. In some late onset cases it has not been detected until 29 GWs. Fetal CHB is considered a dynamic condition, because some patients with second-degree or complete atrioventricular block (AVB) exhibit a normal atrioventricular interval or first-degree AVB in initial screening during pregnancy. Fetuses with severe conditions usually exhibit a significant reduction in ventricular rate, between 50 and 70 beats per minute (2), and in such cases there is a higher likelihood of fetal heart failure, edema, premature delivery, abortion, or intrauterine death.

Current evidence indicates that CHB is almost universally associated with transplacental transfer of Ro and La antibodies, which causes cardiac conduction tissue damage that can occur as early as 11 GWs (3). To date however, the underlying molecular mechanisms of CHB pathogenesis have not been fully elucidated. Approaches to CHB management are still controversial, and decisions are always made based on the advantages and disadvantages of administration. Emerging evidence suggests that early diagnosis and proper treatment can improve the prognosis and survival rate of affected fetuses, and some common opinions pertaining to the prenatal management of CHB have emerged (1, 4).

Herein we present a review of the autoimmune antibodies related to CHB. The review is an attempt to emphasize the practical data and opinions on the pathogenesis and prenatal diagnosis of fetal immune-associated CHB, particularly with respect to fetal intervention and prognosis.

PATHOGENESIS

Autoimmune-associated CHB occurs in 2–5% of pregnancies in anti-Ro/SSA and anti-La/SSB antibody-positive women. It has a recurrence rate of 12–25% in a subsequent pregnancy after the birth of a baby with neonatal lupus (5–7). Anti-Ro/SSA and anti-La/SSB are both antinuclear antibodies that can cross the placenta via Fc γ Rn, and induce autoimmunity. Studies have confirmed that autoimmune-associated CHBs often co-occur in a variety of maternal autoimmune disorders, including Sjögren's

syndrome, systemic lupus erythematosus, rheumatoid arthritis, and undifferentiated connective tissue disease (8–10).

Ro/SSA antigen is a ribonucleoprotein complex. It is composed of Ro52 and Ro60 peptides. Early demonstration of the presence of immunoglobulin deposition in the heart samples of human fetuses that died from CHB provided the first evidence of an association between maternal anti-Ro antibodies and fetal CHB initiation (11, 12). It is now known that there is a strong association between anti-Ro/SSAs and the occurrence of CHB (13). A mouse model with Ro60 and La antibodies exhibits first-degree AVB in offspring (14).

In the fetal heart, Ro52, Ro60, and La antigens are located in the nuclei of cardiomyocytes and cardiac conduction cells. Ro52 is involved in the regulation of interferon regulatory factor-mediated immune responses (15–17), whereas Ro60 is thought to play a role in RNA regulation (18). Ambrosi and Wahren-Herlenius (19) proposed a biphasic model incorporating the apoptosis hypothesis and the cross-reactivity hypothesis to explain how autoantibodies from the maternal circulation lead to fetal immune-associated CHB. The apoptosis hypothesis suggests that antibodies may bind to the surfaces of cells undergoing programmed apoptosis after entering the fetal cycle, resulting in apoptosis fragmentation. These apoptotic fragments are phagocytized by macrophages via podsolization, triggering a series of proinflammatory and fibrotic substrates downstream. The “waterfall effect” of cell factors involves the recruitment of white blood cells and complement, induces an immune inflammatory response and a fibrotic response, and ultimately leads to cardiac conductive cell damage, resulting in fetal immune-associated CHB. Single cell RNA-sequencing has been used to analyze the cellular characteristics of cardiomyocytes and non-cardiomyocytes in hearts of fetal CHB. Increased and heterogeneous interferon responses were identified in varied cell types of the CHB heart, and gene expression enriched in extracellular matrix organization and fibrosis formation (20). The cross-reactivity hypothesis suggests that fetal CHB is primarily associated with maladjustment of calcium channels. In previous studies IgG extracted from the sera of mothers whose fetuses suffered immune CHB could inhibit L-type and T-type calcium channels of ventricular cardiomyocytes, sinoatrial node cells, and atrioventricular node cells, affecting electrical activity (21). Overexpression of L-type calcium channels in mice can reportedly partially ameliorate CHB due to anti-Ro/La antibody exposure (22). Abnormal electrical activity of the atrioventricular node in the heart caused by a cross-reaction would lead to chronic inflammation and fibrogenesis, which is thought to be one of the primary mechanisms of CHB induction. Thus, it has been suggested that the pathophysiological process of fetal CHB is aggressive and irreversible, due to localized cardiomyocyte and conduction cell damage and fibrosis formation (19).

In some recent studies CHB only developed in a small proportion of anti-Ro-positive pregnancies, with various outcomes (4). Given the low penetrance, it appears reasonable that other risk factors may affect the autoimmune damage to the fetal heart conduction system, such as genetic background variants and placental dysfunction. The protein sequences of the arginine 308 allele in the tumor necrosis factor alpha promoter

and the leucine 10 allele in transforming growth factor beta in fetuses were significantly associated with the prevalence of immune CHB (23). Clancy et al. (24) demonstrated that 17 human leukocyte antigen gene mutation sites were significantly correlated with cardiac damage in children with neonatal lupus erythematosus. Several polymorphic sites of MHC-I polypeptide-related sequence B and tumor necrosis factor alpha are reportedly significantly associated with CHB (24). Maternal age, thyroid function, and gestational season are also evidently associated with the occurrence of immune CHB (25).

PRENATAL DIAGNOSIS

Several methods have been used to definitively diagnose fetal CHB, including fetal echocardiography, fetal electrocardiography, and fetal magnetocardiography. In theory, fetal electrocardiography can accurately reflect fetal cardiac electrical activity, but is not commonly used in clinical practice because it is compromised by fetal movement which can lead to loss of signal, and it is also difficult to distinguish fetal signals from maternal signals. Fetal magnetocardiography is another non-invasive technique that can detect cardiac electrophysiological activity, but its application is limited by the need for expensive and specialized equipment, thus it is only available in a few regions. But magnetocardiography, likely to be a useful future diagnostic tool. In recent years, magnetocardiography has become one of the new research hotspots in the field of fetal arrhythmia because of its non-contact, high accuracy. Fetal sebaceous glands and maternal factors such as cervical insulation often affect the accuracy of fECG and fetal echocardiography results. Because magnetic conduction is not limited, fMCG can penetrate the cervix and sebaceous glands to separate the fetal magnetic signals from the maternal magnetic signals, and more directly reflect the fetal magnetic conditions. Multiple studies had shown that fMCG can provide a long-term rhythm analysis, show the mechanism of abnormal rhythm, measure the ventricular repolarization time, assess the risk of fetal sudden death and help clinicians choose more appropriate drugs (26–29).

Fetal echocardiography is currently the most common and effective approach used to identify fetal CHB. It assesses the mechanical consequences of arrhythmia rather than directly detecting the conduction signaling itself, and this can be achieved via M-mode and/or the Doppler method. M-mode echocardiography can simultaneously measure atrial motion (a wave) and ventricular motion (v wave) through the sampling line to determine the time sequence of atrial and ventricular motion, and atrioventricular conduction can then be inferred. Due to the limitations of atrioventricular contraction, peak value, and the variable position of the fetus during pregnancy however, it is still difficult to accurately measure the sampling line perpendicular to the atrioventricular node (30). Pulsed wave Doppler echocardiography (PD) can measure simultaneous flow across the mitral and aortic valve, and in cases of complete CHB it can demonstrate dissociation of atrial inflow and ventricular outflow (30). This can also be evaluated with simultaneous

Doppler flows in the superior vena cava and aorta, as well as in the pulmonary vein and pulmonary artery (31). In patients with first-degree and second-degree heart block, measurements of differences between the onset of atrial and ventricular waveforms are used to calculate mechanical atrioventricular intervals (32). Tissue Doppler imaging (TDI) can directly record the mechanical activity of the atria and ventricles during the cardiac cycle, facilitating more accurate measurement of cardiac intervals (31–33).

The above-described fetal echocardiography techniques are sufficient for distinguishing and interpreting certain types of fetal CHB, particularly second-degree and third-degree AVB. In terms of the diagnosis of fetal first-degree AVB however, differences between PD and TDI need to be further balanced because the different methods to determine fetal atrioventricular intervals and the reference data of atrioventricular intervals vary significantly between the two approaches (30). The measurement of atrioventricular intervals using PD can be performed in two ways; from the mitral valve to the aorta (MV-Ao), and from the beginning of the retrograde venous a wave in the superior vena cava to the beginning of the aortic ejection wave (SVC-Ao) (31), which is influenced by fetal position and orientation. The TDI-derived atrioventricular interval is measured from the atrial contraction to the isovolumetric contraction (Aa-IV) or from the atrial contraction to the ventricular systole (Aa-Sa) at the right ventricular free wall (32). It has been reported that the atrioventricular interval of PD-derived SVC-Ao is shorter than that of MV-Ao however, and the TDI-derived Aa-IV is likely to be shorter than Aa-Sa (31, 32), and Aa-IV measurement underestimates the PR interval (33). Nii et al. concluded that TDI-derived Aa-IV tracks the PR interval more closely than PD, and may be a more accurate ultrasound method for assessing fetal atrioventricular conduction (32). In another study, the difference between TDI and PD was less evident, and atrioventricular times measured via TDI were longer, and it was suggested that applying the proposed cut-off value (> 150 ms) would lead to over-diagnosis and over-treatment of many fetuses at risk (33). It has also been reported that the atrioventricular interval is positively correlated with gestational age (32). A fixed cut-off independent of gestational age and method of measurement is not useful for diagnosing fetal first-degree AVB (33). Therefore, the prenatally accurate diagnosis of first-degree AVB remains poorly defined and it is crucial to diagnose fetal AVB using an adequate screening strategy with appropriate reference ranges.

PRENATAL MANAGEMENT

After maternal serum autoantibodies cross the placenta into the fetal circulation, which begins at 11 GWs, an immune inflammatory response can cause fetal CHB. Thus, in pregnant women whose serum antibody test is positive, prenatal management has important clinical implications with regard to fetal prognosis. The objective of prenatal management is to prevent exacerbation of the early cardiac inflammatory response, and improve the survival rate and prognosis, as well as promoting the safety of pregnant women.

Accurate prenatal diagnosis is the cornerstone of prenatal monitoring and therapy. Pregnant women with clinical and subclinical autoimmune diseases should be referred for fetal cardiac morphology screening and heart function evaluation. If fetal CHB is suspected, the fetal cardiac conduction interval (AV interval) should be closely monitored (34). In a recent study (35) fetal atrioventricular intervals were a poor predictor of CHB progression, but CHB surveillance does facilitate the detection of fetuses with second-degree and third-degree AVB shortly after its development, potentially resulting in timely treatment initiation and a better outcome. As stated above, fetal magnetocardiography is a very useful tool and can provide much detailed information.

The management of CHB relies on interpretation of the atrioventricular interval. The normal range of the fetal atrioventricular interval varies depending on GW and fetal heart rate. It has previously been proposed that if the fetal atrioventricular interval is > 140 ms more frequent fetal echocardiography should be scheduled, and if it is > 150 ms first-degree AVB should be diagnosed (34).

First-Degree AVB

Balancing the benefits and adverse effects of treatment for fetal first-degree AVB is important because little is known about the natural progression of the condition. As indicated by the cases summarized in **Table 1** (35–46), there is still limited evidence on the effects of fluorinated steroids such as dexamethasone on the course of first-degree immune-mediated AVB *in utero*. Progression to third-degree AVB occurred in 2/25 (8%) treated fetuses in whom postnatal pacemakers were not implanted (36). For unclear reasons however, a permanent, rate-modulated, single-chamber, ventricular endocardial pacemaker was implanted a few days after birth in an infant who had undergone sinus conversion prior to delivery. Interestingly, none of the untreated fetuses developed high-degree AVB (36). Persistent first-degree AVB was present in 6/25 (24%) treated fetuses and 10/27 (37%) untreated fetuses before birth. Persistent first-degree AVB was detected in 4/25 (16%) infants who had undergone treatment *in utero* and 3/27 (11%) infants who were untreated. Due to the very small number of cases and the consequent lack of statistical power, there are no statistically significant associations between dexamethasone therapy and any of the observed outcomes. A few studies have investigated the administration of hydroxychloroquine (HCQ), but as yet it is not yet possible to conclude whether HCQ has therapeutic or preventative effects on heart block regression (40, 41, 46).

It has been reported that in some studies untreated patients with first-degree AVB exhibited spontaneous recovery. We calculated a review of previous reports, 16/27 patients (59%) who did not receive treatment spontaneously converted to a normal sinus rhythm, which was maintained until delivery (**Table 2**). Overall the prenatal and follow-up ratios of conversion to sinus rhythm in the treated group were higher than in the untreated group (68 vs. 59% before birth, 72 vs. 63% during follow-up), but the differences were not statistically significant. Given that lower-degree AVB can progress to higher-degree AVB within 24 h, and that the adverse outcomes of third-degree AVB are

irreversible, it is still recommended that the treatment of fetal first-degree AVB be considered with the agreement of guardians in cases where the mother and/or fetus exhibit life-threatening conditions. Transplacental dexamethasone (4–8 mg per day) and/or HCQ (200 mg two times per day) can be supplied for 4 weeks, and re-evaluation of the atrioventricular interval and fetal development is also required after treatment. A reduced dosage of dexamethasone should be administered after the first 2 weeks, and dexamethasone can be terminated if the atrioventricular interval decreases. If fetal development, as determined by the length of the femur and the biparietal diameter, has been identified as 2 weeks behind that of fetuses in the same GW, a more aggressive dexamethasone dosage reduction should be considered in an effort to maintain fetal growth.

Second-Degree AVB

Immune-associated second-degree AVB should be treated to avoid progression and adverse outcomes. Of the different degrees of AVB, treatment procedures for second-degree AVB have attracted the least debate. After a diagnosis of second-degree AVB has been made the therapeutic strategy involves oral administration of dexamethasone and HCQ, and intravenous immunoglobulin (IVIG). The IVIG should be administered four times at a dosage of 1 g/kg, within a period of 2 weeks. Additional administration should be continued once a month at a dosage of 1 g/kg if the initial treatment time is between the 16th and 30th GWs. The administration of dexamethasone and HCQ is the same as the strategy for first-degree AVB. Transplacental dexamethasone (4–8 mg per day) should be administered for 4 weeks, and HCQ (200 mg two times per day) should be considered for fetuses at all GWs. After 4 weeks of treatment echocardiography-based re-evaluation should be performed. If the atrioventricular interval is reduced, dexamethasone should be reduced or even terminated. The most important thing is to assess heart function. Second-degree AVB can lead to cardiac dysfunction. A β -sympathomimetic agent (terbutaline 2.5 mg every 8 h or salbutamol 2.4 mg every 8 h) can be used to increase heart rate, but there is currently insufficient evidence to support the administration of digoxin. Thus, the use of digoxin is an alternative that can be administered after due consideration. Preterm delivery of fetuses with normal heart function should be avoided.

Third-Degree AVB

In fetuses with third-degree AVB the most important thing is to predict both fetal and maternal gestational outcomes. The avoidance of extremely adverse maternal effects should be afforded top priority. Lesions of the heart itself should be screened because the mortality rate is increased by $> 50\%$ in fetuses with endocardial fibroelastosis or dilated cardiomyopathy, and increased by nearly 100% when both lesions are present. Notably however, heart rate should be assessed first. If it is extremely low (55 bpm could work as a potential predictive value) the patient will die with severe heart dysfunction if there is accompanying endocardial fibroelastosis or dilated cardiomyopathy. In such cases pregnancy termination should be considered, to avoid adverse outcomes. Notably

TABLE 1 | Literature summary about the initial diagnosis of fetal first-degree autoimmune-associated congenital heart block.

References	Cases n.	Diagnosed methods	GWs	Mother's disease	Mother's positive autoantibody (titer)	Fetal AV intervals (ms) [z-scores]	Time of first therapy after diagnosis	Therapy medicines (dose)	Treated duration	Potential adverse effects of steroids	Prenatal results before birth	Postnatal therapy	Follow-up time	Follow-up results
Vesel et al. (36)	Case 1	PD	25	SS	Anti-SSA/Ro	185	0	Dex. (4 mg/day)	To 29 w	Severe oligohydramnio	1	Ventricular endocardial pacemaker	4 m	1
Friedman et al. (34, 37)	Case 2	PD MV-Ao	20	SLE/UAS/asym.?	anti-SSA/Ro (Mean 16128)	165	0	Dex. (4 mg/day)	Until delivery	–	1	–	1 y	1
	Case 3	PD MV-Ao	22	SLE/UAS/asym.?	Anti-SSA/Ro (Mean 16128)	160	0	Dex. (4 mg/day)	To 26w	Oligohydramnio	2	–	1 y	1
Rein et al. (38)	Case 4	FKCG	21	SLE	anti-SSA/Ro anti-SSB/La	149 [≥2]	0	Dex. (4 mg/day)	Until delivery	–	1	Pre. (0.1 mg/kg for 6 w)	1–6 y	1
	Case 5	FKCG	25	SS	Anti-SSA/Ro Anti-SSB/La	126 [≥2]	0	Dex. (4 mg/day)	Until delivery	–	1	Pre. (0.1 mg/kg for 6 w)	1–6 y	1
	Case 6	FKCG	26	SLE	Anti-SSA/Ro Anti-SSB/La	110 [≥2]	0	Dex. (4 mg/day)	Until delivery	–	1	Pre. (0.1 mg/kg for 6 w)	1–6 y	1
	Case 7	FKCG	34	SLE	Anti-SSA/Ro Anti-SSB/La	134 [≥2]	0	Bet.+Dex. (4 mg/day)	Until delivery	–	1	Pre. (0.1 mg/kg for 6 w)	1–6 y	1
	Case 8-A	FKCG	32	MCTD	Anti-SSA/Ro Anti-SSB/La	115 [≥2]	0	Dex. (4 mg/day)	Until delivery	–	1	Pre. (0.1 mg/kg for 6 w)	1–6 y	1
	Case 8-B		33			115 [≥2]				–	1	Pre. (0.1 mg/kg for 6 w)	1–6 y	1
Jaeggi et al. (39)	Case 9	Fetal echo	23	–	Anti-SSA/Ro (>100 U/ml)	250 [+14]	0	Steroids +IVIG	–	–	2	–	–	5
Izmirly et al. (40)	Case 10-11	–	–	SLE	Anti-SSA/Ro Anti-SSB/La	–	–	Dex.	–	–	1	–	–	1

(Continued)

TABLE 1 | Continued

References	Cases n.	Diagnosed methods	GWs	Mother's disease	Mother's positive autoantibody (titer)	Fetal AV intervals (ms) [z-scores]	Time of first therapy after diagnosis	Therapy medicines (dose)	Treated duration	Potential adverse effects of steroids	Prenatal results before birth	Postnatal therapy	Follow-up time	Follow-up results
Tunks et al. (41)	Case 12	PD MV-Ao	22	SLE	Anti-SSA/Ro (mean 443)	–	0	Dex. (4 mg/day)	–	–	3 (2 days later)	–	–	3
	Case 13	PD MV-Ao	–	–	Anti-SSB/La (mean 334.7)	150–160?	0	Dex.	Until delivery	–	1	–	–	1
	Case 14		–	–	Anti-Smith (mean 40.3)	150–160?	0	Dex.	Until delivery	–	1	–	–	1
	Case 15		–	–	Anti-RNP (mean 57)	150–160?	0	Dex.	Until delivery	–	2	–	–	2
	Case 16		19+	SS, Hypothyroidism		–	0	Steroids+ IVIG+dex.	Until delivery	–	2	–	–	2
Cuneo et al. (42)	Case 17	PD MV-Ao	19+	–	Anti-SSA/Ro	–	0	Dex.	–	–	1	–	–	1
Sonesson et al. (35)	Case 18	PD MV-Ao +SVC-Ao	23	SS	Anti-Ro52	165 [6.3]	0	Bet. (4 mg/day)	Until delivery	–	2	–	1 y	2
	Case 19		24	SS		16.4 [6.0]	0	Bet. (4 mg/day)	Until delivery	–	2	–	4 y	2
Sonesson et al. (43)	Case 20	PD MV-Ao +SVC-Ao	18–24?	–	Anti-SSA/Ro Anti-SSB/La	–	0	No therapy	Until delivery	–	3 (in 6 days when beta. started)	–	–	3
	Case 21–2	PD MV-Ao +SVC-Ao	18–24?	–	Anti-SSA/Ro Anti-SSB/La	–	–	No therapy	–	–	2	–	Few weeks	1
	Case 24–26					–	–	No therapy	–	–	1	–		1
Friedman et al. (37)	Case 27	PD MV-Ao	32	SLE/UAS/ asym.?	Anti-SSA/Ro (Mean 16128)	170	–	No therapy	–	–	1	–	3 y	2
Skog et al. (44)	Case 28–30	Fetal echo	18–24?	SLE/SS/ other?	Anti-Ro52	–	–	No therapy	–	–	2	–	1 m	1
	Case 31–35					–	–	No therapy	–	–	1	–		1

(Continued)

TABLE 1 | Continued

References	Cases n.	Diagnosed methods	GWs	Mother's disease	Mother's positive autoantibody (titer)	Fetal AV intervals (ms) [z-scores]	Time of first therapy after diagnosis	Therapy medicines (dose)	Treated duration	Potential adverse effects of steroids	Prenatal results before birth	Postnatal therapy	Follow-up time	Follow-up results
Jaeggi et al. (39)	Case 36	Fetal echo	22	-	Anti-SSA/Ro (55 U/ml)	-	-	No therapy	-	-	5	-	-	5
Izmirly et al. (40)	Case 37	-	-	SLE	Anti-SSA/Ro Anti-SSB/La	-	-	No therapy	-	-	2	-	3 y	2
Krishnan et al. (45)	Cases 38-41	PD MV-Ao	16- 28?	-	Anti-SSA/Ro	> 140?	-	No therapy	-	-	1	-	-	5
	Case 42										2	-	-	5
	Case 43		16- 28?	-		160- 170?	-	No therapy	-	-	2	-	-	5
Doti et al. (46)	Case 44	Fetal echo	25+	-	Anti-SSA/Ro Anti-SSB/La	-	-	No therapy	-	-	2	-	-	2
Cuneo et al. (42)	Case 45	PD MV-Ao	26+	-	Anti-SSA/Ro	-	-	No therapy	-	-	1	-	-	1
	Case 46		25+	-		-	-	No therapy	-	-	1	-	-	1
	Case 47		21+	-		-	-	No therapy	-	-	1	-	-	1

GWs, gestational weeks; AVB, atrioventricular block; PD: pulsed-wave Doppler echocardiography; MV-Ao, from the intersection of the mitral E- and A-waves to the onset of the ventricular ejection wave in the aortic outflow; SVC-Ao, from the beginning of the retrograde venous a-wave in the SVC to the beginning of the aortic ejection wave; TDI, tissue Doppler imaging; echo: echocardiography; Aa-Sa, between atrial contraction and ventricular systole; FKCG, fetal kinetocariogram, SLE, systemic lupus erythematosus; UAS, undifferentiated autoimmune syndrome; asym., asymptomatic; SS, Sjögren syndrome; CTD, connective tissue disease; MCTD, mixed CTD; Dex., dexamethasone; Bet., betamethasone; Pre., prednisone; echo, echocardiogram; HCQ, hydroxychloroquine; IVIG, intravenous immune globulin; FGR, fetal growth restriction; LBW, low birth weight.

?: the specific value was not determine and only the range was provided in original study.

The bold type of word: highlighting the cases about the progression of the first-degree AVB to third-degree AVB.

Time from initiation to therapy: 0 mean that the therapy for fetal first-degree AVB was started immediately after the diagnosis was made.

Prenatal results: 1: conversion to sinus rhythm; 2: not progressed and sustained first-degree AVB; 3: progressed to third-degree AVB (complete AVB); 4: died; 5: alive but the detail conditions cannot be obtained from original study.

Follow-up results: 1: healthy and normal development; 2: sustained first-degree AVB; 3: progressed to high-degree AVB; 4: died; 5: alive but the detail conditions cannot be obtained from original study probably attributed to loss of follow-up.

however, in some cases in which the heart rate has dropped below 55 bpm the patient has been kept alive by treatment with a combination of dexamethasone and β -agonists, with improved survival at 1 year and reduced morbidity (47). Such cases are critical though, and each needs to be carefully considered on an individual basis. However, the observational and therapeutic attempts are still considerable to under a totally agreement with mothers. Otherwise, immune-associated complete AVB should be treated via the same therapeutic strategy as second-degree AVB. Repeated echocardiography should be performed to facilitate detailed assessment of heart function and activity (34).

Sinus Bradycardia

More and more studies found that the transplacental effects of anti-SSA/Ro and anti-SSB/La on fetal or neonatal cardiac rhythm are not only manifested as congenital atrioventricular block, but also manifested as sinus bradycardia. Chockalingam et al. observed that sinus bradycardia occurred after birth in neonates with positive maternal anti-SSA/Ro antibodies, and continued to exist in childhood (48). The treatment of sinus bradycardia without heart malformation is mainly observation and monitoring. Fetus with positive maternal anti-SSA/Ro and anti-SSB/La antibodies should be closely monitored for the changes of PR interphase to prevent the irreversible conduction bundle immune injury (34, 49, 50). For the symptoms of fetal bradycardia, pregnant women can use oral sympathetic adrenergic drugs to increase fetal heart rate and can use intravenous salbutamol followed by oral terbutaline maintenance treatment. Fetal heart rate can be increased by 15–25%. Isoproterenol has no significant effect on fetal heart rate (51, 52). Pacing therapy after birth is an option for children with slow heart rate. And since Carpenter had performed the first implantation of a fetal pacemaker (53), some researchers have been exploring fetal pacing therapy. For example a micro-pacemaker to treat severe fetal bradycardia is currently undergoing design which may become an option in the future (54).

Controversies on Therapeutic Strategy

According to Diagnosis and Treatment of Fetal Cardiac Disease: A Scientific Statement from the American Heart Association (55), dexamethasone can prevent fetal cardiomyopathy or death from immune-associated second-degree and third-degree AVB with inflammatory symptoms. During treatment, fetal growth and development should be considered a key parameter when deciding whether to continue or terminate treatment. Depending on clinical follow-up observations, steroids can reverse the extension of the atrioventricular interval during treatment for first-degree AVB (38). Notably however, the risks may outweigh the benefits. Long-term administration of oral dexamethasone (56) is associated with increased body mass, hypertrichosis, mood changes, insomnia, hypertension, abnormal glucose tolerance, infection, cataracts, and bone mineral density problems in pregnant mothers, along with reduced amniotic fluid, growth restriction, and compromised neurodevelopment in the fetus. The administration of dexamethasone should thus be considered with caution, and treatment for first-degree AVB should be

TABLE 2 | The comparison of fetal outcomes of autoimmune-associated first-degree AVB diagnosed initially between treated cases and untreated cases.

Characterizes	Treated group (n = 20, 41.67%)	Untreated group (n = 28, 58.33%)	P-value
Prenatal outcomes			
Conversion to sinus rhythm	13 (65.00%)	16 (57.14%)	0.583
Persistence of first-degree AVB	6 (30.00%)	10 (35.71%)	0.679
Progression to second-/high-degree AVB	0	0	–
Progression to third-degree AVB	1 (5.00%)	1 (3.57)	1.000
Death <i>in utero</i>	0	0	–
Alive but unknown details	0	1 (3.57%)	–
Adverse effects on fetus	2 (10.00%)	–	–
Postnatal outcomes			
Sinus rhythm	14 (70.00%)	17 (60.71%)	0.507
First-degree AVB	4 (20.00%)	3 (10.71%)	0.429
Second-/high-degree AVB	0	0	–
Third-degree AVB	1 (5.00%)	1 (3.57%)	1.000
Death due to AVB or AVB therapy	0	0	–
Alive but unknown details in original study	1 (5.00%)	7 (25.00%)	–

AVB, atrioventricular block.

administered after due evaluation of the potential adverse effects and benefits of dexamethasone. The use of drugs should be reduced or even stopped in the event of conversion or failure to achieve therapeutic goals. The RRNL report demonstrated the failure of dexamethasone administration to prevent disease progression, reduce mortality, and avoid pacemaker implantation and cardiomyopathy in cases of second-degree and third-degree AVB (57). Similar results have been observed in a series of studies conducted by different research groups, challenging the therapeutic role of dexamethasone in fetal CHB management (6, 58). According to a systematic review and meta-analysis of maternal steroid therapy for fetuses with second-degree immune-mediated congenital AVB that was reported in 2018 (59), there is still limited evidence of the advantages of steroid administration with respect to fetal outcomes. A recent meta-analysis demonstrated fluorinated steroids were not superior to any treatment to ameliorate the outcome of autoimmune associated CHB (60).

β -agonists such as terbutaline and salbutamol have favorable transplacental transfer rates and β -agonist action, and they can increase fetal heart rate and mediate a positive inotropic effect on the fetal myocardium. They are usually administrated in

combination with dexamethasone. According to the follow-up results of 37 cases of fetal complete AVB treated by Jaeggi et al. (47), hormone or sympathetic drug administration could improve the survival rate of fetal complete AVB, reduce the incidence of immune-related complications, and improve prognoses. Conversely however, Lopes et al. (61) reported the results of long-term treatment and follow-up of 116 fetal AVB patients, and in that cohort steroids and sympathetic drugs did not reverse fetal AVB or improve the survival rate.

IVIg can reduce transplacental autoantibody transfusion and increase the release of anti-inflammatory factors. It can therefore be used in combination with dexamethasone in the event of endocardial fibroelastosis or impaired cardiac systolic function (57, 62, 63), but there is no solid evidence on the optimal dosage or duration of IVIg administration. A flowchart summarizing the durations and dosages used in the most promising study reported by Trucco et al. (64) in which 80% of patients remained alive 2.9 years after birth without heart transplantation, as well as experiences from our own clinical practice, is shown in **Figure 1**.

The toll-like receptor blocker HCQ can reduce the risk of immune-associated fetal AVB progression, and in pregnant women who took HCQ the prevalence of fetal CHB was lower than it was in those who did not (65). Subsequent RRNL studies suggested a protective role of HCQ by way of reducing fetal CHB in subsequent babies by 64% (66). In one study HCQ was strongly associated with maintaining healthy babies in anti-Ro-positive mothers with systemic autoimmune diagnoses, including systemic lupus erythematosus and Sjögren's syndrome (67). Most recently, Izmirly et al. published an important research on the prevention role of HCQ in fetuses of anti-SSA/Ro positive mothers, which revealed HCQ reduced the recurrence of CHB below the historical rate by >50%, and this drug is recommended for the secondary prevention of fetal cardiac disease in anti-SSA/Ro-exposed pregnancies (68). In the absence of adverse events, HCQ administration could be recommended until delivery (66, 69, 70).

To date many therapeutic studies on fetal immune-associated CHB have been reported, and herein we have summarized the general treatments and assessments utilized in a flowchart of hierarchical management and monitoring regimens. Due to the low incidence of fetal immune-associated CHB it is difficult to conduct large-sample prospective randomized controlled trials, and there are many biases in retrospective studies. Therefore, the conclusions of clinical studies with large samples are sometimes inconsistent, and controversy over treatment strategies still exists. Large prospective studies are necessary to comprehensively evaluate the efficacy of therapy with different drugs and combinations thereof.

COMPLICATIONS AND PROGNOSIS

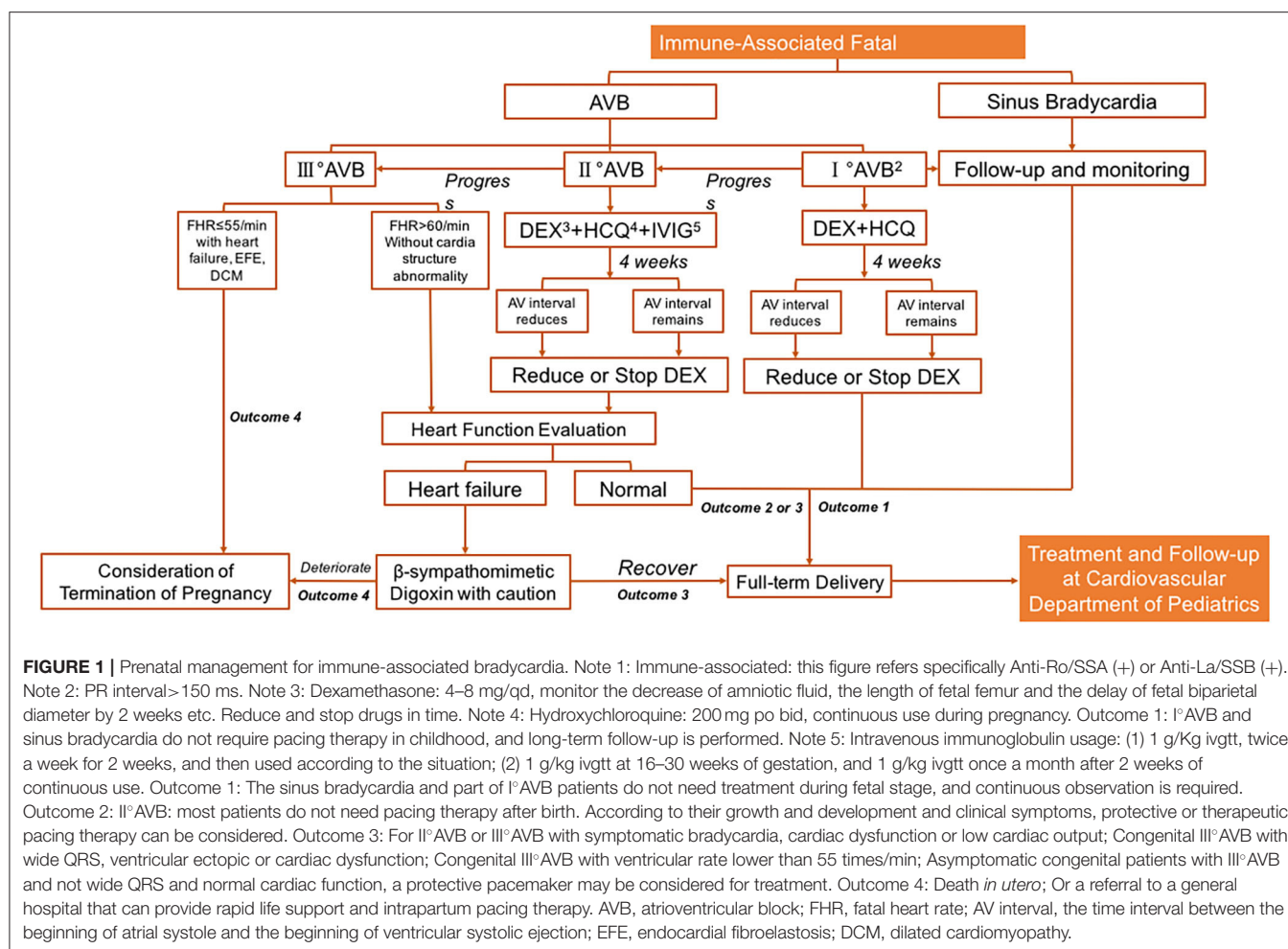
The prognosis of fetal immune-associated bradycardia depends on the severity of the disease, the extent of its effects on cardiac function, and whether it is associated with cardiomyopathy and endocardial fibroelastosis. Generally, increased anti-Ro52 (≥ 50 U/mL), cardiac malformation, early onset (< 20 GWs), advanced

AVB, lower atrial rate (≤ 120 /min), reduced ventricular rate (≤ 55 /min) with heart dysfunction, edema, cardiomyopathy, or endocardial fibroelastosis are associated with unfavorable prognoses and increased mortality (39, 61, 63). To improve the overall survival rate and prognoses of perinatal children with immune-associated CHB, optimal and timely drug interventions are necessary during the fetal period. Pacemaker implantation and integrative medication management of newborns are also important, in order to save lives. In subsequent pregnancies in mothers of affected babies, the therapeutic strategy should involve integrated administrative coordination among obstetrics, ultrasound, and pediatric departments from the prenatal period to the postnatal period, and potentially preventive treatment.

PERSPECTIVE: WHETHER TRANSPLACENTAL MEDICATION ADMINISTRATION BENEFITS THE FETUS

In past decades, extensive studies have investigated autoimmune-associated fetal CHB, and the knowledge they have yielded has facilitated a better understanding of multiple aspects of the condition. Severe fetal AVB results in fetal death. In 40–50% of patients fetal CHB is associated with transplacental transfusion of maternal autoantibodies. Patients with immune-related CHB will potentially benefit from anti-immune treatment, but hierarchical fetal immune-associated CHB management strategies remain a subject of debate. The current review has discussed the accessible practical clinical approaches to fetal immune-associated CHB management based on the degree of AVB and fetal heart function. Previous guidelines on prenatal cardiovascular disease management are predominantly focused on the diagnosis of fetal cardiac malformation, fetal heart failure, and fetal arrhythmia, with little attention paid to the progression of clinical management and prenatal follow-up duration prior to reaching a decision with regard to delivery or termination.

To date no standardized therapy for first-degree AVB has been established, and maternal dexamethasone or HCQ administration usually cannot reverse it. Notably however, sometimes treatment can evidently prevent progression of first-degree AVB to AVB of a higher degree. So that, we could not strongly positive on aggressive supplying dexamethasone and HCQ to such patients as there is no available indicator to predict the progress of severity of fetal AVB, as the adverse effects from steroids administration are still calling attention. Above we have summarized the currently available data and suggested consideration of the administration of dexamethasone and HCQ in some circumstances, but all therapeutic interventions should be monitored very closely and evaluated within 4 weeks. Immune-related second-degree AVB should be treated to avoid progression and adverse outcomes, and patients with second-degree AVB are the most likely to derive advantages from transplacental medication therapy. Notably however, current evidence does not support the administration of anti-immune treatment to fetuses with third-degree AVB. Medication to maintain heart function is recommended. This review is an attempt to discuss the limitations of prenatal management



strategies, and clearly present specific accessible hierarchical therapeutic and fetal monitoring protocols.

Currently, the pathogenesis of such heart conduction disorder is still unknown well, as only a few parts could be identified positive for auto-immune antibodies. And the treatment of dexamethasone, HCQ and IVIG is partially efficient. Echocardiography has been widely used to diagnose fetal arrhythmias, but it does not accurately reflect electrical activity, which leads to limitations with regard to indicators for predicting rapid progression of CHB. This limits the administrative decisions that can be made based on it, in fetuses with first-degree AVB. Moreover, fetuses cannot be fitted with pace-makers, so almost nothing can be done for those with third-degree AVB.

Based on our understanding, a large amount of research remains to be done and no definitive end-point is envisaged in the near future. Future research may mainly be focused on accurate diagnostic methods based on the electrical activity of the fetal cardiac conduction system. Early predictors to identify rapid progression of AVB and heart dysfunction remain elusive. Most importantly, our knowledge of postnatal development in such patients—particularly with regard to neuro-motor and cardiac function restoration as well as the

maturation of cardiomyocytes—is very limited. Further research investigating such issues is urgently required. The physiological processes involved in maternal antibody transportation to the fetal environment also warrant further research, as do genetic risk factors that affect placental morphology and functional development.

The aforementioned future research directions have already been launched. Current treatments still require detailed validation via large-cohort observational studies and other types of evidence-based medical studies. Notably however, there is an ethical issue with respect to conducting randomized clinical trials involving such patients. Several groups have attempted to underline the outcomes on different reasons inducing fetal AVB, including the impacts on prognosis from fetal hydrops. But the etiological guiding management strategy is required. Functional magnetic resonance imaging has been used to investigate whether such patients are likely to suffer neurological disorders (symptomatic or asymptomatic). Recent studies indicate that placental barrier function is impaired under adverse maternal conditions, and the medication transplacental ratio mainly depends on transporters. Accordingly, it is important to identify the characteristics of

placental transporters for dexamethasone, HCQ, and digoxin to release the precious personal therapeutic administration with maternal gestational complications (diabetes, hypertension, obesity, etc.).

In the near future, large cohort studies will provide more detailed information on hierarchical management strategies. Greater understanding of the molecular basis of pathogenesis will lead to the development and administration of more effective medications. Lastly, a better understanding of the role of the placenta in disease and drug transportation may facilitate more individualized treatment regimens based on specific fetal and maternal conditions.

CONCLUSION

Fetal immune-associated CHB is a life-threatening condition. Anti-Ro/SSA and anti-La/SSB antibodies are currently considered to be the predominant antibodies that contribute to the most common causes of fetal immune-associated CHB. Fetal echocardiography is a comparatively reliable method for diagnosing immune-associated CHB prenatally. Recent studies partially support positive treatment approaches for first-degree and second-degree AVB. Transplacental administration of dexamethasone, IVIG, and HCQ evidently cannot reverse

third-degree AVB, but benefit the fetal prognosis. Treatment to maintain heart function via digoxin and β -agonists can evidently benefit delivery outcomes, but such patients always require pacemaker implantation soon after birth. This review is the first to summarize clinically accessible strategies for the management of autoimmune-associated CHB in a hierarchical manner, focusing on controversies surrounding the benefits of transplacental medication administration according to degrees of AVB.

AUTHOR CONTRIBUTIONS

HL, CT, and LQ wrote the paper. CT, CW, and HL performed the collected the data. YH and KZ provided supervision and administration for this manuscript. CW and YL reviewed and approved the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Low-Level Stimulation and Ethanol Ablation of the Vein of Marshall Prevent the Vagal-Mediated AF

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Background: The mechanisms for the vein of Marshall (VOM) mediated atrial fibrillation (AF) are not completely understood. We sought to evaluate the contribution of the intrinsic cardiac autonomic nervous system in VOM mediated AF.

Method: Seven mongrel dogs were administered propranolol and continuously exposed to left superior ganglionated plexi (LSGP) stimulation, LSGP + low-level VOM stimulation, LSGP + atropine administration, LSGP + VOM filling with ethanol separately. The effective refractory period (ERP) and window of vulnerability (WOV) at the left superior pulmonary vein (LSPV), left inferior pulmonary vein (LIPV) and left atrial appendage (LAA) were measured.

Result: LSGP stimulation significantly shortens the ERP and prolonged the ERP dispersion and WOV in LSPV, LIPV, and LAA. Interestingly, low-level VOM stimulation, atropine administration, or VOM filling with ethanol were able to attenuate the effects of LSGP in all sites.

Conclusion: VOM as an inter-communication pathway of ganglionated plexis plays an important role in the development of vagal-related AF.

Keywords: vein of Marshall, vagal atrial fibrillation, ganglionated plexi, low-level stimulation, ethanol ablation

INTRODUCTION

The concept of vagal atrial fibrillation (AF) was first put forward by Coumel, who suggested that the autonomic nervous system plays a pathophysiological role in a subset of patients with AF (1, 2). This was further supported in experiments by Liu and Nattel, whose work demonstrated a shortening of the action potential and refractory period creating heterogeneity across the atrial wall and a substrate for re-entrant arrhythmogenesis (3). Subsequent evidence has further shown that vagal stimulation causes potent increases in the heterogeneity of the atrial effective refractory period (ERP), far over that caused by sympathetic stimulation (3). Recently, intrinsic cardiac nerves (ICNs) and their associated ganglionated plexi (GP) have emerged as potential anatomical sites for the initiation and maintenance of AF by involving the parasympathetic nervous system. Stimulation of these areas can trigger AF in humans (4, 5). Besides, high-frequency stimulation of GP at the pulmonary vein can induce ectopy and AF (1). Other studies in human subjects documented shifts in autonomic tone toward vagal predominance in the initiation of paroxysmal AF (potentially following an initial sympathetic surge), and eventually enhances the occurrence of ectopy at the site of the pulmonary veins (6).

The vein of Marshall (VOM) and ligament of Marshall (LOM) have been implicated in the pathogenesis of AF. Both VOM and LOM, sources of initiating triggers (7), and vehicles of parasympathetic (8) and sympathetic (9) innervations that modulate electrical properties of atrial tissue, contribute to AF maintenance (10, 11). Animal and human studies have shown that the VOM and LOM is a potential therapeutic target (12–14) for AF. In the past, high-frequency stimulation (HFS) in the LOM (without exciting the atrial myocardium) led to induction of AF, and this induction was inhibited by esmolol and atropine administration, suggesting the involvement of autonomic mediation (15, 16). Here, we hypothesized that VOM, as a key inter-communication pathway of GP, may play an important role in the development of vagal-related AF. Therefore, this study was carried out to reduce AF vulnerability by evaluating the effect of VOM ethanol infusion to the intrinsic cardiac autonomic nervous system.

METHODS

Animal Model Preparations

The Institutional Animal Care and Use Committee of Dalian Medical University approved the experimental protocol in advance. Seven adult mongrel dogs (10–15 kg each) were anesthetized with sodium pentobarbital (150 mg/kg intravenously). The dogs were ventilated with a constant volume-cycled respirator through a cuffed endotracheal tube, and blood oxygen saturation was maintained above 95%. The temperature and illumination of the operating room were kept stable throughout the experiment. Standard ECG leads II and aVR was continuously monitored (Prucka 7000; GE Healthcare, Milwaukee, WI). All dogs were administered propranolol, initially a loading dosage of 2-mg/kg bolus, and subsequently a maintenance dosage of 2 mg/kg per hour to inhibit sympathetic activity. All the mongrel dogs continuously underwent the standard procedure as represented in **Figure 1**.

Catheter Positioning

All underwent a left lateral thoracotomy at the fourth intercostal space and the pericardium was opened to expose the left atrial appendage (LAA) and the base of the left ventricle. At the lateral aspect of the LAA, the LOM as a prominent fold was clearly visualized. A mapping electrode catheter was positioned, and sutured to fix it in position at the left superior pulmonary vein (LSPV), left inferior pulmonary vein (LIPV), and LAA to promote the recording of the signals to estimate the effective refractory period (ERP) and window of vulnerability (WOV). In addition, electrodes were deployed for stimulation purposes at the VOM and left superior ganglionated plexi (LSGP). All catheters were manufactured by Cordis, Biosense Webster, Inc. (Diamond Bar, CA) as shown in **Figure 2**.

Abbreviations: AF, atrial fibrillation; ERP, effective refractory period; GP, ganglionated plexi; ICN, intrinsic cardiac nerves; VOM, vein of Marshall; LOM, ligament of Marshall; HFS, High-frequency stimulation; LSPV, left superior pulmonary vein; LIPV, left inferior pulmonary vein; LAA, left atrial appendage; LSGP, left superior ganglionated plexi; LLS, low-level VOM stimulation; ATR, atropine; ALC, alcohol.

LSGP Stimulation

Two pairs of electrodes were embedded in the caudal end of the LSGP for stimulation. A rectangular pulse was delivered through a constant voltage stimulator at 20 Hz with a pulse width of 2 ms by a programmable stimulator (RST-2, Huanan Medical, Hunan, China). The LSGP stimulation threshold was defined as the voltage level that could decrease the heart rate (HR) by 30% or result in a 2:1 atrioventricular block. The threshold voltage $\times 2$ was used to test the atrial ERP.

Low-Level VOM Stimulation

We first defined the stimulation threshold for each dog by stimulating the VOM at 13 Hz (450- μ s pulse duration). The stimulus amplitude (V) that elicited an abrupt decrease of heart rate by $>20\%$ from baseline or caused AV conduction block was defined as the stimulation threshold. We then programmed the pacemaker output to 1 V below the stimulation threshold and confirmed that this stimulus voltage (4 ± 2 V, range 1–6 V) did not cause any changes in heart rate. The stimulation parameters chosen resulted in no serious adverse reactions (17).

VOM Ethanol Infusion

Selective cannulation of the VOM was reliably achieved by introducing a left internal mammary artery catheter in the coronary sinus, engaging the VOM ostium. Through the catheter, an angioplasty wire and a balloon catheter (2 mm \times 8 mm) were advanced into the VOM. The balloon was inflated at the ostium of the VOM and selective VOM venograms were obtained. Substantial anatomical variations were present in the length and caliber of the VOM. The angioplasty balloon was then inflated at the ostium of the VOM and 5 cc of 100% ethanol was delivered over 2 min through the balloon lumen.

ERP and WOV Measurement

ERP and WOV at LSPV, LIPV, and LAA were measured under baseline, LSGP stimulation alone, LSGP stimulation + low-level VOM stimulation, LSGP stimulation + atropine administration, VOM ethanol infusion alone, and VOM ethanol infusion + LSGP stimulation respectively. ERP was measured by an incremental technique, with 2-ms steps at basic drive cycle lengths of 500 ms for eight beats. ERP was measured with a drive train of eight stimuli ($S_1 = 400$ ms) at twice the diastolic threshold, followed by a single premature stimulus (S_2) coupled with increments of 10 ms until local ERP was reached. Atrial ERP was defined as the longest S_1 - S_2 coupling interval that failed to produce a propagated response. We used the WOV as a quantitative measure of AF inducibility. During ERP measurements, if AF was induced by decremental S_1 - S_2 stimulation, the longest and the shortest S_1 - S_2 interval (in ms) at which AF was induced were then determined. The difference between the two was designated as the WOV. AF was defined as ≥ 2 s of atrial activity appearing as fibrillatory waves on the surface ECG. Whereas, ERP dispersion was defined as the coefficient of variation (standard deviation/mean) of the ERP at LSPV, LIPV, and LAA Sites.

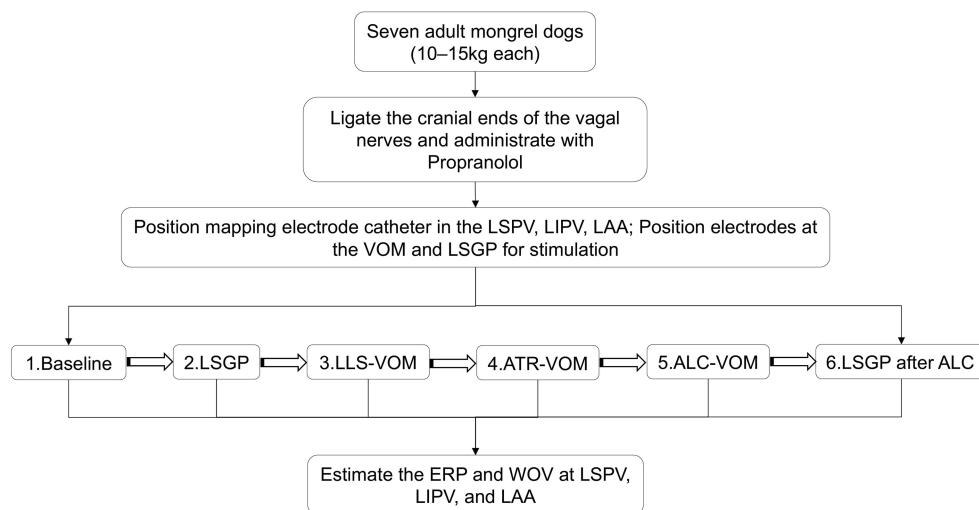


FIGURE 1 | Schematic of the study design.



FIGURE 2 | Anatomical location and catheter positioning. LAA, left atrial appendage; VOM, vein of Marshall; LSGP, left superior ganglionated plexi.

Statistical Analysis

A paired *t*-test was used for comparisons of ERP and WOV before and after stimulations. ANOVA for repeated measurements was used for comparisons of ERP or WOV among different stimulation strategies and followed by *post hoc* testing (least significant differences) for comparisons of the ERP and WOV at the end of each subsequent stimulation strategy vs. ERP and WOV in the baseline state. Statistical significance was defined as $P \leq 0.05$.

RESULTS

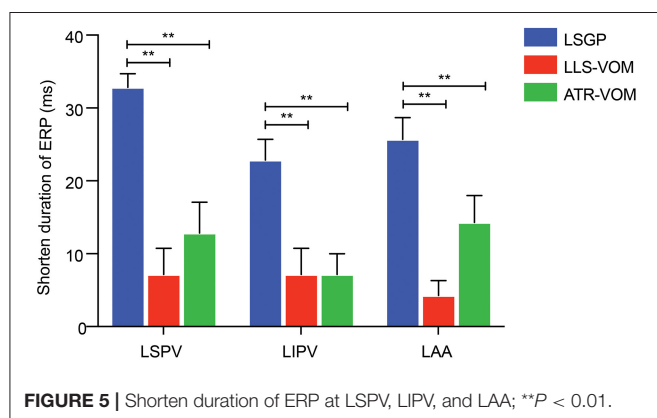
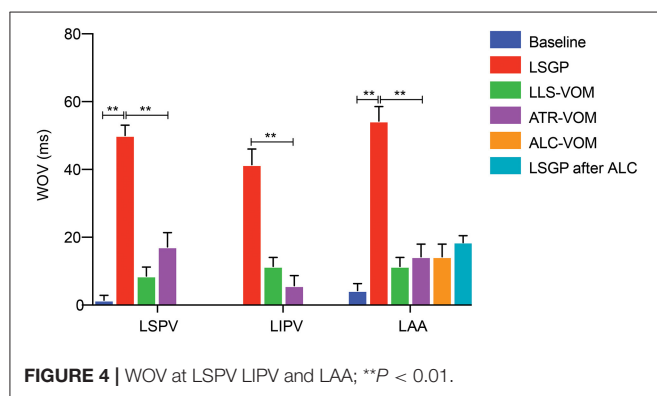
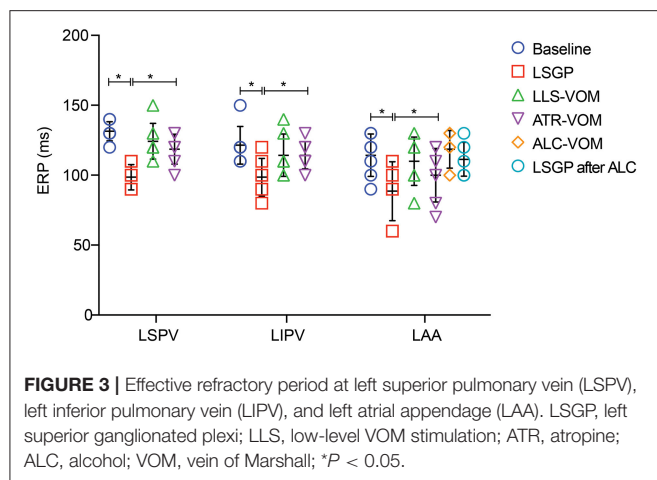
Effect of LSGP Stimulation Mimics Vagal Stimulation

As shown in **Figure 3**, LSGP stimulation markedly decreased the ERP in LSPV (98.6 ± 9.0 ms vs. 131.43 ± 6.9 ms, $p < 0.001$), LIPV (98.6 ± 13.5 ms vs. 121.4 ± 13.5 ms, $p = 0.016$), and LAA (88.6 ± 21.2 ms vs. 114.3 ± 15.1 ms, $p = 0.067$). Meanwhile,

the ERP dispersion and WOV were increased significantly after LSGP stimulation (**Figure 4**). The ERP dispersion increased from 0.10 ± 0.04 to 0.16 ± 0.08 with a *P*-value of 0.032. Also, the WOV at LSPV, LIPV, and LAA was significantly prolonged after LSGP stimulation from 1.4 ± 3.8 ms, 0 ms, to 4.3 ± 5.4 ms in the baseline state to 50 ± 8.2 ms, 41.4 ± 12.2 ms, and 54.3 ± 11.3 ms, respectively.

LSGP + Low-Level VOM Stimulation Attenuates the Effects of LSGP

Compared to the LSGP right before VOM stimulation, the shorten duration of ERP was less in LSPV (98.6 ± 9.0 ms vs. 124.3 ± 12.7 ms, $p < 0.001$), LIPV (98.6 ± 13.5 ms vs. 114.3 ± 15.1 ms, $p = 0.140$), and LAA (88.6 ± 21.2 ms vs. 110.0 ± 17.3 ms, $p = 0.183$), when LSGP combined with low-level VOM stimulation (**Figure 5**). Similarly, the ERP dispersion was hardly increased (0.13 ± 0.03). However, the WOV at LSPV (8.6 ± 6.90 ms), LIPV



(11.4 ± 6.90 ms), and LAA (11.4 ± 6.90) were less prolonged with low-level stimulation in VOM.

LSGP + Atropine Administration Attenuates the Effects of LSGP

In comparison with the ERP measured from LSGP alone, the addition of atropine to LSGP slightly shorten the ERP from 131.4 ± 6.90 ms, 121.4 ± 13.5 ms, and 114.3 ± 15.2 ms to 118.6 ± 10.7 ms, 114.3 ± 9.8 ms, and 100.0 ± 19.2 ms in LSPV, LIPV, and LAA respectively. However, there were no significant differences

in ERP. The growth rate in ERP dispersion (0.12 ± 0.06) and WOV was significantly reduced when atropine administration was added to LSGP stimulation.

LSGP + VOM Filling With Alcohol Attenuates the Effects of LSGP

There was no significant difference in baseline ERP in LAA before (114.3 ± 15.1 ms) and after VOM filling. Likewise, LSGP fails to significantly shorten the ERP in LAA (107.1 ± 18.6 ms) when VOM was filled with alcohol. Also, the baseline WOV was not significantly changed after VOM filling in LSPV (1.4 ± 3.8 vs. 0 ms) and LIPV (0 vs. 0 ms). However, the WOV slightly increased in LAA after VOM filling (14.3 ± 9.8 vs. 4.3 ± 5.4 ms). LSGP stimulation was also unable to significantly prolong the VOM in LSPV (0 vs. 0 ms), LIPV (0 vs. 0 ms), and LAA (14.3 ± 9.8 vs. 18.6 ± 7.0).

DISCUSSION

The present study demonstrated that low-level stimulation, atropine mediation, or VOM filling with alcohol could attenuate the GP-activation-induced shortening of atrial ERP and the increase of AF inducibility, suggesting VOM is being involved in the development of vagal-related AF as an inter-communication pathway of GPs.

The intrinsic cardiac nervous system harbors clusters of neurons, the GP, which innervate the neighboring atrial myocardium and control their electrophysiological properties (18). As shown in our study, LSGP stimulation markedly decreased the ERP while significantly increased the ERP dispersion and WOV, which is consistent with previous studies. The result of our study demonstrates that cardiac autonomic nerves and GPs play an important role in the initiation and maintenance mechanisms of AF (19). GP, as a target during AF ablation, has been reported to be useful to improve the outcomes (20). However, the benefit of GP ablation is still controversial. There are complex interneural communications between different ICN (16), and GP ablation that could damage various nerve structures, myocardium, and other non-neuronal structures is not highly recommended as it leads to proarrhythmia in return (21).

The VOM, coming from the ligament of Marshall running between the left atrial appendage and left pulmonary vein antrum, is an intracardiac structure connects with the coronary sinus. Several studies showed the nervous system forms an interconnected neural network composed of GPs and VOM (15, 22). Valderrabano demonstrated that the VOM contains ICN that connects with the AV node and can trigger AF and retrograde ethanol infusion in the VOM reliably eliminates local ICN responses (14). Lin indicated that the VOM and ILGP function as the “integration centers” that modulate the autonomic interactions between extrinsic and intrinsic cardiac ANS on AV nodal function (16). ICN reached by VOM ethanol infusion may include not only those ICN clustered in the VOM-posterolateral left atrial ganglionated plexus but also those associated with the inferior left ganglionated plexus (23). In our study, we found that the LSGP stimulation result in short

ERP and long ERP dispersion and WOV. Importantly, the changes were alleviated when VOM was filled with alcohol. These phenomena confirm that the VOM is an important inter-communication pathway of GPs, and its participation in the development of vagal-related AF.

The vagus nerve has been proved to exert inhibitory control over the autonomic ganglia and autonomic neuromodulation. A shred of evidence reported that low-level vagus nerve stimulation could inhibit the activity of the autonomic ganglia and reverses acute electrical atrial remodeling during rapid atrial pacing (17, 24, 25). Similarly, our study found low-level stimulation of VOM markedly attenuates the effect of LSGP, which may also contribute to autonomic inhibition. Although the exact downstream targets of LLVNS remain to be determined, there is preliminary evidence that the involvement of the antiadrenergic neuropeptide vasostatin-1 (26), the nitric oxide signaling pathway (27), and up-regulation of the calcium-activated potassium channel 2 in the left stellate ganglion (28). Thus, the potential mechanism for the low-level stimulation of VOM as a treatment for AF needs to be further investigated.

LIMITATION

There are several limitations to the present study. Firstly, the small sample size; secondly, the study was carried out only in animal model thus data on cell line and human is limited. Thirdly, sequential application of different interventions may result in carry over effects, which may limit interpretation of the results.

CONCLUSION

VOM as an inter-communication pathway of GPs is involved in the development of vagal-related AF. Mediation with either

low-level stimulation or atropine administration could attenuate the GP-activation-induced shortening of atrial ERP and the increase of AF inducibility. Destroyed VOM with alcohol further confirmed the critical role of VOM.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by Ethics Committee of First Affiliated Hospital of Dalian Medical University.

AUTHOR CONTRIBUTIONS

XYi and YX designed this study. YS and XYu were in charge of data analysis and critical revision of the article. FL and WS drafted the article. FL, WS, and YL conducted the animal experiments and data collection. All authors have read and approved the final manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Risk Stratification for Atrial Fibrillation and Outcomes in Tachycardia-Bradycardia Syndrome: Ablation vs. Pacing

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Background: Catheter ablation of atrial fibrillation is an alternative treatment for patients with tachycardia-bradycardia syndrome (TBS) to avoid pacemaker implantation. The risk stratification for atrial fibrillation and outcomes between ablation and pacing has not been fully evaluated.

Methods: This retrospective study involved 306 TBS patients, including 141 patients who received catheter ablation (Ablation group, age: 62.2 ± 9.0 months, mean longest pauses: 5.2 ± 2.2 s) and 165 patients who received pacemaker implement (Pacing group, age: 62.3 ± 9.1 months, mean longest pauses: 6.0 ± 2.3 s). The primary endpoint was a composite of call cause mortality, cardiovascular-related hospitalization or thrombosis events (stroke, or peripheral thrombosis). The second endpoint was progress of atrial fibrillation and heart failure.

Results: After a median follow-up of 75.4 months, the primary endpoint occurred in significantly higher patients in the pacing group than in the ablation group (59.4 vs. 15.6%, OR 6.05, 95% CI: 3.73–9.80, $P < 0.001$). None of deaths was occurred in ablation group, and 1 death occurred due to cancer. Cardiovascular-related hospitalization occurred in 50.9% of the pacing group compared with 14.2% in the ablation group (OR: 4.87, 95% CI: 2.99–7.95, $P < 0.001$). More thrombosis events occurred in the pacing group than in the ablation group (12.7 vs. 2.1%, OR 6.06, 95% CI: 1.81–20.35, $P = 0.004$). Significant more patients progressed to persistent atrial fibrillation in pacing group than in ablation group (23.6 vs. 2.1%, $P < 0.001$). The NYHA classification of the pacing group was significantly higher than that of the ablation group (2.11 ± 0.83 vs. 1.50 ± 0.74 , $P < 0.001$). The proportion of antiarrhythmic drugs and anticoagulants used in the pacing group was significantly higher than that in the ablation group (41.2 vs. 7.1%, $P < 0.001$; 16.4 vs. 2.1%, $P = 0.009$).

Conclusion: Catheter ablation for patients with TBS was associated with a significantly lower rate of a composite end point of cardiovascular related hospitalization and thromboembolic events. Furthermore, catheter ablation reduced the progression of atrial fibrillation and heart failure.

Keywords: atrial fibrillation, tachycardia-bradycardia syndrome, long pauses, catheter ablation, pacing, long outcome

INTRODUCTION

Tachycardia-bradycardia syndrome (TBS) is a common clinical arrhythmia used to describe a special subtype of sick sinus syndrome (SSS), with a long pause (RR intervals > 3 s) on termination of atrial fibrillation (AF) (1, 2). Patients with TBS are at a higher risk of amaurosis, syncope, and even sudden death (3). Guidelines determined that catheter ablation could be used as an alternative treatment for patients with TBS to avoid pacemaker implantation and the evidence recommendation level is IIa (4, 5). However, both treatment options for patients are at potential risk. AF or device related problems may remain even after pacemaker implantation, such as (1) the effect of antiarrhythmic drugs on atrial fibrillation is very limited and the incidences of arrhythmic effects and extracardiac adverse effects are high (6); (2) the incidence of atrial fibrillation-related symptoms, rehospitalization, stroke, progression of atrial fibrillation, and atrial fibrillation-mediated cardiomyopathy persists (7–9); and (3) pacemaker-related complications, such as infections and pacemaker-mediated cardiomyopathy are issues (10).

Previous studies only compared the feasibility and safety between ablation and pacing strategy in TBS patients. Studies showed that >85% of the patients may avoid the pacemaker when ablation for atrial fibrillation was performed. However, the long outcome of ablation for atrial fibrillation superior to pacing is not clear. In our study, we conducted a large-scale retrospective analysis involving 306 patients with TBS with an average follow-up time of 6 years, to evaluate whether catheter ablation improved the long-term outcome of the patients with TBS compared with cardiac pacing.

METHOD

Study Population

This single-center retrospective study was approved by the Ethics Committee of the First Affiliated Hospital of Dalian Medical University, Liaoning Province, China. We retrospectively analyzed 1,371 patients undergoing pacemaker implantation and 795 patients underwent catheter ablation due to atrial fibrillation from 2012 to 2017. A total of 306 patients with TBS were ultimately selected, including 165 patients with pacemaker implantation (Pacing group) and 141 patients with catheter ablation (Ablation group).

In this study, TBS diagnosis was in accordance with the diagnostic criteria of BM Kaplan (11), which define TBS as paroxysmal atrial fibrillation, flutter, or tachycardia followed by sinoatrial block or sinus arrest resulting in Stokes-Adams attacks. Patients who had both atrioventricular block/structural heart disease/heart failure, and/or had received radiofrequency ablation or pacemaker implantation in the past were excluded from the study (the screening process is shown in **Figure 1**).

Abbreviations: AAD, Antiarrhythmic drug; AVN, Atrioventricular node; AF, Atrial fibrillation; AUC, Area-under-the-curve; CI, Confidential interval; HF, Heart failure; LAD, Left atrial diameter; LVD, Left ventricular diameter; LVEF, Left ventricular ejection fraction; NOAC, New oral anticoagulants; NYHA, New York Heart Association; TBS, Tachycardia-bradycardia syndrome; Vit K, vitamin K.

Operation Strategy

We provided two treatment options and listed the pros and cons to the patient prior to procedure. And then the patient chose one strategy. In the ablation group, the TBS patients were all diagnosed with paroxysmal atrial fibrillation and received pulmonary vein isolation (PVI) only without addition lesion sets as the ablation strategy. The ablation procedures for this group are as described in a previous study (12). In brief, PV isolation was performed by ablation catheter Navistar Thermocool 3.5-mm D-F curve with Smart Touch technology (Biosense Webster) using contiguous circumferential lesions guided by (lasso™, Biosense and Webster, Inc., CA, USA). RF energy was applied in a power-controlled mode with a power limited of 35 W (30 W at the posterior wall) and a maximal temperature of 45°C. At each point, a radiofrequency current was applied until a voltage of <0.1 mV was achieved, with a maximum of 30 s per point. In the pacing group, the TBS patients all had paroxysmal atrial fibrillation and received DDD or DDDR pacing, and the procedures were as described by Dong et al. (13).

Study Endpoint

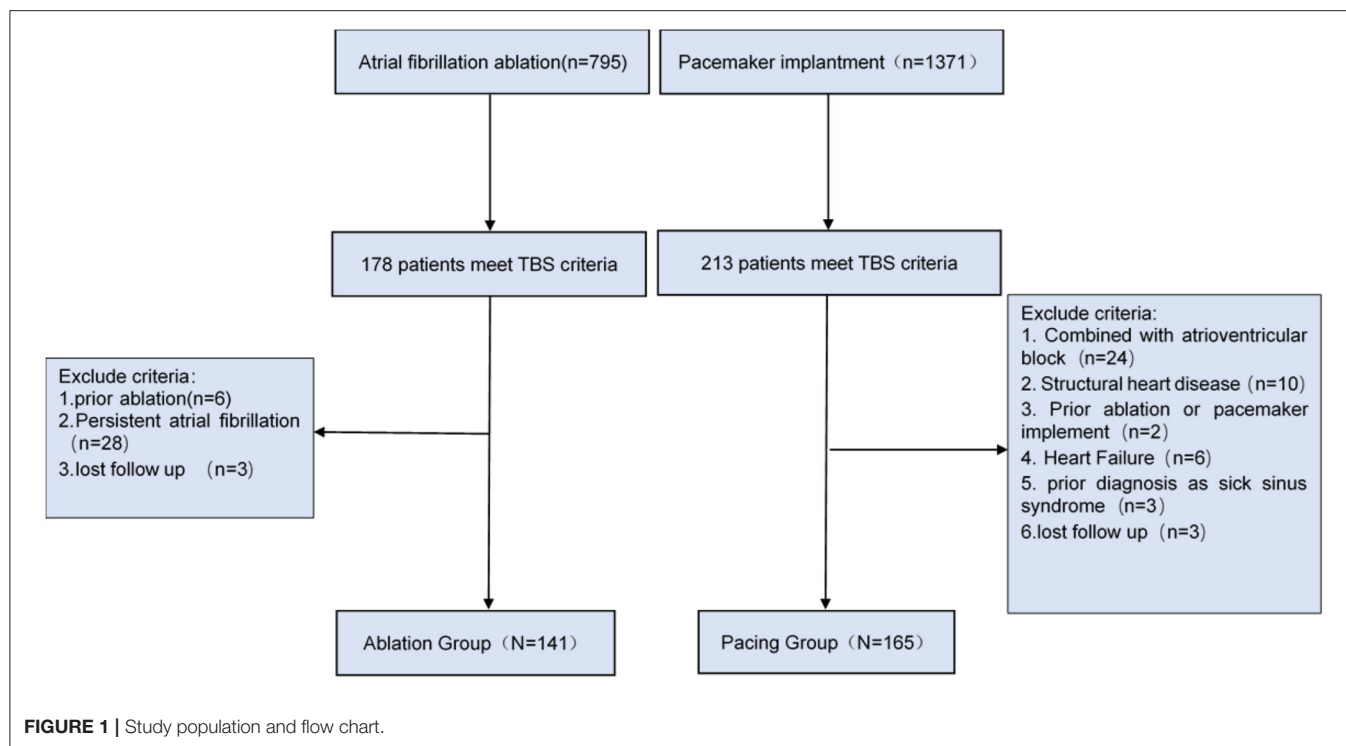
The primary endpoint of this study was a composite endpoint, consisting of all cause mortality cardiovascular rehospitalization and thromboembolic events. Cardiovascular rehospitalization was defined as patients who were re-hospitalized for cardiovascular diseases, including tachycardia, bradycardia, coronary atherosclerotic heart disease (i.e., angina pectoris and/or myocardial infarction), and heart failure. The definition of thrombotic events referred to the occurrence of stroke and/or peripheral thrombotic events (i.e., pulmonary embolism, mesenteric artery embolism, and lower extremity arterial embolism). The definition of the progression of heart failure, we are mainly concerned with NYHA cardiac function grade and left ventricular enlargement or ejection fraction decrease.

Follow-Up

Patients in the ablation group and the pacing group underwent follow-up for an average of 73.2 ± 17.0 months and 77.6 ± 21.3 months, respectively. The follow-ups were completed by a designated follow-up clinic. Patients in the ablation group had follow-ups in the postoperative months 3, 6, and 12, followed by once every 12 months after the operation via telephone and outpatient visit. Patients in the pacing group were followed up by retrospectively reviewing patient pacemaker programmed records, as well as through telephone and outpatient visits. Twenty-four-hour holter or ECG in the ablation group and pacing group were performed to detect the recurrence of AF at 3, 6, and 12 months visit and annually visit during follow up period. Patient data such as symptoms, recurrence of atrial fibrillation, repeated pacing or catheter ablation, usage of medication, rehospitalization occurrence, and the reasons for cardiovascular rehospitalization or thromboembolic events were collected during follow-ups.

Statistical Analysis

The continuous variables are presented as mean \pm standard deviation and compared using an independent sample *t*-test, and



the categorical variables are presented as count and percentage and analyzed using the chi-square test or odds ratio (OR) value. $P < 0.05$ was considered to indicate a significant difference between the groups. The Kaplan–Meier curve was used to compare the incidences of cardiovascular-related rehospitalization, stroke, and/or peripheral thromboembolism, and the log-rank test was used for evaluation. SPSS 23.0 software (IBM Corp., Armonk, NY, USA) was used for statistical analysis in this study.

RESULTS

General Characteristics of the Study Subjects

The clinical characteristics of the patients with TBS are shown in **Table 1**. A total of 306 patients with TBS were selected, including 141 patients in the ablation group, with women accounting for 53.2% and an average age of 62.7 ± 8.8 years, and 165 patients in the pacing group, with women accounting for 52.7% and an average age of 62.4 ± 8.4 years. The longest pauses after termination of atrial fibrillation in the pacing group was slightly longer than that in the ablation group, but this difference was not significant (6.0 ± 2.4 vs. 5.2 ± 2.2 s, $P = 0.081$). The total heart rate per 24 h of the pacing group were lower than that of the ablation group ($89,311 \pm 19,422$ vs. $97,179 \pm 16,888$, $P = 0.030$), but the average heart rate had no statistically significant difference between the two groups ($P = 0.283$). There was no significant difference in CHA₂DS₂-VASc score between the two groups (1.65 ± 1.0 vs. 1.75 ± 1.2 , $P = 0.469$) (**Table 2**).

Comparison of Therapeutic Results Between Ablation Group and Pacing Group

After an average follow-up of 75.5 ± 19.1 months, 116 patients (82.3%) in the ablation group maintained sinus rhythm. In addition, 16 patients (11.4%) in the ablation group had pacemaker implantation due to recurrence of atrial fibrillation with long pauses, and another 6 patients (4.3%) in the ablation group had recurring atrial fibrillation, but no long pauses and without pacemaker implantation. In the pacing group, only 31 patients (18.8%) maintained sinus rhythm, and 8 patients (4.8%) received ablation. Compared with the ablation group, more patients in the pacing group progressed to persistent atrial fibrillation [39 (23.6%) vs. 3 (2.1%), $P < 0.001$], and more patients used antiarrhythmic drugs and anticoagulants [68 (41.2%) vs. 10 (7.1%), $P < 0.001$] and [27 (16.4%) vs. 3 (2.1%), $P < 0.001$]. The New York Heart Association (NYHA) functional classification grade of the pacing group was significantly higher than that of the ablation group (2.11 ± 0.83 vs. 1.50 ± 0.74 , $P < 0.001$). According to CHA₂DS₂-VASc score, 65 patients in pacing group need long-term anticoagulation therapy, but only 27 people actually insist on oral anticoagulation. 5 patients stopped oral anticoagulants due to bleeding events, 28 patients stopped taking anticoagulants without authorization, and 5 patients took anticoagulants irregularly. Fifty-nine people in ablation group need anticoagulation, but only 3 patients actually insist on oral anticoagulation. Atrial fibrillation was cured in 50 patients, 4 patients personally stopped taking anticoagulants, and 2 patients took anticoagulants irregularly. No significant difference in the incidence of surgery-related complications was found between the ablation and pacing groups ($P > 0.05$; **Table 3**).

TABLE 1 | Characteristics of the study subjects.

	TBS patients (n = 306)
Female (n, %)	162 (52.9%)
Age (mean ± SD, y)	62.6 ± 8.6
Diabetes (n, %)	75 (24.5%)
Hypertension (n, %)	137 (44.7%)
Coronary heart disease (n, %)	61 (19.9%)
Stroke (n, %)	5 (1.6%)
AF duration (Mean ± SD, y)	4.6 ± 3.4
Total heart rate (mean ± SD, beats/24 h)	91,486 ± 13,341
Mean heart rate (mean ± SD, beats/min)	64.9 ± 8.4
Longest pause (mean ± SD, s)	5.6 ± 2.3
Symptom	
Amaurosis (n, %)	111 (36.2%)
Syncope (n, %)	97 (31.7%)
LAD (mean ± SD, mm)	38.4 ± 4.18
LVD (mean ± SD, mm)	45.42 ± 4.16
LVEF (mean ± SD, %)	57.7 ± 2.38
CHA ₂ DS ₂ -VASc score (14)	1.7 ± 1.1
NYHA classification (mean ± SD)	1.4 ± 0.5
Ablation therapy (n, %)	141(46%)
Pacing therapy (n, %)	165(54%)
Outcomes	
Cardiovascular related hospitalization (n, %)	104 (34.0%)
Stroke (n, %)	18 (5.9%)
Peripheral thrombosis (n, %)	6 (2.0%)

Comparison of Endpoint Between Ablation Group and Pacing Group

Compared with the ablation group, the pacing group had a higher incidence of the primary study endpoint (59.4 vs.14.2%, OR 6.05, 95% CI: 3.73–9.80, $P < 0.001$). The risk of cardiovascular related hospitalization in the pacing group was 4.87-fold that of the ablation group (95% CI: 3.57–11.01, $P < 0.001$). None of deaths was occurred in ablation group, and 1 death occurred due to cancer. A total of 84 cardiovascular-related hospitalization events occurred in the pacing group, primarily due to tachycardia (35.7%), heart failure (27.7%), coronary heart disease (4.2%). There were only 20 cardiovascular-related hospitalization events occurred in the ablation group, which were primarily due to bradycardia (7.8%), tachycardia (2.1%), heart failure (2.1%), and coronary heart disease (2.1%). A total of 9 patients (5.4%) hospitalized due to tachycardia underwent cardioversion therapy. The risk of thromboembolic events in the pacing group was 6.06-fold that of the ablation group (95% CI: 1.81–20.35, $P < 0.001$). A total of 15 strokes and 6 peripheral vascular embolization occurred in the pacing group, while only 3 strokes occurred in the ablation group (Table 4, Figures 2–4). After correcting hypertension, diabetes, stroke history, anticoagulation and CHA₂DS₂-VASc score by cox regression, there was still significant difference in Primary end point between the two groups ($p < 0.001$).

TABLE 2 | Characteristics of the two groups.

	Ablation group (n = 141)	Pacing group (n = 165)	P
Female (n, %)	75 (53.2%)	87 (52.7%)	0.935
Age (mean, y)	62.7 ± 8.8	62.4 ± 8.4	0.790
Diabetes (n, %)	30 (21.3%)	45 (27.3%)	0.224
Hypertension (n, %)	60 (42.6%)	77 (46.7%)	0.471
Coronary heart disease (n, %)	24 (17.0%)	37 (20.6%)	0.238
Stroke (n, %)	2 (1.4%)	3 (1.8%)	0.783
Total heart rate (mean ± SD, beats/24 h)	97,179 ± 16,888	89,311 ± 19,422	0.030
Mean heart rate (mean ± SD, beats/min)	65 ± 7	64 ± 8	0.283
AF duration (mean ± SD, y)	4.3 ± 2.96	5.0 ± 3.78	0.065
Longest pause (mean ± SD, s)	5.2 ± 2.2	6.0 ± 2.3	0.081
Symptom			
Amaurosis (n, %)	47 (33.3%)	64 (38.8%)	0.323
Syncope (n, %)	43 (30.5%)	54 (32.7%)	0.676
CHA ₂ DS ₂ -VASc score (mean ± SD)	1.65 ± 1.0	1.75 ± 1.2	0.469
NYHA classification (mean ± SD)	1.37 ± 0.48	1.45 ± 0.49	0.130
LAD (mean ± SD, mm)	37.96 ± 3.91	38.78 ± 4.37	0.086
LVD (mean ± SD, mm)	45.18 ± 3.86	45.45 ± 4.05	0.553
LVEF (mean ± SD, %)	57.95 ± 2.58	57.52 ± 2.17	0.116

TABLE 3 | Comparison of therapeutic results between ablation group and pacing group.

	Ablation group (n = 141)	Pacing group (n = 165)	P
Symptoms			
Amaurosis (n, %)	9 (6.3%)	0	NS
Syncope (n, %)	7 (4.9%)	0	NS
Freedom from AF (n, %)	116 (82.3%)	31 (18.8%)	<0.001
AF progression (n, %)	3 (2.1%)	39 (23.6%)	<0.001
Heart failure progression (n, %)	4 (2.8%)	18 (10.9%)	0.006
NYHA class (mean ± SD)	1.50 ± 0.74	2.11 ± 0.83	<0.001
AADs use (n, %)	10 (7.1%)	68 (41.2%)	<0.001
Anticoagulation (n, %)	3 (2.1%)	27 (16.4%)	<0.001
Crossover therapy	16	8	0.035
Pacemaker implement (n, %)	16 (11.3%)	–	NS
Cather ablation (n, %)	–	8 (4.8%)	NS
Operation complications (n, %)	2 (1.4%)	4 (2.4%)	0.527

NS, None Significant.

DISCUSSION

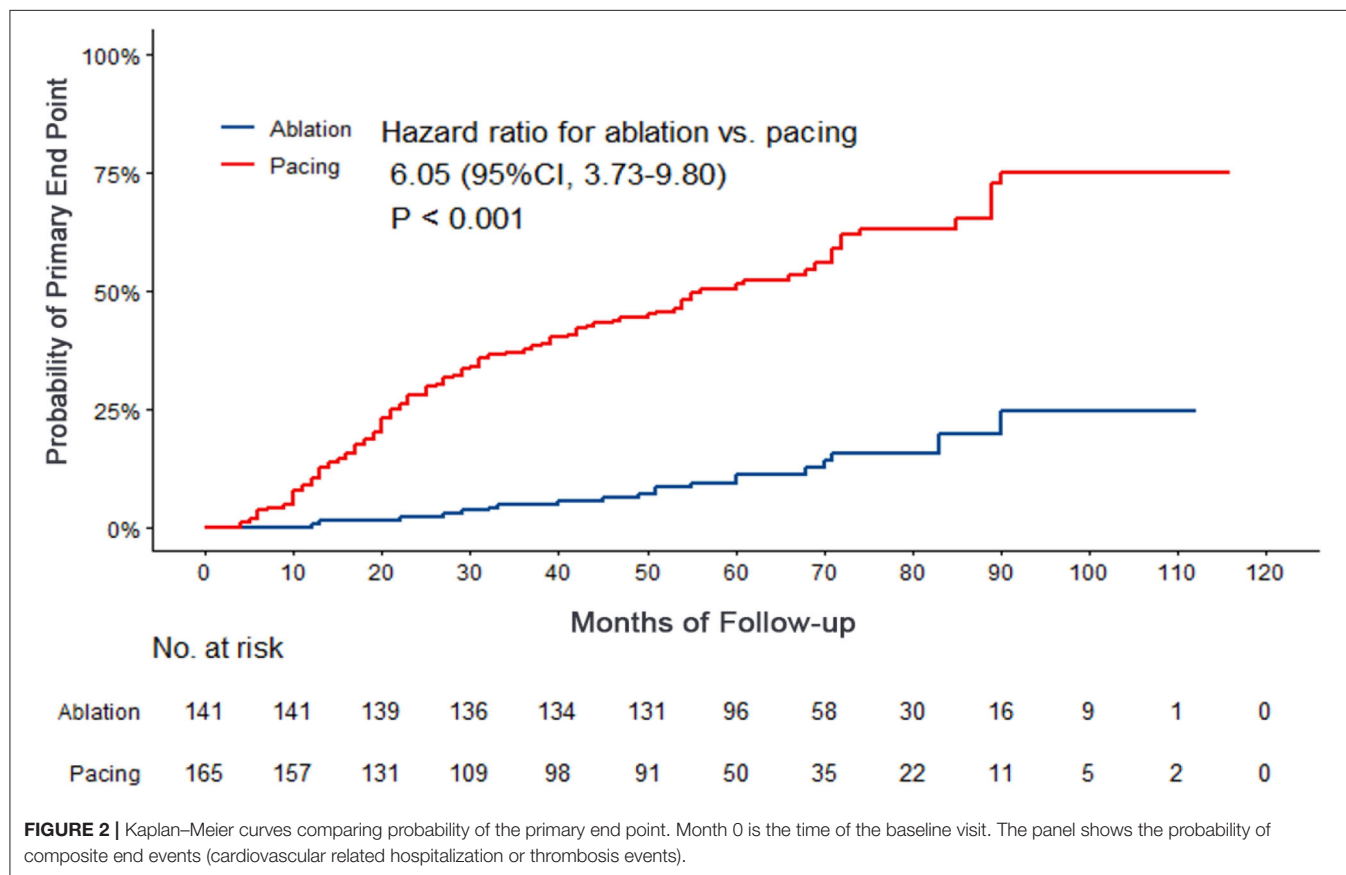
Main Research Findings

This large-scale retrospective study involved 306 patients with TBS, including 141 patients in the ablation group and 165 patients in the pacing group, with an average follow-up of nearly 6 years. The use of ablation for atrial fibrillation in TBS patients was associated with a significantly lower rate of a composite of cardiovascular hospitalization and thrombosis than pacing

TABLE 4 | Comparison of the primary end point between ablation group and pacing group.

	Ablation group N (%)	Pacing group N (%)	HR (95% CI)	P	P-adj
Primary end point	20 (14.2%)	98 (59.4%)	6.05 (3.73–9.80)	<0.001	<0.001
All-cause mortality	0 (0%)	1 (0.6%)	NS	NS	NS
Cardiovascular related hospitalization	20 (14.2%)	84 (50.9%)	4.87 (2.99–7.95)	<0.001	<0.001
Thrombosis events	3 (2.1%)	21 (12.7%)	6.06 (1.81–20.35)	0.001	0.009
Stroke	3 (2.1%)	15 (9.1%)	4.60 (1.30–16.23)	0.010	–
Peripheral thrombosis	0 (0.0%)	6 (3.6%)	NS	0.022	–

NS, None Significant.

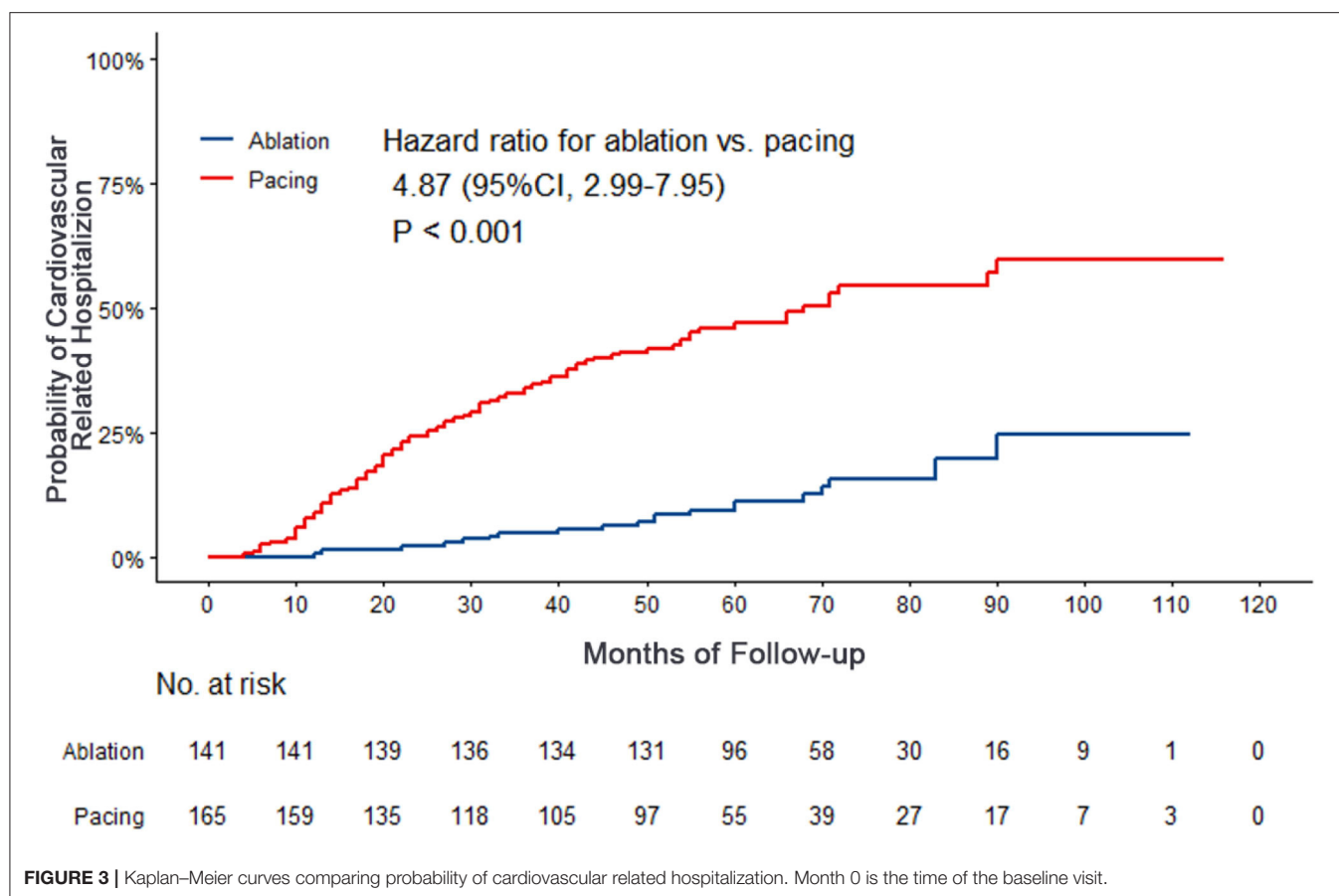


therapy. Furthermore, catheter ablation reduced the progression of atrial fibrillation and heart failure. To our knowledge, this study was the first to compare the effects of catheter ablation and cardiac pacing on the long-term prognosis in TBS patients, TBS patients may be benefit from ablation therapy vs. pacing therapy.

Pacing Therapy in TBS

Kaplan and Langendorf were the first to describe TBS in 1973 (11). Patients with TBS often suffer from syncope, syndrome, and even sudden death due to a long pause on termination of atrial fibrillation. Cardiac pacing effectively corrects long pauses after atrial arrhythmia to avoid the occurrence of symptoms, and is recommended by guidelines as the primary treatment plan. However, clinical problems related to atrial fibrillation

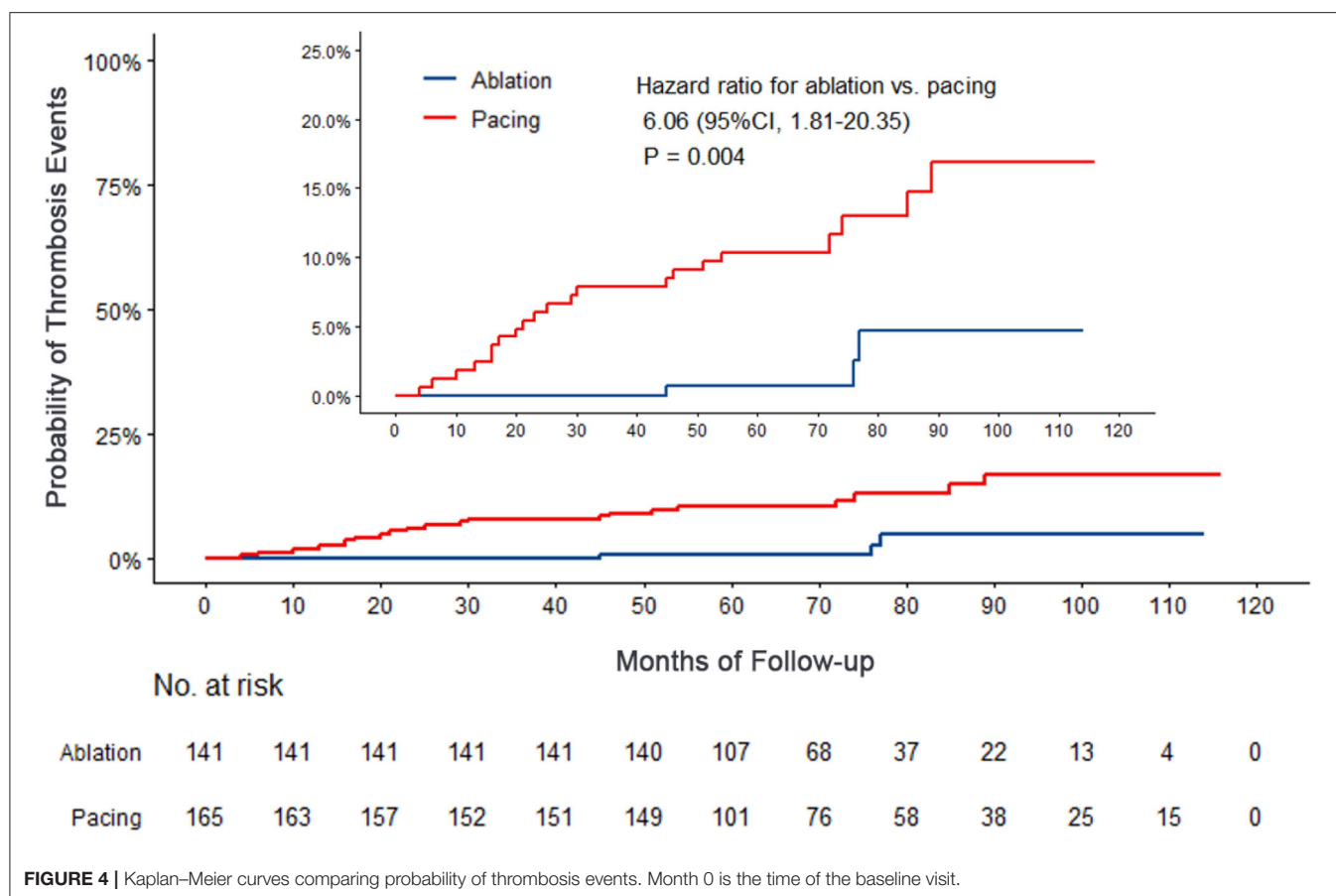
are still unresolved, and related problems caused by pacing are also occurred. A randomized controlled trial conducted by Lau et al. included 385 patients with paroxysmal atrial fibrillation combined with sinus node dysfunction (SSS), and compared the effects of right atrial appendage pacing and right atrial septum pacing on atrial fibrillation (15). The follow-up of the study lasted 3.1 years, and 25.8% of the patients progressed to persistent atrial fibrillation regardless of the pacing positions and patterns. The Danish Multicenter Randomized trial on single-lead atrial pacing vs. dual-chamber placing in sick sinus syndrome (DANPACE) trial is a randomized controlled trial comparing the effect of single-lead atrial pacemaker (AAIR) pacing or dual chamber pacemaker (DDDR) pacing on long-term prognosis in SSS patients, including 1,348 patients with a follow-up of 5.4 years (16). Of these patients, 25.7% developed atrial fibrillation. In



addition, 11.2% of the patients progressed to chronic atrial fibrillation. In our study, 23.6% of the patients progressed to persistent atrial fibrillation, which was consistent with the results of the previous study. In addition, thromboembolic events were a main risk for patients with pacemakers. Brandt et al. (16) conducted a registered trial to evaluate the impact of AAI and DDD pacing modes on rehospitalization or stroke in patients with SSS. In the study the incidence of stroke was nearly 10% regardless of which pacing mode was used. In the present study, the incidence of stroke and peripheral thrombosis was 12.7%. The study conducted by Kristensen et al. (17) showed that atrial fibrillation was an independent risk factor for thrombotic events in patients undergoing pacing (RR: 7.5, 95% CI: 1.6–36.2, $P = 0.01$). Oral anticoagulants are an effective strategy to prevent thromboembolism. However, in this study, only 16.4% of our patients received anticoagulation therapy, but these patients had a relatively high CHA₂DS₂-VASC score. Insufficient anticoagulation may be one of the reasons for the high risk of thrombotic events. In fact, there are still challenges in requiring strict anticoagulation therapy for these patients. For example, bleeding, pacemaker pocket infections, and patient compliance may be the primary concerns. For patients with TBS undergoing pacemaker implantation, it is inevitable that anticoagulation, rhythm control, and ventricular rate control of atrial fibrillation will need to be addressed again.

Catheter Ablation for Atrial Fibrillation in TBS Patients

Numerous studies have confirmed that catheter ablation is an effective and safe method for treating paroxysmal atrial fibrillation, with a success rate of >82% in >5 years (18). A retrospective study conducted by Hada et al. included 65 patients with SSS and atrial fibrillation (SSS-AF). After an average of 1.4 ablations, the patients underwent a 3-year follow-up, showing a success rate of 80.6% (19). Osaka et al. also conducted a study of catheter ablation in patients with SSS-AF and pacemaker implantation ($n = 51$, followed up for 5 years) (20). The success rate of catheter ablation in the study was 86.3%. Inada et al. (21) performed a study to define the potential role of successful ablation in patients with TBS. During the 5.8 years (range: 5–8.7 years) follow up, 86% patients remained free from AF after the last procedure. Only 8% patients required pacemaker implantation of the study by Chen et al. (22) evaluated the effectiveness and safety of catheter ablation in patients with TBS. Although only 43 patients with TBS were included in the study, 41 (95.3%) patients, who underwent follow-up for 20 months, did not require pacemaker implantation. Kim et al. (23) conducted a larger sample retrospective study involving 121 patients with TBS and followed up for 20 months. They found that 90.9% of the patients with TBS did not need pacemaker implantation. In this study, 88.6% of the patients with TBS



undergoing catheter ablation avoided pacemaker implantation, suggesting that catheter ablation can prevent nearly 90% of the patients with TBS from requiring an implanted pacemaker.

Should Catheter Ablation Be the First Line Therapy for TBS Patients?

Cardiovascular related hospitalization, thromboembolic events, and heart failure progression are essential endpoints for evaluating the prognosis of TBS patients. However, few studies have evaluated the hard endpoints of catheter ablation vs. pacing in patients with TBS. Chen et al. (22) showed that catheter ablation significantly reduced the rate of rehospitalization related to atrial fibrillation. However, no significant difference in cardiovascular rehospitalization rate was found, which may be due to the small sample size of that study. One of our previous studies (24) showed that catheter ablation significantly reduced the risk of new strokes in patients with TBS compared with the pacing group (15.1 vs. 5.4%, $P < 0.05$). In this study, after 5.9 years of follow-up, the risk of cardiovascular-related hospitalization and thrombosis in the TBS patients undergoing cardiac pacing was 6.05-fold higher than that of the patients undergoing catheter ablation (95% CI: 3.73–9.80, $P < 0.001$). The risk of cardiovascular related hospitalization of the pacing group was 4.87-fold than that of the ablation group (95% CI: 2.99–7.95, $P < 0.001$), and the thromboembolic event risk of the pacing

group was 6.06-fold than that of the ablation group (95% CI: 1.81–20.35, $P = 0.001$). The long-term outcome data suggested that catheter ablation significantly reduced cardiovascular-related hospitalizations, strokes, and peripheral thromboembolic events, and also effectively reduced atrial fibrillation burden and heart failure progression. Our findings supported that pacing may be a risk factor for worse prognosis in TBS patients. In this cohort, patients received the RV pacing. Considering the impact of right ventricular pacing on heart function, the results may be bias. Recent years, His bundle pacing (HBP) or left bundle branch pacing can achieve the physiological pacing via directly stimulating the His-Purkinje conduction bundle, which can significantly reduce pacing induced cardiomyopathy (25). Future evidence was needed to verify the results in the TBS patients.

Limitations

This was a retrospective study, so the clinical evidence level is low, and a prospective randomized controlled study is needed to verify our findings. TBS patients in the pacing group had a higher proportion of anticoagulation but inadequate, suggesting that an increase in anticoagulation rate may effectively reduce the incidence of thromboembolic events in the pacing group. Lower use of anticoagulation in the entire group may limit the applicability of the data. Adequate use of anticoagulation in TBS patients may reduce the difference of the prognosis between the

two therapy strategies. In our series these patients had relatively normal left atrial size. This data may not be applicable to patients with moderate or severely dilated left atria. Additionally, there may have been a selection bias during the selection of treatment strategies in this retrospective study.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committee of First Affiliated Hospital of Dalian Medical University. Written informed consent for

participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

YX, LG, and XYi contributed to conception and design of the study. YW, ZW, and MY organized the database. XYu and XX performed the statistical analysis. YW wrote the first draft of the manuscript. RZ wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Ventricular Tachyarrhythmia Risk in Paediatric/Young vs. Adult Brugada Syndrome Patients: A Territory-Wide Study

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Introduction: Brugada syndrome (BrS) is a cardiac ion channelopathy with a higher prevalence in Asia compared to the Western populations. The present study compared the differences in clinical and electrocardiographic (ECG) presentation between paediatric/young (≤ 25 years old) and adult (> 25 years) BrS patients.

Method: This was a territory-wide retrospective cohort study of consecutive BrS patients presenting to public hospitals in Hong Kong. The primary outcome was spontaneous ventricular tachycardia/ventricular fibrillation (VT/VF).

Results: The cohort consists of 550 consecutive patients (median age of initial presentation = 51 ± 23 years; female = 7.3%; follow-up period = 83 ± 80 months), divided into adult ($n = 505$, mean age of initial presentation = 52 ± 19 years; female = 6.7%; mean follow-up period = 83 ± 80 months) and paediatric/young subgroups ($n = 45$, mean age of initial presentation = 21 ± 5 years, female = 13.3%, mean follow-up period = 73 ± 83 months). The mean annual VT/VF incidence rate were 17 and 25 cases per 1,000 patient-year, respectively. Multivariate analysis showed that initial presentation of type 1 pattern (HR = 1.80, 95% CI = [1.02, 3.15], $p = 0.041$), initial asymptomatic presentation (HR = 0.26, 95% CI = [0.07, 0.94], $p = 0.040$) and increased P-wave axis (HR = 0.98, 95% CI = [0.96, 1.00], $p = 0.036$) were significant predictors of VT/VF for the adult subgroup. Only initial presentation of VT/VF was predictive (HR = 29.30, 95% CI = [1.75, 492.00], $p = 0.019$) in the paediatric/young subgroup.

Conclusion: Clinical and ECG presentation of BrS vary between the paediatric/young and adult population in BrS. Risk stratification and management strategies for younger patients should take into consideration and adopt an individualised approach.

Keywords: Brugada syndrome, paediatric, risk stratification, ventricular arrhythmia, sudden cardiac death

INTRODUCTION

Cardiac channelopathies are primary electrophysiological disorders that predispose spontaneous ventricular tachycardia/fibrillation (VT/VF) and sudden cardiac death (SCD) in the absence of structural abnormalities (1–3). Brugada syndrome (BrS), congenital long QT syndrome, and catecholaminergic ventricular tachycardia are the most common hereditary cardiac ion channelopathies (4–6). Although SCD in young people is more commonly caused by hypertrophic cardiomyopathy in the United States and arrhythmogenic right ventricular cardiomyopathy in parts of Europe, cardiac ion channelopathies often underlie juvenile cases of SCD without pre-existing comorbidities, which can cause great distress toward patients' families and the general public (7, 8).

Of these conditions, BrS is the most prevalent ion channelopathy found in Asia (9–12). BrS typically manifests in the fourth to fifth decades of life, but those presenting in childhood are deemed to be at high risk of SCD if symptomatic (13, 14). Due to the small population of paediatric BrS patients, it can be challenging to identify the specific differences between the paediatric and adult populations. As a result, the application of adult-based risk stratification criteria upon the paediatric population may result in misinterpretation of SCD risk. The present study aims to demonstrate the difference in clinical and electrocardiographic (ECG) presentation between paediatric/young and adult BrS patients.

METHODS

Study Population

This study was approved by The Joint Chinese University of Hong Kong—New Territories East Cluster Clinical Research Ethics Committee. The cohort included consecutive patients diagnosed with BrS between January 1st, 1997 and June 20th, 2020 by public hospitals of Hong Kong. Centralised electronic health records from the Hospital Authority were reviewed for patient identification and data extraction. The diagnosis of BrS was made initially by the case physicians. They were confirmed by G.T. and N.S.M. through the review of case notes, documented ECGs, treadmill test results, and genetic reports. Diagnosis of BrS was made based on the 2017 criteria proposed by the Expert Consensus Statement, as used in previous studies by our group (15). These patients fulfilled either criteria of (1) presentation of type 1 Brugada ECG pattern (BrP), or; (2) presentation of type 2 BrP with positive flecainide challenge test or VT/VF-induced on the electrophysiological study (EPS). Patients ≤ 25 years old were categorised into the paediatric/young subgroup, with the remainder of patients categorised into the adult subgroup. The age cut-off was adopted from Gonzalez et al.'s study on the risk stratification amongst young Brugada patients (14).

Clinical and Electrocardiographic Data Collection

The baseline clinical data extracted from the electronic health records include: (1) sex; (2) age of first characteristic ECG presentation and last follow-up; (3) follow-up duration; (4)

family history of SCD and BrS; (5) syncope manifestation and its frequency; (6) presentation of sustained VT/VF and its frequency; (7) ECG details as mentioned below; (8) performance of EPS, 24-h Holter study, genetic testing, and the respective results; (9) performance of echocardiogram; (10) presence of other arrhythmias; (11) implantation of implantable cardioverter-defibrillator (ICD); (12) occurrence, cause, and age of death; (13) period between the initial presentation of characteristic ECG and the first post-diagnosis VT/VF episode; (14) initial disease manifestation (asymptomatic, syncope, VT/VF). In the present study, symptoms refer to syncope and VT/VF, thus asymptomatic indicates freedom from both presentations. Other arrhythmias include sick sinus syndrome, atrioventricular block, atrial tachyarrhythmias, and supraventricular tachyarrhythmias. Positive EPS is defined as the induction of VT/VF that either sustained a minimum of 30 s or produced hemodynamic collapse.

In addition to the aforementioned details, the following clinical data on BrP were extracted: (1) the presence of fever and type of BrP; (2) any presentation of type 1 BrP during follow-up; (3) evolution in BrP type during follow-up; (4) performance and results of the flecainide challenge. The presence of type 1 BrP and the evolution of BrP types were identified by G.T. and S.L. through reviewing all documented ECGs from the BrS cohort.

The following automatically measured indices from the baseline ECG was extracted: (1) heart rate; (2) P wave duration (PWD) and PR interval; (3) QRS duration; (4) QT and QTc interval; (5) P, QRS, and T wave axis; (6) amplitude of R and S wave from leads V5 and V1, respectively; (7) presence of 1st degree atrioventricular block, defined as PR-interval >200 ms; (8) presence of interventricular delay, defined as QRS-interval ≥ 110 ms. Baseline ECG is the documented ECG with the initial characteristic ECG presentation. All ECG parameters, except for the amplitude of R and S wave from leads V5 and V1, respectively, were averaged across the 12 leads. These indices were selected as they reflect BrS-associated electrocardiographic changes, such as electrical axis deviation, and electrocardiographic indices that are used for risk stratification, such as depolarization parameters including prolonged QRS, 1st-degree atrioventricular block, positive R wave in lead V1, and QTc prolongation (16–19).

Statistical Analysis

Given the Shapiro–Wilk's normality test shows that all parameters were not normally distributed with $P < 0.05$, non-parametric tests were adopted. Subgroup differences of categorical variables were compared through Fisher's exact test and reported as total number (percentage), whilst discrete and continuous variables were compared by the Mann–Whitney *U*-test (median \pm interquartile range [IQR]). The annual VT/VF and case incidence rate of each subgroup was calculated by dividing the number of sustained VT/VF episodes and the number of patients with VT/VF during follow-up, respectively, by the sum of the follow-up duration in the subgroup. Cox regression was used to identify independent predictors of time to first post-diagnosis sustained VT/VF. The hazard ratio (HR) and 95% confidence interval (CI) were reported for Cox regression. Univariate predictors with $P < 0.05$ were selected

TABLE 1 | Baseline characteristics of the Brugada syndrome cohort.

Characteristic	Overall (n = 550)	Adult (n = 505)	Paediatric/Young (n = 45)	P-value
Demographics and clinical presentation				
Female	40 (7.27)	34 (6.73)	6 (13.3)	0.126
Onset age	51 ± 23	52 ± 19	21 ± 5	-
Current age	58 ± 23	60 ± 19	27 ± 9	-
Initial type 1 BrP	341 (62.0)	312 (61.8)	29 (64.4)	0.631
Type 1 BrP	413 (75.1)	381 (75.4)	31 (71.1)	0.716
Evolution of BrP type	188 (34.2)	171 (33.9)	17 (37.8)	0.513
Fever	87 (15.8)	74 (14.7)	13 (28.9)	0.018
Family history of BrS	17 (3.09)	12 (2.38)	5 (11.1)	0.009
Family history of SCD	45 (8.18)	40 (7.92)	5 (11.1)	0.401
Syncope	237 (43.1)	213 (42.2)	24 (53.3)	0.160
Syncope frequency	1.49 ± 16.4	1.54 ± 17.1	0.933 ± 1.19	0.162
VT/VF	86 (15.6)	77 (15.2)	9 (20.0)	0.394
Sustained VT/VF frequency	0.77 ± 4.16	0.80 ± 4.33	0.42 ± 1.01	0.294
Initial asymptomatic	332 (60.4)	312 (61.8)	20 (44.4)	0.026
Initial symptomatic	218 (39.6)	193 (38.2)	25 (55.5)	0.023
Initial syncope	175 (31.8)	154 (30.5)	21 (46.7)	0.030
Initial VT/VF	43 (7.82)	39 (7.72)	4 (8.89)	0.771
Initial diagnostic evaluation				
Flecainide challenge	234 (42.5)	209 (41.4)	25 (55.6)	0.083
Positive flecainide challenge	204 (87.2)	185 (88.5)	19 (76.0)	0.114
EPS	112 (20.4)	108 (21.4)	4 (8.89)	0.052
Positive EPS	76 (67.9)	74 (68.5)	2 (50.0)	0.596
Holter study	153 (27.8)	139 (27.5)	14 (31.1)	0.605
Arrhythmia in Holter Study	64 (41.8)	62 (44.6)	2 (14.3)	0.048
Other arrhythmias	81 (14.7)	79 (15.6)	2 (4.44)	0.046
Genetic test	53 (9.64)	45 (8.91)	8 (17.8)	0.064
Positive genetic test	18 (34.0)	13 (28.9)	5 (62.5)	0.104
Echocardiogram	259 (47.1)	236 (46.7)	23 (51.1)	0.641
EEG	61 (11.1)	55 (10.9)	6 (13.3)	0.619
Positive EEG	16 (26.2)	16 (29.1)	0 (0.00)	0.325
Treatment and outcomes				
ICD	143 (26.0)	135 (26.7)	8 (17.8)	0.218
Death	39 (7.09)	38 (7.52)	1 (2.22)	0.356
BrS death	6 (1.09)	5 (0.99)	1 (2.22)	1.00
Follow-up duration	83 ± 80	83 ± 80	73 ± 83	0.314
Baseline ECG characteristics				
Heart rate	79 ± 26	78 ± 26	86 ± 26.5	0.018
P-wave duration	113 ± 17	113 ± 16	108 ± 15.8	0.160
PR interval	166 ± 31	166 ± 32	158 ± 30	0.022
QRS interval	103 ± 16	104 ± 16	103 ± 17.3	0.883
QT interval	368 ± 48.0	369 ± 48.0	355 ± 48.5	0.004
QTc interval	415 ± 35.0	415 ± 34.5	410 ± 43.8	0.172
P axis	64 ± 24	64 ± 24	60 ± 23	0.508
QRS axis	60 ± 47	58 ± 47	75 ± 39	0.017
T axis	56 ± 28.0	56 ± 27.3	57.5 ± 30.3	0.617
V5 R wave amplitude	1.42 ± 0.76	1.42 ± 0.77	1.25 ± 0.60	0.139
V1 S wave amplitude	0.54 ± 0.44	0.54 ± 0.439	0.49 ± 0.98	0.614
1st degree AV block	55 (10.0)	52 (10.3)	3 (6.67)	0.602
Interventricular delay	149 (27.1)	138 (27.3)	11 (24.4)	0.856

For discrete variables, the table presents the number of patients (patient percentage concerning the cohort or subgroup). Bold text indicates $P < 0.05$.

for the multivariate analysis to avoid overfitting. To check for collinearity, variance inflation factor (VIF) is computed for the parameters in the multivariate analysis. $VIF \geq 5$ indicates the presence of collinearity and the variable in question would be removed. Separate models with and without the inclusion of predictors from the baseline ECG were established. Kaplan–Meier estimator curves were constructed for comparing the time-to-first VT/VF between paediatric/young and adult subgroups, and were compared using the log-rank test. All statistical analysis was performed using R Studio (Version: 1.3.1073). Statistical significance was defined as $P < 0.05$.

RESULTS

Baseline Characteristics

The baseline characteristics of BrS cohort and subgroups are presented in **Table 1**. The BrS cohort consists of 550 consecutive patients (age of initial presentation = 51 ± 23 years; female = 7.3%; follow-up period = 83 ± 80 months), divided into adult ($n = 505$, mean age of initial presentation = 52 ± 19 years; female = 6.7%; mean follow-up period = 83 ± 80 months) and paediatric/young subgroups ($n = 45$, mean age of initial presentation = 21 ± 5 years, female = 13.3%, mean follow-up period = 73 ± 83 months). Gender ($p = 0.126$) and follow-up duration ($p = 0.314$) did not differ significantly between the two subgroups. There was no significant intergroup difference in both the overall ($p = 0.716$) and initial ($p = 0.631$) presentation of type 1 BrP. There was a significantly greater proportion of paediatric/young patients presenting with fever at the onset of BrP ($p = 0.018$), or with a family history of BrS ($p = 0.009$). There are 143 patients with ICD implanted, which consists of eight paediatric/young patients. Amongst the 35 patients who received at least one appropriate shock, three cases belong to the paediatric/young subgroup, whilst two of the 24 patients who received inappropriate shocks were in the paediatric/young subgroup.

Outcomes and Follow-Up

A total of seven paediatric/young and 59 adult patients suffered from incident VT/VF on follow-up. This is equivalent to an incidence rate of 0.052 (7 cases per 135 person-days) and 0.0149 (59 cases per 3,965 person-days) for these groups, respectively, yielding an incidence rate ratio of 3.48 (95% confidence interval: 1.34–7.64). Furthermore, the overall manifestation of syncope ($p = 0.160$) and VT/VF ($p = 0.394$), in addition to their respective frequencies (syncope = 0.162, sustained VT/VF = 0.294), had no statistically significant difference between the two groups using the Mann–Whitney U -test. There was a greater proportion of adults with arrhythmias other VT/VF ($p = 0.046$), and arrhythmia detected during Holter monitoring ($p = 0.048$). At the initial onset of BrP, a significantly greater proportion of adult patients were diagnosed asymptotically ($p = 0.026$), whilst paediatric/young patients were more commonly diagnosed after the manifestation of syncope ($p = 0.030$). Amongst the seven initially asymptomatic paediatric/young patients with fever and type 1 BrP, no one experienced VT/VF during follow-up.

TABLE 2A | Multivariate Predictors for post-diagnosis VT/VF-free survival in BrS excluding baseline ECG parameters.

Parameter	HR	Variance inflation factor	95% CI	P-value
Adult ($n = 505$)				
Initial type 1 BrP	1.80	1.06	[1.02, 3.15]	0.041
Initial asymptomatic	0.53	1.28	[0.26, 1.07]	0.076
Initial VT/VF	1.37	1.25	[0.75, 2.52]	0.311
Paediatric/Young ($n = 45$)				
Age	0.94	1.24	[0.83, 1.07]	0.368
Initial VT/VF	19.4	1.24	[1.59, 237]	0.020

Bold text indicates $P < 0.05$.

TABLE 2B | Multivariate predictors for post-diagnosis VT/VF-free survival in BrS including baseline ECG parameters.

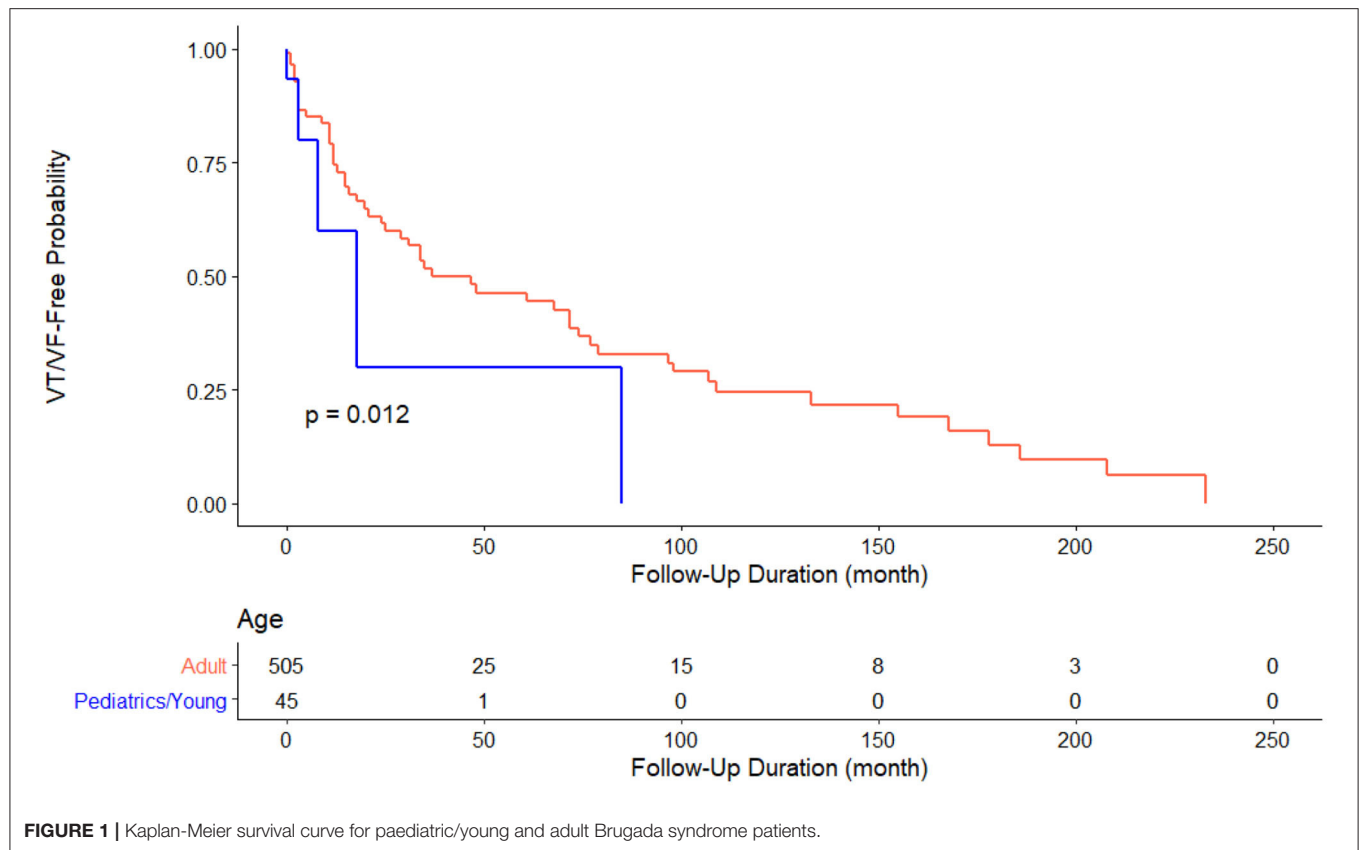
Parameter	HR	Variance inflation factor	95% CI	P-value
Adult ($n = 220$)				
Initial type 1 BrP	2.74	1.47	[0.98, 7.65]	0.054
Initial asymptomatic	0.26	1.11	[0.07, 0.94]	0.040
Initial VT/VF	1.06	1.28	[0.45, 2.52]	0.897
P Axis	0.98	1.26	[0.96, 0.999]	0.036
Lead V5 R wave amplitude	0.60	1.34	[0.23, 1.58]	0.303
Lead V1 S wave amplitude	0.39	1.38	[0.07, 2.18]	0.286
Paediatric/Young ($n = 38$)				
Age	0.95	2.86	[0.78, 1.17]	0.648
Initial VT/VF	13.1	1.79	[0.65, 265.00]	0.093
QTc interval	1.01	3.85	[0.96, 1.05]	0.832

Bold text indicates $P < 0.05$.

There was no statistically significant intergroup difference in all-cause mortality ($p = 0.356$) and BrS-related mortality ($p = 1.00$). In terms of baseline ECG indices, paediatric/young patients had a significantly higher heart rate ($p = 0.018$), which can contribute to a shorter QT interval ($p = 0.004$) in addition to the influence of age (20). The paediatric/young subgroup also had a significantly higher QRS axis than the adult subgroup ($p = 0.017$).

Spontaneous VT/VF Predictors

Different predictors for time-to-first post-diagnosis VT/VF-free survival were found for the adult and paediatric/young subgroups on both univariate and multivariate analysis, as displayed on **Tables 2A,B**. For the adult subgroup, the following significant predictors were found on univariate analysis: (1) initially asymptomatic (HR = 0.53, 95% CI = [0.28, 0.98], $p = 0.042$); (2) initial VT/VF presentation (HR = 1.85, 95% CI = [1.07, 3.19], $p = 0.027$); (3) P-wave axis (HR = 0.99, 95% CI = [0.97, 1.00], $p = 0.033$); (4) R-wave amplitude in lead V5 (HR = 0.40, 95% CI = [0.17, 0.92], $p = 0.030$); (5) S-wave amplitude in lead V1 (HR = 0.20, 95% CI = [0.05, 0.83], $p = 0.027$). Under multivariate analysis, initial presentation of type 1 BrP is predictive when baseline ECG predictors were excluded (HR = 1.80, 95% CI = [1.02, 3.15], $p = 0.041$). When baseline ECG predictors were included, both the initial asymptomatic presentation (HR = 0.26, 95% CI = [0.07, 0.94], $p = 0.040$) and



higher P axis (HR = 0.98, 95% CI = [0.96, 1.00], $p = 0.036$) were found to be protective against spontaneous VT/VF.

For the paediatric/young subgroup, age (HR = 0.88, 95% CI = [0.79, 0.97], $p = 0.015$), initial VT/VF (HR = 31.9, 95% CI = [3.31, 308.00], $p = 0.003$), and baseline QTc interval (HR = 1.04, 95% CI = [1.01, 1.06], $p = 0.008$) were identified as significant predictors under univariate analysis. Only the initial presentation of VT/VF is found to be predictive when baseline ECG predictors were excluded (HR = 19.40, 95% CI = [1.59, 237.00], $p = 0.020$). Additionally, paediatric/young status was found to be predictive of shorter post-diagnosis VT/VF-free survival (HR = 2.67, 95% CI = [1.20, 5.95], $p = 0.016$). **Figure 1** illustrates the significant intergroup difference in VT/VF-free survival with the Kaplan-Meier survival curve ($p = 0.012$). The drop in patient number is solely due to the occurrence of VT/VF during follow-up, with no patients lost to follow-up.

DISCUSSION

This is the first territory-wide cohort study, to the best of our knowledge, comparing paediatric/young and adult patients of BrS patients from Asia. There are several major findings for the present study: (1) there are significant differences in clinical and ECG presentation amongst adult and paediatric patients of BrS; (2) paediatric/young BrS patients have a higher risk for spontaneous VT/VF; (3) different predictors

for spontaneous VT/VF were found between adult and paediatric/young BrS patients.

Brugada Syndrome in the Young

Elevated risks of VT/VF occurrence amongst paediatric/young BrS patients have been reported by existing studies (14, 21–23). In a multi-centre study from 15 French tertiary centres including 1,613 patients, age at diagnosis changes the clinical presentation of BrS (23). The authors found that children present the highest risk of SCD (23). Whilst ICD therapy has been reported to be an effective treatment against potentially lethal arrhythmia in >25% young patients, it is frequently associated with complications and inappropriate shocks. Thus, risk stratification for SCD is particularly important amongst young patients (24). Furthermore, the greater proportion of fever-induced BrP amongst young patients was reported by other studies from the Survey on Arrhythmic Events in Brugada Syndrome (SABRUS) registry (22, 25). In a study of 128 young BrS patients (≤ 25 -year-old), the VT/VF event rate was 4.5% per year, the presence of spontaneous type 1 BrP, atrial arrhythmias and conduction abnormalities identified as significant predictors for ventricular arrhythmic events (14). In another study, the significant predictors were sinus node dysfunction, atrial arrhythmias, intraventricular conduction delay, and large S-wave in the paediatric subgroup, whereas only the presence of SCN5A mutations was predictive for the adolescent subgroup (22). Conte et al. (26) reported that children

experience more frequent episodes of sinus node dysfunction comparing to older subjects, with a comparable incidence of atrial tachyarrhythmia. The change in predictiveness of mutation may be due to a later presence of hormonal and autonomic triggers in life for the ECG phenotype to appear (27). In our study, the incidence rate for VT/VF is 17 and 25 cases per 1,000 patient-years for the adult and paediatric/young subgroups, respectively.

Initial presentation with VT/VF is a significant predictor of incident VT/VF, however spontaneous type 1 BrP was not a predictive factor in the paediatric population. This may be attributed to a greater proportion of females in the relatively small paediatric population, because spontaneous type 1 BrP did not predict spontaneous VT/VF amongst females (28). Previously, it was found that female BrS patients had a lower arrhythmic risk (29). This may be due to the role of testosterone in BrS, where hypertestosteronemia was reported to be positively associated with the Brugada phenotype (30).

In terms of ECG features, conduction abnormalities were shown to be predictive of spontaneous VT/VF on follow-up in paediatric BrS patients (14, 22). In contrast to these findings, our study identified repolarization (prolonged QTc interval) but not conduction abnormalities (PR interval, QRS interval, 1st-degree atrioventricular block) as significant predictors of VT/VF for the paediatric/young subgroup. This would suggest altered repolarization playing an important role in mediating ventricular arrhythmogenesis in this subgroup (31–34). It is hypothesised that QTc prolongation reflects the increased dispersion in transmural ventricular repolarization, thus increase the risk of VT/VF (35–42). Moreover, we found that paediatric/young patients had a higher QRS axis, in keeping with previous demonstrations of right axis deviation in younger patients but this variable was not a predictor of arrhythmic events. However, there is a lack of significant predictors for spontaneous VT/VF after the inclusion of baseline ECG predictors, likely due to the small sample size ($n = 38$) with a small number of events ($n = 7$), hence there is insufficient statistical power for the identification of significant predictors.

Brugada Syndrome in Adults

Several factors contribute to the differences in clinical and ECG presentation between paediatric/young and adult BrS patients. Testosterone has been found to play a significant role in the male predominant adult BrS population (30). Since testosterone is found to increase the risk of atrial arrhythmias, particularly amongst men, this explains the increased incidence of atrial arrhythmias within the male subgroup, possibly through increased adrenergic activity (43–45). Furthermore, ST-elevation and the resulting BrP may only become apparent later in life, despite a lifelong elevated SCD risk, which may explain the intergroup differences in ECG indices (46). The inherent difference in paediatric and adult ECG also contributes to the ECG subgroup differences, such as the lengthening of PR-interval as age increases (47).

Furthermore, our study found that higher R-wave and S-wave amplitudes were significant predictors of lower VT/VF in the adult subgroup. Indeed, a lower “minimum late R” and S-wave

duration,” reflecting a reduced voltage, was associated with a higher incidence of VT/VF (48). Moreover, a higher P-wave axis was associated with a lower likelihood of VT/VF in the adult subgroup. Abnormal P-wave axis outside the normal range of 0–75 degrees is known to be associated with atrial fibrillation and myocardial ischemia (49, 50). Whilst these changes increase the risk of cardiovascular mortality in the general population, in Brugada patients it may reflect a longer survival that allows the development of these degenerative changes.

Strengths and Limitations

The major strengths of the present study include: (1) this is the first study that compared the characteristics of paediatric/young and adult patients in BrS; (2) predictors of post-diagnosis VT/VF-free survival were derived for adult and paediatric/young patients; (3) holistic differences in clinical and ECG aspects of adult and paediatric/young patients were evaluated; (4) the study cohort was followed-up for a substantial length of time.

Several limitations should be noted for the present study. First, the retrospective nature of the study is inherently subjected to selection and information bias. However, consultations were performed at least annually for most patients, hence the patients were closely followed up. Also, it should be noted that the documented syncope may not be of cardiogenic origin, hence it may be unrelated to BrS. Moreover, the heterogeneity of age within the paediatrics population may limit the statistical power in the identification of VT/VF predictors. Therefore, multinational registries on the paediatric population are needed to expand the cohort size and homogenise the classes of age in paediatric studies. Furthermore, changes in guidelines for investigations and diagnostic tests throughout follow-up introduced inevitable inconsistency in indications for different tests. Due to the limited availability of public genetic service, not all BrS patients included in this study underwent genetic screening, and hence genotype-phenotype correlations could not be established with greater degrees of certainty.

CONCLUSION

Clinical and ECG presentation of BrS vary between the paediatric/young and adult population in BrS. Risk stratification and management strategies for younger patients should take into consideration and adopt an individualised approach.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Joint Chinese University of Hong Kong—New Territories East Cluster Clinical Research Ethics Committee. Written informed consent from the participants’ legal guardian/next of kin was not required to participate in this

study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

SL and GT: study conception, data acquisition, database building, statistical analysis, manuscript drafting, and manuscript revision. WW, IW, CM, NM, and TL: data

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Circulating Soluble Suppression of Tumorigenicity 2 Predicts Recurrence After Radiofrequency Ablation of Persistent Atrial Fibrillation

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Objective: A more extensively fibrotic left atrium contributes to atrial fibrillation (AF) occurrence, persistence, and recurrence. The soluble suppression of tumorigenicity 2 (sST2) has emerged as a ventricular fibrotic biomarker for patients with heart failure. The present study is to investigate associations between circulating sST2 and risk of recurrence after ablation in AF patients.

Methods: We measured the baseline plasma level of sST2 from patients with persistent AF ($n = 117$) and paroxysmal AF ($n = 93$) patients. Patients were followed up for 15 months after ablation. The relationship between circulating sST2 and recurrence was assessed by multivariable Cox regression. The cutoff value of sST2 was determined by receiver operating characteristic curve. The relationship between baseline sST2 level and left atrial volume index (LAVI) was assessed by multivariate linear regression analysis. Serial sST2 measurements were also conducted after 24 h, 6 months, and 15 months of ablation. ST2 localization was examined in left atrial appendages of persistent AF patients by immunohistochemistry and Western blot.

Results: Baseline sST2 positively associated with LAVI in the persistent AF group, and elevated sST2 (≥ 39.25 ng/ml) independently increased the risk of recurrence after ablation (area under the curve = 0.748), with hazard ratio of 1.038 (95% confidence interval 1.017–1.060, $P < 0.001$) when adjusted for co-variables. In contrast, elevated sST2 cannot predict recurrence in paroxysmal AF.

Conclusions: In persistent AF patients, increased sST2 serves as a marker of recurrence after radiofrequency ablation. Patients with sST2 ≥ 39.25 ng/ml are more likely to develop recurrence within a year.

Keywords: atrial fibrillation, atrial fibrosis, sST2, biomarker, recurrence

INTRODUCTION

Atrial fibrillation (AF) is the most common arrhythmia and contributes significantly to mortality and morbidity (1, 2). Large clinical trials have shown that there are no currently available anti-arrhythmic medications in the treatment for AF (3, 4). Despite the success of using catheter ablation in the control of AF, recurrence rates still remain high, ranging from 30 to 60% across

reports (5, 6). A more extensively fibrotic left atrium contributes to AF occurrence, persistence, and recurrence (7, 8). However, a valuable circulating marker, which can predict atrial fibrosis or recurrence following ablation is very limited.

Suppression of tumorigenicity 2 (ST2) belongs to the Toll-like/IL-1 receptor superfamily. ST2 has two main isoforms, the soluble ST2 (sST2) and the membrane-bound ST2 (ST2L), due to differential promoter binding (9, 10). Binding of ST2L and IL-33 promotes NF- κ B pathway activation (9). sST2 lacks the transmembrane and cytoplasmic domains in comparison with ST2L, and functions as a decoy receptor to prevent the binding between ST2L and IL-33 (11). Membrane receptor ST2L is mainly expressed on hematopoietic cells and involved in inflammatory responses (9). sST2 is induced in fibroblasts or cardiomyocytes subjected to stimulations (12). sST2 has emerged as a ventricular fibrotic biomarker for patients with heart failure or myocardial infarction, and the initial sST2 level was independently associated with the incidence of mortality (13, 14).

Blood sST2 concentration in AF patients was higher than that of healthy controls, and sST2 was likely a biomarker, which can predict AF patients' risk of emergency admission (15). Circulating sST2 can predict the risk of occurrence of the new-onset AF with coronary artery disease (16). Okar et al. also reported that sST2 was an independent parameter for predicting AF recurrence in patients with non-valvular paroxysmal AF who have undergone cryoballoon catheter ablation (17). Moreover, Walek et al. reported that sST2 was a predictor of successful electrical cardioversion and long-term maintenance of sinus rhythm (SR) in persistent AF patients (18). Wang et al. reported that sST2 levels were higher in paroxysmal AF patients with left atrial low-voltage zone (LVZ) > 20% compared with those with a smaller LVZ, and elevated sST2 levels served as a novel predictor of paroxysmal AF recurrence rate in patients who had undergone ablation (19). Persistent AF patients normally have a more severe fibrotic atrium than paroxysmal AF patients (20), which promote us to investigate the diagnostic value of sST2 in the setting of persistent AF.

In the present study, we measured the baseline circulating level of sST2 in patients with paroxysmal AF or persistent AF, and examined whether sST2 could be an important indicator of AF recurrence following ablation. Moreover, additional experiments were also performed to explore the potential cellular sources of sST2 in AF patients.

METHODS

Study Population

The current study was reviewed and approved by the Institutional Review Board of the First Affiliated Hospital of Dalian Medical University, and conducted in accordance with the Declaration of Helsinki. All the participants signed an informed consent. Initially, a total of 356 consecutive patients with drug-refractory AF who underwent their primary radiofrequency catheter ablation procedure at our hospital were recruited to the study between January 2015 and December 2018. AF patients were categorized as paroxysmal when episodes self-terminated within 7 days or as persistent when episodes lasted over 7 days or

required electrical cardioversion. Patients were excluded from the study if (1) they had received a previous endovascular, surgical AF ablation or a maze surgery, (2) they had untreated hyperthyroidism, (3) they had heart failure [left ventricular ejection fraction (LVEF) < 50% or B-type natriuretic peptide (BNP) > 400 ng/ml], coronary heart disease, stroke, pulmonary diseases, and hepatic or renal dysfunction, (4) they were in chronic inflammatory status or had acute infection, and (5) patients needed cardioversion post ablation. Finally, a total of 210 patients were enrolled for the follow-up study.

Radiofrequency Catheter Ablation

Oral anticoagulation therapy was stopped at least 1 day before ablation and bridged with low molecular weight heparin. Patients were heparinized to activate clotting time > 250 s, after double transseptal puncture. Circumferential pulmonary vein isolation (CPVI) using irrigated radiofrequency ablation is the initial ablation approach used in all AF patients who underwent ablation. For persistent AF patients, additional substrate modification, including left atrial linear ablation and isthmus ablation, were performed followed by CPVI. The endpoint of CPVI was defined as the absence of any pulmonary vein spike potential in the spiral-mapping catheter inside the lateral PVs. Ablation of complex fractionated atrial electrograms (CFAE) guided by an LA CFAE map was performed if SR could not be achieved after CPVI. If frequent atrial premature beats or atrial tachycardia occurred, superior vena cava isolation was performed. A cavotricuspid isthmus ablation was performed if familiar atrial flutter occurred (21).

Assessment of Soluble Suppression of Tumorigenicity

Fresh blood samples (2 ml) were obtained by venipuncture from AF patients prior to ablation (baseline), 24 h, 6 months, and 15 months after ablation. Levels of sST2 were measured by using human sST2 enzyme-linked immunosorbent assay (ELISA) kit (Presage ST2 Assay, Critical Diagnostics, USA).

Follow-Up

After the ablation, all patients were followed up for 15 months (the first 3 months is blanking period). In total, of 210 people initially recruited, 92.8% of the participants had their data measured at the end of this study. One patient was lost to follow-up at 8 months, and further 14 patients (6.66%) were lost to follow-up between 12 and 15 months. Amiodarone or propafenone were administered after ablation if not contraindicated to prevent the early recurrence of AF. Anticoagulation treatment was prescribed for at least 3 months and thereafter according to the CHA₂DS₂-VASC score. Patients were evaluated by 24-h Holter monitor if symptoms of arrhythmia occurred. Routine medical examination, including the 24-h Holter monitoring, was performed for patients without any symptoms at 3, 6, 9, 12, and 15 months post-ablation. Recurrence was defined as AF, atrial tachycardia, or atrial flutter \geq 30 s in duration after a 3-month blanking period according to the 2012 Heart Rhythm Society consensus document.

Immunohistochemistry

Atrial appendages from persistent AF patients with valvular disease were collected during valvular replacement surgery and then fixed in 4% paraformaldehyde ($n = 6$). In the control group, heart samples were taken from body donors who died in an accident and were anatomically confirmed to have no cardiovascular diseases ($n = 6$). After embedding in paraffin, samples were cut into 4- μ m sections for HE staining, Masson staining, and IHC.

IHC was used to determine the contents of ST2 or α -SMA in the atrial sections. Briefly, antigen retrieval was conducted by immersing in the citrate-EDTA buffer and then in microwave oven for 5 min at high power. Non-specific staining was blocked by using 3% H_2O_2 and then followed by 10% goat serum. After blocking, 50 μ l of diluted primary antibodies, sST2 (1/100 dilution, 11920-1-AP, Proteintech, Wuhan, China) or α -SMA (1/100 dilution, 14395-1-AP, Proteintech, Wuhan, China) were applied onto each section for 1 h. After incubation with biotin-conjugated-goat anti-rabbit secondary antibody (1/1,000 dilution, B-2770, Thermo Fisher, USA) sections were incubated with Avidin and NeutrAvidin™ Biotin-Binding complex (A2666, Thermo Fisher, USA) for 30 min. Finally, sections were visualized by DAB staining (A34002, Thermo Fisher, USA) for two (α -SMA) or 3 min (sST2).

Western Blotting

Total protein from atrial appendages was isolated with radioimmunoprecipitation assay (RIPA) buffer (Beytime, China). Equal amounts (30 μ g) of protein from samples were loaded on SDS-Page and run at constant voltage about 80 V for 1 h through the resolving gel and at 120 V for 2.5 h through the stacking gel. After transfer, the membranes were blocked with 5% skim milk for 1 h and then incubated in diluted primary antibody ST2 (1/1,000 dilution, 11920-1-AP, Proteintech, Wuhan, China) at 4°C overnight. After the primary antibody incubation, membranes were washed three times (each for 10 min) in TBST and then incubated in a goat-anti rabbit secondary antibody conjugated with horseradish peroxidase (1/10,000, A16096, Thermo Fisher, USA) for 2 h at room temperature. Membranes were then washed three times before exposure using Immun-star HRP chemiluminescent substrate kit (1705040, Bio-Rad, USA). After exposure, the antibodies loaded on the membranes were stripped in stripping solution (0.0625 M Tris-Cl pH 6.8; 2% SDS, 0.7% β -mercaptoethanol) for 30 min at 60°C. Then membranes were re-probed with α -tubulin (1/5,000, ab4074, Abcam, USA) at 4°C overnight to verify loading consistency.

Statistical Analysis

Continuous data were tested for normality and expressed as mean \pm standard deviation (SD). Qualitative variables were expressed as percentages (%), and Fisher's exact test and χ^2 test were used for comparison between groups. Hazard ratios (HR) with 95% confidence intervals (CI) were presented. Associations between circulating sST2 and left atrial volume index (LAVI) were assessed by multivariable linear regression models after adjusting for co-variables. Univariate and multivariable Cox proportional hazard regression were used to identify significant

TABLE 1 | Clinical characteristics in all atrial fibrillation (AF) patients.

Variables	Recurrence ($n = 43$)	No recurrence ($n = 167$)	P-value
Age (years)	57.74 \pm 11.45	58.49 \pm 9.95	0.674
Male (n , %)	26 (60.5%)	117 (70.1%)	0.271
History of AF (months)	4.84 \pm 4.17	4.76 \pm 4.86	0.837
Persistent AF (n , %)	33 (57.9%)	84 (54.9%)	0.756
Smoking (n , %)	15 (34.9%)	47 (28.1%)	0.454
Medical history			
Hyperlipidemia (n , %)	14 (32.6%)	38 (22.8%)	0.234
Hypertension (n , %)	20 (46.5%)	68 (40.7%)	0.494
T2DM (n , %)	2 (4.7%)	24 (14.4%)	0.118
Medication			
VKA (n , %)	14 (32.6%)	35 (21.0%)	0.156
Amiodarone (n , %)	22 (51.2%)	92 (55.1%)	0.732
Beta blockers (n , %)	10 (23.3%)	18 (10.8%)	0.043
ACEI (n , %)	3 (7.0%)	6 (3.6%)	0.394
Lab tests of plasma			
sST2 (ng/ml)	38.65 \pm 17.78	32.44 \pm 10.34	0.033
Cre (μ mol/L)	79.00 \pm 47.71	71.98 \pm 14.27	0.346
UA (μ mol/L)	366.43 \pm 122.69	360.71 \pm 79.97	0.756
FBG (g/L)	5.82 \pm 1.42	5.91 \pm 1.17	0.673
TC (mmol/L)	4.78 \pm 1.04	4.77 \pm 1.08	0.982
HDL (mmol/L)	1.25 \pm 0.26	1.23 \pm 0.25	0.603
HCY (μ mol/L)	12.83 \pm 5.79	14.59 \pm 7.92	0.177
TSH (mU/L)	2.37 \pm 1.84	2.37 \pm 1.78	0.988
Echocardiography			
LVEF (%)	63.38 \pm 6.31	61.60 \pm 7.11	0.137
LAVI (ml/m ²)	30.74 \pm 7.54	27.29 \pm 7.76	0.009
Ablation time (min)	88.56 \pm 20.74	94.59 \pm 24.23	0.136

T2DM, type 2 diabetes mellitus; VKA, vitamin K antagonist; ACEI, angiotensin-converting enzyme inhibitors; sST2, soluble suppression of tumorigenicity 2; Cre, creatinine; UA, uric acid; FBG, fibrinogen; TC, total cholesterol; HDL, high-density lipoprotein; HCY, homocysteine; TSH, thyroid stimulating hormone; LVEF, left ventricular ejection fraction; LAVI, left atrial volume index.

predictors of the primary outcomes. The associations between sST2 level and AF recurrence were analyzed by multivariable Cox regression analysis. Receiver operating characteristic curve (ROC) curves were developed using a probability-weighted Cox model. Recurrence rate in AF patients following ablation was visualized using Kaplan–Meier survival curves and Log-rank tests. Serial measurements of sST2 were analyzed using repeated measurements analyses of variance. All values were two-tailed, and differences were considered statistically significant at $P < 0.05$. Gpower software version 3.1 (Gpower Kiel, Germany) was used to perform power analysis; the total estimated sample size in our study was 172. Power, alpha, and effect size were set at 90%, 0.05, and 0.5, respectively. The statistical analysis was performed with SPSS software version 13.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Demographic and Clinical Characteristics

Table 1 summarizes the baseline characteristics of all the participants. A total of 210 AF patients (117 persistent and

TABLE 2 | Clinical characteristics in persistent AF patients.

Variables	Recurrence (n = 23)	No recurrence (n = 94)	P-value
Age (years)	57.70 ± 12.89	59.03 ± 9.60	0.578
Male (n, %)	16 (69.6%)	73 (77.7%)	0.423
History of AF (years)	4.79 ± 4.47	4.60 ± 5.18	0.871
Smoking	7 (30.4%)	26 (27.7%)	0.800
Medical history			
Hyperlipidemia (n, %)	6 (26.1%)	17 (18.1%)	0.390
Hypertension (n, %)	10 (43.5%)	39 (41.5%)	0.862
T2DM (n, %)	0 (0%)	9 (9.6%)	0.202
Medication			
VKA (n, %)	13 (56.5%)	28 (29.8%)	0.027
Amiodarone (n, %)	18 (78.3%)	74 (78.7%)	0.961
Beta blockers (n, %)	6 (26.1%)	11 (11.7%)	0.100
ACEI (n, %)	3 (13.0%)	5 (5.3%)	0.189
Lab tests of plasma			
sST2 (ng/ml)	46.38 ± 20.58	33.46 ± 10.32	0.007
Cre (μmol/L)	87.05 ± 63.25	72.56 ± 13.26	0.286
UA (μmol/L)	403.35 ± 124.28	380.56 ± 77.44	0.408
FBG (g/L)	5.42 ± 0.72	5.77 ± 0.96	0.112
TC (mmol/L)	4.73 ± 1.24	4.85 ± 1.24	0.685
HDL (mmol/L)	1.22 ± 0.24	1.23 ± 0.25	0.837
Echocardiography			
LVEF (%)	61.18 ± 5.50	59.45 ± 7.30	0.359
LAVI (ml/m ²)	33.82 ± 6.62	28.52 ± 8.34	0.005
Ablation time (min)	101.30 ± 19.37	108.61 ± 22.81	0.126

T2DM, type 2 diabetes mellitus; VKA, vitamin K antagonist; ACEI, angiotensin-converting enzyme inhibitors; sST2, soluble suppression of tumorigenicity 2; Cre, creatinine; UA, uric acid; FBG, fibrinogen; TC, total cholesterol; HDL, high-density lipoprotein; HCY, homocysteine; TSH, thyroid stimulating hormone; LVEF, left ventricular ejection fraction; LAVI, left atrial volume index.

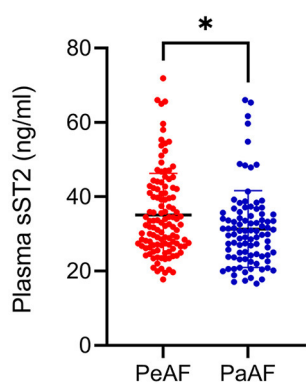


FIGURE 1 | Enhanced plasma soluble suppression of tumorigenicity 2 (sST2) was found in persistent atrial fibrillation (AF) patients. Plasma levels of sST2 were measured by enzyme-linked immunosorbent assay (ELISA) kit and presented by dot plots. PaAF, paroxysmal AF; PeAF, persistent AF. * $P < 0.01$ vs. PeAF.

93 paroxysmal AF patients) were enrolled in this study. AF recurrence (20.5%) was observed between 3 and 15 months after ablation. The occurrence of AF was predominant in male. The

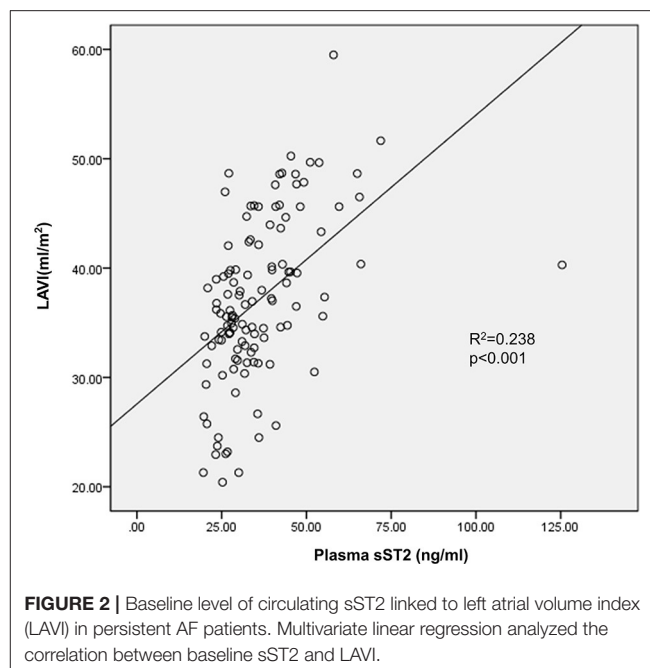


FIGURE 2 | Baseline level of circulating sST2 linked to left atrial volume index (LAVI) in persistent AF patients. Multivariate linear regression analyzed the correlation between baseline sST2 and LAVI.

most prevalent comorbidity was hypertension (41.9%), followed by hyperlipidemia (24.8%) and T2DM (12.4%). Significant difference in the use of beta blockers and the plasma level of sST2 was observed. **Table 2** summarizes the baseline characteristics of the persistent AF patients. During a total of 15-month follow-up period, 80.3% (94/117) persistent AF patients were successfully converted to SR. We further confirmed that circulating sST2 from persistent AF patients was significantly increased compared with that from paroxysmal AF patients at baseline level (**Figure 1**).

Baseline Soluble Suppression of Tumorigenicity 2 Correlated to Left Atrial Volume Index and Atrial Fibrillation Recurrence After Ablation in Persistent Atrial Fibrillation Patients

Both univariate and multivariate linear regression models were used to evaluate relationships between baseline sST2 and LAVI in persistent AF patients (**Figure 2**, **Tables 3, 4**). Only baseline level of sST2 reached a P -value of <0.05 during the univariate linear regression analysis. For the multivariate linear regression model, all covariates listed in **Table 1** were adjusted. Baseline sST2 was strongly associated with LAVI during the univariate linear regression analysis (**Table 4**). The relationships between sST2 and LAVI were also evaluated in paroxysmal AF patients, with no significant differences (**Table 5**).

The associations between baseline sST2 level and all AF recurrence were analyzed by multivariable Cox regression analysis based on adjusted for nothing (Model 1); sex and age (Model 2); and sex, age, hypertension, type 2 diabetes mellitus (T2DM), uric acid (UA), total cholesterol (TC), and beta blockers (Model 3) (**Table 6**). The associations between baseline sST2 and paroxysmal AF or persistent AF were based on adjusted for sex,

TABLE 3 | Univariate linear regression analyzed the correlations between baseline sST2 and LAVI in persistent AF patients.

Variables	Unstandardized coefficients		Standardized coefficients	t	P	95% CI for B	
	B	SE				Lower	Upper
Age (years)	0.058	0.068	0.079	0.848	0.398	−0.077	0.192
Male (n, %)	−0.824	1.631	−0.047	−0.505	0.614	−4.056	2.407
History of AF (months)	−0.124	0.139	−0.083	−0.891	0.375	−0.398	0.151
Smoking (n, %)	−1.575	1.541	−0.095	−1.022	0.309	−4.628	1.479
Hyperlipidemia (n, %)	−0.224	1.753	−0.012	−0.128	0.899	−3.696	3.249
Hypertension (n, %)	−1.149	1.408	−0.076	−0.816	0.416	−3.938	1.641
T2DM (n, %)	0.213	2.615	0.008	0.82	0.935	−4.966	5.393
VKA (n, %)	2.618	1.440	0.167	1.818	0.072	−0.234	5.47
Amiodarone (n, %)	0.289	1.700	0.016	0.170	0.865	−3.077	3.656
Beta blockers (n, %)	3.437	1.951	0.162	1.761	0.081	−0.428	7.301
ACEI (n, %)	2.503	2.751	0.085	0.91	0.365	−2.946	7.952
sST2 (ng/ml)	0.264	0.044	0.488	5.995	< 0.001	0.177	0.351
Cre (μmol/L)	0.004	0.023	0.017	0.186	0.853	−0.041	0.050
UA (μmol/L)	0.003	0.008	0.041	0.435	0.664	−0.012	0.019
FBG (g/L)	0.551	0.746	0.070	0.738	0.462	−0.928	2.029
TC (mmol/L)	0.133	0.567	0.022	0.235	0.815	−0.990	1.256
HDL (mmol/L)	−1.087	2.783	−0.037	−0.390	0.697	−6.601	4.428
HCY (μmol/L)	−0.090	0.093	−0.091	−0.968	0.335	−0.275	0.994
TSH (mU/L)	0.455	0.396	0.108	1.149	0.253	−0.330	1.241
LVEF (%)	−0.093	0.104	−0.088	−0.892	0.374	−0.299	0.113

T2DM, type 2 diabetes mellitus; VKA, vitamin K antagonist; ACEI, angiotensin-converting enzyme inhibitors; sST2, soluble suppression of tumorigenicity 2; Cre, creatinine; UA, uric acid; FBG, fibrinogen; TC, total cholesterol; HDL, high-density lipoprotein; HCY, homocysteine; TSH, thyroid stimulating hormone; LVEF, left ventricular ejection fraction; LAVI, left atrial volume index.

TABLE 4 | Multivariate linear regressions for the correlations between baseline sST2 and LAVI in persistent AF patients.

Variables	Unstandardized coefficients		Standardized coefficients	t	P	95% CI for B	
	B	SE				Lower	Upper
sST2 (ng/ml)	0.257	0.055	0.499	0.711	< 0.001	0.149	0.366

sST2, soluble suppression of tumorigenicity 2.

age, hypertension, T2DM, UA, TC, and beta blockers (Model 4 and Model 5; **Table 6**). After adjusting for potential confounders, we found that sST2 was an independent predictor of recurrence for all AF patients (HR = 1.026, 95% CI 1.007–1.046, $P < 0.001$), especially in the persistent AF group (HR = 1.038, 95% CI 1.017–1.060, $P < 0.001$) (**Table 2**), but not in paroxysmal AF patients.

ROC analysis demonstrated that plasma sST2 ≥ 39.25 ng/ml was predictive of recurrence after a single ablation, with 74% sensitivity and 77% specificity. The area under the ROC curve was 0.748 (**Figure 3A**). Kaplan–Meier analysis showed a significantly higher rate of recurrent AF in sST2 high (17 of 39, 43.6%) vs. sST2 low group (6 of 78, 7.7%) (**Figure 3B**).

Serial Soluble Suppression of Tumorigenicity 2 Measurements at Different Time Points

sST2 levels in persistent AF patients were measured at four different time points. After 24 h of ablation, sST2 was dramatically increased. After 6 months of ablation, sST2 level

dropped below the baseline level. There was no change between 6 and 15 months of post ablation in sST2 levels (**Figure 4**).

Accumulation of Soluble Suppression of Tumorigenicity 2 Was Observed in Atria of Persistent Atrial Fibrillation Patients

Atria from persistent AF patients displayed high level of fibrotic areas, as indicated by Masson, HE, and IHC staining (α -SMA) (**Figure 5A**). Interestingly, IHC results revealed that ST2 was largely found in the fibrotic area, that co-localized with myofibroblasts (**Figure 5A**), indicating that cardiac ST2 was mainly derived from myofibroblasts. Western blot confirmed that cardiac ST2 was mainly in the form of sST2, not ST2L, according to the molecular weight (**Figures 5B,C**).

DISCUSSION

The major findings in the current study were (1) baseline sST2 from persistent AF patients was elevated compared with paroxysmal AF patients; (2) baseline sST2 positively correlated

TABLE 5 | Univariate linear regression analyzed the correlations between baseline sST2 and LAVI in paroxysmal AF patients.

Variables	Unstandardized coefficients		Standardized coefficients	<i>t</i>	<i>P</i>	95% CI for B	
	B	SE	B			Lower	Upper
Age (years)	0.128	0.068	0.195	1.895	0.061	−0.006	0.262
Male (<i>n</i> , %)	1.218	1.418	0.09	0.859	0.393	−1.599	4.034
History of AF (months)	0.136	0.163	0.087	0.834	0.407	−0.188	0.46
Smoking (<i>n</i> , %)	−1.742	1.505	−0.12	−1.157	0.25	−4.732	1.248
Hyperlipidemia (<i>n</i> , %)	2.255	1.498	0.156	1.506	0.136	−0.72	5.23
Hypertension (<i>n</i> , %)	1.027	1.419	0.076	0.723	0.471	−1.793	3.846
T2DM (<i>n</i> , %)	1.182	2.092	0.059	0.565	0.574	−2.974	5.337
VKA (<i>n</i> , %)	−1.972	2.497	−0.08	−0.790	0.432	−6.931	2.987
Amiodarone (<i>n</i> , %)	1.184	1.648	0.075	0.718	0.474	−2.09	4.458
Beta blockers (<i>n</i> , %)	−1.59	2.169	−0.077	−0.733	0.465	−5.898	2.718
ACEI (<i>n</i> , %)	1.825	6.808	0.028	0.268	0.789	−11.698	15.347
sST2 (ng/ml)	0.103	0.072	0.149	1.439	0.153	−0.039	0.246
Cre (μmol/L)	−0.032	0.046	−0.073	−0.696	0.488	−0.123	0.059
UA (μmol/L)	−0.005	0.009	−0.052	−0.501	0.617	−0.023	0.014
FBG (g/L)	−0.198	0.478	−0.043	−0.413	0.681	−1.15	0.754
TC (mmol/L)	0.512	0.86	0.062	0.596	0.553	−1.196	2.221
HDL (mmol/L)	1.846	2.775	0.070	0.665	0.508	−3.666	7.358
HCY (μmol/L)	0.011	0.107	0.011	0.102	0.919	−0.201	0.223
TSH (mU/L)	0.858	0.445	0.199	1.931	0.057	−0.025	1.742
LVEF (%)	−0.249	0.116	−0.222	−2.150	0.034	−0.479	−0.019

T2DM, type 2 diabetes mellitus; VKA, vitamin K antagonist; ACEI, angiotensin-converting enzyme inhibitors; sST2, soluble suppression of tumorigenicity 2; Cre, creatinine; UA, uric acid; FBG, fibrinogen; TC, total cholesterol; HDL, high-density lipoprotein; HCY, homocysteine; TSH, thyroid stimulating hormone; LVEF, left ventricular ejection fraction; LAVI, left atrial volume index.

TABLE 6 | Associations between sST2 and AF recurrence by Cox Regression.

	HR (95% CI)	<i>P</i> -value
Model 1	1.030 (1.013–1.047)	<0.001
Model 2	1.033 (1.016–1.050)	<0.001
Model 3	1.026 (1.007–1.046)	0.008
Model 4	0.978 (0.929–1.031)	0.41
Model 5	1.038 (1.017–1.060)	<0.001

Model 1 adjusted for nothing.

Model 2 adjusted for sex and age.

Model 3 adjusted for sex, age, hypertension, T2DM, UA, TC, beta blockers, and LAVI.

Model 4 adjusted for sex, age, hypertension, T2DM, UA, TC, beta blockers, and LAVI in PaAF.

Model 5 adjusted for sex, age, hypertension, T2DM, UA, TC, beta blockers, and LAVI in PeAF.

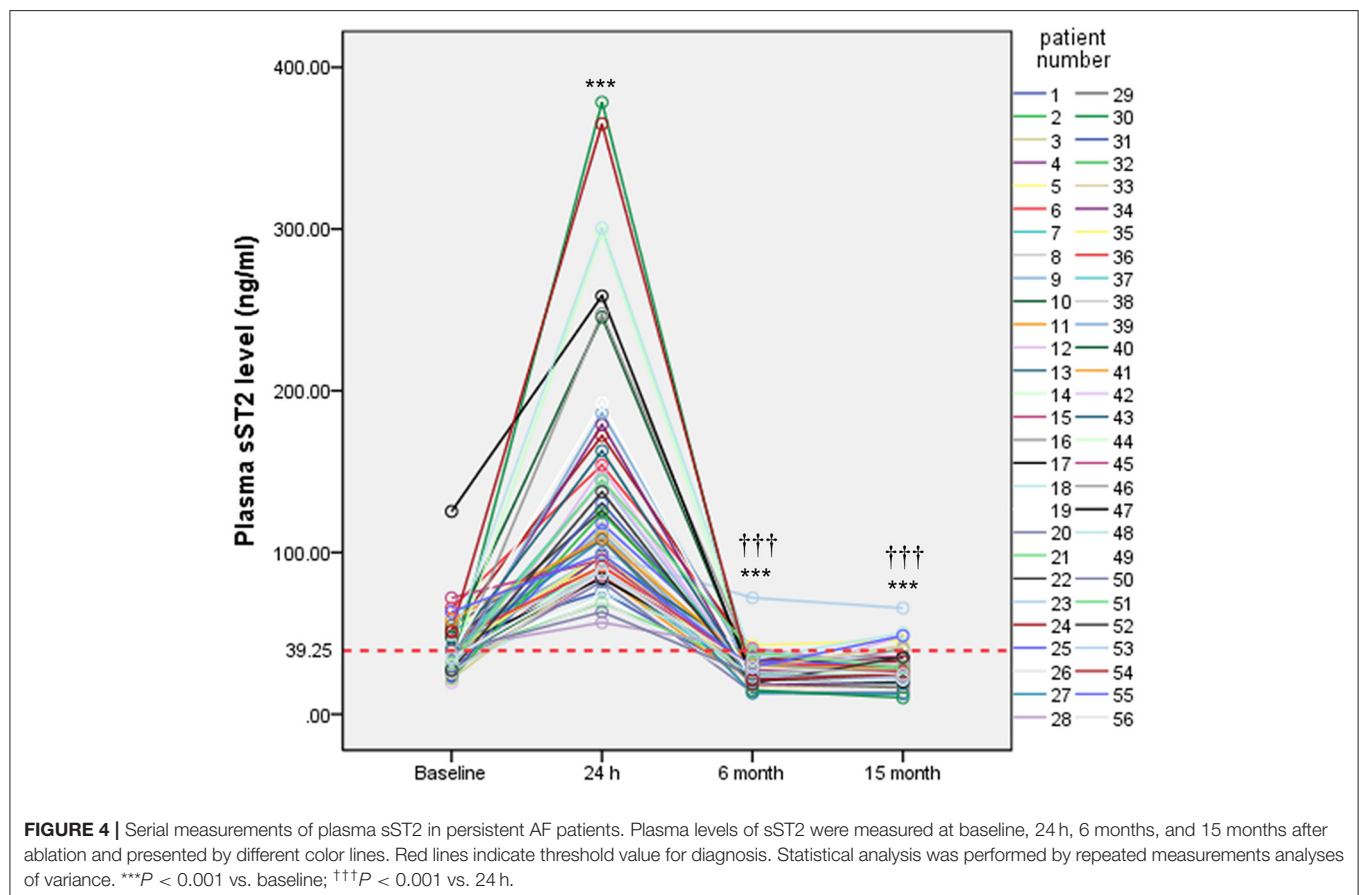
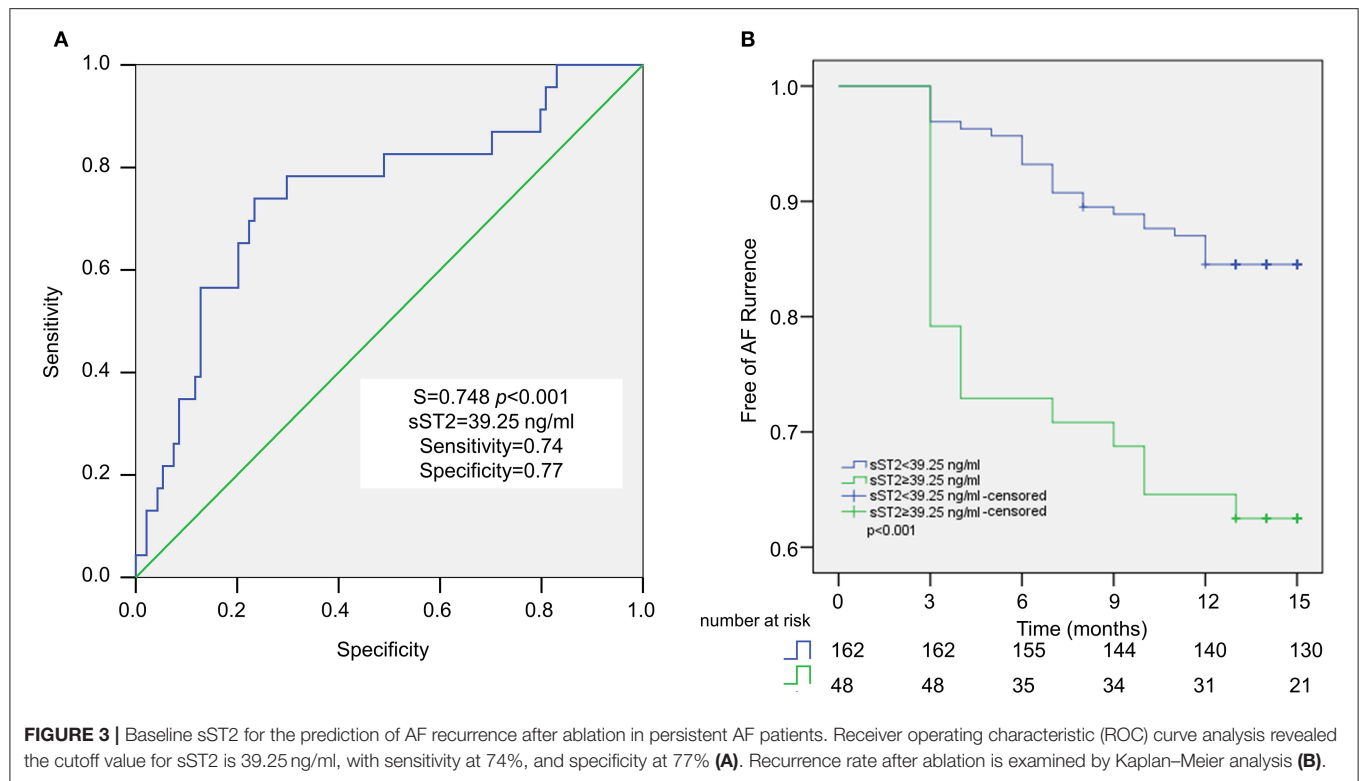
HTN, hypertension; T2DM, Type 2 diabetes mellitus; UA, uric acid; TC, total cholesterol; PaAF, paroxysmal atrial fibrillation; PeAF, persistent atrial fibrillation; LAVI, left atrial volume index; HR, hazard ratios; CI, confidence intervals.

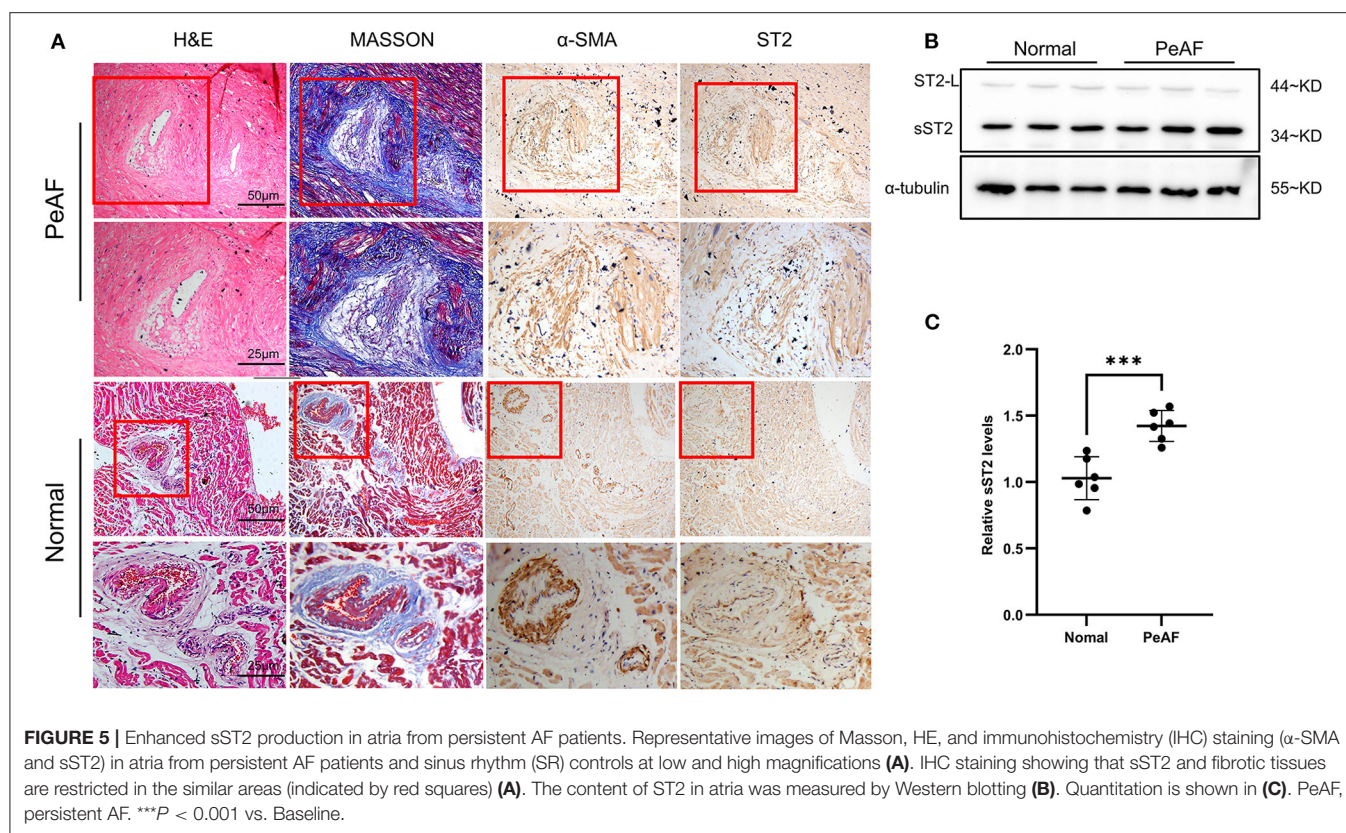
with LAVI and was able to predict recurrence after ablation in persistent AF patients; and (3) atrial fibroblasts were likely a cellular source of circulating sST2. Our results provide a useful circulating marker for assessing response to catheter ablation and advance our understanding of the mechanisms of LA fibrosis.

Mechanistically, clinical and experimental studies have shown that atrial fibrosis is a prominent feature in AF and is considered as the main mechanism of AF occurrence, persistence, and recurrence (7, 8). Therefore, circulating biomarkers, which reflect

the degree of fibrosis could be considered as biomarkers of relapse after ablation. sST2 served as a ventricular fibrotic biomarker for patients with heart failure or myocardial infarction, and the initial sST2 level was independently associated with the incidence of mortality (13, 14). In the present study, we observed that baseline sST2 was elevated in persistent AF patients, and related to atrial structural remodeling, which was assessed by LAVI. Additionally, sST2 served as a strong predictor of recurrence for persistent AF patients, but not in paroxysmal AF. Liu et al. found that sST2 had poor correlation with the preexisted abnormalities during endocardial mapping, but well with the dynamic abnormalities during endocardial mapping (22). Their findings suggest that baseline sST2 level might have a role in distinguishing whether recurrence is originated from pulmonary vein or atrial fibrosis, and help in refining the future approach to AF ablation. In our study, whether the recurrent persistent AF patients who with high level of sST2 have additional abnormalities than recurrent persistent AF patients without, still needs further investigations.

Both ST2 forms are expressed in cardiac tissues (12). It was previously thought that the increase in circulating sST2 in AF patients is caused by higher heart rate and atrial pressure (23). In the present study, we confirmed that sST2 in the atria of persistent AF patients was much higher than the form of ST2L. Furthermore, we found that cardiac sST2 was mainly located in atrial myofibroblasts, suggesting that the increment of baseline sST2 in persistent AF may be a result of myofibroblast activation rather than higher heart rate and atrial pressure. Persistent





AF patients are normally associated with more severe atrial fibrosis than paroxysmal AF patients (24), indicating that the number and activity of myofibroblasts are enhanced in the atria from persistent AF patients. This finding somehow explains why sST2 is preferential in predicting persistent AF rather than paroxysmal AF.

Furthermore, serial measurements of sST2 were conducted at three different time points in persistent AF patients. The value of sST2 at 24 h after ablation was dramatically increased compared with baseline value. Such a sudden increase was largely due to myocardial strain or damage during the ablation procedure. sST2 value dropped below baseline after 6 and 15 months of ablation, indicating that ablation was useful in repressing the release of sST2 from the heart. One study reported that measuring baseline sST2 would be a simple method in identifying which AF patients are at high risk of heart failure (23). Further research is needed to determine sST2 at which time points could be used as a biomarker for predicting heart failure in the future.

ST2L and sST2 exert opposite functions driven by binding with IL-33 (25, 26). Both cardiomyocytes and cardiac fibroblasts expressed IL-33 and sST2, and were increased by biomechanical strain and Ang II stimulation (12). In the mouse study, pressure overload model enhanced IL-33 protein production in the fibroblasts of left ventricle (12). Deletion of ST2 in mouse with pressure overload enhanced hypertrophy and cardiac fibrosis (12). Addition of IL-33 prevented cardiac fibrosis, improved cardiac function and survival after ischemia–reperfusion in rats through induction of anti-apoptotic proteins in cardiomyocytes

(12). These results indicate that IL-33/ST2L system is cardiac protective. As a decoy receptor, sST2 blocks the binding between IL-33 and ST2L, which brings detrimental effects to the heart (26, 27). Treatment of rat cardiac fibroblasts with IL-33 was found to repress the migratory activity of fibroblasts (12, 28). In our present study, we found that atrial myofibroblast-derived sST2 was increased in persistent AF patients, compared with SR controls, which raised the possibility that the ST2/IL-33 system might regulate fibroblast activity directly, although the mechanisms need to be further investigated.

It is well-known that AF is associated with both regional and systemic inflammatory responses (29), which is characterized by upregulation of inflammatory mediators and enhanced leukocyte activity, especially monocytes/macrophages (29–31). Human macrophages constitutively expressed both ST2L and sST2. However, shifting these macrophages toward an M2 phenotype by using IL-4 and IL-13 increased the expression of ST2L but not sST2 (32). Activation of ST2L/IL-33 signaling enhanced activation of mouse peritoneal macrophages (33). Whether elevated circulating sST2 in AF patients could affect monocyte/macrophage phenotype or activity still needs further investigation.

LIMITATIONS

We have several limitations in the present study. *First*, our study was carried out in one center and only included a small number of

patients. *Second*, the IHC and WB experiments were done on left atrial appendages taken from patients presenting with a different pathology (AF combined with valvular heart disease). *Third*, although echocardiography was used to evaluate atrial structural remodeling, there is no direct assessment of atrial fibrosis by using MRI.

CONCLUSION

In persistent AF, circulating sST2 ≥ 39.25 ng/ml was predictive of recurrence after primary ablation. Furthermore, atrial myofibroblasts were likely a cellular source of circulating sST2, which might serve as an important biomarker for the degree atrial fibrosis. Inhibiting circulating sST2 might be useful as an adjuvant treatment to improve outcomes of catheter ablation for persistent AF.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committee of the First Affiliated Hospital of Dalian Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XYi and Y-LX designed the research. RT, HY, XH, XYa, and YL carried out the experiments. RT, XH, XYa, and YL analyzed the results. XYi and Y-LX wrote the paper. RT, YL, and XYi revised the manuscript. All authors contributed to the article and approved the submitted version.

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Clinical and Functional Genetic Characterization of the Role of Cardiac Calcium Channel Variants in the Early Repolarization Syndrome

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Background: Early repolarization syndrome (ERS) is an inherited sudden cardiac death (SCD) syndrome. The present study investigates the role of genetic variants in cardiac calcium-channel genes in the pathogenesis of ERS and probes the underlying mechanisms.

Methods: Polymerase chain reaction-based next-generation sequencing was carried out using a targeted gene approach. Unrelated ERS probands carrying calcium-channel variants were evaluated clinically and compared with matched healthy controls. Wild-type (WT) and mutant *CACNA1C* genes were coexpressed with *CACNB2b* and *CACNA2D1* in HEK293 cells and studied using whole-cell patch-clamp techniques and confocal fluorescence microscope.

Results: Among 104 ERS probands, 16 carried pathogenic variants in calcium-channel genes (32.2 ± 14.6 years old, 87.5% male). The symptoms at diagnosis included syncope (56.3%), ventricular tachycardia/fibrillation (62.5%), and SCD (56.3%). Three cases (18.8%) had a family history of SCD or syncope. Eight patients (50.0%) had a single calcium gene rare variant. The other half carried rare variants in other ERS-susceptible genes. Compared with controls, the heart rate was slower (72.7 ± 8.9 vs. 65.6 ± 16.1 beats/min, $*p < 0.05$), QTc interval was shorter (408.2 ± 21.4 vs. 386.8 ± 16.9 ms, $**p < 0.01$), and Tp-e/QT was longer (0.22 ± 0.05 vs. 0.28 ± 0.04 , $***p < 0.001$) in single calcium mutation carriers. Electrophysiological analysis of one mutation, *CACNA1C*-P817S (c.2449C>T), revealed that the density of whole-cell calcium current (I_{Ca}) was reduced by $\sim 84.61\%$ compared to WT (-3.17 ± 2.53 vs. -20.59 ± 3.60 pA/pF, $n = 11$ and 15 , respectively, $**p < 0.01$). Heterozygous expression of mutant channels was associated with a 51.35% reduction of I_{Ca} . Steady-state inactivation was shifted to more negative potentials and significantly accelerated as well. Confocal microscopy revealed trafficking impairment of *CACNA1C*-P817S (peripheral/central intensity: 0.94 ± 0.10 in WT vs. 0.33 ± 0.12 in P817S, $n = 10$ and 9 , respectively, $**p < 0.01$).

Conclusions: ERS associated with loss-of-function (LOF) genetic defects in genes encoding the cardiac calcium channel represents a unique clinical entity characterized by decreased heart rate and QTc, as well as increased transmural dispersion of repolarization. In the case of *CACNA1C*-P817S, impaired trafficking of the channel to the membrane contributes to the LOF.

Keywords: early repolarization syndrome, calcium channel, gene mutation, trafficking, sudden cardiac death

INTRODUCTION

An early repolarization pattern (ERP), defined as J-point elevation ≥ 1 mm in ≥ 2 contiguous inferior and/or lateral leads of a standard 12-lead electrocardiogram (ECG), was traditionally considered as a normal electrocardiographic variant with a benign outcome for several decades (1–4). However, in 2008, Haïssaguerre et al. demonstrated a definitive relationship between ERP and idiopathic ventricular fibrillation (IVF), supporting the hypothesis of Gussak and Antzelevitch in 2000 that ERP is not always benign and may be malignant in some cases (5, 6). Numerous clinical and experimental observations have since confirmed the association between ERP and fatal arrhythmias (7–9), giving rise to the term *early repolarization syndrome* (ERS). Thus, ERP in a patient resuscitated from otherwise unexplained ventricular fibrillation (VF)/polymorphic ventricular tachycardia (VT) is referred to as ERS (1).

ERS has a propensity for heritability (10, 11). Eight ion-channel genes (*KCNJ8*, *ABCC9*, *SCN5A*, *SCN10A*, *KCND3*, *CACNA1C*, *CACNB2b*, and *CACNA2D1*) have been associated with ERS in recent years (12–17). Brugada syndrome (BrS) patients carrying calcium-channel mutations have been reported to have briefer QTc intervals and greater risk for cardiac events and sudden cardiac death (SCD) (18). Only a small fraction of calcium mutations associated with ERS have been functionally analyzed to ascertain causality and establish a plausible contribution to pathogenesis. Thus, the contribution of cardiac calcium-channel mutations to the etiology of ERS remains unclear.

The L-type calcium channel (LTCC) is a multisubunit protein complex composed of four subunits: the main pore-forming $\alpha 1$ (Cav1.2) subunit encoded by *CACNA1C*, which determines the main biophysical and pharmacologic properties of the channel, and three auxiliary subunits, including a cytoplasmic β subunit encoded by *CACNB*, $\alpha 2\delta$ subunit encoded by *CACNA2D*, and a γ subunit encoded by *CACNG* (19–21).

We sought to identify genetic variations in the $\alpha 1$, $\beta 2$, and $\alpha 2\delta 1$ subunits of LTCC among probands diagnosed with ERS and to investigate the potential underlying mechanism. Clinical characterization and functional genomic identification are investigated in detail.

METHODS

Clinical Analysis

This study was approved by Renmin Hospital of Wuhan University Institutional Review Board and performed in

accordance with the Declaration of Helsinki. The study population consisted of 104 cases diagnosed with ERS and 150 healthy controls with no family history of cardiac arrhythmias. All participants underwent clinical and genetic studies after obtaining informed consent. Patients were diagnosed with ERS based on established criteria (1, 2). Gender, age at diagnosis, clinical presentation, family history of SCD, and results of genetic screening were assessed. ECG parameters, including P wave duration, PR interval, QT interval, rate corrected QT interval, and QRS duration, were measured from lead II of 12-lead ECGs. ERP in the lateral leads is referred to as ERS type 1, in inferolateral leads is type 2, and with a global pattern (inferolateral + anterior or right ventricular leads) is type 3.

Mutation Analysis

Genomic DNA was extracted from peripheral blood leukocytes of patients with a standard protocol and amplified by polymerase chain reaction (PCR) on GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). A panel designed using the tool Array (Agilent Technologies, Inc.) was used to directly sequence targeted genes previously associated with cardiac arrhythmias, including ERS. PCR products were purified with reagent (ExoSAPIT, USB, Cleveland, OH, USA) and directly sequenced from both directions using an ABI PRISM 3730 Automatic DNA Analyzer (Applied Biosystems). The sequencing results were analyzed by Mutation Survey V4.0.8 software (Softgenetics, USA) and reconfirmed by the above procedures. Novel variants considered to be pathogenic were either (1) stop/frameshift variants; (2) missense mutations located in the amino acid conservative region across species; (3) splice-site variations meeting the GT-AT rules; (4) minimum allele frequency (MAF) in the control population ≤ 0.003 ; or (5) predicted to be “possibly damaging” or “disease-causing” by the bioinformatics programs of SHIFT, PolyPhen-2, PROVEAN (Protein Variation Effect Analyzer) and MutationTaster2.

Site-Directed Mutagenesis and Transfection of the HEK293 Cell

For the patch-clamp study, site-directed mutagenesis was performed on full-length human wild-type (WT) and mutant *CACNA1C* cDNA cloned in pCDNA3.1 vector tagged with enhanced yellow fluorescent protein (EYFP). cDNA of WT-*CACNB2b* and WT-*CACNA2D1* genes both cloned in pCDNA3.1 vector. Mutated genes were functionally expressed in human embryonic kidney (HEK293) cells as previously described (17). cDNAs of the three LTCC subunits were cotransfected

with a 1:1:1 molar ratio using Lipofectamine 2000 reagent (Invitrogen™, Carlsbad, CA, USA). Electrophysiological studies were performed after 48 to 72 h of incubation.

Electrophysiology Study

Calcium currents were recorded in HEK293 cells using whole-cell, patch-clamp techniques at room temperature (20–24°C) with Axon-700B patch-clamp amplifiers and pCLAMP10.4 software (Axon Instruments, Sunnyvale, CA, USA). HEK293 cells were placed in the experimental groove over an inverted microscope (IX70; Olympus, Tokyo, Japan) and perfused with a corresponding external solution containing the following (in mmol/L): glucose 10, CaCl₂ 2, MgCl₂ 1, HEPES 10, and TEA 150, pH 7.35 with CsOH). Patch pipettes, made from 1.5-mm OD borosilicate glass capillaries, were filled in a solution containing the following (in mmol/L): CsCl 110, CaCl₂ 0.1, HEPES 10, EGTA 10, MgATP 2, and TEA 10 (pH 7.3 with CsOH), with uncompensated access resistances of $1.5 \pm 0.7 \text{ M}\Omega$. All currents were filtered at 1 kHz and digitized at 5 kHz with an eight-pole Bessel filter. Series resistance was electronically compensated at 70–80%.

Whole-cell calcium current (I_{Ca}) was constructed with voltage steps by applying 400-ms pulses from a holding potential of -90 mV , to potentials ranging between -60 and $+60 \text{ mV}$, in 10-mV steps. Voltage dependence of the steady-state voltage-dependent inactivation of I_{Ca} was evoked by a dual-pulse protocol with a 400-ms conditioning pulse from -90 mV to potentials between -100 and $+20 \text{ mV}$. A Boltzmann function was fitted to the conductance-voltage and inactivation or activation curves, yielding the midpoint ($V_{1/2}$) and slope (k) value of the curves.

Localization of Ca²⁺ Channels

Channel trafficking was assessed using Ca²⁺ channels tagged with EYFP by confocal microscopy, as previously described (18). Cells were experimented 48 h after transfection by a Leica confocal laser scanning microscopy (Leica Microsystems, Heidelberg, Germany). EYFP-labeled cells were analyzed in the XYZ configuration. A region of interest was restricted within 2 μm of the plasma membrane, and the average pixel intensity within this region was referred to as peripheral staining, with average pixel intensity for the remaining part of the cell defined as central staining. The ratio of peripheral to central intensity was calculated.

Statistical Analysis

Statistical analysis was carried out using GraphPad software version 8.0. A normality test was performed to assess the distribution of the data before applying a parametric test. Continuous variables were expressed as the mean \pm standard error and evaluated using the Student *t*-test between two groups and one-way analysis of variance between multiple groups. Descriptive statistics for categorical variables were presented as count and percentages. Statistical significance was defined as $p < 0.05$.

TABLE 1 | Clinical characteristics of ERS probands carried calcium mutation.

Index	ERS cases
Age (years)	32.2 \pm 14.6
Gender	
Male, <i>n</i> (%)	14 (87.5)
Female, <i>n</i> (%)	2 (12.5)
Symptom, <i>n</i> (%)	
Syncope	9 (56.3)
SCD/ASCD	9 (56.3)
VT/VF	10 (62.5)
Atypical symptom	6 (37.5)
Asymptomatic	0
AF, <i>n</i> (%)	3 (18.8)
Bradycardia, <i>n</i> (%)	8 (50.0)
CCD, <i>n</i> (%)	5 (31.3)
Family history of SCD/syncope, <i>n</i> (%)	3 (18.8)
Type, <i>n</i> (%)	
ERS1	1 (6.3)
ERS2	5 (31.3)
ERS3	10 (62.5)
Probands with calcium mutations	
Single calcium mutation*	8 (50.0)
With additional mutation(s) [#]	8 (50.0)

*Variants from CACNA1C, CACNB2b, CACNA2D1.

[#]Combined with extra mutations in SCN5A, SCN10A, ABCC9, KCNJ11, etc.

RESULTS

Clinical Characterization

Among the 104 unrelated patients diagnosed with ERS who underwent genetic screening for ion-channel gene mutations, 16 (15.4%) were found to carry a variant in one of the calcium-channel genes. The clinical characteristics of patients displaying a calcium-channel gene variant were summarized in **Table 1**. The average age at diagnosis of ERS was 32.2 ± 14.6 years; 87.5% were males. The symptoms at diagnosis included syncope (56.3%), VT/VF (62.5%), SCD (56.3%), and other atypical symptoms (37.5%); none were asymptomatic. Three cases (18.8%) had a family history of SCD or syncope. Three presented with atrial fibrillation (AF), five (31.3%) with cardiac conduction disease (CCD), and four (25.0%) with bradycardia. Of the 16 ERS patients carrying calcium-channel variant(s), 1 displayed J-point elevation localized to the lateral leads (ERS1), 5 localized to inferior leads (ERS2), and 10 presented with a global pattern (ERS3).

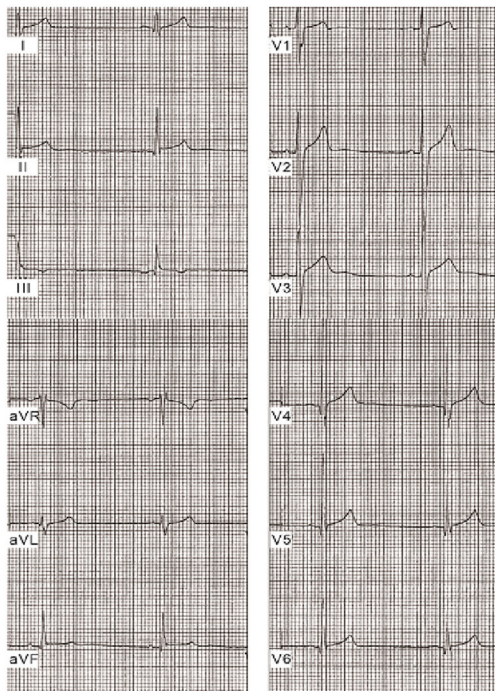
Compared with healthy controls, heart rate was significantly slower [72.7 ± 8.9 beats/min (bpm), 65.6 ± 16.1 bpm, 60.9 ± 9.6 bpm; $p < 0.05$, $p < 0.001$ vs. controls, respectively]; QTc interval was significantly shorter ($408.2 \pm 21.4 \text{ ms}$, $386.8 \pm 16.9 \text{ ms}$, $389.5 \pm 23.6 \text{ ms}$; $p < 0.01$, $p < 0.05$ vs. controls, respectively), and Tp-e/QT was significantly longer (0.22 ± 0.05 vs. 0.28 ± 0.04 vs. 0.26 ± 0.07 ; $p < 0.01$, $p < 0.05$ vs. controls, respectively) in both single calcium mutation carriers and cases with additional mutation(s) (**Table 2**). **Figure 1** shows

TABLE 2 | ECG Parameters of ERS probands with calcium mutations and healthy control.

Index	Healthy control (n = 150)	ERS probands with calcium mutation (n = 16)			
		Single calcium mutation (n = 8)	P-value	With additional mutation(s) (n = 8)	P-value
HR (bpm)	72.7 ± 8.9	65.6 ± 16.1	0.0378	60.9 ± 9.6	<0.001
P wave (ms)	87.6 ± 9.1	90.9 ± 13.5	0.3318	89.4 ± 19.4	0.6133
PR interval (ms)	170.7 ± 18.7	178.0 ± 51.0	0.3448	184.3 ± 32.5	0.0567
QRS duration (ms)	89.4 ± 14.6	94.6 ± 16.1	0.3302	96.3 ± 25.9	0.2154
QTc interval (ms)	408.2 ± 21.4	386.8 ± 16.9	0.0061	389.5 ± 23.6	0.0177
Tp-e	82.3 ± 9.9	104.7 ± 18.5	<0.001	102.0 ± 27.6	<0.001
Tp-e/QT	0.22 ± 0.05	0.28 ± 0.04	0.0011	0.26 ± 0.07	0.0324

P-value is presented in bold if $P < 0.05$.

A Patient 1



B Patient 2

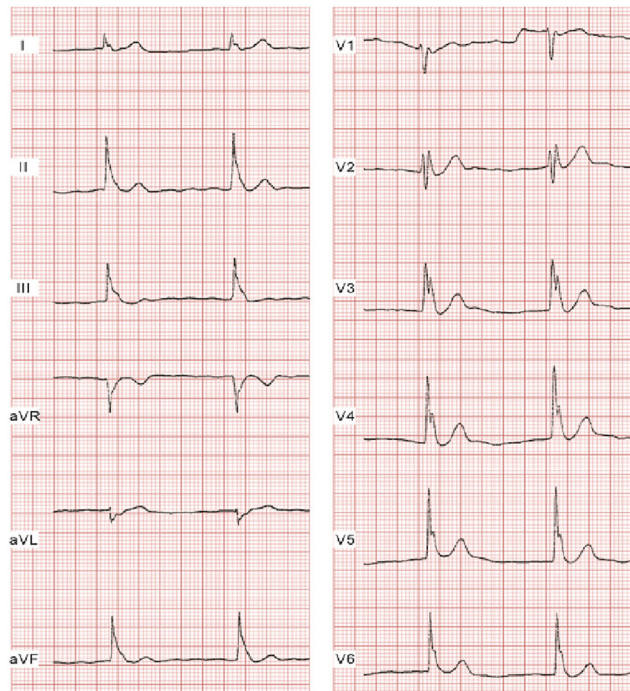


FIGURE 1 | Representative electrocardiograms of two ERS probands with *CACNA1C*. **(A)** Patient 1 was a 20-year-old male patient, identified with a mutation, P817S, in *CACNA1C*. He suffered syncopal episodes twice while exercising. ECGs showed bradycardia (HR = 46 bpm) and an ERP with notching in the inferior leads, leading to a diagnosis of ERS type 2. **(B)** Patient 2, a 17-year-old young male patient, presented with spontaneous VF on the background of syncope. ECGs exhibited sinus bradycardia (HR = 50 bpm) and an ERP with notching/slur in a global pattern, thus diagnosed with ERS type 3.

a representative 12-lead ECG from an ERS case. Patient 1, whose mutation we studied in greater detail, was a 20-year-old male patient, who had experienced syncopal episodes twice while exercising. Physical examination was normal, 2D echo showed mild right atrial and right ventricular (RV) dilation with normal left ventricular and RV ejection fraction. ECG (**Figure 1A**) showed bradycardia [heart rate (HR) = 46 bpm] and an ERP with notching in the inferior leads, leading to a diagnosis of ERS type 2. J-point elevation ≥ 2 mm only can be seen in III and aVF, and an upsloping ST-segment elevation ≥ 1 mm was detected in V₃-V₅ (QTc, 367 ms; Tp-e/QT, 0.3). Patient 2 was a 17-year-old male patient presenting with spontaneous VF

and episodes of syncope. His ECG (**Figure 1B**) exhibited sinus bradycardia (HR = 50 bpm) and an ERP with notching/slur in global leads, J point elevating prominently (≥ 2 mm) in leads II, III, aVF, and V₁-V₆, leading to a diagnosis of ERS type 3 (QTc, 363 ms; Tp-e/QT, 0.3).

Genetic Screening

Genetic analysis revealed 16 probands (15.4%) with variants in *CACNA1C*, *CACNB2*, or *CACNA2D1* genes. Eight of these (50.0%) carried additional variants in other genes, including *KCNJ8*, *SCN5A*, *SCN10A*, *ABCC9*, and other genes related to inherited arrhythmias. The remaining eight cases

TABLE 3 | Summary of single calcium mutation in ERS probands.

Variant	CACNA1C				CACNB2b			
	P817S	G37R	G490R	E850del	S160T	A170V	S503L	R571C
Reported ID	rs112532048	rs34534613	rs121912775	rs575583988	rs149253719	NA	rs137886839	rs1060499847
Type	Missense	Missense	Missense	Frameshift	Missense	Missense	Missense	Missense
Change in nucleotide	2449C>T	109G>A	1468 G>A	2548-2550del	479G>C	509C>T	1508C>T	1711C>T
Exon location	17	2	10	19	5	6	13	13
MAF								
GnomAD	0.003488	0.004555	0.000162	0.00042	0.000581	NA	0.001099	NA
ExAC	0.003534	0.007385	0.000809	0.000526	0.001007	NA	0.001162	NA
1000 Genomes	0.001597	0.000399	0.000399	NA	0.001797	NA	0.001398	1.62686e-05
SIFT								
Score	0.212	0	0.133	NA	0.01	0.062	0.001	0
Prediction	Tolerated	Damaging	Tolerated	NA	Damaging	Tolerated	Damaging	Damaging
MetaLR								
Score	0.839	0.9028	0.83	NA	0.3893	0.5276	0.5305	0.6806
Prediction	Damaging	Damaging	Damaging	NA	Tolerated	Damaging	Damaging	Damaging
MutationTaster								
Score	0.99975	1	0.999727	NA	1	1	0.999999	1
Prediction	Disease causing	Disease causing	Disease causing	NA	Disease causing	Disease causing	Disease causing	Disease causing
PolyPhen2								
Score	0.999	1	1	NA	0.787	0.007	0.996	1
Prediction	Probably damaging	Probably damaging	Probably damaging	NA	Probably damaging	Benign	probably damaging	Probably damaging
FATHMMM								
Score	−3.83	−3.88	−3.33		−1.67	−1.81	−1.85	−2.17
Prediction	Damaging	Damaging	Damaging		Damaging	Damaging	Damaging	Damaging

carried only one calcium-channel variant *CACNA1C*(4) and *CACNB2*(4), and none in *CACNA2D1* (Table 3). Patient 1 was identified carrying a missense mutation in *CACNA1C*. PCR-based sequencing analysis uncovered a double peak in the sequence of exon 17 of *CACNA1C* (C to T transition at nucleotide 2449), predicting substitution of proline by serine at codon 817 (P817S). In the proposed topology of the Cav1.2 channel, the *CACNA1C*-P817S variant was located in a conserved site among different species in the cytoplasmic linker between domains II and III. And the mutation was indicated as “disease causing” or “damaging” by MutationTaster (0.99975), MetaLR (0.839), and FATHMMM (−3.83) with GlobalMAF 0.002.

Functional Expression

To explore the molecular consequences of the mutation, we cotransfected *CACNA1C*-P817S and WT with the other two subunits (*CACNB2b* and *CACNA2D1*) forming the LTCC into HEK293 cells and performed whole-cell patch-clamp experiments. Typical I_{Ca} tracings of voltage-dependent activation from WT, P817S+WT, and P817S mutation are shown in Figure 2A. Analysis of the current–voltage relationship (I–V curves) reveals that P817S significantly reduced the peak current density of I_{Ca} with 84.61% reduction at +10 mV, compared to WT (-3.17 ± 2.53 vs. -20.59 ± 3.60 pA/pF, $p < 0.01$;

Figures 2B,C). Heterozygous expression of mutant also led to a 51.35% reduction.

The activation conductance variables (I/I_{max}), obtained from the I–V curves, were further fitted with a Boltzmann function to obtain the half activation voltage $V_{1/2}$, which displayed no significant difference among the three groups (WT vs. P817S+WT vs. P817S: -11.66 ± 0.41 vs. -13.05 ± 0.60 vs. -14.32 ± 1.24 , $p > 0.05$; Figures 3A,B). Steady-state inactivation curve was also fitted by Boltzmann function, showing a significant acceleration in both P817S and P817S+WT groups (P817S vs. P817S+WT vs. WT: -40.76 ± 2.05 vs. -36.63 ± 1.98 vs. -30.53 ± 1.40 , $p < 0.01$, $p < 0.05$; Figures 3A,C). The results above indicated that the *CACNA1C*-P817S mutation causes a “loss of function” (LOF) in cardiac calcium-channel activity.

To evaluate whether the mutation-caused LOF was due in part to a trafficking defect, we assessed the intracellular expression pattern of WT or P817S channels tagged with EYFP by using the confocal microscopic technique. XYZ scans of WT channels on the confocal microscope showed both a central and peripheral pattern of fluorescence (Figures 4A,C), whereas the staining of P817S channels was restricted in intracellular organelles (Figures 4D,F). In the total overlapping, the ratio of peripheral/central intensity of P817S diminished (WT vs. P817S: 1.10 ± 0.16 vs. 0.58 ± 0.15 , $p < 0.05$), with the decrease in the middle section more dramatic (WT vs. P817S: 0.94 ± 0.10 vs. 0.33

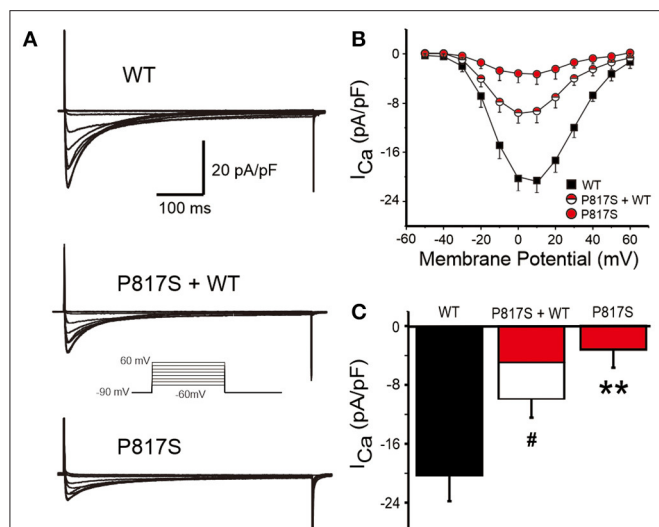


FIGURE 2 | Analysis of whole-cell calcium current (I_{Ca}) recorded from HEK293 cells expressing WT, P817S+WT, and P817S ($n = 15, 10, 11$, respectively). **(A)** Representative whole-cell I_{Ca} traces from WT, P817S+WT, and P817S. **(B)** Current density-voltage relationship for WT, P817S+WT, and P817S. **(C)** Bar graph showing peak I_{Ca} density at 0 mV from WT, P817S+WT, and P817S. # $p < 0.05$, compared with P817S+WT. ** $p < 0.01$, compared with P817S.

± 0.12 , $p < 0.01$), suggesting that P817S channels were trapped in the endoplasmic reticulum and/or Golgi complex, remaining very few localizing at the sarcolemma. These findings suggest that the loss of current observed with P817S is partially due to an impairment in trafficking of mature $Ca_v1.2$ channels from the endoplasmic reticulum/Golgi complex to the cell membrane.

DISCUSSION

Our data show the clinical characterization and functional genetic association of ERS with cardiac calcium-channel variant, providing data in support of mutations in calcium-channel being pathogenic in ERS. In this study, a unique clinical entity in 16 unrelated ERS probands associated with genetic defects in cardiac calcium-channel is discovered. It is characterized by decreased HR and QTc, as well as increased transmural dispersion of repolarization (TDR). While an LOF in I_{Ca} has previously been attributed to ERS using whole-cell patch-clamp techniques, none of them further demonstrated that the impairment of membrane trafficking was the underlying mechanism of the LOF. The present study uses a confocal fluorescence microscope to verify that impaired membrane trafficking of calcium-channel contributes to the LOF in I_{Ca} caused by *CACNA1C*-P817S (Figure 4). This is the largest one for studying calcium-related ERS by far.

ERS shares many similarities in terms of clinical perspective with BrS, such as male predominance, the average age of first arrhythmic events, and condition of arrhythmic episodes, suggesting similar pathophysiology; thus, these two entities are referred to as J-wave syndromes (JWS) (2). In genetics, multiple ion-channel mutations have been linked with ERS. The first

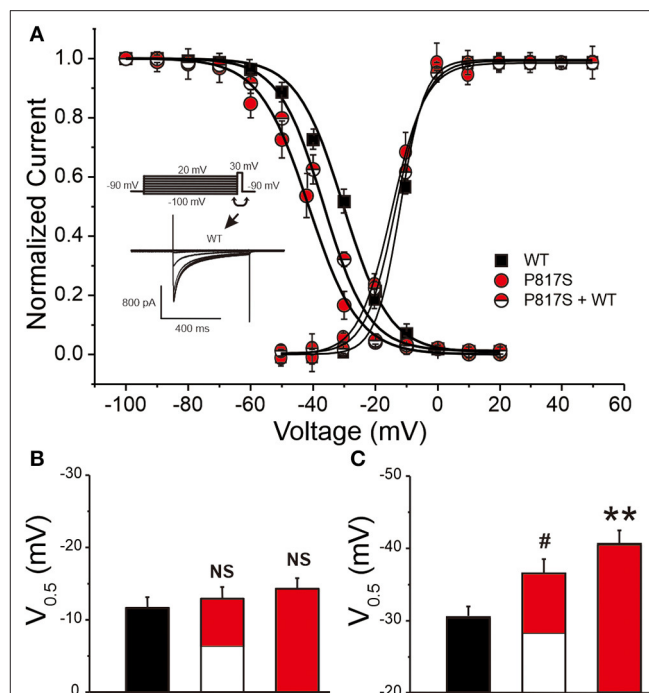
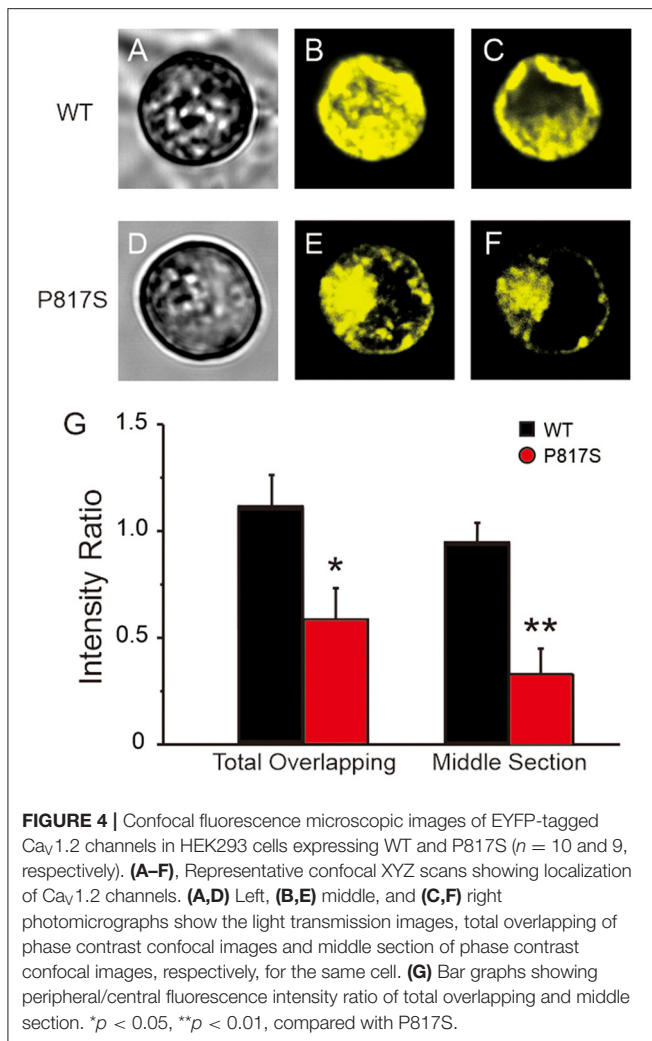


FIGURE 3 | The steady-state activation and inactivation of HEK293 cells expressing WT, P817S+WT, and P817S ($n = 15, 10$, and 11 for activation; $n = 8, 10$, and 7 for inactivation, respectively). **(A)** Normalized activation conductance and inactivation current, fitted to the Boltzmann equation. **(B,C)** Bar graph showing potential for half-maximal activation and inactivation of each group. NS (no significance), # $p < 0.05$, compared with P817S+WT. ** $p < 0.01$, compared with P817S.

gene mutation locates in *KCNJ8* (12, 13), causing a gain-of-function of I_{K-ATP} ; others include LOF mutations in *CACNA1C*, *CACNB2*, *CACNA2D* (17), *SCN5A* (15), and *SCN10A* (14) and gain-of-function mutations in *ABCC9* (14) and *KCNQ3* (16).

CACNA1C lies in chromosome 12, coding for the main pore-forming $\alpha 1$ ($Ca_v1.2$) subunit of the cardiac LTCC. The genetic defects of *CACNA1C* can precipitate many cardiac syndromes, such as Timothy syndrome (TS) (22), long QT syndrome (LQTS) (23), BrS, and ERS (18). The discovery of *CACNA1C*-encoded LTCC mutation in JWSs manifested that LOF perturbations in cardiac LTCC could have drastic phenotypic implications, such as male predominance, accentuated J waves, and ST-segment elevation when bradycardia or pauses happen, relative shortened QTc interval, unexplained syncope, and SCD (18). Since then, studies identify a *CACNA1C* mutation (E850del) that cosegregates with ERS phenotype in a pedigree with ERS-associated SCD and confers a markedly decrease in peak I_{Ca} density (17, 24). Since then, Chen et al. and Liu et al. confirm that the LOF of I_{Ca} caused by *CACNA1C*-R1973P or *CACNA1C*-Q1916R can induce ERS (25, 26). Understanding the role of cardiac calcium-channel mutation in the etiology of ERS is limited.

The initial event of ERP may be cardiac arrest, which is often the presenting episodes of VF, and male predominates among those patients with cardiac arrest related to ERP (>70%) (5).



Nevertheless, VF is rare, whereas ERP is a relatively common electrocardiographic phenomenon; it might not happen until the third decade of life, perhaps due to relatively higher testosterone levels in this stage (5, 27, 28). A history of syncope at rest has a strong association with ERS, which is due to pause-dependent augmentation of J waves and ST-segment elevation ahead of VF, so does bradycardia (27). Report discovers ERP has evidence for a heritable basis in the general population, or ERP owns a heritability (10). Family history of SCD is important information we should pay attention to in clinical practice. However, only 12.5% of ERS probands are found to have a familial history of SCD in our study, not far from what Haïssaguerre et al. found (16%) (5). Nevertheless, if a positive family history of unexplained SCD at a young age is discovered, systematic evaluations of all surviving family members are necessary regardless of the presence or absence of ERP. Besides, we found three patients (18.8%) present with AF, a higher prevalence than in the general population. Watanabe et al. reported that AF is found in 23% of their cases with ERS, and AF has been reported in 15% of ERS patients in the investigation of Hwang et al. and 22% in the research of Kamakura et al., implying

high overlapping between AF and ERS (29–31). A report also demonstrates that ERP is more common in patients younger than 60 years with lone AF than healthy controls and declares that ERP may indicate susceptibility to AF (32). Causative genes of ERS encode ion channels including sodium, calcium, and potassium channels. Given the affected protein is present in ventricular and atrial myocardium, the repolarization abnormalities of the atrial and ventricular may be potentially related. Interestingly, all of the causative genes of ERS have also been associated with AF. There may be common genetic background for AF and VF related to ERP (32).

TDR has been proposed to underlie arrhythmogenesis in JWS, including ERS and BrS (33). Electrocardiographic markers reflecting TDR include Tp-e interval and Tp-e/QT ratio and increased Tp-e interval and Tp-e/QT ratio have been associated with IVF development in ERS and therefore may be considered as non-invasive markers of arrhythmogenesis (34–36). In the process of looking for JWS candidate genes, Antzelevitch et al. found three (60%) of five BrS probands who present with a short QT interval carried a calcium-channel mutation, which points to genetic heterogeneity for this phenotype, being the first to propose LTCC genes as cause for BrS associated with short QT. A decrease in I_{Na} or I_{Ca} or augmentation of any one of outward currents, including I_{Kr} , I_{Ks} , and I_{to} , can cause a net repolarizing current, resulting in an accentuated action potential dome, manifesting as the augmentation of the J wave or appearance of ST-segment elevation in ECG. A further increase in net repolarizing current can result in partial or complete loss of the action potential dome, leading to a transmural voltage gradient that manifests as greater ST-segment elevation. $I_{Ca,L}$ is the main current of phase 1 of the action potential; a decrease of $I_{Ca,L}$ may shorten the action potential duration (37, 38). In our data, the average QTc is 386.8 ± 16.9 ms among those who carry calcium mutation alone, which falls into the shorter side of the range of QTc interval. As to the half with additional variation(s), 62.5% carry variations in previously reported QT-prolonging genes, including *SCN5A* (39) and *SCN10A* (40). Slower HR, shorter QTc, and longer Tp-e/QT are also found in those compound mutations carriers. That implies the unique clinical phenotype or entity aroused by cardiac calcium mutation is dominant in ERS cases.

The majority of ERP subjects will remain asymptomatic in practice with a relatively low prevalence of arrhythmic events or SCD. Rosso et al. indicate that the presence of J wave on the ECG increases the probability of VF from 3.4:100,000 to 11:100,000 (27). Thus, careful attention should be devoted to risk assessment for the development of life-threatening arrhythmias in potential ERS cases. Risk stratifications may encompass but are not limited to the following: high-amplitude J waves (≥ 0.2 mV), J waves with horizontal or downsloping ST segment, documented VF or documented polymorphic VT, dynamic changes in J-wave amplitude, positive family history of SCD or arrhythmic syncope, prolonged Tp-e interval, identified gene mutations, and so on. First, either slurred or notched J-point elevation ≥ 0.2 mV appears to be associated with an increased risk, although relatively rare in the general population (9). And horizontal or descending ST segment following J-point elevation is associated

with a worse prognosis than an ascending ST segment (41). Furthermore, J-point elevation in IVF is of greater amplitude and wider ECG lead distribution than those with an established cause of cardiac arrest (8). The family history of SCD in subjects with ERP has been proven to be another risk factor (42). The study also suggests that an increased Tp-e/QT ratio, which reflects TDR in subjects with ERP, may be a specific ECG marker for increased arrhythmic risk (43). In the present study, we found 10 (62.5%) suffered IVF, 4 of them (40.0%) presented with a marked J-point elevation (≥ 2 mm), and 3 (30.0%) showed a global pattern (ERS3). Our patient 2 displays all these traits and presents with severe clinical manifestation, which calls for a close follow-up. These patients are at higher risk of life-threatening arrhythmias, and more active therapies should be administered.

Further genetic analysis detects a missense mutation, P817S-*CACNA1C*, in patient 1, pointing to genetic heterogeneity for this phenotype. Heterologous expressions and patch clamping techniques are conducted to illustrate the disease-causing ability of the mutation; we found out that the P817S mutant dramatically decreases peak current density by 84.61%, and the steady-state inactivation is also significantly accelerated, displaying an LOF of I_{Ca} . Further confocal microscopy study reveals a decreased trafficking of $Ca_v1.2$ protein caused by P817S mutation. Decreased $I_{Ca,L}$ leads to augmented net outward currents and shortens the cardiomyocyte repolarization period. Because of the transmural discrepancies of outward potassium currents (I_{to} , I_K , etc.), the enhancement in net outward current results in partial or complete loss of the action potential dome, leading to a transmural voltage gradient that manifests as J waves. Otherwise, accelerated repolarization caused by the mutation could lead to a pursuant short QT interval in the surface ECG. Boczek et al. revealed that *CACNA1C*-P857A induces LQTS by causing a gain-of-function of $I_{Ca,L}$ and an increase of $Ca_v1.2$ membrane trafficking and speculates the modulation might be because the mutation locates at the conserved proline, glutamic acid, serine, and threonine rich (PEST) domain of $Ca_v1.2$ II-III loop, which acts to proteolytic signaling through the cellular “quality control” system (PEST1 is S446/459, and PEST3 is S840/861) (23). However, as early as 2007, *CACNA1C*-A39V inducing LOF due to a trafficking defect in BrS is found by Antzelevitch et al. (18). Yet, it still remains unclear why *CACNA1C* mutations can cause the trafficking decrease/defect of the channels to the plasma membrane. Trafficking defects are considered to involve misfolding or improper assembly of the protein structure, leading to its retention in the endoplasmic reticulum and degeneration without transport to the Golgi complex by the “quality control” system, in which lectin-like endoplasmic reticulum chaperones play a key role. Substitution of proline with serine has significant meanings for protein folding and thereby causes trafficking abnormalities and degradation. Tertiary post-translational processing may be affected and therefore influences trafficking to the cell membrane.

Study Limitations

One limitation of our study may be the small number of affected individuals, which precludes us from reaching a more definitive conclusion, although this cohort has already been the largest one for studying calcium-related ERS by far. Another one is the

mechanism of trafficking decrease caused by *CACNA1C*-P817S. To observe the progressive nature of the disease, we plan to further follow up with these patients, especially those with a high risk of life-threatening arrhythmias. The work led by our coauthors has demonstrated the first human induced pluripotent stem cell (hiPSC) model of ERS recently (44). hiPSCs from the ERS probands with calcium-related mutation are also projected in our schedule, to replicate the phenotype and perform further research on stem cell level.

CONCLUSIONS

The present study demonstrates that ERS caused by cardiac calcium-channel variant presents unique clinical features, including decreased HR and QTc, as well as increased transmural heterogeneity. Further functional investigations provide solid evidence that *CACNA1C*-P817S is a mutation causing impaired membrane trafficking of $Ca_v1.2$ protein, inducing an LOF of I_{Ca} , thus leading to the manifestation of ERS phenotype.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Renmin Hospital of Wuhan University Institutional Review Board and performed in accordance with the declaration of Helsinki. Written informed consent to participate in this study was provided by the participants or their legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

HB-M and DH designed the study performed clinical phenotyping of study subjects, supervised, and coordinated the genetic laboratory work. XC, HB-M, HX, BY, HJ, CA, and DH coordinated the clinical evaluations. XC, HB-M, ZZ, and DH performed electrophysiology and confocal study. XC, HB-M, ZZ, GC, and DH organized and summarized the database. XC, HB-M, and DH analyzed the data. GC, BY, HJ, and DH developed the conceptual approaches to data analysis. XC, HB-M, CA, and DH wrote the manuscript. All authors contributed to editing the manuscript.

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SUPPLEMENTARY MATERIAL

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Age Is a Predictor for the Syncope Recurrence in Elderly Vasovagal Syncope Patients With a Positive Head-Up Tilt Test

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Background: Valid predictors of the syncope recurrence in vasovagal syncope (VVS) patients with a positive head-up tilt test (HUTT) are currently lacking. The goal of this study was to identify the predictive performance of age for the recurrence of syncope in VVS patients with a positive HUTT.

Methods: In total, 175 VVS patients with a positive HUTT were observed for 6–32 months, and the recurrence of ≥ 1 syncope or typical pre-syncope prodromal episodes during follow-up was considered syncope recurrence. The population was divided into 2 groups, namely, a syncope recurrence group (44 patients) and a no syncope recurrence group (131 patients). The baseline clinical data, haemodynamic parameters, and classification of VVS on the HUTT were analyzed. Logistic regression was used to analyse the effect size and confidence interval for age. A receiver operating characteristic (ROC) curve analysis was used to assess the predictive performance and investigate the predictive value of age by the area under the curve (AUC).

Results: The median age of the syncope recurrence group was older than that of the no syncope recurrence group [60.0 (47.8, 66.0) years > 53.0 (43.0, 62.0) years], and there was a significant difference between the two groups ($P < 0.05$). The trend for syncope recurrence changed with advancing age, and the logistic regression model adjusted by sex showed that older patients had an increased risk of syncope recurrence in VVS with a positive HUTT [OR value: 1.03, 95% confidence interval (CI): 1.008–1.061, $p < 0.05$]. Age was a valid predictor for the recurrence of syncope in elderly VVS patients with a positive HUTT (AUC: 0.688; 95% CI: 0.598–0.777, $p < 0.05$). The cut-off value was 53.5 years, and the sensitivity and specificity were 72.7 and 52.7%, respectively.

Conclusions: Age may be a valid predictor for syncope recurrence in elderly VVS patients with a positive HUTT. The rate of syncope recurrence increased with advancing age, especially in females.

Keywords: age, predictor, syncope recurrence, vasovagal syncope, head up tilt test

BACKGROUND

Syncope is a transient loss of consciousness due to transient global hypoperfusion characterized by rapid onset, short duration, and spontaneous complete recovery. The pre-syncope prodrome includes dizziness, headache, sweating and amaurosis (1). Vasovagal syncope (VVS) is challenging to treat as a heterogeneous disorder. The quality of life and mental health of patients could be seriously affected by recurrent VVS (2, 3). Most clinical studies on the treatment of syncope recurrence in VVS patients were mainly based on head-up tilt test (HUTT) results and the VVS classification. However, results of these studies have been inconsistent (4–8). Severe and refractory syncope is usually treated with medical or device therapy. Thus, valid prediction of recurrent syncope is urgently needed to guide the choice of treatment options. Factors that can best predict syncope recurrence after a positive HUTT are few despite numerous randomized trials. Sheldon et al. found that the most powerful predictor of syncope recurrence was the logarithm of the number of preceding syncopal spells (9). We attempted to identify whether age is an important factor affecting syncope recurrence in VVS patients.

METHODS

Subjects

In total, 479 inpatient and outpatients who were clinically suspected neuro-mediated syncope (NMS) underwent a HUTT in Ningbo Hospital, Zhejiang University from April 2016 to June 2018, which was performed in accordance with the 2018 ESC Guidelines for the diagnosis and management of syncope (1); All patients underwent a complete physical examination, chest X-ray, biochemical serum tests, echocardiography, electrocardiogram (ECG) or Holter (if necessary), etc. to exclude severe diseases related to the central nervous, cardiovascular, and metabolic systems, such as sinus node or conduction system disease etc. Two hundred sixty-five patients were excluded due to not meet inclusion criteria (5 cases), incomplete data (7 cases), not signing the informed consent (3 cases), with a negative HUTT (243 cases), orthostatic hypotension (4 cases), or psychogenic syncope (3 cases) who were not tilted for the full 45 min period (**Figure 1**). A total of 214 inpatients and outpatients with a positive HUTT were diagnosed with VVS. Then, they were followed up for between 6 and 32 months. Three patients were treated pharmacologically (small doses of metoprolol or theophylline drugs, per os), 2 patients received pacemaker treatment (just DDD pacemaker), and 34 patients were lost to follow-up (**Figure 1**). The data for 175 patients with a median age of 55.0 years [interquartile range: 44.0, 63.0 years, range from 15 to 82 years] were finally enrolled in the analysis; data from patients who were lost to follow-up or received medication or pacing were excluded from the analysis. The study

Abbreviations: VVS, vasovagal syncope; HUTT, head-up tilt test; BHUTT, baseline HUTT; SNHUTT, sublingual nitroglycerin HUTT; ROC, receiver operating characteristic; AUC, area under the curve; ANS, autonomic nervous system; Epi, epinephrine; NE, norepinephrine; CO, cardiac output; SVR, systemic vascular resistance.

was approved by the ethics committee of Ningbo First Hospital and carried out in accordance with the Declaration of Helsinki.

HUTT (1) Preparation

The HUTT was performed strictly in accordance with the protocol of the European Society of Cardiology. Cardiovascular active drug treatments were stopped for at least a 5 half-life period. Drugs and foods that could have affected normal autonomic nervous system function were avoided before the test. The HUTT was performed in a quiet, softly lit, temperature-controlled (20~25°C) room equipped with medical resuscitation facilities, such as a defibrillation apparatus and atropine and other resuscitative drugs. The patients were fasted for at least 4 h before the HUTT. Basic clinical data related to cerebrovascular diseases (including cerebral ischemia, cerebral infarction, epilepsy, etc.), hypertension, cardiovascular disease, diabetes (including type 1 or type 2), natural course of disease and number of spells in lifetime was needed to be available for inclusion in the study.

Baseline HUTT

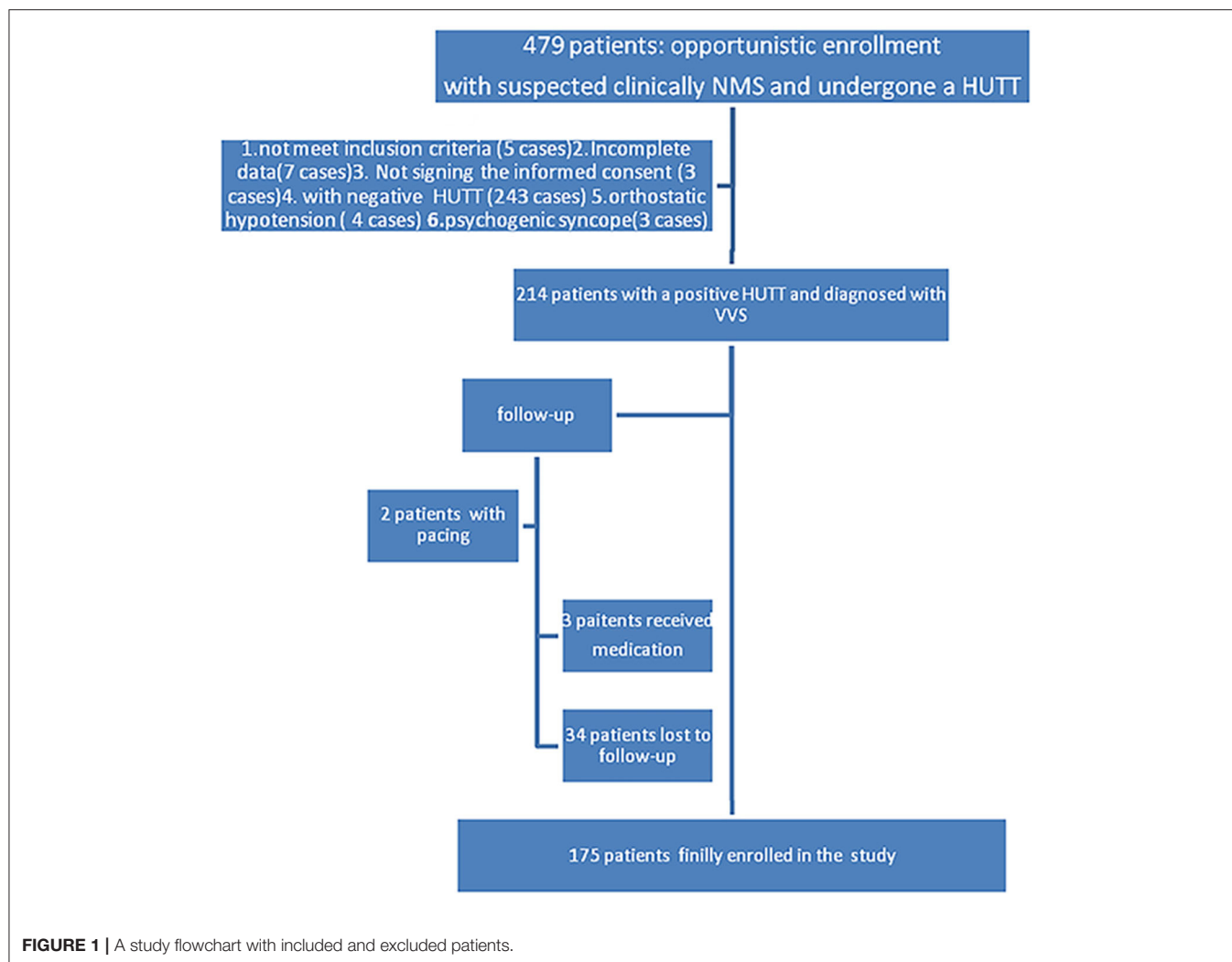
The patients were secured to the electric head-up tilt test table with a manual control board (ST-721, Beijing Juchi Medical Technology Co. Ltd), and a 12-lead synchronous ECG monitor (CASE, General Electric Company) and an automated sphygmomanometer (Tango M2, SunTech Medical Inc) was used for continuous monitoring of heart rate, rhythm, and blood pressure. Barring a positive response or loss of consciousness, the patients were asked to lie supine for at least 10 min with supine systolic blood pressure, supine diastolic blood pressure, and heart rate recorded, and then the table was tilted to 70°. The HUTT was terminated at any time when a positive reaction or orthostatic hypotension or psychogenic syncope was occurrence, or the patient requested to terminate because of intolerance; Otherwise, the tilted position would be maintained for full 45 min. Blood pressure, heart rate, and rhythm were recorded every 5 min and every 1 min in the initial 3 min to rule out orthostatic hypotension during the test. Symptoms were recorded in real-time. The patients were returned to a supine position as soon as a positive response occurred. The test was terminated if the HUTT was positive in the basic stage; otherwise, the test was continued with the sublingual nitroglycerin HUTT.

Sublingual Nitroglycerin HUTT

The process of sublingual nitroglycerin HUTT (SNHUTT) was the same as that in the basic stage assessment but followed the administration of sublingual nitroglycerin (300–400 µg, 0.5 mg/tablet of Xinyi Pharmaceutical Co., Ltd.) in the baseline HUTT-negative patients.

Diagnostic Criteria

Syncope or pre-syncope prodrome accompanied by any decreases in blood pressure or changes in heart rate with an electrocardiogram showing sinus arrest, nodal or ventricular escape, atrioventricular block or cardiac arrest ≥ 3 s was a positive HUTT response characteristic.



Positive VVS Response Types

Type 1 (Mixed Type)

Type 1 is characterized as a heart rate fall but not to <40 beats per minute (bpm) for <10 s at the time of syncope with or without asystole <3 s. The blood pressure decrease occurs prior to the heart rate fall.

Type 2 (Cardioinhibitory Type) Is Classified Into 2 Subtypes

Type 2A (Without Asystole)

Type 2A is characterized as a ventricular rate below 40 bpm for longer than 10 s and asystole <3 s. The blood pressure decrease occurs later than the heart rate fall.

Type 2B (With Asystole)

Type 2B is characterized as asystole >3 s. The heart rate fall coincides with or precedes the blood pressure decrease.

Type 3 (Vasoinhibitory Type)

Type 3 is characterized as systolic blood pressure (SBP) or mean pressure decrease $\geq 20 \sim 30$ mmHg or SBP $\leq 60 \sim 80$ mmHg (1

mmHg = 0.133 kPa). The heart rate does not fall more than 10% from its peak value at the time of syncope.

Counseling and Advice (Conservative Treatment)

All patients with a positive HUTT were provided with an overview of the VVS cause and its overall benign outcome and coached on how to avoid some situations that might provoke syncope, such as fatigue, late nights, particular emotional states, etc. They were also asked to sit down or lie in a supine position if syncope was unavoidable. They also received advice about increasing dietary salt and fluid intake unless contraindicated.

Follow-Up Protocols

The duration of follow-up ranged from 6 to 32 months after the HUTT by individual telephone calls to all patients. The patients or their parents were asked about syncope recurrence, which was defined as the recurrence of ≥ 1 syncope or typical pre-syncope prodrome that occurred during the follow-up. Patients who

underwent pacing or related drug therapy, such as metoprolol treatment, were excluded during the follow-up study.

Statistical Analysis

All reported levels of significance are 2 sided. A $P \leq 0.05$ was considered statistically significant. Statistical analysis was carried out with SPSS 23.0 software. Continuous variables with normal distributions are expressed as the mean \pm standard deviation (SD), and comparisons of normally distributed parameters between the two groups were performed with a t -test for independent samples. The non-normally distributed parameters are reported as the median and interquartile range (25–75%) and compared by the Mann-Whitney U -test. Categorical variables

are reported as frequencies and percentages. The data were compared by Pearson's chi-square test, and the exact probability method was used when the theoretical frequency was $<20\%$. Logistic regression was used to analyse the effect size and confidence interval of the individual or multiple factors with statistical significance for syncope recurrence based on the independent sample t -test or Pearson's chi-square test. The predictive performance of age was evaluated by prediction probability. The receiver operating characteristic (ROC) curve was utilized to evaluate the predictive value of the predictors, and the area under the curve (AUC) represented the predictive value. A 95% CI of the AUC that did not contain 0.5 or a $P < 0.05$ confirmed that the factor was a reliable predictor of recurrent

TABLE 1 | Baseline characteristics of the study population and follow-up duration.

Cases ($n = 175$)	Syncope recurrence group ($n = 44$)	No syncope recurrence group ($n = 131$)	$t/X^2/Z$ -value	P -value
Males/Females (n)	13/31	58/73	2.964	0.085
Age (yrs)	60.0 (47.8, 66.0)	53.0 (43.0, 62.0)	-2.346	0.019
BMI (kg/m ²)	23.3 \pm 3.1	22.4 \pm 2.9	1.622	0.107
LVEF (%)	60.4 \pm 4.7	61.4 \pm 5.4	1.100	0.273
LVDd (mm)	44.9 \pm 2.9	44.1 \pm 2.8	-1.618	0.107
Patients with comorbidities (n)	19 (43.2%)	49 (37.4%)	0.463	0.496
Hypertension (n)	11 (25.0%)	36 (27.5%)	0.103	0.748
Cardiovascular disease (n)	3 (6.8%)	7 (5.3%)	0.000	1.000
Diabetes (n)	3 (6.8%)	4 (3.1%)	0.433	0.511
Cerebrovascular disease (n)	1 (2.3%)	3 (1.5%)	0.000	1.000
Calcium channel blockers	6 (13.6%)	13 (9.9%)	0.164	0.686
Diuretics	3 (6.8%)	10 (7.6%)	0.000	1.000
ACE-I/ARB	5 (11.4%)	11 (8.4%)	0.083	0.773
β-blockers	3 (6.8%)	7 (5.3%)	0.000	1.000
Nitrates	2 (4.5%)	4 (3.1%)	0.000	1.000
Duration of symptoms (<2 years)	25 (56.8%)	84 (64.1%)	0.748	0.387
Number of spells in lifetime (more than 3 times)	22 (50.0%)	52 (39.7%)	1.433	0.231
Follow-up duration (months)	18.0 (14.0, 25.0)	16.0 (11.0, 23.0)	-1.482	0.138

TABLE 2 | Baseline positive HUTT characteristics of the study population.

Cases ($n = 175$)	Syncope recurrence group ($n = 44$)	No syncope recurrence group ($n = 131$)	t/X^2 -value	P -value
Positive BHUTT/Positive SNHUTT	5/39	6/125	1.550	0.213
HR (bpm)	71.1 \pm 15.2	70.47 \pm 12.1	0.263	0.793
Supine systolic BP (mmHg)	126.3 \pm 19.1	124.7 \pm 18.8	0.482	0.630
Supine diastolic BP (mmHg)	78.3 \pm 9.9	75.5 \pm 11.2	1.469	0.144
Supine heart rate (bpm)	71.1 \pm 15.2	70.5 \pm 12.1	0.263	0.793
Induced Syncope (n)	19	58	0.016	0.899
Arrhythmic events (n)	4	18	0.648	0.421
Type 1 (mixed type) (n)	19	69	1.187	0.276
Type 2 (cardioinhibitory type) (n)	3	10	0.000	1.000
Type 2A (n)	1	5	0.000	0.993
Type 2B (n)	2	5	0.000	1.000
Type 3 (vasodepressor type) (n)	22	52	1.433	0.231

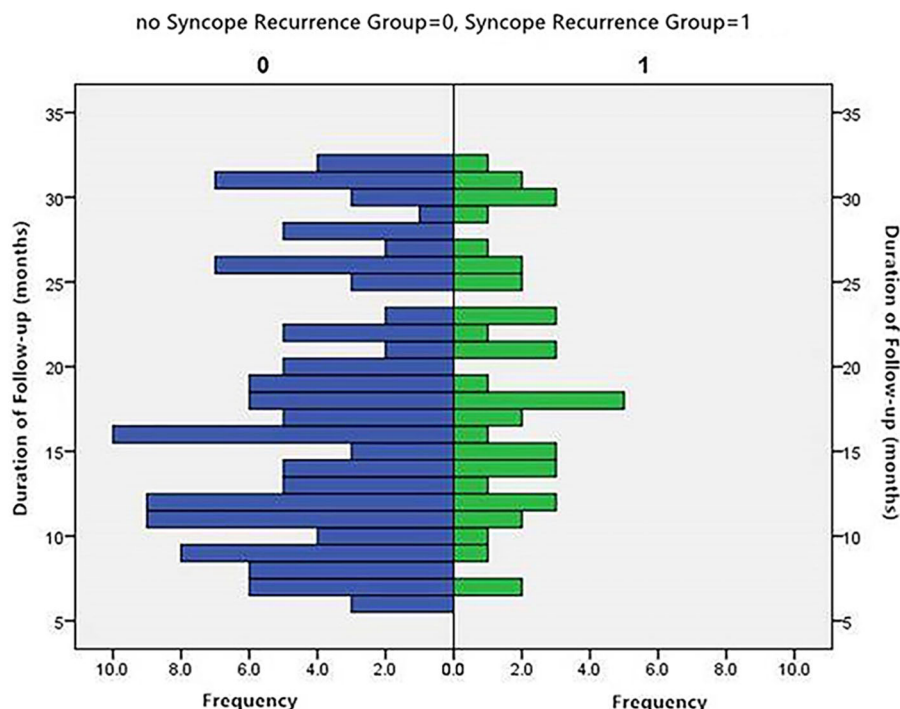


FIGURE 2 | Distribution of patients by months of follow-up.

syncope in VVS patients with a positive HUTT. The optimal cut-off value was determined as the maximum Youden index, which was defined as the sensitivity plus specificity minus 1, where sensitivity and specificity were calculated as proportions.

RESULTS

Patient Population

The valid data of 175 VVS patients with a positive HUTT were included in the analysis. Forty-four (25.1%) VVS patients had ≥ 1 recurrence of syncope or typical pre-syncope prodrome during follow-up.

Baseline Characteristics

The baseline characteristics of the two groups are shown in Table 1. The syncope recurrence group was older than the no syncope recurrence group ($P < 0.05$). Other characteristics were not significantly different between the groups ($P > 0.05$).

HUTT Characteristics

The HUTT characteristics of the two groups are shown in Table 2. None of the HUTT characteristics were significantly different between the groups ($P > 0.05$).

Distribution of Patients by Months of Follow-Up

The distribution of patients by month of follow-up is shown in Figure 2. The distribution was inconsistent between the two groups. The average rank of the data

shown in Table 2 was not significantly different between the groups ($P > 0.05$).

Trend in Syncope Recurrence Changed With Advancing Age

The study population was classified based on age into 8 groups (<20 , 20–30, 31–40, 41–50, 51–60, 61–70, 71–80, and >80 years old), and the graph shows that the rate of syncope recurrence increased with advancing age (Table 3, Figure 3).

Predictive Performance of Age

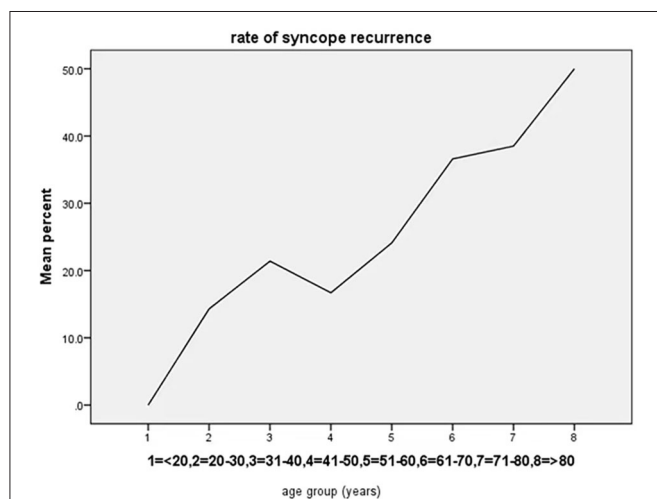
Age, sex, BMI, and supine diastolic BP (all $p < 0.15$ in the univariate analysis) were included in the logistic regression analysis. Only age, which was statistically significant in the univariate analysis, (OR value: 1.034, $p = 0.011$) and sex (OR value: 2.302, $p = 0.032$) were independently associated with syncope recurrence in VVS with a positive HUTT in the multivariable model (Table 4).

Predictive Ability of Age According to the ROC Curve Analysis

The ROC curve of age for the prediction of syncope recurrence in VVS patients with a positive HUTT had an AUC of 0.688 (95% CI: 0.598–0.777, $p < 0.05$; vs. the null hypothesis AUC of 0.5). A cut-off value of 53.5 years of age yielded high sensitivity (72.7%) and specificity (52.7%) (Figure 4).

TABLE 3 | Sample sizes for all ages in the study population.

Age (years)	<20	20–30	31–40	41–50	51–60	61–70	71–80	>80
Recurrence (n)	0	2	3	5	13	15	5	1
No recurrence (n)	7	12	11	25	41	26	8	1
Percent (%)	0	14.3	21.4	16.7	24.1	36.6	38.5	50

**FIGURE 3 |** Age-trend for syncope recurrence based on the percentage within each age group. The rate of syncope recurrence increased with advancing age.

DISCUSSION

The main findings of this study are as follows: (1) the total rate of syncope recurrence was 25.1% (44/175 cases) in VVS patients (median age: 55.0 years) with a positive HUTT, and it increased with advancing age during follow-up in our study. (2) We found a significant relationship between age and syncope recurrence. Elderly patients had an increased risk of syncope recurrence in VVS with a positive HUTT, and the cut-off value was 53.5 years, especially in females. Age as a predictor of syncope recurrence fulfilled all 3 criteria for the highest level of statistical significance according to single factor analysis, binary logistic regression, and receiver operating characteristic (ROC) curve analysis. In contrast, other factors, such as Vasovagal Syncope International Study (VASIS) classification and the number of syncope episodes, were not statistically significant.

VVS with atypical features is often diagnosed by a HUTT and is known to have a benign prognosis; however, recurrent VVS can seriously affect the quality of life and mental health of patients, particularly older adults who usually experience atypical VVS, which may have little or no prodrome (10). Therefore, valid predictors for the recurrence of syncope in VVS, especially in VVS patients with a positive HUTT, are very important for the design of efficient, economical, individualized treatment approaches. Sumner et al. proposed that the number of syncope episodes in the year preceding

presentation was the most powerful predictor of the time to the recurrence of syncope in a referral-based VVS population (11). There was a similar phenomenon in which the rate of ≥ 3 lifetime syncopal spells in the syncope recurrence group tended to be higher than that in the no syncope recurrence group (50.0 vs. 39.7%, respectively) in our study, although this difference was not significant. Another important finding was that age adjusted by sex was significantly associated with syncope recurrence. The results indicated that agedness was a valid predictor of syncope recurrence in VVS patients with a positive HUTT.

Previous reports have focused their attention on the research of patient history, the number of preceding syncopal spells, arterial baroreflex sensitivity, serum biochemical parameters, etc. (9, 12, 13). HUTT is usually used to define the syncope recurrence in those reports. In this study, we enrolled older patients and defined recurrence of syncope in nature state rather than HUTT. In addition, the patients enrolled in this study received professional guidance from the clinician including ensuring sufficient sleep, the relief from anxiety and fatigue, avoiding exciting habits, and activities and proper response measures in the event of pre-syncope. All of those could help to reduce the syncope recurrence in patients with VVS.

The results may be attributed to the following, although the reason for this is not fully clear:

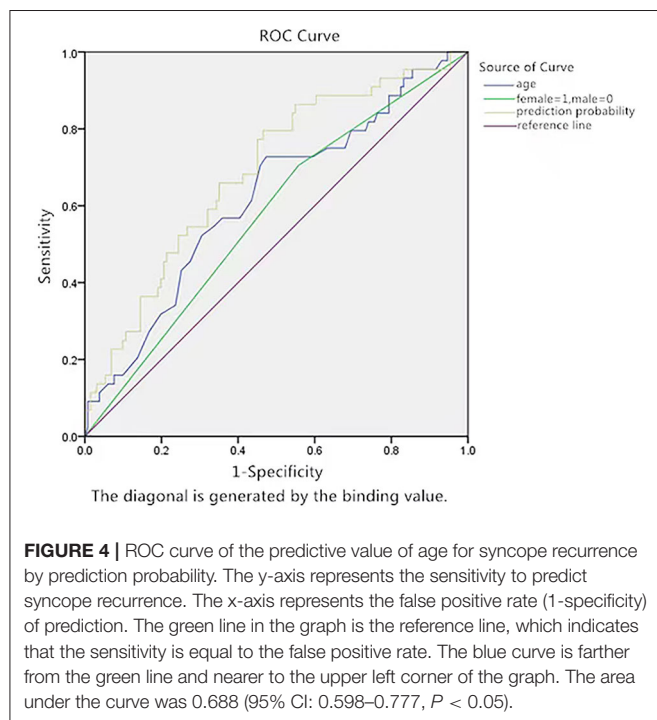
First, autonomic control of the cardiovascular system could be influenced by both age and sex (14, 15). Maria et al. found attenuation of the autonomic nervous system (ANS) in the regulation of cardiac function in older essential hypertensive patients (16), and Benditt et al. discovered a lower epinephrine (Epi)/norepinephrine (NE) ratio in older patients than in younger patients with syncope during tilt-induced VVS (17). NE is the junctional transmitter between sympathetic ganglion cells and effectors. Indeed, a decrease in cardiac sympathetic and parasympathetic components was observed in the older population. The autonomic control of blood pressure appears to decline with aging (18). These observations could reflect a decreased sensitivity of cardiac beta-adrenoceptors with aging. The effect of aging might be related to the parallel age-related reduction in sympathetic vasomotor responsiveness. Attenuation of this process results in autonomic nerve function damage, and ANS imbalance may be one of the underlying mechanisms (19).

Second, in adults, a decrease in cardiac output (CO) is the dominant hypotensive mechanism, and it is not vasodilatation because systemic vascular resistance (SVR) always remains above baseline levels during VVS (20). Yamaguchi et al. proposed that among patients with a positive HUTT, the syncope recurrence rate after the HUTT in those with LV dysfunction

TABLE 4 | Determinants of syncope recurrence in the study population.

Factor	B value	S.E value	Wald value	P-value	Exp(B)	95%CI of Exp(B)
Sex	0.834	0.389	4.598	0.032	2.30	1.074–4.932
Age	0.034	0.013	6.427	0.011	1.03	1.008–1.061

CI, confidence interval.



was higher than that in those with normal LV function (21), and relatively older patients often have LV dysfunction because of cardiovascular comorbidities. Interestingly, our study also showed that the rate of comorbidities in the syncope recurrence group tended to be higher than that in the no syncope recurrence group (43.2 vs. 37.4%, respectively), although the difference was not significant.

In addition, sustained orthostatic hypotension and delayed BP recovery are more common and are risk factors for falls, injuries, and cognitive decline in the older population. Hence, a mild orthostatic hypotension response during orthostatic stimulation may take part in syncope recurrence despite being diagnosed with VVS in the HUTT.

Based on our findings, recurrent syncope is more common in older patients with a positive HUTT, but that the likely results from multiple mechanisms, not just VVS. Greater clinical vigilance and other considerations may effectively decrease the frequency of syncope recurrence in old VVS patients (older than 53.5 years) with a positive HUTT, such as continued antihypertensive therapy and active treatment of ventricular dysfunction.

Limitations

The present study also has limitations. First, our study was an open-label, observational, and single-center retrospective study, and selection bias is inevitable. Second, the inability to know the cause of recurrent syncope, detailed confounding comorbidities in the older patients and the lack of data on LV function at the time of recurrent syncope resulted in the inability to stratify the population. Third, the interval from the HUTT to the first recurrence of syncope was observed during follow-up, and the duration of follow-up varied. In addition, there are known limitations to the AUC statistical method. Randomized controlled studies are essential to assess the predictive value of age.

CONCLUSIONS

Age may be a valid predictor for the recurrence of syncope in elderly VVS patients with a positive HUTT. The rate of syncope recurrence increased with advancing age, especially in females. VVS patients who were older than 53.5 years with a positive HUTT had a greater recurrence possibility despite receiving non-pharmacological measures other than pacing. This intriguing novel finding deserves further study.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committee of Ningbo First Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

YG participated in the design of the study, data collection, and drafting of the manuscript. XC conceived the study and participated in its design. TZ, LW, and LC participated in the design of the study and data collection. All authors read and approved the final manuscript.

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A Review of Biomarkers for Ischemic Stroke Evaluation in Patients With Non-valvular Atrial Fibrillation

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Atrial fibrillation (AF) is the most prevalent cardiac arrhythmia worldwide and results in a significantly increased ischemic stroke (IS) risk. IS risk stratification tools are widely being applied to guide anticoagulation treatment decisions and duration in patients with non-valvular AF (NVAf). The CHA₂DS₂-VASc score is largely validated and currently recommended by renowned guidelines. However, this score is heavily dependent on age, sex, and comorbidities, and exhibits only moderate predictive power. Finding effective and validated clinical biomarkers to assist in personalized IS risk evaluation has become one of the promising directions in the prevention and treatment of NVAf. A number of studies in recent years have explored differentially expressed biomarkers in NVAf patients with and without IS, and the potential role of various biomarkers for prediction or early diagnosis of IS in patients with NVAf. In this review, we describe the clinical application and utility of AF characteristics, cardiac imaging and electrocardiogram markers, arterial stiffness and atherosclerosis-related markers, circulating biomarkers, and novel genetic markers in IS diagnosis and management of patients with NVAf. We conclude that at present, there is no consensus understanding of a desirable biomarker for IS risk stratification in NVAf, and enrolling these biomarkers into extant models also remains challenging. Further prospective cohorts and trials are needed to integrate various clinical risk factors and biomarkers to optimize IS prediction in patients with NVAf. However, we believe that the growing insight into molecular mechanisms and in-depth understanding of existing and emerging biomarkers may further improve the IS risk identification and guide anticoagulation therapy in patients with NVAf.

Keywords: atrial fibrillation, non-valvular atrial fibrillation, ischemic stroke, biomarker, CHA₂DS₂-VASc score

INTRODUCTION

Atrial fibrillation (AF) is the most prevalent cardiac arrhythmia in clinical practice (1). Results from the famous Framingham Heart Study and Atherosclerosis Risk in Communities (ARIC) cohort showed that the lifetime risk to develop AF was up to one in three (2, 3). It is estimated that AF will affect >8 million people in America by 2050, and 18 million people in Europe by 2060 (1). Hence,

AF poses a markedly increasing burden worldwide. Meanwhile, AF is a well-recognized risk factor for ischemic stroke (IS), heart failure (HF), cognitive decline, and is associated with substantial morbidity, disability, and mortality (4). The risk of IS among patients with AF is ~5% per year and is up to 5-fold higher than the general population (5). AF is reported to contribute to almost 15–20% of all stroke cases, and AF-related stroke has higher mortality and permanent disability than strokes from other etiologies (6). Therefore, IS prevention is the central pillar of AF management.

Oral anticoagulants effectively prevent IS and improve outcomes among patients with AF (7). However, prior to anticoagulation, stroke risk assessment is the first and the most vital step to maximize the benefits of anticoagulant drugs. Clinicians should identify patients at high-risk for IS, who will benefit in the first line from anticoagulation, or rather determine patients at low-risk of IS, in whom anticoagulation may not be warranted. In current clinical practice, the CHA₂DS₂-VASc score is recommended by the most influential guidelines as the primary means of stratifying patients with non-valvular AF (NVAf) (8–10). The major advantage of the CHA₂DS₂-VASc score is its perspicuity and simplicity of use, as it is a clinical risk-factor-based prediction score. However, it also has several drawbacks, such as widely ranged stroke rates of non-anticoagulated AF patients in different populations, and a limited predictive ability of stroke events (11, 12).

In recent years, biological markers (biomarkers) have been constituted a very powerful tool in the early diagnosis, risk stratification, prognosis prediction, and guiding therapy in many cardiovascular diseases (13, 14). According to the definition of the Biomarkers Definitions Working Group, “any characteristic that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” is belonged to a biomarker (15). With the advancements in medicine, the contents of biomarkers have also continuously extended. A number of studies have explored differentially expressed biomarkers in NVAf patients with and without IS, and the potential role of various biomarkers for the prediction or early diagnosis of IS in patients with NVAf. Some previous publications have also summarized these biomarkers (16–21). However, in recent years, research into the possible biomarkers capable of predicting the IS events in patients with NVAf is constantly growing. Hence, our present updated review will focus on the current status of clinical biomarkers beyond the CHA₂DS₂-VASc score for the assessment of IS in patients with NVAf, which might provide a basis for the future perspectives of clinical application.

CHA₂DS₂-VASc SCORE AND ITS LIMITATIONS

In 2001, Gage et al. created the CHADS₂ index that included five variables: congestive HF, hypertension, age, diabetes, and stroke, for a maximum of 6 points, and has been well-validated in the National Registry of AF, which showed high prediction

performance (c-statistic of 0.82) (22). However, later studies indicated that a CHADS₂ score of 0–1 has poor identification of NVAf patients at truly low risk of IS (23, 24). Moreover, this score ignored several potential clinical risk factors for IS. Thus, in 2010, the CHA₂DS₂-VASc score was developed by re-stratifying the risk of IS based on the CHADS₂ score, which incorporated three additional components: vascular disease, age 65–74, and female sex (25). A national prospective study has confirmed that the predictive ability for low risk of IS with the CHA₂DS₂-VASc score is significantly superior to the CHADS₂ score, which provides more reliable guidance to determine whether or not anticoagulation treatment is required in patients with NVAf (23). The above two scores exhibited similar predictabilities in meta-analytic data, but CHA₂DS₂-VASc score had the important advantage of identifying extremely low-risk patients (26). Altogether, the CHA₂DS₂-VASc score is currently considered as a core risk stratification model for IS assessment in patients with NVAf.

Despite the simplicity and practicality, certain limitations exist in the CHA₂DS₂-VASc score. First, the contribution of the individual component to the risk of IS in patients with NVAf is unequal, but most components carry equal weight, and only two risk factors, age and prior stroke/transient ischemic attack (TIA), are assigned with different points (27, 28). Second, cardiovascular complications screening varies in practice by country and region. For example, ankle-brachial index (ABI), an indicator of peripheral arterial disease (PAD), is not routinely assessed in developing countries, which might potentially lead to an underestimation of the overall IS risk. Third, racial/ethnic differences may exist in IS risk prediction in NVAf, and the CHA₂DS₂-VASc score may not be validated in an ethnically diverse population (29). For example, the determination of the age threshold of IS risk assessment may vary with different populations (30). Fourth, several other identified risk factors or biomarkers not included in the score, which lead to a suboptimal predictive performance in selected populations (e.g., patients with renal insufficiency) (31). Moreover, evidence from a recent systematic review shows that this score has not ideal predictive power (c-statistic of 0.6–0.7) (12). It is, thus, essential to improve the prediction accuracy of this model.

OTHER RISK STRATIFICATION MODELS

Several recent studies have attempted to refine the CHA₂DS₂-VASc score system. Maheshwari et al. found that abnormal P-wave axis (aPWI) can predict the occurrence of IS independent of CHA₂DS₂-VASc variables, and proposed P₂-CHA₂DS₂-VASc score to assess the risk of AF-related stroke, in which aPWI was scored with 2 points (32). A study from China indicated that urine albumin was an independent predictor of thromboembolism (TE) events for NVAf patients, and the new CHA₂DS₂-VASc-UA₂ score system showed better performance in predicting TE events compared with the CHA₂DS₂-VASc score (33). Similarly, an analysis of the national health insurance database of more than 460,000 AF patients shows that the addition of African-American race (1 point) to CHA₂DS₂-VASc

score (CHA₂DS₂-VAsC-R score) significantly improved stroke prediction (34). In a recent study analyzing Korean NVAf populations, chronic kidney disease (CKD) but not female sex is an independent predictor of TE events (35). The authors proposed a CHA₂DS₂-VAK score in which “S”ex “c”ategory was replaced by “K”idney disease, the new score system enhanced discrimination of low to intermediate TE risk in NVAf patients (35). In another Asian study, a modified mCHA₂DS₂-VAsC score, which assigned one point for patients aged 50–74 years, demonstrated a better predictive performance than the CHA₂DS₂-VAsC in Taiwan AF population (36). Patients with a mCHA₂DS₂-VAsC score of 1 (males) or 2 (females) obtained positive net clinical payoffs from anticoagulation therapy (36).

Furthermore, investigators are also proposing new prognostic models, such as ATRIA score, GARFIELD-AF model, and ABC stroke score (Table 1). Similar to the CHA₂DS₂-VAsC score, these models all incorporate age and common clinical risk factors of IS. The ATRIA score takes into account the interaction between age and previous stroke (37). The GARFIELD-AF model is composed of more than 30 clinical risk factors (38). The ABC stroke risk score is a biomarkers-based nomogram, which include age, N-terminal prohormone of brain natriuretic peptide (NT-proBNP), cardiac troponin I (cTnI), and prior stroke/TIA (39). Therefore, the calculation of these scores is complicated, making it impractical for clinical use. Moreover, a recently published systematic review, which summarized current risk stratification tools for IS prediction in patients with NVAf, did not show a better prediction role for the above scores in improving the ability of IS events compared with CHADS₂ and CHA₂DS₂-VAsC score (12). Taken together, despite the limitations of the CHA₂DS₂-VAsC score, no strong evidence has been able to show that these novel or modified risk scores can replace it.

CLINICAL BIOMARKERS BEYOND THE CHA₂DS₂-VAsC SCORE FOR IS EVALUATION IN NVAf

While IS risk prediction scores have been heavily weighted by well-established clinical factors, findings from randomized controlled trials (RCTs) and community-based cohorts showed that various biomarkers can improve predictive accuracy and risk assessment. Numerous studies have examined the utility of AF characteristics, cardiac imaging and electrocardiogram (ECG) markers, atherosclerosis-related markers, circulating biomarkers, and novel genetic markers (Figure 1) in IS prediction in patients with NVAf. These biomarkers, whether new or old, may enhance our understanding of the pathophysiology of AF-related IS and help us to find new therapeutic targets.

AF CHARACTERISTICS

The type, duration, and burden of AF are the most frequent clinical characteristics assessed by clinicians. AF has been conventionally categorized into “valvular” or “non-valvular” on the basis of the presence or absence of valvular heart disease. Besides, the types of AF can be classified as first

diagnosed, paroxysmal, persistent, long-standing persistent, and permanent AF in light of the presentation, duration, and spontaneous termination of AF episodes. The secondary analysis of several RCTs which examined the clinical efficacy of novel oral anticoagulants or aspirin in AF patients demonstrated that persistent and permanent AF increased the risk of IS compared to paroxysmal AF in patients taking anticoagulation therapy, as well as patients taking antiplatelet therapy (40–43). Similarly, in one Japanese cohort study, a lower incidence of stroke/systemic embolism was observed in paroxysmal AF compared with sustained AF regardless of oral anticoagulant uses (44). A meta-analysis of 12 studies containing 99,996 patients showed that non-paroxysmal AF is associated with an increase in TE events [hazard ratio (HR) = 1.384, 95% confidence intervals (CI):1.191–1.608, $P < 0.001$] compared with paroxysmal AF, and in subgroup analyses, this difference was present for both patients on oral anticoagulants and not on oral anticoagulants (45).

Although ECG and 24-h Holter are commonly applied in patients with AF, it still requires a longer period of continuous monitoring to obtain AF burden and duration. Current studies that assess the burden of AF and stroke risk are mostly based on patients with cardiovascular implantable electronic device (CIED) implantation. Atrial high rate episodes (AHREs) were exactly described as the unknown AF with a fast atrial episode (>180 bpm) recorded on CIED for at least 5 min (46). Epidemiological data reported the incidence of AHRE reached ~25–35% during 2-year follow-up in patients without a natural history of AF (47). Current evidence supported the elevated AHRE burdens increased the risk of adverse cardiovascular prognoses such as myocardial infarction, HF, and ventricular arrhythmia (48). Additionally, the association was being drawn between AHRE and increased stroke risk by a growing number of clinical trials. Early in 2003, the MOST (Mode Selection Trial) investigators prospectively evaluated the association between AHREs and clinical outcomes in sinus node dysfunction patients with pacemaker therapy. After the adjustments of prognostic and baseline variables, AHRE was reported as the independent risk factor of death or non-fatal stroke (HR = 2.79, 95% CI:1.51–5.15, $P = 0.0092$) by Cox proportional hazards analysis (49). The ASSERT trial on the larger sample size detected the AHREs in the population without diagnostic AF ($n = 2,580$) for 3 months after ICD implantation. Such subclinical AF, a confirmed predictor of stroke, contributed to the increased risk of IS or systemic embolism (HR = 2.49, 95%CI:1.28–4.85, $P = 0.008$) (50). Meanwhile, the correlation between asymptomatic AF and high TE risk has been illustrated by the EORP-AF Pilot General Registry (51). Compared with healthy controls, asymptomatic AF patients potentially progressed to permanent AF, and may lead to higher systemic ischemic events (52, 53). A proof-of-concept study found that machine-learned signatures of AF burden could provide prognostic information on the near-term risk of stroke in patients with CIED (54). However, a recent cohort study that included 384 CIED implanted patients without anticoagulation showed that the burden and duration of AF were not associated with IS/TIA, and only the CHA₂DS₂-VAsC score can predict IS/TIA (55). The inconsistency in the aforementioned results may

TABLE 1 | Influential IS risk stratification models/scores for NVAf.

Model/Score	Components	Points	Range of stroke risk stratification	Validation studies
CHADS ₂ score	Heart failure, hypertension, age, diabetes, stroke	0 to 6	Low (0 point), moderate (1 point), high (≥ 2 points)	Yes
CHA ₂ DS ₂ -VASc score	Heart failure, hypertension, age ≥ 75 , diabetes, stroke, vascular disease, age 65–74, female sex	0 to 9	Low (0 point), moderate (1 point), high (≥ 2 points)	Yes
ATRIA score	Age, prior stroke, female sex, diabetes, heart failure, hypertension, proteinuria, eGFR < 45 or ESRD	0 to 15	Low (0–5 points), moderate (6 points), high (7–15 points)	Yes
GARFIELD-AF model	Age, pulse, systolic blood pressure, vascular disease, history of bleeding, heart failure, renal disease, use of OAC*	Machine learning model	—	Yes
ABC stroke score	Age, prior stroke/transient ischemic attack, NT-proBNP, cTnl	Nomogram	—	Yes

*Components of the simplified GARFIELD-AF risk model.

IS, ischemic stroke; NVAf, non-valvular atrial fibrillation; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; OAC, oral anticoagulation; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; cTnl, cardiac troponin I.

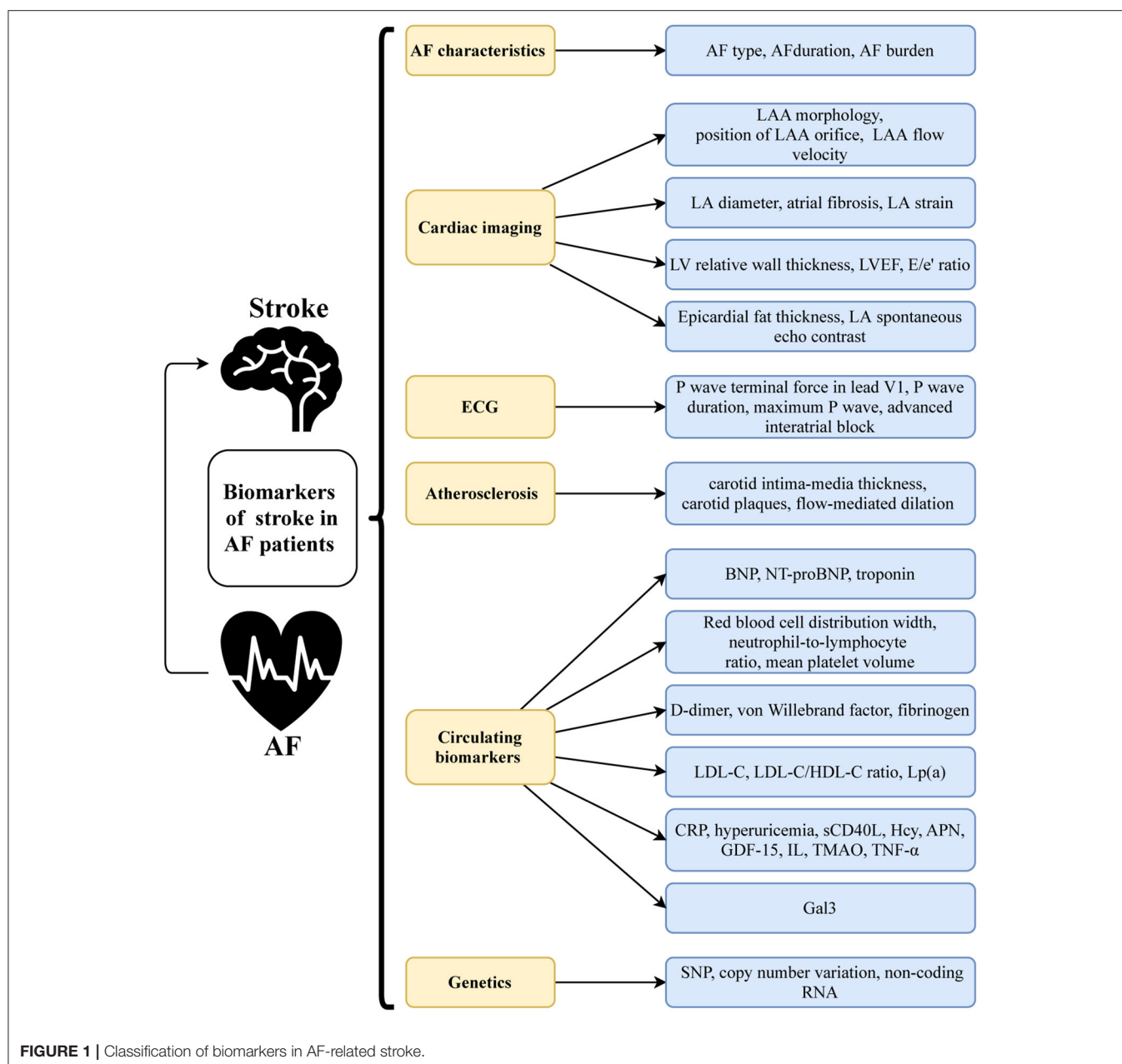
be caused by the difficult screening of subclinical ischemic brain lesions (IBLs), which results in an underestimated embolic rate. A Spanish research group prospectively assessed the relationship of AHRE and IBLs through the computed tomography scan in patients with CIED implantations, and results showed that AHRE was an independent predictor for silent IBL both in the overall population and in patients without a history of AF or stroke (56). Similar results were observed in patients with cardiac resynchronization therapy (57). However, there is no sufficient evidence to reveal the distinct temporal relevance between AHRE and subsequent events. As shown in TRENDS study, an AHRE episode is able to be recorded before, during, or after the stroke event (58). Based on current cognition, whether the AHRE performs a cause or merely a biomarker of TE should be interpreted more prudently. In addition, with the development of science and technology, the increasing using of wearable devices and apps in daily life and clinical practice may be useful and convenient to quantify AF burden (59). We consider that more research is needed in the future to explore the role of wearables-detected AF burden in evaluating IS events.

CARDIAC IMAGING AND ECG BIOMARKERS

According to Virchow's triad, there are three pivotal factors to venous thrombosis: vascular damage, blood stasis, and hypercoagulability. With the progression of AF, progressive atrial dilatation, endocardial denudation, and oedematous or fibroelastic infiltration of the extracellular matrix will lead to abnormal blood flow patterns through the atrium and the formation of intra-atrial thrombus (60). Therefore, using parameters that reflect cardiac structural and functional remodeling to predict the risk stratification of TE events in NVAf is very meaningful. In fact, a large number of studies have investigated the role of cardiac, especially atrial structure, function, electrocardiography, and cardiac circulating biomarkers in AF-related IS.

Left Atrial Appendage (LAA) Structure and Function

LAA is an embryological remnant of the primordial left atrium (LA). As early as two decades ago, LAA was reported to be closely related to atrial thrombus formation in NVAf patients because of its hooked morphology and "low flow state" (61). In 2012, Di Biase et al. (62) firstly divided LAA morphology into four types: Chicken wing, Cactus, Windsock, and Cauliflower, and reported that NVAf patients with non-Chicken Wing (CW) LAA morphologies were more likely to occur TE events than CW patients after controlling for comorbidities and CHADS₂ score. A later meta-analysis included in 12 studies showed that the risk of cerebrovascular accident in AF patients with CW morphology was reduced by 41% relative to non-CW patients (63). However, this subjective classification of LAA morphology is not well-quantifiable and can be widely influenced by clinicians and reviewers. In one study conducted in Fuwai Hospital, the consensus of LAA morphology was only reached in 28.9% among three experienced reviewers (64). A retrospective study revealed that the classification of with or without clearly lobulated structure of LAA, a relatively concise classification strategy, was independently associated with LAA thrombosis in NVAf patients (OR = 4.216, 95% CI: 1.825–9.740, $P = 0.001$) (65). Several recent studies investigated the role of quantitative assessments of LAA, such as the angle bend from the proximal/middle portion of the LAA (66) and statistical shape analysis of LAA (67) in predicting stroke. Nevertheless, large-scale validation is needed to further verify these preliminary findings. Additionally, Zhao et al. (68) showed that higher position of LAA orifice had a strong relationship with thrombus formation after adjusting for confounding factors in AF patients, which was consisting with Nedios et al. (69), who reported that higher position of the superior LAA-takeoff in NVAf patients was paralleled with increasing TE events after catheter ablation. Furthermore, the Stroke Prevention in Atrial Fibrillation (SPAF-III) Study trial revealed that low LAA flow velocity (< 0.2 m/s), reflecting the systolic function of LAA, was associated with TE events in AF patients (70). Similar results were shown in a Korean study, in which increased orifice size and decreased flow velocity of LAA



were related to IS risk in patients with NVAF (71). In a word, the structural and functional characteristics of LAA contributed to the assessment of IS risk in NVAF patients.

LA Structure and Function

The structure and function of LA could also assist in IS prediction in patients with NVAF (72, 73). In Fushimi AF Registry, a large community-based cohort study of Japanese NVAF patients, larger LA diameter (LAD) was a strong predictor of stroke/TE whether oral anticoagulant was used or not (HR = 1.74, 95% CI: 1.25–2.42, $P < 0.01$) (74). This was paralleled with the result from the Framingham Heart Study (75), in which LA enlargement remained a significant predictor of stroke in male AF patients.

Paciaroni et al. (76) reported that severe LA dilation (defined by $LAD \geq 5.0 \text{ cm/m}^2$ or $LAVi \geq 40 \text{ ml/m}^2$) was associated with the incidence of TE events (OR = 2.05, 95% CI: 1.08–2.87, $P = 0.027$). Additionally, the functional status of LA also needs to pay more attention. It has been established that atrial fibrosis, which could be assessed with late gadolinium enhancement (LGE) on cardiac magnetic resonance imaging (MRI), was independently associated with the higher risk of TE events (77, 78). In a mean of 7.9 years follow-up study of 1,361 first diagnosis of AF patients, P-wave to A' duration on tissue Doppler imaging, reflecting total atrial conduction time, was independently associated with IS risk in a fully-adjusted model including CHA₂DS₂-VASc score, age, and anticoagulant use (79). Furthermore, previous studies have

documented that LA strain was also associated with LA fibrosis, and the independent relationship between the reduced LA strain, strain rate, and IS were subsequently certificated (80–82).

Left Ventricular (LV) Structure and Function

Parameters of LV structure have shown closely related to stroke events in AF patients, the underlying pathophysiologic mechanism lies in that elevated LV filling pressure would lead to LV hypertrophy and subsequent LA dilation (73). In the ARAPACIS Study, the prevalence of LV hypertrophy in patients with NVAf is higher, which is consistent with the higher risk of TE risk in these patients (83). Meantime, in a large community-based prospective study, Tezuka et al. (84) manifested that after adjustment for various potential confounders, high LV relative wall thickness (RWT) was independently associated IS in NVAf patients (HR = 1.81, 95% CI: 1.34–2.47, $P < 0.01$), indicating the vital role of LV morphology in contributing to TE. Additionally, LV systolic function categorized by LV ejection fraction (LVEF) was thought of as a key TE event predictor in NVAf. In fact, as the most important diagnostic indicator of HF, LVEF has already been included in the CHA₂DS₂-VAsC score. In 1992, Asinger et al. (85) found that LV dysfunction was associated with TE events in 568 AF patients (RR = 2.0, 95% CI: 1.0–4.0, $P < 0.05$). Result from 3 RCTs including 1,066 NVAf patients also showed that moderate to severe LV systolic dysfunction was a strong independent predictor of stroke (RR = 2.5, 95% CI: 1.5–4.4, $P < 0.01$) (86). As a more reliable and stable index of LV dysfunction, we speculate that LV strain could provide a significant advantage in predicting IS compared with LVEF. However, as far as we know, there was no current study certificated that the LV strain could be used as a predictor of stroke in NVAf. Besides, previous studies have documented the evident relationship between chronic LV diastolic dysfunction and LA enlargement, promoted the AF occurrence and thrombi formation (73). Among LV diastolic parameters, E/e' ratio had an independent association with stroke in NVAf patients (OR = 1.21, 95% CI: 1.08–1.37, $P = 0.002$) (87). Therefore, structural and functional parameters of LV also play an important role in predicting IS in patients with NVAf.

Other Echocardiographic Indicators

In addition to the above markers, other echocardiographic indicators also deserve considerable attention. In a matched cross-sectional study, the role of epicardial fat thickness in AF patients with and without acute IS was analyzed, and higher epicardial fat thickness (OR = 7.356, 95% CI: 3.880–13.947, $P < 0.0001$) independently predicted acute IS (88). Early studies demonstrated that LA spontaneous echo contrast (LASEC), a frequent finding on transesophageal echocardiography, was thought to a marker of the hypercoagulable state (89). A prospective cohort study showed that NVAf patients with IS had higher grades and video intensity value of LASEC compared with patients without IS, and the video intensity value of LASEC had a better predictive performance of IS in NVAf patients than LA thrombus, CHADS₂ score, and CHA₂DS₂-VAsC score (90).

ECG Markers

The P wave results from electric activity in the atrium and is an indicator of atrial depolarization (91). Thus, P wave indices could be used to evaluate the LA abnormalities, which might further be associated with increased risk of IS in AF patients. A recent systematic review reported that several common P wave indices, including P wave terminal force in lead V1, P wave duration, and maximum P wave area were predictors of IS (92). As earlier mentioned, an abnormal P-wave axis was associated with increased IS risk independent of CHA₂DS₂-VAsC score in AF patients (32). In addition, some small sample studies have shown that advanced interatrial block, diagnosed upon the duration of the P wave and morphology in limb lead ECG, could serve as a marker of atrial electromechanical dysfunction and a surrogate for LA strain reduction, and might act as a predictor of IS in AF patients (93, 94).

In summary, parameters of cardiac MRI, echocardiography, and ECG could reflect the atrial and ventricular structure and function and could be used as risk predictors of IS in NVAf. Moreover, in the last years, innovations in multi-modality imaging can offer a comprehensive evaluation of cardiac remodeling, which we believe could be further used for accurate IS prediction in AF patients (95).

ARTERIAL STIFFNESS AND ATHEROSCLEROSIS-RELATED MARKERS

Atherosclerosis is a well-recognized risk factor for IS in the general population. Vascular diseases, including a history of myocardial infarction, aortic plaque, and PAD, is a component of the CHA₂DS₂-VAsC score. However, as previously mentioned, PAD and aortic plaques examinations are not routinely performed for AF patients in many instances. Therefore, many studies have explored the predictive role of other arteriosclerosis-related indicators such as carotid intima-media thickness (cIMT), carotid plaques, and flow-mediated dilation (FMD) with IS in NVAf.

Our previous hospital-based study showed that carotid plaque was detected in more than half of patients with NVAf (96). In fact, a growing number of studies across the globe have shown that carotid plaque is more common in patients with AF than in those without AF (97). Result from ARAPACIS study showed that the combination of vascular diseases and carotid plaque was independently associated with stroke in patients with NVAf (HR = 1.78, 95% CI: 1.05–3.01, $P = 0.0318$) (98). Similarly, two studies from Korea (99) and the USA (100) both demonstrated that the addition of cIMT and carotid plaque in the CHA₂DS₂-VAsC score can better predict the occurrence of IS in patients with AF. In addition, a case-control study suggested that the stability of carotid plaques was also associated with IS in patients with NVAf (101). A recently published systematic review identified available data and confirmed an association of carotid atherosclerosis with the risk of IS and TIA in patients with AF (102). In a 2-year follow-up study, low FMD was associated with an increased composite endpoint for cardiovascular events including IS in NVAf patients (103). It is noteworthy that

evidence from a meta-analysis revealed that the use of statins, the most common clinically anti-atherosclerotic agent, reduces mortality in AF patients, which might, on the other hand, illustrate that atherosclerosis has a detrimental role contributing to IS in patients with AF (104). In general, given its simplicity and stability, we believe that carotid plaque is a promising marker for improving classification in CHA₂DS₂-VAsC score in NVAf patients.

CIRCULATING BIOMARKERS

Cardiac Biomarkers

There is a broad consensus that elevated B-type natriuretic peptide (BNP) and NT-proBNP are the hallmarks of HF (105). BNP is mainly produced by cardiac myocytes as a response to increased end-diastolic pressure and/or volume expansion, and then enzymatically cleaved to the NT-proBNP (106). Recent studies suggested that BNP and NT-proBNP might also have the effect of predicting stroke in AF patients. One possible reason is that the increased pressure of atrial myocytes can lead to the increased secretion of BNP, and thus reflecting atrial dysfunction (16). An early small-sample research indicated that elevated BNP level was significantly associated with TE events in AF patients treated with warfarin (107), since increased levels of BNP was observed at the acute stage of IS (108) and in patients with a history of TE or echocardiographic evidence of thrombus (109) of NVAf patients. *Post-hoc* analyses of ARISTOTLE trial (110), RE-LY trial (111), and ENGAGE AF-TIMI 48 trial (112) similarly showed that NT-proBNP was independently associated with the increased risk of IS, and adding NT-proBNP to the CHA₂DS₂-VAsC score could improve C-statistics. Similarly, a single-center study showed that incorporating NT-proBNP into the CHA₂DS₂-VAsC score increased the ability of IS/systemic embolism risk prediction in anticoagulated patients with AF by 17% (113). Result of the Hokuriku-Plus AF Registry illustrated that high levels of BNP were also increased the risk of TE events in NVAf patients (114), which was corresponded with the findings of Paulin et al. (115). Evidence from a multicenter, prospective observational study (Fushimi AF Registry) showed that BNP was associated with IS and TE events in patients with AF without HF, and the addition of BNP into CHA₂DS₂-VAsC score as a new risk prediction model can better predict IS risk (116). In addition, high levels of BNP can be used as a predictive marker for recurrent IS in IS survivors with AF (117). Moreover, BNP can also be used as an etiological diagnosis indicator of acute IS in patients with AF. Sakamoto et al. (118) reported that low levels of BNP (<130 pg/mL) were associated with non-cardiogenic IS, while high levels of BNP were associated with cardiogenic IS, which may be due to the fact that BNP could promote intracardiac thrombosis.

Troponin is a marker of myocardial injury and is widely used in the diagnosis and prognosis of acute coronary syndrome (105). As the most widely applied biomarker in cardiovascular disease, emerging evidence suggested that troponin could also predict stroke in patients with AF. In a retrospective study of 199 NVAf patients, elevated hs-cTnI was independently associated with abnormal anatomy of the LA, defined as LAA flow velocity

<20 cm/s or dense spontaneous echo contrast, and the incidence of IS increases with higher cTnI levels (119). Similar to NT-proBNP, the *post-hoc* analyses of ARISTOTLE trial (120), RE-LY trial (111), and ENGAGE AF-TIMI 48 trial (112) also indicated a positive association between cTnI/cTnT level and risk of TE/IS, and integrating troponin to the CHA₂DS₂-VAsC score could improve C-statistics. In a real-world cohort study, after adjusting for CHA₂DS₂-VAsC score, a high level of cTnT (≥ 8.04 ng/L) was shown to be associated with IS/TIA in patients with AF (HR = 2.44, 95%CI:1.13–5.26, $P = 0.023$) (121). In a study validating the performance of ABC risk score, cTnT has also been shown to be correlated with IS/systemic embolism in patients with AF (122). A recent meta-analysis which focused on the correlation between hs-cTnT and risk of stroke showed that the HR value of IS in AF patients with a high level of hs-cTn was 1.95 (95% CI: 1.29–2.62), suggesting that hs-cTn could be used as a marker of IS risk stratification in AF patients (123).

To sum up, the BNP, NT-proBNP, and cTn are shown to be effective to improve risk stratification in addition to the current CHA₂DS₂-VAsC score. They are widely used and readily accessible in clinics, and are easy to popularize in daily clinical practice. Further, the dynamic evolution of each cardiac marker must not be overlooked. In a very recent result from ENGAGE AF-TIMI 48 trial, there were quite a large number of AF patients who experienced dynamic changes of NT-proBNP and hs-cTnT in the follow-up, and upward changes in these markers were associated with increased risk of IS/systemic TE (124). This might increase the burden of these markers on clinical application.

Markers of Routine Blood Test

The complete blood cell count is one of the most frequently ordered laboratory tests in clinical practice. Previous findings suggested that several parameters in routine blood tests, such as red blood cell distribution width (RDW), neutrophil-to-lymphocyte ratio (NLR), and mean platelet volume (MPV) might relate to the evaluation of IS in patients with NVAf.

RDW is a quantitative measurement of differences in the size and volume of circulating red blood cells, and increased RDW reflects the existence of erythrocytopenia, caused by impaired erythropoiesis or erythrocyte degradation, which reflect underlying chronic inflammation and high levels of oxidative stress state (125). In a cross-sectional study, RDW was independent correlated with the increase of CHADS₂ and CHA₂DS₂-VAsC score in patients with NVAf, suggesting that RDW could predict the risk of TE (126). Likewise, a high RDW (>13.16%) was shown to be associated with LA thrombosis in patients with NVAf in another study (127). In an up-to 5.2-year follow-up “real-world” retrospective cohort study, increased RDW value was independently associated with TE events in patients with NVAf (128). The result of a national study showed that the cumulative stroke incidence in AF patients not taking anticoagulants at baseline increased across RDW quartiles, and after adjusting for known conventional clinical risk factors, RDW was independently associated with stroke (129).

NLR is a marker of systemic inflammation. Specifically, the high neutrophil count reflects subclinical inflammation, while the decrease of lymphocyte count reflects an impairment of the

adaptive immune system and poor general health status (130). A preliminary study showed that NLR levels were significantly correlated with CHA₂DS₂-VASc score in NVAF patients (131). A small sample study showed that the level of NLR in patients with NVAF complicated with IS was higher than that of non-stroke patients (132). A subsequent large sample cohort study further revealed that the incidence rate of stroke increased across NLR quartiles in patients with AF, and NLR refined the risk of stroke across all CHA₂DS₂-VASc score strata (133).

MPV is considered to indicate the intensity of the inflammatory process and risk of thrombotic complications (134). Emerging evidence also supports the use of MPV as a biomarker for predicting IS risk in patients with AF. An earlier study showed that MPV was a predictive marker for stroke in patients with AF; its predictive power for stroke was independent of age, gender, and other CHADS₂ score components (135). In a study composed of 352 NVAF patients, high MPV was found to be an independent predictor of the composite of IS event and incidental LA thrombus (136). Gul et al. (137) found that MPV levels were significantly higher in acute IS patients with NVAF than those without NVAF. A study of NVAF patients who did not receive anticoagulant therapy showed that MPV was an independent predictor of IS in this population, and the combination of MPV and CHA₂DS₂-VASc score had improved predictive value and sensitivity, suggesting MPV could be used as a powerful tool for risk stratification of IS in patients with NVAF (138).

Coagulation Markers

As a component of Virchow's triad, hypercoagulability is considered an integral mechanism in the pathogenesis of thrombosis (139). Therefore, indicators of coagulation tests might have potential value in predicting AF-related IS. Studies have shown that D-dimer, von Willebrand factor (vWF), and fibrinogen may become new therapeutic targets or auxiliary diagnostic means to assist the risk stratification in AF-related IS.

D-dimer is a specific degradation product of cross-linked fibrin, and a biomarker indicating the activation of coagulation and fibrinolysis (140). In a cross-sectional study, D-dimer levels were positively associated with LA enlargement in anticoagulation-naïve patients with an acute IS and NVAF, suggesting that D-dimer could be helpful as a potential surrogate and predictive marker for adverse cardiovascular events in NVAF patients (141). Sub-analysis of several large RCTs testing the efficacy of direct oral anticoagulants vs. warfarin showed that greater levels of D-dimer were associated with higher frequencies of IS or systemic TE events (112, 142, 143). In a study of 509 NVAF patients, D-dimer level in combination with clinical risk factors could effectively predict subsequent TE events even when treated with warfarin (144). In a prospective observational study, AF patients with high levels of D-dimer have increased an risk of composite cardiovascular endpoint (myocardial infarction, stroke or TIA, and arterial embolic events) (145). In a retrospective study, the correlational analysis revealed that D-dimer levels are directly related to stroke volume, severity, and prognosis in patients with NVAF (146). However, a study of 323 NVAF patients, who did not receive anticoagulant

therapy, revealed that only the D-dimer level at stroke onset were independent risk factors for IS, while baseline D-dimer levels was not an independent risk factor for IS (147). Therefore, the dynamic detection of D-dimer levels might be necessary for patients with NVAF.

vWF is a plasma glycoprotein synthesized by endothelial cells during endothelial cell activation or injury, which promotes platelet adhesion and aggregation at the site of vascular injury, and is a definite marker of endothelial injury or dysfunction (148). Elevated vWF levels were found in patients with AF compared with healthy controls in an early study (149). A later cross-sectional study showed that raised plasma vWF was associated with four recognized risk factors for IS in AF patients (advancing age, prior IS, HF, and diabetes) (150). In a prospective study, plasma vWf levels were a significant predictor of stroke in NVAF patients taking aspirin, however, after adjustment for other clinical predictors, the relationship between vWf and stroke became non-significant (151). In a 3-year follow-up study, Pinto et al. (152) pointed that baseline vWF was a predictor of new-onset IS in patients with chronic NVAF. In another study over a median follow-up of 5.4 years, elevated plasma vWF was an independent risk factor for IS and all-cause death in patients with NVAF (153).

It has been already observed that the levels of fibrinogen were significantly higher in AF patients than in sinus rhythm patients (154). In patients with AF, those with a higher CHA₂DS₂-VASc score had increased fibrinogen level compared with those with a low risk of IS (155). Plasma fibrinogen level was associated with a history of stroke in NVAF patients in a case-control study (156). In addition, fibrinogen was also independently and positively associated with leukoaraiosis and periventricular hyperintensity in patients with stroke and AF (157).

Lipid Markers

Dyslipidemia is closely associated with cardiovascular disease and is the important predictor and therapeutic target of cardiovascular risk. After optimizing the stratification of risk factors other than CHA₂DS₂-VASc scores, a recently published meta-analysis showed that the levels of low-density lipoprotein cholesterol (LDL-C) and total cholesterol in the IS group were higher than those in the non-stroke group in NVAF patients (28). A case-control study showed that LDL-C is an independent predictor of IS in patients with NVAF and could improve stroke risk stratification (158). However, another large sample retrospective cohort study did not reach a consistent conclusion. The study carried by Omelchenko et al. (159) showed that LDL-C levels were not associated with the risk of IS in NVAF patients treated with oral anticoagulants, and they interpreted this lack of association as the high selectivity of patients (patient taking oral anticoagulants) and the high proportion of TE etiology for IS in AF patients. On the other hand, compliance with statins was associated with a reduced risk of recurrent IS in patients with AF, suggesting that AF status should not be a condition for excluding statins as a condition for secondary stroke prevention in patients with IS (160). High-density lipoprotein cholesterol (HDL-C) is an anti-atherosclerotic lipoprotein and is reported negatively correlated with the risk of IS (161). Our previous

case-control study has shown that the LDL-C/HDL-C ratio is a predictor of IS in patients with NVAF (162). Elevated LDL-C/HDL-C ratio may suggest that the imbalance between atherosclerotic and anti-atherosclerotic components, and the increase of pro-inflammatory components, which might both affect the occurrence and development of IS. In a small-sample study, NVAF patients with IS have higher levels of lipoprotein (a) [Lp(a)], and Lp(a) ≥ 30 mg/dL is associated with TE events in patients with NVAF (163). However, results from ARIC cohort showed that high Lp(a) levels were associated with increased IS risk, primarily among individuals without AF but not in those with AF (164). Overall, at present, the results of the relevant observational studies on blood lipids and IS in patients with NVAF are contradictory. High-quality evidence is lacking. Further studies are still needed to confirm the relationship between blood lipids and IS in patients with NVAF.

Oxidative Stress and Inflammation Biomarker

Oxidative stress and inflammation are tightly linked to AF (165). Therefore, markers of inflammation might be identified as predictors of AF-related IS. Numbers studies have shown that various inflammation markers, such as C-reactive protein (CRP), hyperuricemia, soluble CD40 ligand (sCD40L), homocysteine (Hcy), adiponectin (APN), growth differentiation factor 15 (GDF-15), circulating interleukins (IL) might be useful biomarkers for predicting AF-related IS.

CRP is the most commonly used measure of the inflammatory response. A high CRP level was associated with LA enlargement and depression of contractile function of LA in paroxysmal AF patients (166). Secondary analysis of SPAF III clinical trial (167) and RE-LY trial (168) showed that CRP was positively correlated to stroke risk in AF patients taking aspirin or oral anticoagulant.

Hyperuricemia is a known independent competing risk factor for AF (169). Recent studies also demonstrated that hyperuricemia was associated with IS among AF patients. Several case-control studies showed that uric acid level was closely associated with LA stasis (composed of LA thrombus, LASEC) in patients with NVAF (170–173). In a study of NVAF patients at clinically low-intermediate risk (CHA₂DS₂-VASc score = 0 or 1), uric acid levels were higher in those with transesophageal echocardiography (TEE) thromboembolic risk than in those without TEE risk (174). Hyperuricemia was shown to dependently predict IS after adjusting for CHA₂DS₂-VASc score and other comorbidities in a cohort study, and could further stratify low-risk patients into 2 groups with different stroke rates (175).

sCD40L has been considered as a marker of thrombosis and inflammation in several diseases. In patients with AF, the presence of LA thrombus was associated with significantly increased levels of sCD40L (176). In a study of 44 consecutive outpatients with chronic NVAF, plasma sCD40L was the independent variable for LASEC or LA thrombus formation, and for cerebrovascular events (177). Another larger study came to a similar conclusion, which enhanced soluble CD40L level was a predictor of fatal and non-fatal IS in patients with NVAF (178).

In an early study, hyperhomocysteinemia is associated with the presence of LA thrombus in stroke patients with NVAF (179). A later study showed that increased fasting Hcy levels were independently associated with a history of IS in NVAF patients hospitalized for cardiac reasons (180). AF and elderly patients were shown to have elevated Hcy levels, which might result in the correlation between high levels of Hcy and stroke in the elderly AF patients (181).

APN possesses anti-inflammatory and antiatherogenic effects. In a cross-sectional study, APN levels were higher in anticoagulated AF patients with LASEC, a LA thrombus, or a LAA thrombus (182). Additionally, AF patients at high risk of stroke disclosed low levels of APN (183). However, in a study of 918 stable anticoagulated outpatients with NVAF, APN was neither predictive of stroke/TE in both male and female patients (184).

GDF-15 is a peptide hormone and a divergent member of the transforming growth factor-beta superfamily (185). In a cross-sectional study, elevated GDF-15 was associated with the presence of LA/LAA thrombus in NVAF patients without anticoagulation (186). Insights from ARISTOTLE trial (187) and ENGAGE AF-TIMI 48 (122) also showed that GDF-15 was a risk factor for stroke in AF patients with anticoagulation therapy.

At present, researchers are also focusing on the correlation between other markers of inflammation and AF-related IS. In a recent pilot study, trimethylamine N-oxide (TMAO) was an independent predictor in IS in AF patients, and the level of TMAO was correlated with the CHA₂DS₂-VASc score (188). Results of a two-sample Mendelian randomization study showed a positive association of IL-1ra with cardioembolic stroke and inverse associations of IL-6 with cardioembolic stroke (189). In a 3-year follow-up study, baseline plasma levels of TNF- α and IL-6 are predictors of new-onset IS at follow-up in patients with chronic NVAF (152). In addition, evidence from a meta-analysis showed that increased circulating plasminogen activator inhibitor-1 and thrombin-antithrombin levels were significantly associated with subsequent stroke in patients with AF (190).

In general, many studies have shown that indicators of inflammatory could predict stroke in NVAF patients. However, these biomarkers are diverse and lack specificity. Therefore, more research is needed to find reliable inflammatory markers.

Fibrosis Markers

Cardiac (especially atrial) fibrosis is a critical feature of myocardial remodeling. The imaging manifestations of cardiac fibrosis, such as increased LAD, LA strain, and LGE, have been confirmed to be associated with an increased risk of IS in patients with AF as described previously. Several studies have also shown that circulating fibrosis biomarkers are associated with AF-related IS. High Gal-3 level was closely related to LAA flow velocity and occurrence of LAA thrombus in patients with NVAF (191). However, peripheral levels of circulating fibrosis biomarkers are susceptible to non-cardiac fibrosis, and might not be representative of the severity of cardiac fibrosis (192). Therefore, more research is required to explore the usefulness of circulating fibrosis in predicting AF-related IS in the future.

TABLE 2 | Major verified biomarkers adding in stroke/TE risk stratification beyond CHA₂DS₂-VASC score in AF patients.

Category	Biomarker	Supportive findings	Study population	DOI
ECG markers	Abnormal P-wave Axis	P ₂ -CHA ₂ DS ₂ -VASC score improved the C-statistic for CHA ₂ DS ₂ -VASC score. In ARIC study: C-statistic was 0.67 vs. 0.60, NRI = 0.25 (0.13, 0.39); In MESA study: C-statistic was 0.75 vs. 0.68 for CHA ₂ DS ₂ -VASC, NRI = 0.51 (0.18, 0.86).	AF patients	10.1161/CIRCULATIONAHA.118.035411
Cardiac imaging markers	Parameters of LAA shape	LAA shape parameters + CHA ₂ DS ₂ -VASC score increased the area under the ROC curve from 0.640 to 0.778 (<i>P</i> = 0.003).	AF patients	10.1007/s10554-021-02262-8
	LA strain	LA strain had an incremental value over the CHA ₂ DS ₂ -VASC score (<i>P</i> < 0.0001).	AF patients	10.1016/j.echo.2014.03.010
	Video intensity value of LASEC	Video intensity value of LASEC had better performance than CHA ₂ DS ₂ -VASC (0.844 ± 0.041 vs. 0.720 ± 0.065).	NVAF patients	10.1038/srep27650
	Left ventricular relative wall thickness	CHA ₂ DS ₂ -VASC + RWT increased the area under the ROC curve from 0.614 (0.5734–0.6562) to 0.624 (0.5823–0.6667), NRI = 0.25 (0.11–0.40).	NVAF patients	10.1093/ehjqqco/qcaa003
Atherosclerotic markers	cIMT, carotid plaque	C-statistics increased from 0.648 (95% CI, 0.538–0.757) to 0.716 (95% CI, 0.628–0.804) in the CHA ₂ DS ₂ -VASC score model after the addition of cIMT and carotid plaque as a vascular component (<i>P</i> = 0.013).	AF patients	10.3904/kjim.2019.099
	cIMT, carotid plaque	The addition of cIMT+plaque to the CHA ₂ DS ₂ -VASC score marginally increased the C-statistic from 0.685 (0.623–0.747) to 0.698 (0.638–0.759).	AF patients	10.1161/STROKEAHA.116.013133
Cardiac biomarkers	NT-proBNP	The addition of NT-proBNP to the CHA ₂ DS ₂ -VASC score increased the C-statistic from 0.62 (0.59–0.65) to 0.68 (0.56–0.71), NRI = 0.174 (<i>P</i> = 0.047).	AF patients	10.1161/STROKEAHA.113.003338
	NT-proBNP, cTnI	CHA ₂ DS ₂ -VASC + cTnI + NT-proBNP increased the C-statistic from 0.68 to 0.72 (<i>P</i> < 0.0001).	AF patients	10.1161/CIRCULATIONAHA.111.038729
	NT-proBNP	Adding NT-proBNP levels to the CHA ₂ DS ₂ -VASC score improved C-statistics from 0.62 to 0.65 (<i>P</i> = 0.0009)	AF patients	10.1016/j.jacc.2012.11.082
	BNP	Adding BNP to the CHA ₂ DS ₂ -VASC score improved C-statistics from 0.65 (0.56–0.75) to 0.75 (0.67–0.83), NRI = 0.76.	NVAF patients	10.1253/circ.CJ-17-1085
	Troponin, BNP, D-dimer	Combination of biomarkers had better AUROC for the prediction of stroke than CHA ₂ DS ₂ -VASC (0.378 ± 0.028 vs. 0.410 ± 0.028).	NVAF patients	Int J Health Sci (Qassim), 2019, 13(6): 3-12
	NT-proBNP	Adding NT-proBNP to the CHA ₂ DS ₂ -VASC score improved C-statistics from 0.624 to 0.666, NRI = 0.180.	AF patients	10.1136/heartjnl-2020-317735
	cTnI, NT-proBNP, D-dimer	Adding biomarkers to the CHA ₂ DS ₂ -VASC score improved C-statistics from 0.586 (0.565–0.607) to 0.708 (0.688–0.728), NRI = 0.594 (<i>P</i> < 0.001).	AF patients	10.1001/jamacardio.2016.3311
	cTnT	Adding cTnT to the CHA ₂ DS ₂ -VASC score improved the C statistic from 0.620 to 0.635 (<i>P</i> = 0.0226).	AF patients	10.1016/j.jacc.2013.07.093
Routine blood test markers	NLR	Adding NLR to the CHA ₂ DS ₂ -VASC score increased the AUC from 0.627 (0.612–0.643) to 0.635 (0.619–0.651).	AF patients	10.1111/jth.13006
	MPV, D-dimer	The addition of MPV and D-dimer to the CHA ₂ DS ₂ -VASC score increased the C-statistic from 0.761 to 0.816.	NVAF patients	10.1186/s12872-020-01525-x
Lipid markers	LDL-C	AUCs for CHA ₂ DS ₂ -VASC score and CHA ₂ DS ₂ -VASC score plus LDL-C were 0.591 and 0.674.	NVAF patients	10.1016/j.amjcard.2016.12.031
	LDL-C/HDL-C ratio	AUC of the CHA ₂ DS ₂ -VASC score plus LDL-C/HDL-C was higher than that of the CHA ₂ DS ₂ -VASC score (0.91 vs. 0.89, <i>Z</i> = 3.26, <i>P</i> < 0.01).	NVAF patients	10.1186/s12944-020-01392-7
Genetic markers	Genetic variants	Compared with CHA ₂ DS ₂ -VASC, the integrated tool improved net reclassification (NRI = 2.3%).	AF patients	10.1161/CIRCGEN.120.003168
Urine markers	Urine albumin	AUC of CHA ₂ DS ₂ -VASC-UA ₂ score was larger than that of CHA ₂ DS ₂ -VASC score (0.873 vs. 0.860, <i>P</i> < 0.01).	NVAF patients	10.1016/j.ijcard.2016.07.145

TE, thromboembolism; AF, atrial fibrillation; ECG, electrocardiogram; NRI, net reclassification improvement; LAA, left atrial appendage; LA, left atrium; LASEC, left atrial spontaneous echo contrast; NVAF, non-valvular atrial fibrillation; RWT, relative wall thickness; ROC, receiver operating characteristic curve; cIMT, carotid intima-media thickness; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; cTnI, cardiac troponin I; BNP, B-type natriuretic peptide; AUROC, area under the receiver operating characteristic curve; cTnT, cardiac troponin T; NLR, neutrophil-to-lymphocyte ratio; MPV, mean platelet volume.

NOVEL MARKERS OF GENETICS AND BIOINFORMATICS

At present, the research field of genetics and bioinformatics, and their applications to AF continue to evolve rapidly (193). Existing studies have identified a variety of genetic markers of AF-related IS through single nucleotide polymorphisms (SNP) analysis, genome-wide association study (GWAS), bioinformatic analysis, and omics. In an early study, a genetic risk score of twelve SNPs could identify individuals at increased risk for future AF and stroke (194). Based on the discovery of GWAS, copy number variation and SNPs could be genetic predictors of risk of TE and cardioembolic stroke for patients with AF (195–197). In a recent update study using data from the largest available GWAS in Europeans, a polygenic risk score incorporated of over half a million genetic variants could significantly improve net reclassification compared with CHA₂DS₂-VAsC score in predicting IS in patients with AF (198). Two studies analyzed datasets of Gene Expression Omnibus via bioinformatic analysis, respectively, and identified several genes which were involved in AF-related stroke (199, 200). In addition, studies have shown that abnormal expression of non-coding RNAs, such as lncRNA ANRIL, hsa-miR-22-3p, was associated with functional outcome or prognosis in AF patients, and could potentially serve as potential biomarkers for AF-related IS (201, 202). On the basis of current research, it can be expected that new and promising genetics biomarkers for AF-related IS will be further discovered in the near future.

SUMMARY AND PERSPECTIVE

Biomarkers have become an important integral to the clinical practice of AF. The purpose of this review is to acquaint clinicians and researchers with the progress of biomarkers in AF-related IS. In summary, although a great deal of research has been done on biomarkers for IS prediction in NVAf patients as mentioned above, most potential biomarkers have not yet been translated into clinical use. Nevertheless, these biomarkers can help us to better understand the etiology and pathophysiology of AF-related IS.

An ideal biomarker should be simple, practical, inexpensive, and with high sensitivity. Based on current evidence, we acknowledge that non-paroxysmal AF type, carotid plaque, cardiac troponin, NT-proBNP, and D-dimer are promising biomarkers for IS in NVAf patients since these biomarkers strike a balance between practicality and simplicity. They are easily acquired in clinical practice. Meanwhile, these markers are cardiac-specific or reflecting AF features and pathophysiological processes of stroke. Moreover, the clinical value of these markers has been confirmed by multiple studies.

It is important to recognize, however, that the existing studies have significant limitations. First, most studies are observational studies with small samples size, which limits the clinical value of the identified markers. At the same time, limited by the study design, most studies investigate the correlation between only one biomarker and IS in AF patients, and a

single biomarker might be disturbed by other confounding factors, and only a few studies evaluate the role of multiple biomarkers (203). Second, the majority of the study population was treated patients with anticoagulation therapy, and studies focusing on un-anticoagulated patients and other populations are lacking. Third, the end-points of the studies are not uniform, such as IS, systemic TE, TIA, and the combination of them. Additionally, the inclusion criteria and covariates are inconsistent between the studies. Forth, there may be a time-dependent correlation between some biomarkers and IS outcome, and the assessment of a baseline level of the biomarker may not draw a reliable conclusion. For different research investigating the same biomarker, the cut-off values of the biomarker are often incongruent, which makes it impossible to combine the results of the studies. Fifth, the majority of studies merely find the differentially expressed biomarker in AF patients with stroke compared with those without stroke. Almost all the studies fail to show a valuable improvement in clinical usefulness, although a slightly improved predictive performance for IS compared with the commonly used risk score is shown in some studies (Table 2) (204, 205).

Incorporating biomarkers into existing models may allow improved predictive accuracy and guide individualized anticoagulation treatment, but it brings complexity. Given these uncertainties, what should the clinician do? First, we believe it is still necessary to discover novel biomarkers and verify the predictive value of current markers in large-scale prospective cohort studies. Studies in patients with CHA₂DS₂-VAsC score of 0–1 are encouraged, thereby improving the prognosis of patients who are not provided with a clear indication for oral anticoagulants in current guidelines. In addition, it is important to find biomarkers that could distinguish between ischemic and hemorrhagic stroke, because most existing indicators in CHA₂DS₂-VAsC score indicate both bleeding and ischemia. Second, in view of the complexity and interdependence of pathophysiological pathways for AF-related IS, multi-omics and high-throughput analysis should be used to find multiple biomarkers to discover new therapeutic targets. Third, comprehensive studies are needed to integrate current biomarkers and clinical score to optimize the prevention of IS in patients with NVAf. At last, the effectiveness of the “biomarker plus CHA₂DS₂-VAsC” score-guided treatment strategy in NVAf patients should also be evaluated.

AUTHOR CONTRIBUTIONS

LXS, LZ, YKG, HXS, XXZ, and YKB searched literatures and prepared the manuscript. XHZ and BPT provided funds support, conceived the idea, reviewed the drafts, and provided important information for the completion of this manuscript. All authors contributed to the writing and final approval of the manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Prevalence of Cardiovascular Events and Their Risk Factors in Patients With Chronic Obstructive Pulmonary Disease and Obstructive Sleep Apnea Overlap Syndrome

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Rationale: Chronic obstructive pulmonary disease (COPD) and obstructive sleep apnea (OSA) have been identified as independent risk factors for cardiovascular diseases. However, the impact of COPD and OSA overlap syndrome (OS) on cardiovascular outcomes remains to be elucidated.

Objective: To determine the prevalence of cardiovascular events and their risk factors in OS patients.

Methods: Seventy-four patients who had OS between January 2015 and July 2020 were retrospectively enrolled, and 222 COPD-only patients and 222 OSA-only patients were pair-matched for age and sex from the same period and served as the OS-free control group. The prevalence rates of coronary heart disease (CHD), arrhythmia, heart failure, and pulmonary arterial hypertension (PAH) were compared among the three groups, and multivariable logistic regression models were used to screen the risk factors for specific cardiovascular events.

Results: OS patients had higher prevalence rates of heart failure (10.8 vs. 0.5 and 1.4%, respectively) and PAH (31.1 vs. 4.5 and 17.1%, respectively) than those with OSA alone or COPD alone (all $P < 0.01$). The CHD prevalence was also significantly higher in the OS group than in the COPD-alone group (25.7 vs. 11.7%, $P < 0.01$). There was no significant difference in the prevalence of arrhythmia among the three groups (20.3, 22.5, and 13.1%, respectively, $P > 0.05$). In OS patients, risk factors for CHD included hypertension, diabetes, body mass index, lactate dehydrogenase level, and tidal volume; risk factors for heart failure included diabetes, partial pressure of oxygen, partial pressure of carbon dioxide, maximum ventilatory volume, and neutrophilic granulocyte percentage; and risk factors for PAH included minimum nocturnal oxygen saturation, partial pressure of carbon dioxide, and brain natriuretic peptide and lactate dehydrogenase levels.

Conclusions: OS patients have a higher prevalence of cardiovascular events, which is associated with hypoxemia, hypercapnia, and impaired lung function in these patients.

Keywords: chronic obstructive pulmonary disease, risk stratification, cardiac rhythm abnormalities, obstructive sleep apnea, overlap syndrome, cardiovascular events

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) and obstructive sleep apnea (OSA) are among the most prevalent chronic diseases and represent a major reason for health-care utilization, imposing a heavy health burden worldwide (1–3). The prevalence of COPD in American adults is estimated to be 13.9% (4, 5) compared with 13.7% in Chinese adults aged 40 years or older (6), and the incidence of OSA in adults is close to 9–26% (7). Existing epidemiological data suggest that overlap syndrome (OS), which refers to the coincidence of both COPD and OSA (8), is not a common disease in the general population (incidence ranges from 1 to 3.6%), but patients who already have COPD or OSA exhibit a significant increase in the incidence of overlap syndrome (2.9–65.9%) (9).

COPD is characterized by incomplete reversible airflow obstruction that increases the risk of cardiovascular disease through increased sympathetic nervous activity and persistent low-grade systemic inflammation (10, 11). Obstruction of the upper airway during sleep causes OSA, which leads to periodic apnea and hypopnea, resulting in nocturnal hypoxemia. Patients with OSA usually complain of severe snoring, problems with being awakened by wheezing or suffocation, and daytime sleepiness (12, 13). OSA has also been recognized as a risk factor for cardiovascular diseases. Possible pathophysiological mechanisms include transient and repeated oxygen desaturation, which could induce oxidative stress, systemic inflammation, endothelial dysfunction, and autonomic dysfunction (14).

Under the synergistic effect of COPD and OSA, patients with OS have lower nocturnal oxygen saturation, more profound oxidative stress, more serious systemic inflammation, more severe vascular endothelial dysfunction, and accelerated atherosclerosis (15–17), all of which are believed to be strong risk factors for cardiovascular disease. It is well known that COPD and OSA are independent risk factors for cardiovascular events. However, real-world evidence concerning the prevalence and related risk factors for cardiovascular diseases in patients with COPD and OSA overlap syndrome has rarely been reported. Therefore, this study aimed to evaluate the prevalence of specific cardiovascular events and their risk factors in patients with COPD and OSA overlap syndrome to help improve the management of these patients.

METHODS

Study Design

This is a cross-sectional study, and anonymized clinical data were collected from the Biobank of the First Affiliated Hospital of Xi'an Jiaotong University from January 2015 to July 2020. The Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong

University approved this study (no. XJTU1AF2020LSK-187), and informed consent was obtained. All methods were carried out in accordance with the relevant guidelines and regulations according to the principles expressed in the Declaration of Helsinki.

Subjects

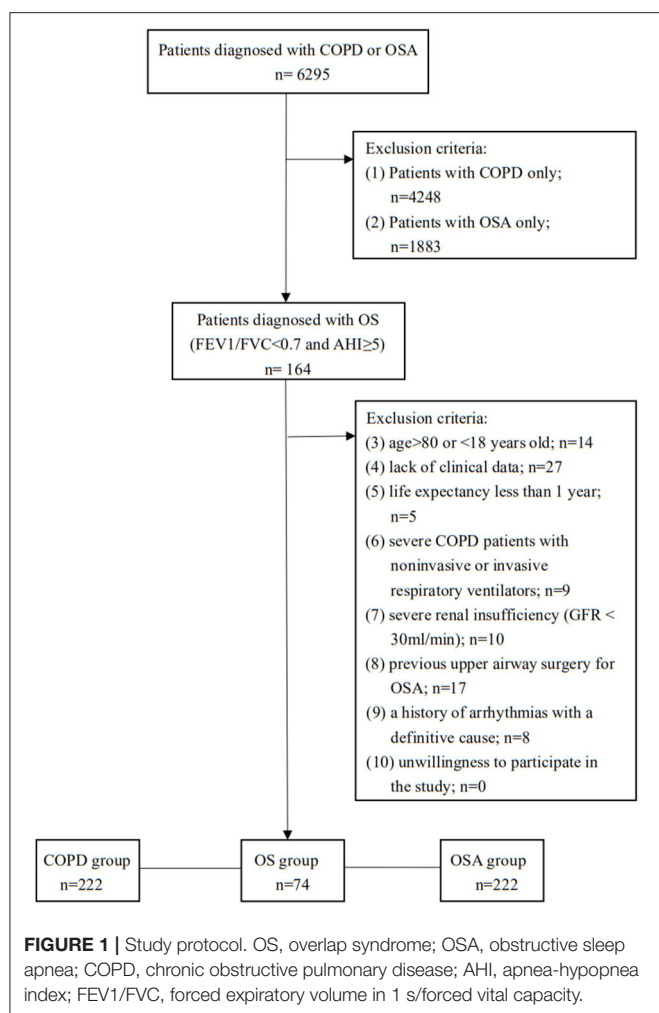
The medical records of all patients who were diagnosed with COPD or OSA were retrospectively analyzed. The diagnoses were based on clinical expert consensus documents. COPD should have been considered in any patient with dyspnea, chronic cough or sputum production, a history of exposure to risk factors for the disease, and a post-bronchodilator forced expiratory volume in 1 s (FEV1)/forced vital capacity (FVC) < 0.70 as measured by spirometry. OSA was defined as symptoms such as excessive daytime sleepiness, recurrent awakenings from sleep, daytime fatigue, and impaired concentration, as well as overnight monitoring demonstrating five or more obstructed breathing events per hour during sleep (apnea/hypopnea index > 5). OS was defined as the simultaneous occurrence of COPD and OSA in the same patient (8, 18, 19). The exclusion criteria were as follows: (1) age > 80 or < 18 years old; (2) lack of clinical data; (3) life expectancy less than 1 year; (4) severe COPD patients with noninvasive or invasive respiratory ventilators; (5) severe renal insufficiency (glomerular filtration rate < 30 ml/min); (6) previous upper airway surgery for OSA; (7) a history of arrhythmias with a definitive cause (unrelated to the disease under study); and (8) unwillingness to participate in the study. The enrolled cohort was then divided into three groups: (1) OS group, (2) OSA-alone group, and (3) COPD-alone group. The two control groups were pair-matched with the OS group with respect to age and sex at a ratio of 1:3 (Figure 1).

Data Collection

All data, including medical history, personal history, and laboratory examination, were obtained from the electronic medical record system of the hospital and recorded using a standardized protocol. Pulmonary function tests were performed according to the guidelines of the American Thoracic Society (20), and full-night polysomnography was performed in accordance with those of the American Academy of Sleep Medicine (21). All clinical diagnoses were made by professional staff according to standardized criteria.

Outcomes Measures

The primary endpoint in this study was to explore the prevalence rates of four specific types of cardiovascular diseases—coronary heart disease (CHD), heart failure, arrhythmia, and pulmonary arterial hypertension (PAH)—and whether there was any difference in prevalence between patients with OS and those



with OSA alone or COPD alone. The secondary endpoint was to screen the risk factors for these diseases.

Statistical Analysis

The Kolmogorov–Smirnov test was used to test the normality of each data distribution. Categorical variables are reported as counts and percentages, whereas continuous variables are reported as the mean (\bar{X}) and standard deviation. When comparing groups, the Kruskal–Wallis test was used if the data were not normally distributed, and analysis of variance with a *post-hoc* test was used if the data were normally distributed for continuous variables. The chi-squared test was used for categorical variables. When the ratio of variables to missing values was less than 5, the median of quantitative variables or the most common attribute values of qualitative data were used as simple assignments. Univariable logistic regression models were used to characterize OS compared with COPD alone or OSA alone, and multivariable logistic regression was used to explore the risk factors for cardiovascular diseases. Statistical analyses were conducted with SPSS v18.0.0. $P < 0.05$ were considered statistically significant.

RESULTS

Characteristics of Study Participants

Of the 6,295 patients initially recruited to the study, 4,412 had COPD as the main diagnosis, and 2047 had OSA. Through a detailed screening, 74 consecutive patients identified to have overlap syndrome were enrolled in the OS group; 222 patients paired-matched for age and sex were enrolled in the OSA-alone group, and the same method was used to form the COPD-alone group (Figure 1).

In this study, the prevalence rate of COPD in OSA patients was 8.01% (164/2047), and the prevalence rate of OSA in COPD patients was 3.72% (164/4412). The clinical characteristics of the study subjects with OS, OSA alone, and COPD alone are described in Table 1. The study cohort was predominantly composed of men (70%), with a mean age of 60.95 ± 9.35 years. The smoking index ($P < 0.05$), body mass index (BMI) ($P < 0.01$), and partial pressure of carbon dioxide (PCO_2) ($P < 0.01$) were higher, and the partial pressure of oxygen (PO_2) ($P < 0.01$) and oxygen saturation (SaO_2) ($P < 0.01$) were lower in subjects with overlap syndrome than in those from the other two groups. The patients with OS had higher serum levels of brain natriuretic peptide (BNP), lactate dehydrogenase (LDH), and neutrophil granulocytes than the patients with OSA alone (all $P < 0.05$). However, the serum troponin levels and the number of patients who smoked or drank alcohol were similar between the three groups (Table 1).

Regarding medications, the number of patients using nifedipine and spironolactone in the OS group was significantly higher than that in the other two groups ($P < 0.01$), and the number of patients using angiotensin-converting enzyme inhibitors/angiotensin receptor blockers and beta-blockers in the OS group was significantly higher than that in the COPD-alone group ($P < 0.01$). The use of medications such as amiodarone, warfarin, or rivaroxaban was not significantly different among the three groups (Table 1).

For comorbidities, the Charlson comorbidity index was significantly higher in the OS group than in the COPD-alone group or OSA-alone group ($P < 0.05$). Six common comorbidities in these cohorts were evaluated: the prevalence of hypertension and pulmonary thromboembolism (PTE) in the OS group was significantly higher than that in the other two groups ($P < 0.01$); the prevalence of stroke in the OS group was higher than that in the COPD-alone group ($P < 0.05$); and the prevalence of venous embolism of the extremities in the OS group was higher than that in the OSA-alone group ($P < 0.05$). However, no significant difference was observed in the prevalence of diabetes among the three groups (Table 2).

Polysomnography

In the overlap syndrome population, most patients were categorized as having mild/moderate OSA (mild = 39.13%, moderate = 34.78%), whereas, in the OSA-alone group, nearly half of the patients (42.68%) had severe OSA (Table 2). In the sleep testing study, the apnea/hypopnea index was much higher (30.67 ± 18.73 vs. 23.04 ± 19.51 , $P < 0.05$), and the mean oxygen saturation (mean SpO_2 ; 89.09 ± 4.43 vs.

TABLE 1 | Characteristics of patients with OS, OSA, and COPD.

	OS	OSA alone	COPD alone	P-value	P-value	P-value
	n = 74	n = 222	n = 222		OS and OSA	OS and COPD
Age (year)	60.95 ± 9.35	60.72 ± 9.34	60.76 ± 9.30	0.984		
Male, n (%)	52 (70)	156 (70)	156 (70)			
BMI (kg/m ²)	26.89 ± 3.39	23.29 ± 3.45	22.53 ± 3.76	< 0.001	<0.001	<0.001
Tobacco use, n (%)	17 (22.97)	30 (13.5)	35 (15.77)	0.155		
Smoking index	958.82 ± 128.35	665 ± 444.43	712.57 ± 380.57	0.049	0.03	0.041
Alcohol use, n (%)	1 (1.4)	8 (3.6)	2 (9.0)	0.351		
Laboratory data						
PO ₂ (mmHg)	59.69 ± 15.06	76.20 ± 17.54	74.66 ± 19.96	<0.001	<0.001	<0.001
PCO ₂ (mmHg)	56.55 ± 14.64	43.94 ± 11.62	47.36 ± 12.65	<0.001	<0.001	0.493
SpO ₂ (%)	86.38 ± 11.74	92.12 ± 9.54	92.87 ± 5.87	<0.001	<0.001	<0.001
D ₂ polymers (mg/L)	1.24 ± 1.50	0.74 ± 1.48	1.50 ± 3.58	<0.001	0.187	0.476
CRP (mg/dl)	15.16 ± 32.79	8.82 ± 37.37	30.79 ± 36.66	<0.001	0.339	0.008
Neutrophil granulocyte (%)	70.40 ± 10.84	63.71 ± 72.14	72.14 ± 12.18	<0.001	<0.001	0.199
TC (mmol/L)	3.94 ± 1.41	3.99 ± 1.14	3.90 ± 0.94	0.65		
TG (mmol/L)	1.55 ± 1.64	1.89 ± 1.33	1.19 ± 0.72	0.006	0.184	0.262
LDL (mmol/L)	2.34 ± 1.07	2.37 ± 0.83	2.04 ± 0.61	0.085		
BNP	1,198.65 ± 2,523.06	434.25 ± 1,316.00	1,147.23 ± 2,930.81	0.017	0.032	0.884
cTnT	0.03 ± 0.03	0.06 ± 0.47	0.10 ± 0.54	0.628		
cTnI	25.43 ± 46.69	11.40 ± 34.51	20.71 ± 38.50	0.298		
CK (U/L)	82.85 ± 108.58	119.53 ± 273.77	86.31 ± 194.15	0.183		
CKMB (U/L)	13.89 ± 5.90	15.41 ± 23.69	14.78 ± 9.70	0.789		
LDH (U/L)	264.19 ± 72.47	213.04 ± 60.11	252.45 ± 111.49	0.000	<0.001	0.329
Medications						
ACEI/ARB, n (%)	30 (40.5)	106 (47.7)	40 (18.0)	<0.001	0.281	<0.001
β-blockers, n (%)	22 (29.7)	95 (42.8)	41 (18.5)	<0.001	0.047	0.04
Nifedipine, n (%)	28 (37.8)	52 (23.4)	45 (20.3)	0.009	0.016	0.002
Antisternerone, n (%)	26 (35.1)	33 (14.9)	36 (16.2)	<0.001	<0.001	0.001
Diuretic, n (%)	18 (24.3)	6 (2.7)	45 (20.3)	<0.001	<0.001	0.461
Amiodarone, n (%)	1 (1.4)	6 (2.7)	9 (4.1)	0.507		
Warfarin, n (%)	3 (4.1)	3 (1.4)	6 (2.7)	0.318		
Rivaroxaban, n (%)	5 (6.8)	9 (4.1)	10 (4.5)	0.604		
Aspirin, n (%)	29 (38.2)	151 (68.0)	45 (20.3)	<0.001	<0.001	0.001
Clopidogrel, n (%)	7 (9.5)	95 (42.8)	28 (12.6)	<0.001	<0.001	0.467
Comorbidities						
CCI	3.43 ± 1.58	2.85 ± 1.55	3.03 ± 1.31	0.012	0.003	0.043
Hypertension, n (%)	45 (60.8)	55 (24.8)	66 (29.7)	<0.001	<0.001	<0.001
SBP (mmHg)	135.18 ± 21.33	138.79 ± 21.52	126.77 ± 19.41	<0.001	0.279	0.016
DBP (mmHg)	82.38 ± 12.80	81.29 ± 13.75	79.93 ± 10.85	0.452		
Diabetes mellitus, n (%)	16 (21.6)	67 (30.2)	27 (12.2)	<0.001	0.156	0.051
Fatty liver, n (%)	2 (2.7)	24 (10.8)	3 (1.4)	<0.001	0.033	0.602
Stroke, n (%)	15 (20.3)	46 (20.7)	23 (10.4)	<0.001	0.93	0.027
PTE, n (%)	4 (5.4)	0	1 (0.5)	<0.001	<0.001	0.004
Venous embolism of the extremities, n (%)	4 (5.4)	1 (0.5)	7 (3.2)	<0.001	0.015	0.476

COPD, chronic obstructive pulmonary disease; OSA, obstructive sleep apnea; OS, overlap syndrome; PCO₂, partial pressure of carbon dioxide; PO₂, oxygen partial pressure; SpO₂, oxygen saturation; CRP, C-reactive protein; TC, cholesterol; TG, triglyceride; LDL, low-density lipoprotein; cTnI, cardiac troponin I; cTnT, cardiac troponin T; CK, creatine kinase; CKMB, creatine kinase-MB; LDH, lactate dehydrogenase; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; SBP, systolic blood pressure; DBP, diastolic blood pressure; CCI, Charlson Comorbidity Index; PTE, pulmonary embolism.

TABLE 2 | Polysomnographic findings of the study population.

	OS <i>n</i> = 74	OSA alone <i>n</i> = 222	<i>P</i> -value
Severity of OSA, <i>n</i> (%)			
Mild	29 (39.13)	89 (40.24)	0.891
Moderate	26 (34.78)	38 (17.07)	0.001
Severe	19 (26.08)	95 (42.68)	0.009
AHI	30.67 ± 18.73	23.04 ± 19.51	0.029
meanSpO ₂ , %	89.09 ± 4.43	91.61 ± 34.03	0.001
minSpO ₂ , %	64.09 ± 20.30	70.66 ± 30.72	0.141
timeSpO ₂ <90%, %	27.92 ± 26.00	25.92 ± 24.76	0.722
Sleep architecture, %TST			
Stage 1 sleep	12.98 ± 14.00	11.3 ± 10.02	0.884
Stage 2 sleep	52.23 ± 18.28	58.14 ± 28.72	0.313
Stage 3 sleep	20.22 ± 15.69	19.54 ± 15.78	0.776
Stage REM sleep	9.85 ± 7.63	10.32 ± 7.36	0.761

OSA, obstructive sleep apnea; OS, overlap syndrome; AHI, apnea-hypopnea index; CT90, percentage of total sleep time with oxygen saturation spent < 90%; TST, total sleep time; REM, rapid eye movement.

91.61 ± 34.03, $P < 0.01$) was much lower in the OS group than in the OSA-alone group, whereas the minimum SpO₂ and sleep architecture were not different between the two groups.

Pulmonary Function Test

There was no significant difference in the distribution of COPD severity between the OS group and COPD-alone group ($P > 0.05$), and FEV1% predicted was also comparable in both groups ($P > 0.05$). However, the FEV1/FVC ratio was significantly lower in the OS group than in the COPD alone group. Vital capacity and maximum ventilatory volume (MVV), which represent lung volume and pulmonary ventilation function, were lower in patients with overlap syndrome than in those with COPD alone (Table 3).

Prevalence of Cardiovascular Events

The prevalence rates of four cardiovascular events in the OS, OSA, and COPD groups were 25.7, 31.5, and 11.7% for CHD, 20.3, 22.5, and 13.1% for arrhythmias, 10.8, 0.5, and 1.4% for heart failure, and 31.1, 4.5, and 17.1% for PAH, respectively. The prevalence of heart failure and PAH in subjects with OS was significantly higher than in those with OSA or COPD alone ($P < 0.01$), and the prevalence of CHD in subjects with OS was higher than in those with COPD alone ($P < 0.01$). However, the prevalence of arrhythmias (such as atrial fibrillation, premature atrial contraction, ventricular premature contraction, and atrioventricular or ventricular tachycardia) was not significantly different between the OS group and OSA or COPD groups (Figure 2 and Table 4).

Risk Factors for Cardiovascular Events

Regarding the risk factors for cardiovascular diseases, multivariate logistic regression showed that hypertension,

TABLE 3 | Pulmonary function tests and GOLD classification of the study population.

	OS <i>n</i> = 74	COPD alone <i>n</i> = 222	<i>P</i> -value
Severity of COPD, <i>n</i> (%)			
GOLD 1	8 (10.81)	27 (12.16)	0.475
GOLD 2	24 (32.43)	93 (41.89)	0.994
GOLD 3	22 (29.72)	46 (20.72)	0.488
GOLD 4	20 (27.02)	56 (25.23)	0.287
FEV1/FVC ratio (%)	62.45 ± 25.51	56.14 ± 19.08	0.001
FEV1 (% predicted)	46.29 ± 24.41	48.06 ± 22.70	0.327
VC (%)	2.11 ± 0.70	2.15 ± 0.96	0.012
TV (%)	1.21 ± 0.61	1.29 ± 0.71	0.202
MVV (cmH ₂ O/L/S)	38.65 ± 21.88	53.43 ± 37.82	< 0.001

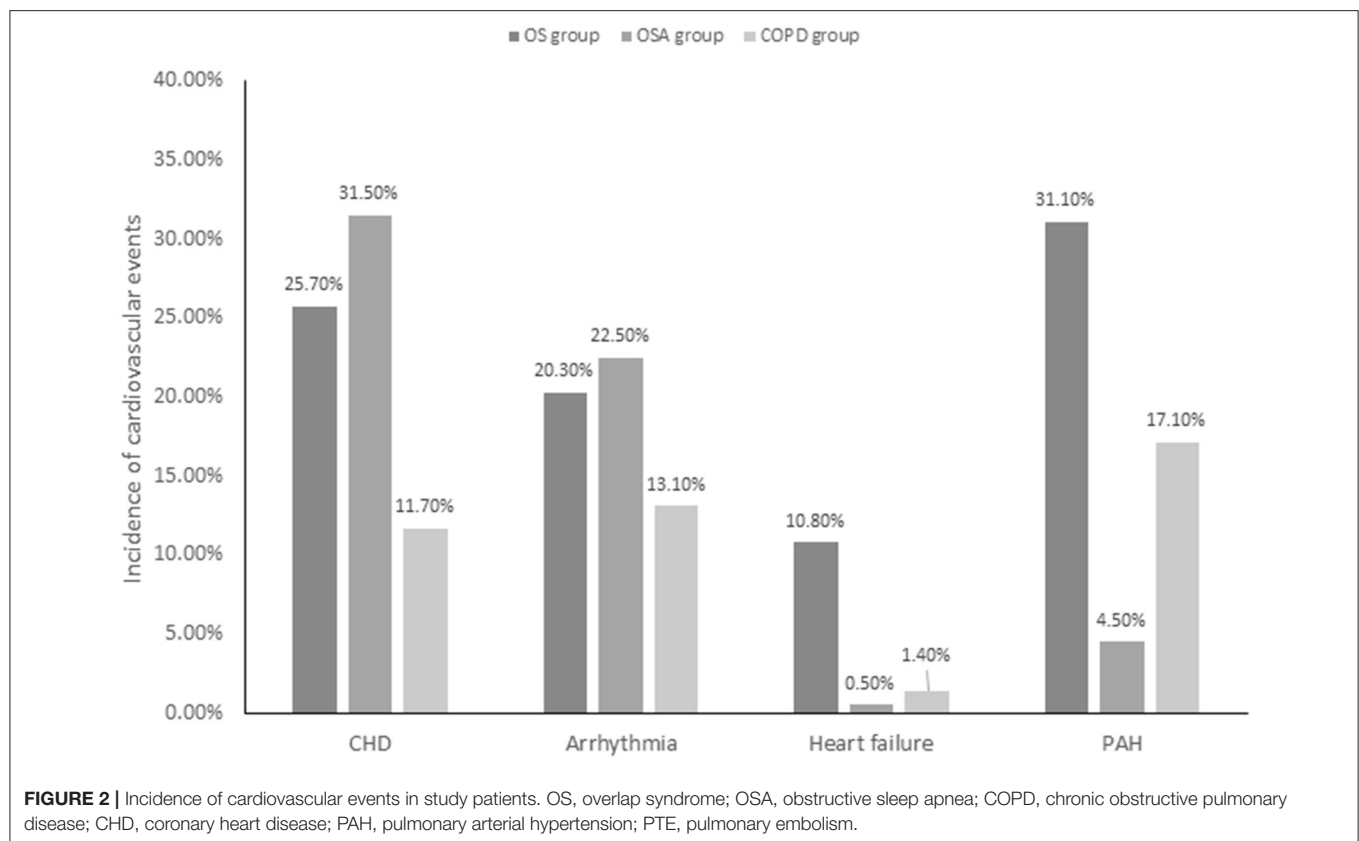
COPD, chronic obstructive pulmonary disease; OS, overlap syndrome; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; VC, vital capacity; TV, tidal volume; MVV, maximum ventilatory volume; GOLD, Global Initiative for Chronic Obstructive Lung Disease.

diabetes, stroke, LDH level, BMI, and tidal volume were positively correlated with CHD prevalence; diabetes, PTE, PCO₂, PO₂, MVV, and neutrophil granulocyte percentage were correlated with heart failure prevalence; and PTE, PCO₂, BNP and LDH levels, and mean SpO₂ were correlated with PAH prevalence (Table 5).

DISCUSSION

In this study, a significant increase in the prevalence of cardiovascular events was observed in the OS population. Patients with OS had a higher prevalence of heart failure and PAH than those with COPD alone or OSA alone ($P < 0.01$), and the prevalence of CHD was also higher in the OS group than in the COPD-alone group ($P < 0.01$). In patients with OS, risk factors for CHD included hypertension, diabetes, stroke, BMI, LDH level, and tidal volume; risk factors for heart failure included diabetes, PTE, PO₂, PCO₂, MVV, and neutrophilic granulocyte percentage; and risk factors for PAH included PTE, PCO₂, minSpO₂, PCO₂, and BNP and LDH levels. To the best of our knowledge, this study initially identifies the prevalence and risk factors for cardiovascular events in OS patients in an Asian population compared with both OSA-alone patients and COPD-alone patients.

Although the underlying mechanisms of exactly how OS contributes to cardiovascular events have not been fully elucidated, there are many pathways by which cardiovascular events occur in the presence of OS. The association between the burden of nocturnal hypoxemia, inflammation, and cardiovascular disease in our study is consistent with that found in other clinical investigations in this field (15, 22). In addition, we found that daytime hypoxemia



and hypercapnia also contribute to the development of cardiovascular disease.

Hypoxemia is thought to act as a cardiovascular risk factor by increasing cardiac load, inducing oxidative stress by concomitant reactive oxygen species release, impairing vascular endothelial function (23), causing electrophysiological instability in the cardiac conduction system (24), and leading to severe metabolic disorder. Chronic hypoxia and hypercapnia caused by COPD and intermittent nocturnal hypoxia and sleep deprivation caused by OSA can lead to a decrease in the sensitivity of the respiratory center to both hypoxemia and hypercapnia stimulation in patients with OS, leading to further aggravated hypoxemia and hypercapnia. Previous studies confirmed that OS patients have more serious nocturnal hypoxemia, which has been considered a risk factor for cardiovascular diseases (22). However, our study evaluated not only nocturnal hypoxemia but also daytime hypoxemia and hypercapnia, and we found that patients with OS also had higher levels of daytime hypoxemia and hypercapnia than the COPD group or OSA group. Therefore, together with nocturnal hypoxemia, daytime hypoxemia and hypercapnia may have a synergistic effect on promoting cardiovascular diseases. This finding may have clinical implications for developing a better strategy for oxygen therapy in patients with OS.

Hypoxia can upregulate the expression of systemic inflammatory mediators (23, 24) and cause systemic

inflammation, which is considered to be another important factor in cardiovascular diseases. There are many common molecular signaling pathways between COPD and OSA, such as C-reactive protein, interleukin 6, and nuclear factor kappa B. Their interaction can cause a systemic inflammatory response and increase body oxidative stress, leading to cardiovascular diseases. Baseline sustained hypoxemia in patients with OS predisposes them to other molecular responses relevant to the mechanisms of cardiovascular disease, especially *via* activation of the transcription factor pathway mediated by hypoxia-inducible factor-1 alpha (25) and related downstream products such as vascular endothelial growth factor. In this study, the level of serum C-reactive protein, a marker of inflammation, was higher in patients with overlap syndrome than in patients with COPD alone, indicating that OSA increased body's inflammatory stress in patients with COPD. There was no significant difference in serum C-reactive protein levels between the OS and OSA-alone groups. One plausible reason is that, in this study, the vast majority of patients in the OSA-alone group were admitted to the hospital for arrhythmias, and these patients already have a higher level of inflammation.

The prevalence of heart failure and PAH in subjects with OS in this study was significantly higher than in those with OSA or COPD alone, and the prevalence of CHD in subjects with OS was higher than in those with COPD alone, which

TABLE 4 | Incidence of cardiovascular events in study patients.

	OS <i>n</i> = 74	OSA alone <i>n</i> = 222	COPD alone <i>n</i> = 222	<i>P</i> -value	<i>P</i> -value OS and OSA	<i>P</i> -value OS and COPD
CHD, <i>n</i> (%)	19 (25.7)	70 (31.5)	26 (11.7)	<0.001	0.341	0.004
Arrhythmia, <i>n</i> (%)	15 (20.3)	50 (22.5)	29 (13.1)	0.011	0.685	0.131
Atrial arrhythmia, <i>n</i> (%)	9 (12.2)	23 (10.4)	15 (6.8)	0.253		
AF	8 (10.8)	16 (7.2)	12 (5.4)	0.280		
PAC	1 (1.4)	4 (1.8)	2 (0.9)	0.874		
AT	0	3 (1.4)	1 (0.5)	0.525		
Ventricular arrhythmias, <i>n</i> (%)	2 (2.7)	9 (4.1)	4 (1.8)	0.397		
VPB	1 (1.4)	7 (3.2)	2 (0.9)	0.247		
PSVT	1 (1.4)	1 (0.5)	1 (0.5)	0.527		
VT	0	1 (0.5)	1 (0.5)	1		
CRBBB, <i>n</i> (%)	1 (1.4)	6 (2.7)	3 (1.4)	0.628		
CLBBB, <i>n</i> (%)	0	1 (0.5)	1 (0.5)	1		
SSS, <i>n</i> (%)	1 (1.4)	0	1 (0.5)	0.266		
AVB, <i>n</i> (%)	0	2 (0.9)	1 (0.5)	1		
Heart failure, <i>n</i> (%)	8 (10.8)	1 (0.5)	3 (1.4)	<0.001	<0.001	0.001
PAH, <i>n</i> (%)	23 (31.1)	10 (4.5)	38 (17.1)	<0.001	<0.001	0.01
Mild	18 (24.3)	6 (2.7)	15 (6.8)	<0.001	<0.001	<0.001
Moderate	4 (5.4)	3 (1.4)	12 (5.4)	<0.001	0.068	1
Severe	1 (1.4)	1 (0.5)	11 (5.0)	<0.001	0.438	0.306

CHD, coronary heart disease; AF, atrial fibrillation; PAC, premature atrial contraction; AT, atrial tachycardia; VPB, ventricular premature beat; PSVT, paroxysmal supraventricular tachycardia; VT, ventricular tachycardia; CRBBB, complete right bundle branch block; CLBBB, complete left bundle branch block; SSS, sick sinus syndrome; AVB, atrioventricular block.

has rarely been reported in real-world studies. No statistically significant difference in the prevalence of arrhythmia between the three groups was found in this study, although there have been studies confirming that patients with OS are at greater risk of AF than those with either COPD or OSA alone (26).

Multivariate logistic regression analysis showed that the prevalence rate of cardiovascular events in this study was correlated with hypertension, diabetes, stroke, PTE, BMI, BNP level, decreased pulmonary function, and nocturnal hypoxemia, which were already believed to be risk factors for cardiovascular diseases (22, 27). In logistic regression analysis, the risk factors for cardiovascular events included daytime hypoxemia and hypercapnia. Therefore, the high incidence of cardiovascular events in patients with OS was speculated to be ultimately caused by hypoxia, hypercapnia, and systemic inflammation. In our study, arterial blood gas analysis was performed in all patients to assess daytime hypoxemia and hypercapnia and is essential in identifying the promoting effect of daytime hypoxemia and hypercapnia on cardiovascular diseases. The benefits of continuous positive airway pressure (CPAP) are now clearly established in patients with OS. Marin and coauthors reported improved long-term survival and a lower rate of hospitalizations in over 200 OS patients treated with CPAP compared with those who were not treated, and the outcomes of those OS patients treated with CPAP were similar to those of patients with COPD alone over a median follow-up period of 9.4 years (28). So, it is

reasonable to hypothesize that a more stringent treatment of hypoxia and hypercapnia may be beneficial for patients with overlap syndrome. A prospective, multicenter trial should be carried out to research the effect of improvement of daytime hypoxemia and hypercapnia on cardiovascular outcomes of patients with OS.

We acknowledge certain limitations in this study that should be highlighted when interpreting the findings. First, given the retrospective nature of the study, clinical information on every aspect of cardiovascular events and their risk factors in the cohort was not available; for example, data by Holter, or telemetry monitoring were missing, and therefore, the prevalence of paroxysmal arrhythmias could not be evaluated. Second, this is a single-center retrospective study with a small population. Third, the low incidence of overlap syndrome may lead to an analysis bias, although we enlarged the sample size of the control groups to increase the credibility of the data.

CONCLUSION

Patients with OS are at greater risk of cardiovascular events than patients with either COPD or OSA alone, which is related to their impaired pulmonary function, severe hypoxemia, and hypercapnia. It is of great importance for clinical physicians to diagnose and treat patients with OS, promote the management of these patients and improve their quality of life.

TABLE 5 | Results of logistic regression used to explore risk factors for cardiovascular events.

Outcomes	Variables	OR	95%CI	P-value
CHD	Hypertension	3.657	2.262–5.911	0
	Diabetes	1.898	1.151–3.132	0.012
	Stroke	2.459	1.425–4.243	0.001
	LDH	1.004	1.001–1.007	0.003
	BMI	1.963	1.132–3.404	0.016
	TV	0.167	0.084–0.332	0
Heart failure	Diabetes	0.306	0.102–0.916	0.034
	PTE	7.354	1.185–45.635	0.032
	PCO ₂	1.049	1.019–1.080	0.001
	Neutrophil granulocyte	1.053	1.024–1.083	0
	PO ₂	0.975	0.951–0.998	0.036
	MVV	2.341	1.282–4.274	0.006
PAH	PTE	7.191	1.109–46.643	0.011
	PCO ₂	1.046	1.017–1.076	0.002
	BNP	1	1.000–1.000	0.029
	LDH	1.004	1.001–1.007	0.013
	meanSpO ₂	0.063	0.007–0.554	0.013

CHD, coronary heart disease; PAH, pulmonary arterial hypertension; PTE, pulmonary embolism; LDH, lactate dehydrogenase; BMI, body mass index; TV, tidal volume; MVV, maximum ventilatory volume; CT90, percentage of total sleep time with oxygen saturation spent <90%; PCO₂, partial pressure of carbon dioxide; PO₂, oxygen partial pressure; SaO₂, oxygen saturation.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University (No. XJTU1AF2020LSK-187). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MT and YL: methodology, writing, and original draft preparation. MT, SL, and XY: data curation and investigation. TS: supervision, writing, reviewing, and editing the manuscript. All authors provided critical review of the manuscript and approved the final draft for publication.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Modulated Calcium Homeostasis and Release Events Under Atrial Fibrillation and Its Risk Factors: A Meta-Analysis

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Background: Atrial fibrillation (AF) is associated with calcium (Ca^{2+}) handling remodeling and increased spontaneous calcium release events (SCaEs). Nevertheless, its exact mechanism remains unclear, resulting in suboptimal primary and secondary preventative strategies.

Methods: We searched the PubMed database for studies that investigated the relationship between SCaEs and AF and/or its risk factors. Meta-analysis was used to examine the Ca^{2+} mechanisms involved in the primary and secondary AF preventative groups.

Results: We included a total of 74 studies, out of the identified 446 publications from inception (1982) until March 31, 2020. Forty-five were primary and 29 were secondary prevention studies for AF. The main Ca^{2+} release events, calcium transient (standardized mean difference (SMD) = 0.49; I^2 = 35%; confidence interval (CI) = 0.33–0.66; p < 0.0001), and spark amplitude (SMD = 0.48; I^2 = 0%; CI = –0.98–1.93; p = 0.054) were enhanced in the primary diseased group, while calcium transient frequency was increased in the secondary group. Calcium spark frequency was elevated in both the primary diseased and secondary AF groups. One of the key cardiac currents, the L-type calcium current (I_{CaL}) was significantly downregulated in primary diseased (SMD = –1.07; I^2 = 88%; CI = –1.94 to –0.20; p < 0.0001) and secondary AF groups (SMD = –1.28; I^2 = 91%; CI = –2.04 to –0.52; p < 0.0001). Furthermore, the sodium–calcium exchanger (I_{NCX}) and NCX1 protein expression were significantly enhanced in the primary diseased group, while only NCX1 protein expression was shown to increase in the secondary AF studies. The phosphorylation of the ryanodine receptor at S2808 (pRyR-S2808) was significantly elevated in both the primary and secondary groups. It was increased in the primary diseased and proarrhythmic subgroups (SMD = 0.95; I^2 = 64%; CI = 0.12–1.79; p = 0.074) and secondary AF group (SMD = 0.66; I^2 = 63%; CI = 0.01–1.31; p < 0.0001). Sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA) expression was elevated in the primary diseased and proarrhythmic drug subgroups but substantially reduced in the secondary paroxysmal AF subgroup.

Conclusions: Our study identified that I_{CaL} is reduced in both the primary and secondary diseased groups. Furthermore, pRyR-S2808 and NCX1 protein expression are enhanced. The remodeling leads to elevated Ca^{2+} functional activities, such as increased frequencies or amplitude of Ca^{2+} spark and Ca^{2+} transient. The main difference identified between the primary and secondary diseased groups is SERCA expression, which is elevated in the primary diseased group and substantially reduced in the secondary paroxysmal AF subgroup. We believe our study will add new evidence to AF mechanisms and treatment targets.

Keywords: atrial fibrillation, calcium handling, calcium release events, Ca^{2+} sparks, primary AF prevention, secondary AF prevention

INTRODUCTION

Atrial fibrillation (AF) is the most common sustained arrhythmia, with markedly increasing prevalence (1, 2). It is associated with significant mortality and morbidity and becomes more challenging to treat as it advances (3–5). AF is mainly managed by primary and/or secondary preventative therapies. Primary prevention includes early detection and intervention on risk factors before AF develops, while secondary prevention involves diagnosing and treating AF (4). However, current pharmacological strategies are often associated with limited efficacy and adverse consequences, mainly due to an incomplete understanding of underlying cellular mechanisms related to AF (4, 6). In particular, calcium (Ca^{2+}) is one of the most crucial ions for cardiac excitation–contraction coupling and Ca^{2+} -dependent signaling pathways for maintaining cardiac function (7, 8).

Intracellular Ca^{2+} release events are exclusively investigated in myocardial physiology and pathophysiology, as they hold the key to understanding how cardiomyocyte Ca^{2+} signaling is regulated by ionic channels and Ca^{2+} proteins (7, 8). In a single cardiac cycle, the L-type calcium channels (LTCCs) localized on the sarcolemma and tubules are first activated (9). The opening of LTCCs results in the movement of Ca^{2+} into the cytosol, which induces the cardiac type 2 ryanodine receptors (RyR2) located on the junctional sarcoplasmic reticulum (SR) to release Ca^{2+} from its stores into the cytosol (9–11). This elementary Ca^{2+} release event is observed as a form of a Ca^{2+} spark, and the process is known as calcium-induced calcium release (12). Increases in highly localized, short-lived Ca^{2+} signals raise intracellular Ca^{2+} [Ca^{2+}]_i, which contributes to global Ca^{2+} waves or transients that propagate through the cell (10, 13). [Ca^{2+}]_i then binds to troponin to allow myosin adenosine triphosphatase (ATPase) to bind to actin in the sarcomere to initiate cardiac contraction (9, 14). Ca^{2+} is mainly recycled back into the SR *via* the SR Ca^{2+} -ATPase (SERCA2a) pump or extruded across the cell membrane through the cardiac sodium–calcium exchanger (NCX1) (15). The reduction in [Ca^{2+}]_i causes Ca^{2+} to dissociate from troponin and terminate myofilament cross-bridge cycling for cardiac relaxation (9, 16). SERCA2a activity is directly modulated by phospholamban (PLN). In its unphosphorylated state, PLN acts as an inhibitor to SERCA2a. When phosphorylated by protein

kinase A (PKA), PLN dislodges from SERCA2a to enable the reuptake of Ca^{2+} (9). Another important signaling protein besides PKA is the Ca^{2+} /calmodulin-dependent protein kinase II (CAMKII), which is responsible for transducing cytosolic Ca^{2+} , and calmodulin, a Ca^{2+} -binding messenger protein that modulates RyR activity and transduces Ca^{2+} signals to other protein kinases or phosphatases (17–19).

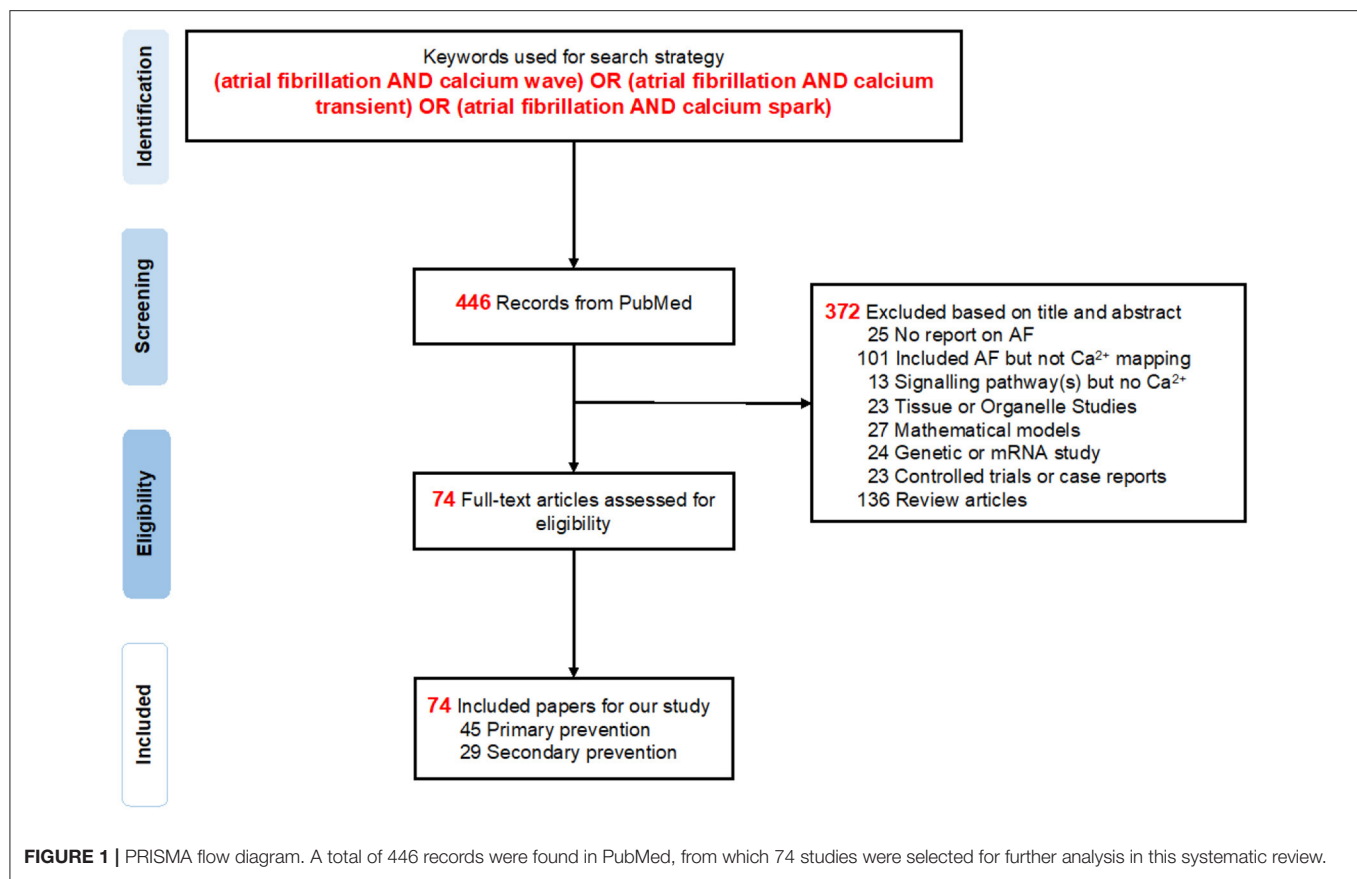
In diseased states, spontaneous Ca^{2+} release events (SCaEs) are observed as spontaneous Ca^{2+} sparks or arrhythmogenic Ca^{2+} waves are substantially enhanced (20, 21). Such defective Ca^{2+} homeostasis often results from remodeled Ca^{2+} -handling proteins (22–24). However, current studies reported conflicting results on how these Ca^{2+} -handling proteins were remodeled in AF and its risk factors, which hinders the development of effective AF treatment and prevention. In this study, we aim to illustrate the precise mechanisms and targeted therapies for AF and AF prevention by investigating the pathophysiological role of Ca^{2+} and its arrhythmogenicity. This systematic review has compared the different Ca^{2+} mechanisms between the primary and secondary AF preventative groups in the existing studies to date.

METHODS

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (refer to the PRISMA 2009 checklist in **Supplementary Table 1**) (25).

Search Strategy and Eligibility

The systematic electronic search was performed using the terms “atrial fibrillation” AND “calcium wave”/“calcium transient”/“calcium spark” in all fields to identify articles in PubMed from inception through March 31, 2020. Based on their titles and abstracts, the searched articles were screened manually for inclusion. Screening criteria included publications that mapped SCaEs in atrial cardiomyocytes in sinus rhythm and/or AF. Publications that did not conduct any experimental studies on atrial cells, such as mathematical modeling, population-based or organ-level studies, review papers, and editorial reports, were excluded. We also excluded papers that focused on genes and/or miRNA, signaling pathways, tissue, and organelle



calcium experimental studies. Two co-authors then reviewed the screened articles in full text for eligibility, and those that met the criteria were selected. Any discrepancies were resolved by a third author through discussion and consensus. Full details of the search terms were presented in **Supplementary Table 1**. The quality of the included studies was assessed according to the Newcastle–Ottawa Scale (**Supplementary Table 2**) (26). A study with a score of 5 and above was considered satisfactory.

Data Extraction

The atrial cellular activities were extracted from selected studies. It included SCaEs [Ca²⁺ spark frequency (CaSpF), Ca²⁺ transient frequency (CaTF), Ca²⁺ spark amplitude (CaSpA), and Ca²⁺ transient amplitude (CaTA)], and Ca²⁺ load and leak. It also included atrial current densities, such as L-type calcium current (I_{CaL}), sodium–calcium exchanger current (I_{NCX}), late sodium current (I_{Na–Late}), and potassium current (I_K), and protein expressions, such as L-type alpha 1C subunit voltage-dependent calcium current (Ca_v1.2), NCX1, RyR2, phosphorylated ryanodine receptor 2 (pRyR2), SERCA2a, PLN, phosphorylated phospholamban (pPLN), CAMKII, phosphorylated Ca²⁺/calmodulin-dependent protein kinase II (pCAMKII), and PKA. We further categorized the above results into two main groups: primary and secondary preventative therapies for AF. Primary prevention was divided into three subclasses: the diseased group (risk factors for AF), the

application of proarrhythmogenic agents or antiarrhythmogenic agents. Secondary prevention was classified into either the paroxysmal or chronic AF group. The analysis was conducted using *R*.

Data Synthesis and Statistical Analysis

Statistical analyses were performed using *R* (27). Dichotomous values were used to calculate 95% confidence interval (CI) of relative risk ratios, and continuous values to quantify standardized mean difference (SMD). Each study was given a weighting factor to determine its importance in the meta-analysis, which was represented by gray boxes in forest plots. When the boundaries of the CI were within the box, a white horizontal line was plotted; otherwise, it was illustrated by a black horizontal line. Studies with the CI not crossing zero were deemed to be statistically significant.

The overall SMD was interpreted using Cohen's guidelines (28), where a value of 0.2–0.49 was deemed to be small, 0.5–0.79 represented medium, and 0.8 and above was large. Statistical heterogeneity was calculated using *I*² for all studies (29). In general, heterogeneity was classified into three main categories, low, medium, and high, when *I*² values were ≤25%, between 25 and 50%, and ≥75%, respectively. Statistical significance was measured with *p*-values. We considered a result to be statistically significant when *p*-value was ≤0.05. We also

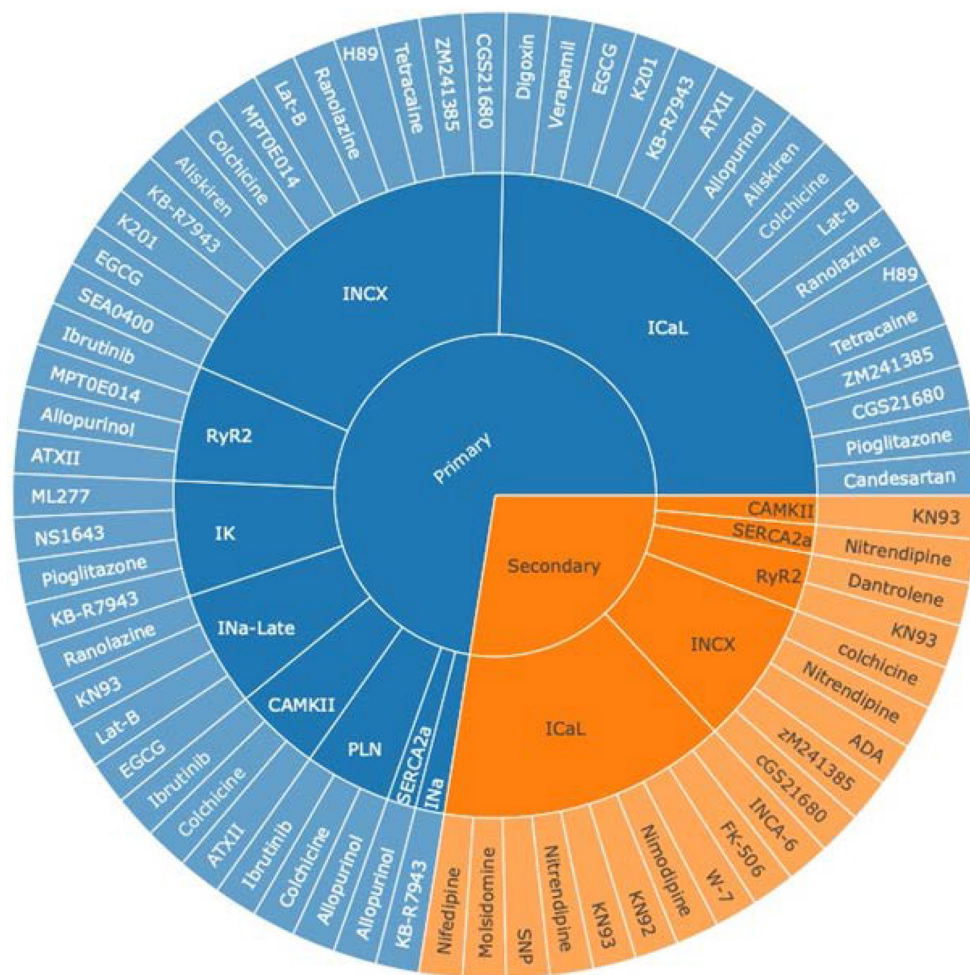


FIGURE 2 | An overview of pharmacologically targeted ionic channels or proteins for AF treatment, grouped by primary (blue) and secondary (orange) prevention. The sunburst plot's core was divided into two groups, primary and secondary prevention for AF, where the middle section represented ion channels, and the outer segment symbolized various drug therapies. I_{NCX} stands for the sodium–calcium exchanger current; I_{CaL} , the L-type calcium current; RyR2, the ryanodine receptor 2; I_K , the potassium current; $I_{Na-Late}$, the late sodium current; CAMKII, the Ca^{2+} /calmodulin-dependent protein kinase II; PLN, phospholamban; SERCA2a, the sarco/endoplasmic reticulum Ca^{2+} -ATPase 2a pump; I_{Na} , the sodium current.

employed influence analysis and graphic display of heterogeneity (GOSH) plots to detect influential studies and remove outliers (27).

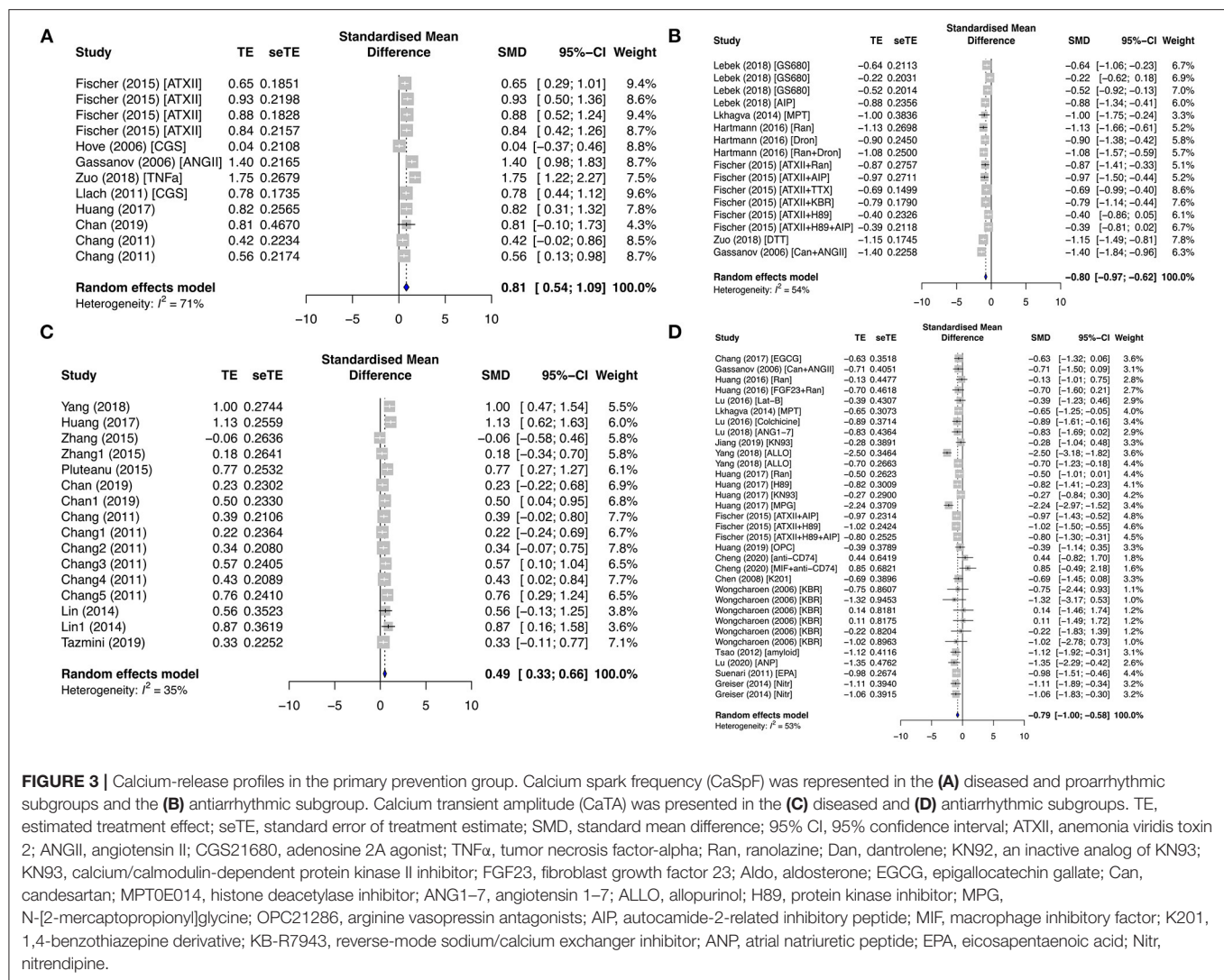
RESULTS

Study Characteristics

Our literature search identified a total of 446 publications from inception (1982) to March 31, 2020 (Figure 1). When screening the titles and abstracts, a total of 372 papers were excluded (Supplementary Table 3): 25 articles focused on other diseases instead of AF, 101 included AF but did not conduct experiments on SCAEs, 13 papers mentioned signaling pathways but not Ca^{2+} , 23 were tissue or organelle studies, another 24 studied mRNA and genes, 27 indicated mathematical models, 23 articles were controlled trials or case reports, and 136

were review articles. Eventually, a total of 74 studies (12, 24, 30–100), consisting of 45 primary and 29 secondary prevention studies for AF, were eligible and included for this systematic review.

Based on the 74 selected studies, pharmacological targets were grouped by their mechanism of action on the ionic channel(s) or protein(s). We discovered that I_{CaL} was the most widely studied current in both primary and secondary pharmacological therapy for AF, followed by I_{NCX} and RyR2 channels (Figure 2). This coincides with the present targeted drug therapies available for AF, where LTCC antagonists are one of the most frequently prescribed drugs for the treatment of hypertension and AF. Figure 2 aids us in understanding and exploring other potential pathways for therapeutic drug discovery, such as the I_{NCX} and RyR2. It is noteworthy that the late sodium current was only commonly studied for primary prevention.



Spontaneous Calcium Release Events

The evolution of cardiac Ca^{2+} waves is influenced by local elevations of $[\text{Ca}^{2+}]_i$, seen as Ca^{2+} sparks. The properties (frequency and amplitude) of these Ca^{2+} release events are key determinants to the arrhythmogenicity of the cardiomyocytes. In our study, key calcium-handling remodeling including ionic currents, calcium release events, and protein expressions was summarized for the primary prevention and secondary AF groups in **Figure 3**. In the primary prevention group, CaSpF was significantly enhanced in the diseased subgroup (SMD = 0.6; I^2 = 0%; CI = 0.30–0.89; p = 0.6601) and proarrhythmic drug subgroup (SMD = 0.89; I^2 = 79%; CI = 0.48–1.30; p < 0.0001) (**Supplementary Figures 1A,B**). When these results were combined from both subgroups, they displayed a similar result (SMD = 0.81; I^2 = 71%; CI = 0.54–1.09; p < 0.0001) (**Figure 3A**). The addition of antiarrhythmic drugs significantly decreased CaSpF (SMD = -0.80; I^2 = 54%; CI = -0.97 to -0.62; p = 0.0054) (**Figure 3B**). A similar trend was observed

for CaTA. CaTA was increased in the diseased subgroup (SMD = 0.49; I^2 = 35%; CI = 0.33–0.66; p < 0.0001) (**Figure 3C**) and reduced by antiarrhythmic drugs (SMD = -0.79; I^2 = 53%; CI = -1.00 to -0.58; p = 0.0002) (**Figure 3D**). In addition, CaSpA was enhanced in the diseased subgroup (**Supplementary Figure 4**).

In contrast, the CaSpF and calcium transient frequency (CaTF) were significantly elevated in both the secondary paroxysmal and chronic AF subgroups, with respective SMD = 0.81; I^2 = 96%; CI = -0.14–1.76; p < 0.0001 (**Figure 4A**), and SMD = 0.85; I^2 = 92%; CI = 0.12–1.57; p < 0.0001 (**Figure 4B**), and high heterogeneities. However, the change in CaSpA was almost negligible in both subgroups (SMD = 0.06; I^2 = 55%; CI = 0.27–0.39; p < 0.0386) (**Supplementary Figure 4G**). Surprisingly, CaTA was unaltered in both paroxysmal (SMD = -0.07; I^2 = 66%; CI = -0.34–0.20; p < 0.0001) and permanent AF (SMD = -0.06; I^2 = 79%; CI = -0.49–0.38; p < 0.0001) (**Supplementary Figures 2C,D**).

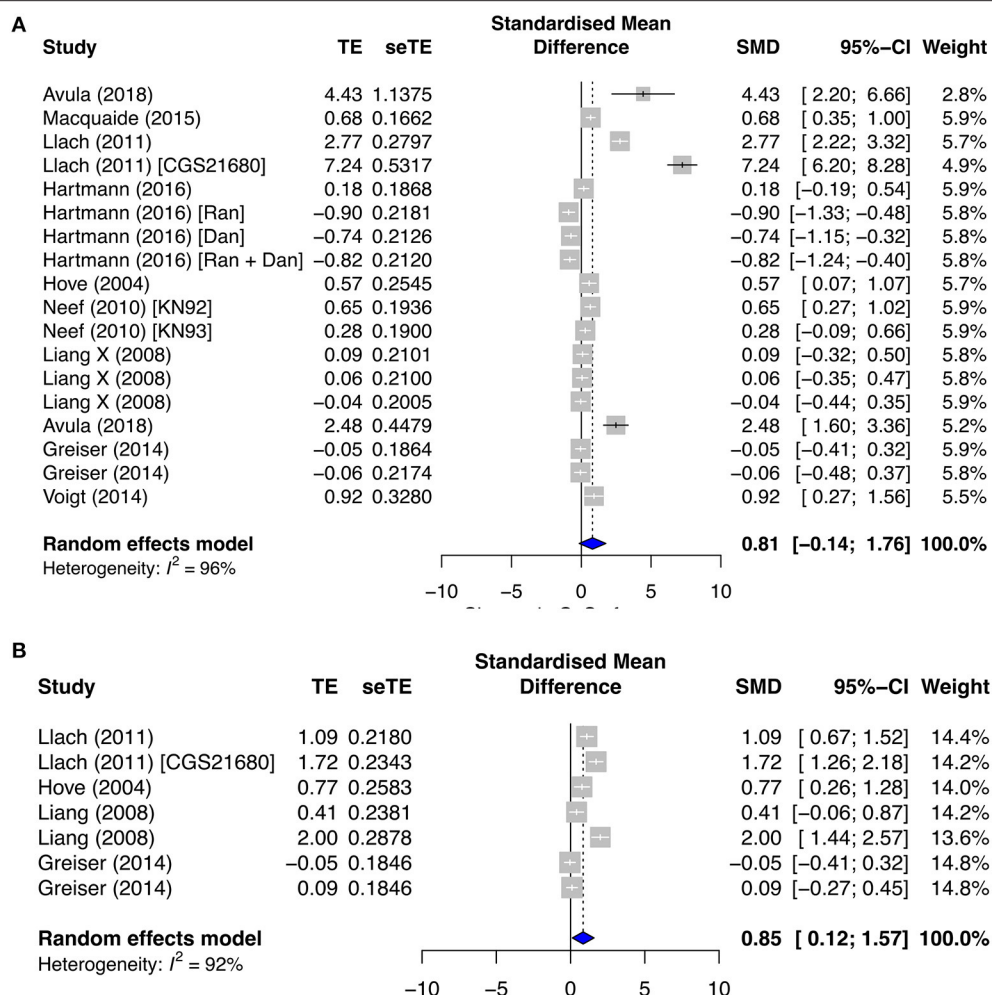


FIGURE 4 | Calcium release profiles in the secondary prevention group. **(A)** Calcium spark frequency (CaSpF) and **(B)** calcium transient frequency (CaTF). TE, estimated treatment effect; seTE, standard error of treatment estimate; SMD, standard mean difference; 95% CI, 95% confidence interval; CGS21680, adenosine 2A agonist; Ran, ranolazine; Dan, dantrolene; KN92, an inactive analog of KN93; and KN93, calcium/calmodulin-dependent protein kinase II inhibitor.

SR Ca^{2+} Leak-Load Relationship

SR Ca^{2+} release is affected by the opening of RyR channels from its stores. In particular, SR Ca^{2+} leak is a major contributor to cardiac arrhythmia. Ca^{2+} load remained relatively unchanged in both the primary and secondary subgroups, except when antiarrhythmic drugs were applied in the primary group (SMD = -0.40; $I^2 = 59\%$; CI = -0.62 to -0.17; $p < 0.0001$) (Figure 5A). No change in Ca^{2+} leak was observed in the secondary prevention group (Supplementary Figure 5), but it was significantly affected by pro- and antiarrhythmic drugs in the primary subgroups. Ca^{2+} leak was raised by proarrhythmic agents (SMD = 0.81; $I^2 = 0\%$; CI = 0.54–1.09; $p = 0.7583$) (Figure 5B) and antagonized by antiarrhythmic agents (SMD = -0.66; $I^2 = 33\%$; CI = -0.81 to -0.50; $p = 0.0932$) (Figure 5C).

Ionic Mechanisms of Atrial Remodeling

One of the most important currents for atrial cardiac action potential generation is I_{CaL} . I_{CaL} was significantly downregulated

in the primary diseased subgroup (SMD = -1.07; $I^2 = 88\%$; CI = -1.94 to -0.20; $p < 0.0001$) (Figure 6A), antiarrhythmic drug subgroup (SMD = -0.96; $I^2 = 61\%$; CI = -1.31 to -0.61; $p < 0.0001$) (Figure 6B), and secondary AF subgroups (SMD = -1.28; $I^2 = 91\%$; CI = -2.04 to -0.52; $p < 0.0001$) (Figure 6C). These results were consistent with $\text{Ca}_v1.2$ protein expression in the primary antiarrhythmic subgroup (SMD = -0.70; $I^2 = 30\%$; CI = -1.25 to -0.16; $p = 0.2027$) (Figure 6D) and secondary permanent AF group (SMD = -1.69; $I^2 = 0\%$; CI = -7.05–3.67; $p < 0.0001$) (Supplementary Figure 6F).

The extrusion of $[\text{Ca}^{2+}]_i$ for Ca^{2+} recycling is *via* the cardiac NCX. I_{NCX} was significantly enhanced in both the primary diseased and proarrhythmic subgroups (SMD = 0.68; $I^2 = 89\%$; CI = 0.01–1.35; $p < 0.0001$) (Figure 7A) and reduced in the primary antiarrhythmic drug group (SMD = -1.03; $I^2 = 76\%$; CI = -1.51 to -0.55; $p < 0.0001$) (Figure 7B). Likewise, NCX1 protein expression was upregulated in the primary diseased and

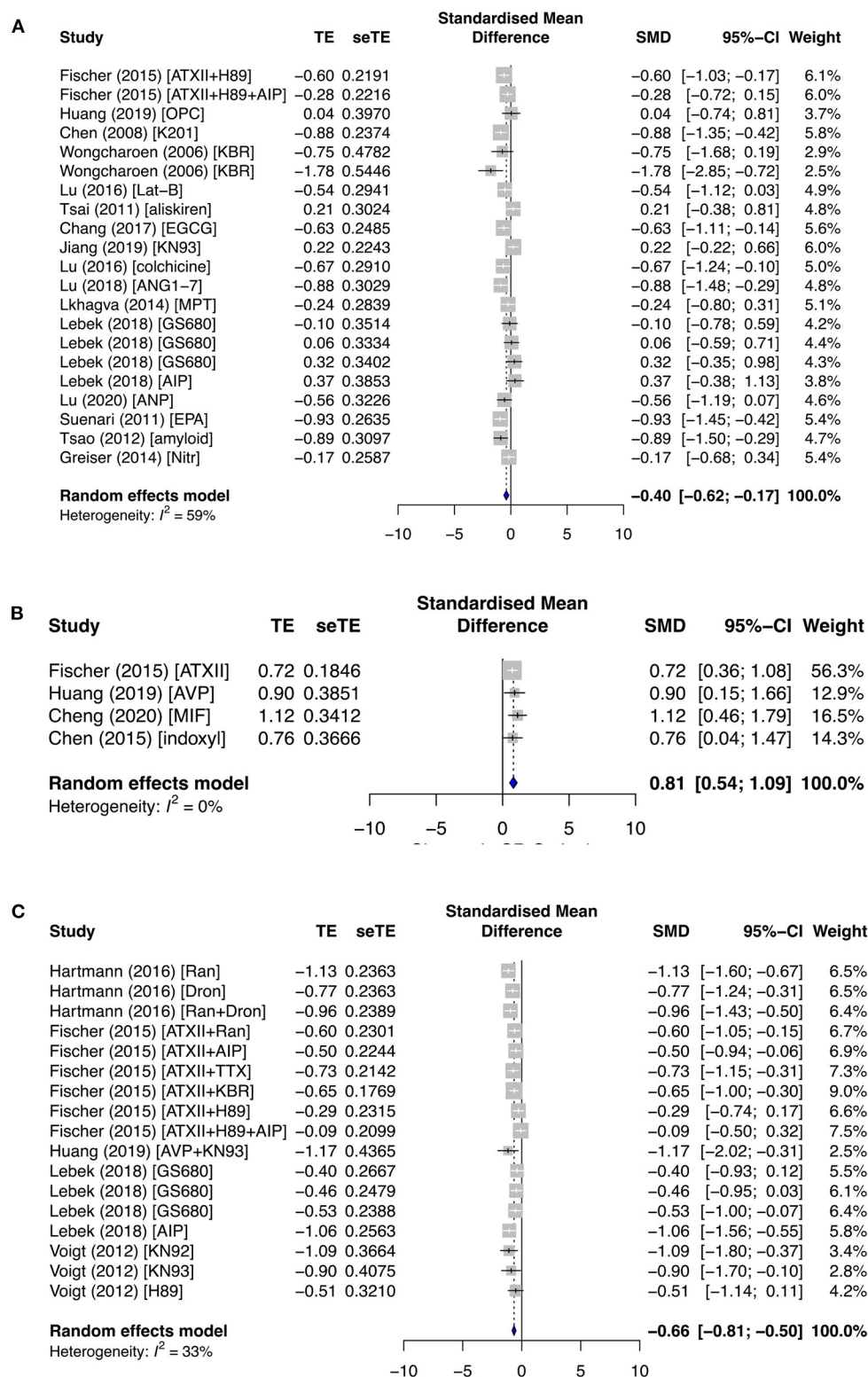
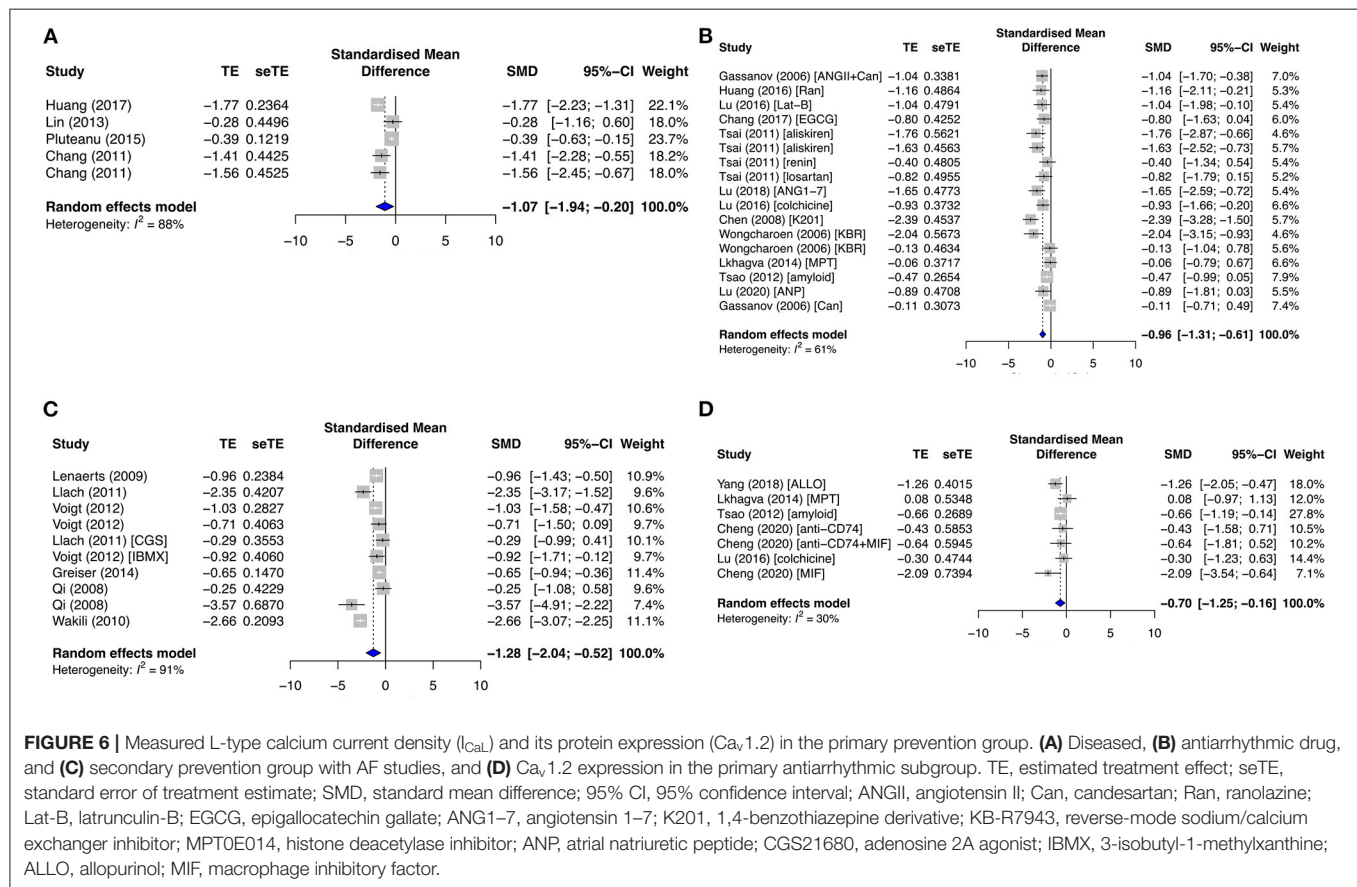


FIGURE 5 | The effect of primary groups on SR calcium leak-load relationship. SR calcium load was affected by (A) antiarrhythmic drugs, and SR calcium leak was influenced by (B) proarrhythmic and (C) antiarrhythmic agents. TE, estimated treatment effect; seTE, standard error of treatment estimate; SMD, standard mean difference; 95% CI, 95% confidence interval; ATXII, anemona viridis toxin 2; H89, protein kinase inhibitor; AIP, autocamide-2-related inhibitory peptide; OPC21286,

(Continued)

FIGURE 5 | arginine vasopressin antagonists; MIF, macrophage inhibitory factor; K201, 1,4-benzothiazepine derivative; KB-R7943, reverse-mode sodium/calcium exchanger inhibitor; Lat-B, latrunculin-B; EGCG, epigallocatechin gallate; ANG1-7, angiotensin 1-7; MPT0E014, histone deacetylase inhibitor; GS680, calcium/calmodulin-dependent protein kinase II inhibitor; EPA, eicosapentaenoic acid; Nitir, nitrendipine; AVP, arginine vasopressin; Ran, ranolazine; Dan, dantrolene; and TTX implies tetrodotoxin.



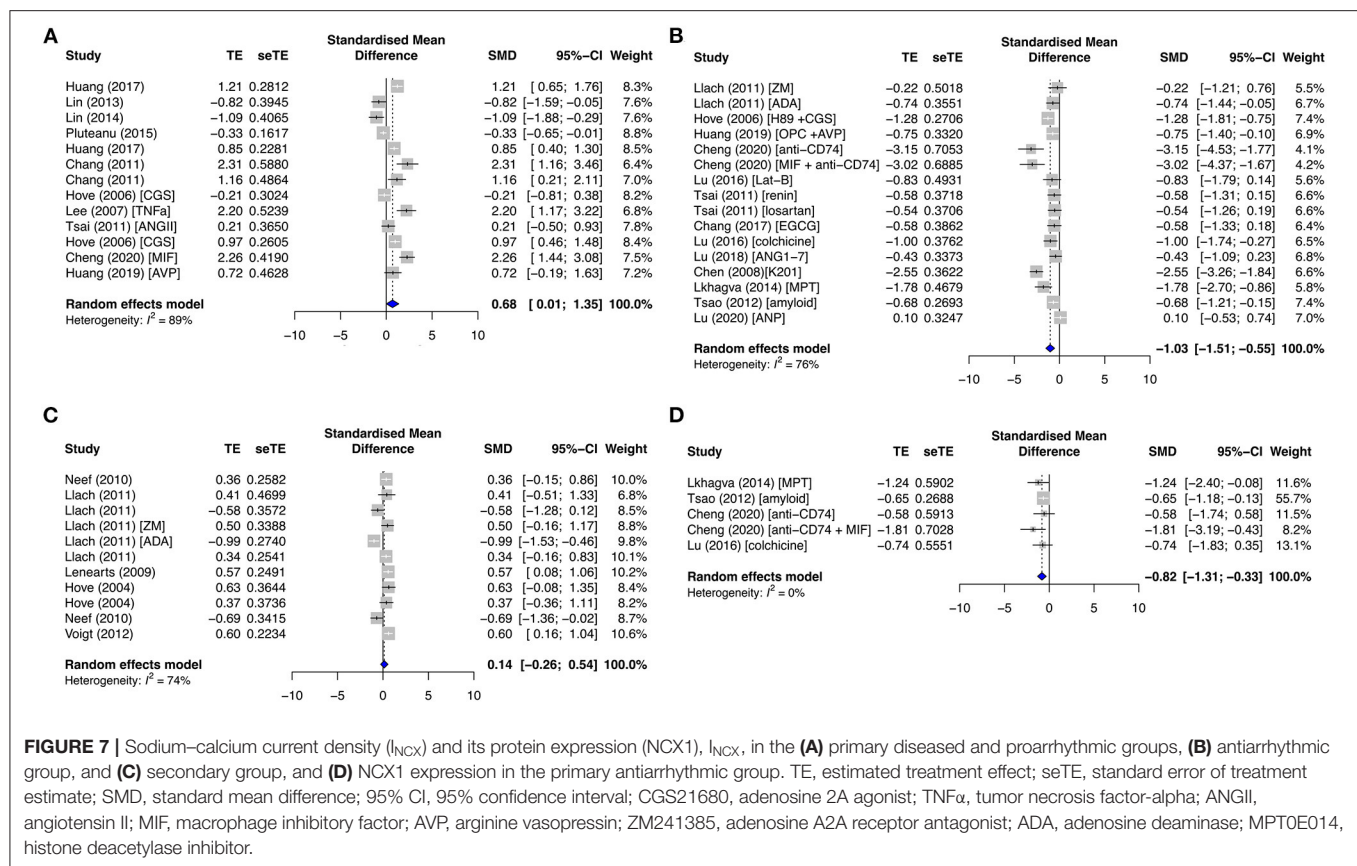
proarrhythmic subgroup (SMD = 0.43; I^2 = 50%; CI = -0.25–1.10; p = 0.0638) (Supplementary Figure 7E) and significantly inhibited in the primary antiarrhythmic subgroup (SMD = -0.82; I^2 = 0%; CI = -1.31 to -0.33; p < 0.0001) (Figure 7D). As opposed to the primary group, AF studies demonstrated that I_{NCX} and NCX1 protein expression had mixed results [SMD = 0.14; I^2 = 74%; CI = -0.26–0.54; p < 0.0001 (Figure 7C), and SMD = 0.62; I^2 = 61%; CI = -0.29–1.54; p = 0.0638 (Supplementary Figure 7H), respectively].

RyR is a major cardiac channel and mediator of the myocardial excitation–contraction coupling. It contains two key phosphorylation sites, serine S2808 (pRyR-S2808) and S2814 (pRyR-S2814). S2808 is phosphorylated by PKA, while S2814 is modulated by CAMKII. Only pRyR-S2808 was significantly affected in both primary and secondary groups, while total RyR (tRyR) and pRyR-S2814 showed no significant changes. pRyR-S2808 expression was increased in the primary diseased and proarrhythmic subgroups (SMD = 0.95; I^2 = 64%; CI = 0.12–1.79; p = 0.074) (Figure 8A) and the secondary AF group (SMD = 0.66; I^2 = 63%; CI = 0.01–1.31; p < 0.0001) (Figure 8C) but

was inhibited by antiarrhythmic drugs (SMD = -1.45; I^2 = 57%; CI = -2.56 to -0.34; p = 0.0315) (Figure 8B).

Another pathway for Ca^{2+} recycling is *via* the SERCA2a pump, in which activity is directly controlled by PLN. Few studies reported on SERCA and PLN expression, resulting in inconsistent and non-significant results. SERCA expression was elevated in the primary diseased and proarrhythmic subgroups but substantially reduced in the secondary paroxysmal AF subgroup. Total PLN (tPLN) remained relatively constant in all primary subgroups but was similarly decreased in paroxysmal AF. The results for phosphorylated PLN at sites serine 16 (pPLN-S16) and threonine 17 (pPLN-T17) in both groups were markedly diverse.

The signaling proteins, CAMKII and PKA, pCAMKII in particular, were affected by proarrhythmic drugs (SMD = 1.58; I^2 = 60%; CI = 0.77–2.40; p = 0.0192) (Figure 9A) and antiarrhythmic therapies (SMD = -0.88; I^2 = 66%; CI = -2.04–0.28; p = 0.0015), and total CAMKII (tCAMKII) by AF groups (SMD = 1.89; I^2 = 63%; CI = 0.47–3.32; p = 0.023) (Figure 9B). Phosphorylated CAMKII had a greater activity than tCAMKII in



most subgroups. Only primary studies reported on the sodium current (I_{Na}), $I_{Na-Late}$, and I_K current densities. However, all data extracted were non-significant and/or inconsistent except for $I_{Na-Late}$. $I_{Na-Late}$ was moderately enhanced in diseased (SMD = 0.74; I^2 = 29%; CI = 0.33–1.15; p = 0.2206) (Figure 9C) and proarrhythmic drugs (SMD = 0.64; I^2 = 0%; CI = 0.36–0.92; p = 0.7447) (Figure 9D) and significantly antagonized by antiarrhythmic agents (SMD = –1.00; I^2 = 11%; CI = –1.29 to –0.70; p = 0.3408) (Figure 9E), with low heterogeneities across all three primary subgroups.

The action of various drugs or reagents in the different experimental models were summarized in Supplementary Tables 4, 5. Additionally, the renin-angiotensin system, targeted by reagents such as angiotensin (ANG) and renin, was most commonly reported. The renin-angiotensin system, which is currently a drug target for hypertension, could also be a potential pharmacological discovery for the treatment and prevention of AF.

DISCUSSION

Main Findings

This preclinical systematic review study analyzed 74 articles identified from 446 searched primary and secondary AF prevention articles. Forty-five publications

were classified as primary AF prevention studies and 29 others as secondary prevention. To our knowledge, this is the largest study of this kind to explore the association between modulated calcium homeostasis and release events for primary and secondary prevention of AF. Our principal findings are summarized as follows (Table 1).

With regard to the key Ca^{2+} channels/proteins/mediators, our study found that I_{CaL} was the most widely studied current in both primary and secondary AF prevention, followed by I_{NCX} and RyR2 channels.

- We showed that I_{CaL} was significantly downregulated in primary and secondary diseased groups, which were largely consistent with our results for $Ca_v1.2$ protein expression. Antiarrhythmic drugs in the primary group further reduced I_{CaL} significantly.
- Furthermore, the NCX1 protein expression was significantly enhanced in both the primary and secondary diseased groups, but I_{NCX} was only elevated in the primary diseased group.
- In addition, our study demonstrated that the key phosphorylation expression for RyR was enhanced at serine 2808 in both the primary diseased and secondary AF groups, and inhibited in the primary antiarrhythmic drug subgroup. On the other hand, the other key RyR phosphorylation expression at serine 2814 showed no significant changes in both the primary and secondary diseased groups.

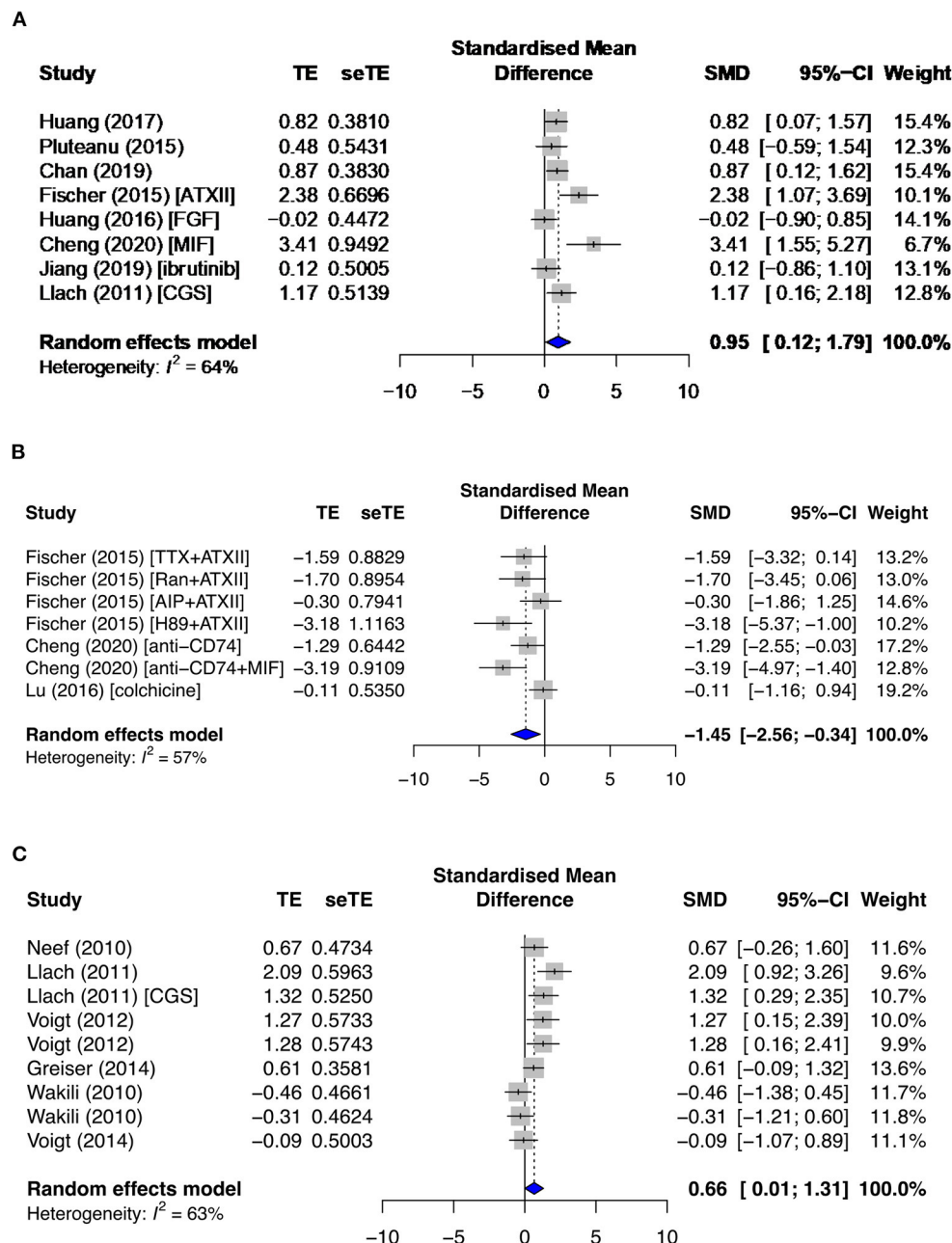


FIGURE 8 | Phosphorylated ryanodine receptor at serine 2808 (pRyR-S2808) protein expression in the **(A)** diseased and proarrhythmic subgroups, **(B)** antiarrhythmic subgroups, and **(C)** secondary AF group. TE, estimated treatment effect; seTE, standard error of treatment estimate; SMD, standard mean difference; 95% CI, 95% confidence interval; ATXII, anemonia viridis toxin 2; FGF23, fibroblast growth factor 23; MIF, macrophage inhibitory factor; CGS21680, adenosine 2A agonist; TTX, tetrodotoxin; Ran, ranolazine; AIP, autocamide-2-related inhibitory peptide; H89, protein kinase inhibitor.

- SERCA expression was elevated in the primary diseased and proarrhythmic drug subgroups but substantially reduced in the secondary paroxysmal AF subgroup. tPLN remained relatively constant in all primary subgroups but was decreased in paroxysmal AF.
- Finally, the Ca^{2+} signaling mediator CAMKII was increased in the secondary AF group. With its phosphorylation activity at threonine 286, pCAMKII-T286 was significantly raised by proarrhythmic drugs and significantly reduced by antiarrhythmic therapies.

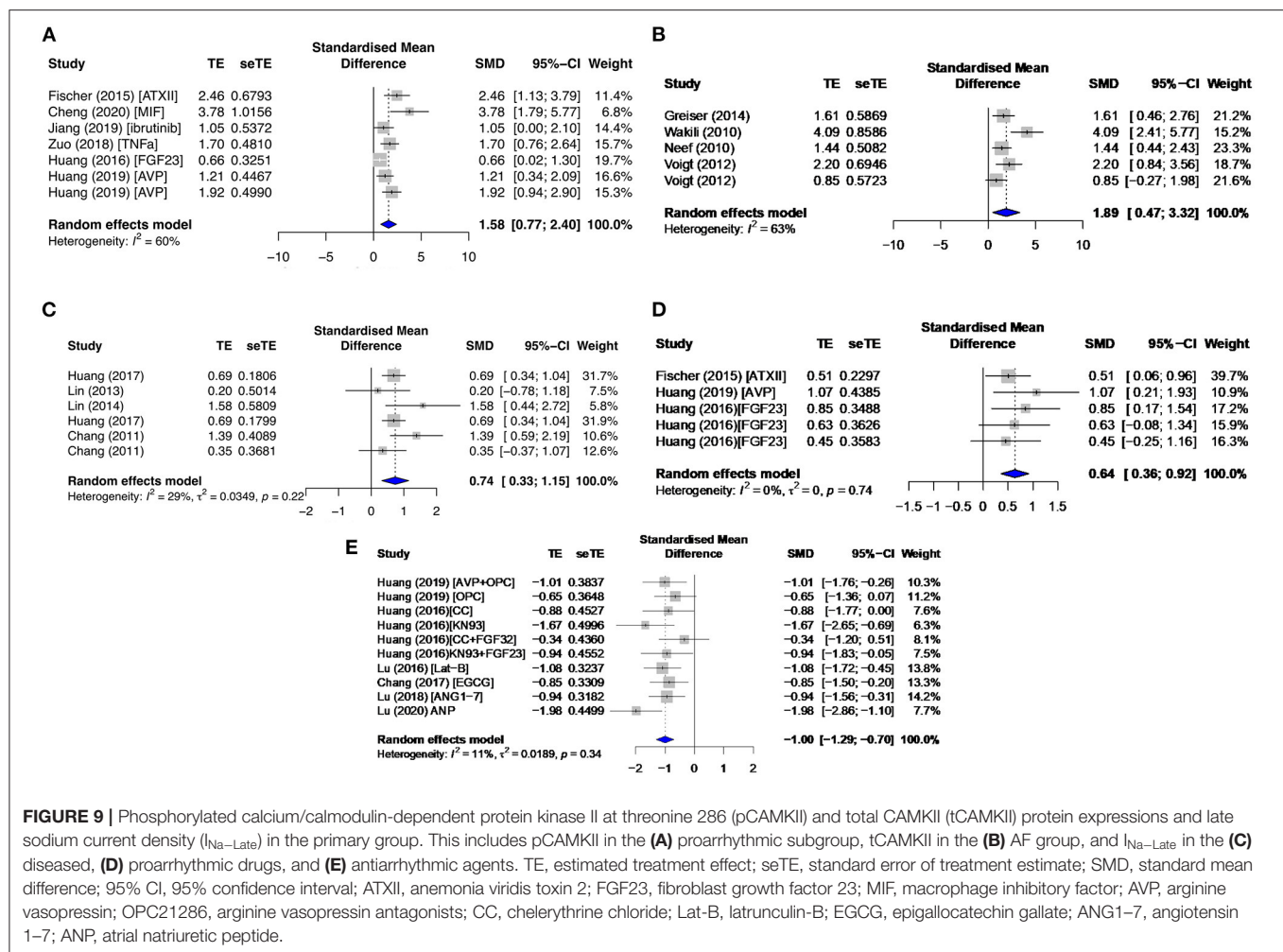


FIGURE 9 | Phosphorylated calcium/calmodulin-dependent protein kinase II at threonine 286 (pCAMKII) and total CAMKII (tCAMKII) protein expressions and late sodium current density ($I_{Na-Late}$) in the primary group. This includes pCAMKII in the (A) proarrhythmic subgroup, tCAMKII in the (B) AF group, and $I_{Na-Late}$ in the (C) diseased, (D) proarrhythmic drugs, and (E) antiarrhythmic agents. TE, estimated treatment effect; seTE, standard error of treatment estimate; SMD, standard mean difference; 95% CI, 95% confidence interval; ATXII, anemionia viridis toxin 2; FGF23, fibroblast growth factor 23; MIF, macrophage inhibitory factor; AVP, arginine vasopressin; OPC21286, arginine vasopressin antagonists; CC, chelerythrine chloride; Lat-B, latrunculin-B; EGCG, epigallocatechin gallate; ANG1-7, angiotensin 1-7; ANP, atrial natriuretic peptide.

It is noteworthy that there is a growing surge of interest for the late sodium current $I_{Na-Late}$ and its direct effect on arrhythmia. Our study identified many primary preventative publications that showed that $I_{Na-Late}$ was moderately enhanced in the primary diseased and proarrhythmic drug subgroups but significantly antagonized by antiarrhythmic agents, with low heterogeneities across all three subgroups.

As a result of atrial remodeling in the ionic channels and protein/signaling expressions in diseased and AF conditions, we observed changed Ca^{2+} functional activities, i.e., Ca^{2+} spark, Ca^{2+} transient, and Ca^{2+} load/leak. In the primary prevention group, CaSpF, CaTA, and CaSpA were significantly enhanced in the diseased subgroup and decreased by antiarrhythmic drug agents. On the other hand, CaTF and CaSpF were significantly elevated in both the secondary paroxysmal and chronic AF subgroups. Interestingly, we discovered that SR Ca^{2+} load and Ca^{2+} leak remained relatively constant in the primary and secondary subgroups, except when SR Ca^{2+} load was reduced when antiarrhythmic drugs were applied in the primary group. Furthermore, we found that Ca^{2+} leak was raised by proarrhythmic

agents and antagonized by antiarrhythmic agents in the primary group.

Potential Mechanisms for Primary AF Diseases

The pathophysiological mechanism that causes spontaneous sarcoplasmic calcium release in the primary group involves the downregulation of I_{CaL} and dysfunction of the Ca^{2+} -handling proteins, in particular pRyR at S2808. The enhanced pRyR-S2808 activity may increase the frequency of the Ca^{2+} spark due to calcium-induced calcium releases. This would eventually lead to a high Ca^{2+} level in the cytosol and enhanced trigger activities *via* the forward mode of I_{NCX} , which was demonstrated in this review as the elevation of I_{NCX} activity. Furthermore, the enhancement of $I_{Na-Late}$ was shown to potentially play a more significant role in the generation of arrhythmia in our review and recent studies (95–97). The reduction in I_{CaL} was predicted to result in reduced SR load and diminished spontaneous activity. On the contrary, the downregulation of $Ca_v1.2$ current and protein expression has resulted in SCAEs, which could possibly be due

TABLE 1 | The key calcium handling remodeling in the primary prevention group and the secondary diseased group.

	Ionic currents and calcium proteins/released events	Primary diseased group	Secondary AF group
Spontaneous calcium-release events	Calcium transient amplitude (CaTA)	↑	↔
	Calcium spark amplitude (CaSpA)	↑	↔
	Calcium transient frequency (CaTF)	n/a	↑
	Calcium spark frequency (CaSpF)	↑	↑
	Sarcoplasmic reticulum (SR) calcium load	↔	↔
	SR calcium leak	n/a	↔
Ionic current densities	L-type calcium current, I_{CaL}	↓	↓
	Sodium–calcium exchanger current, I_{NCX}	↑	↔
	Sodium current, I_{Na}	↔	n/a
	Late sodium current, $I_{Na-Late}$	↑	n/a
	Inward rectifier potassium current, I_{K1}	↔	n/a
	Funny current, I_f	↓	n/a
	Ultrarapid delayed outward rectifier current, I_{Kur}	↔	n/a
	Transient outward potassium current, I_{to}	↔	n/a
	Transient inward potassium current, I_{ti}	↑	n/a
Calcium-handling protein expressions	L-type calcium channel subunit, $Ca_v1.2$	↓	↓
	Total ryanodine receptor, tRyR	↔	↔
	Sodium–calcium exchanger 1, NCX1	↑	↑
	tSERCA	↑	↓
	Total phospholamban, tPLN	↔	↓
	tCAMKII	↔	↑
	pRyR-S2808	↑	↑
	pRyR-S2814	↔	↔
	pPLN-S16	↔	↔
	pPLN-T17	↔	↑
	pCAMKII	n/a	↑
	Total protein kinase A, tPKA	↑	n/a

↑ means increased, ↓ means decreased, ↔ means no significant changes, n/a means no data available.

The red color key means that both the primary and secondary diseased groups have a similar trend in the calcium event, protein, or ionic remodeling.

tSERCA, total sarco/endoplasmic reticulum calcium-ATPase; tCAMKII, total CAMKII, calcium/calmodulin-dependent protein kinase II; pRyR-S2814, ryanodine receptor phosphorylation at serine 2814; pRyR-S2808, ryanodine receptor phosphorylation at serine 2808; pPLN-S16, phospholamban phosphorylation at serine 16; pPLN-T17, phospholamban phosphorylation at threonine 17; pCAMKII, calcium/calmodulin-dependent protein kinase II phosphorylation at threonine 286.

to cell compensation (98, 99). Overall, this interplay led to an overload of Ca^{2+} in the cell, potentially causing AF.

Abnormal Ca^{2+} Activity in AF

The electrophysiological remodeling induced in the fibrillating atria and its molecular basis were extensively reviewed. Recent and past AF studies (95–97) have suggested that I_{CaL} density was downregulated, together with the reduction of its protein expression, $Ca_v1.2$. Strangely, reduced I_{CaL} density did not diminish the SR load; it remained unchanged (96). In contrast to the primary AF disease, consistently reduced SERCA levels were identified, reducing the releasable SR Ca^{2+} in the cytosol (98, 99).

On the other hand, some studies observed that the SR Ca^{2+} leak and activity of RyR were consistently upregulated (97, 100). Their observation justified the increase in SCAEs. The NCX expression was also increased, in contrast to what we saw earlier in the primary mechanism (100). The increased NCX expression could also account for the increase in frequencies of both the Ca^{2+} sparks and Ca^{2+} transients. The overextrusion of Ca^{2+} explains the unchanged CaSpA and CaTA.

We have shown that I_{CaL} and NCX1 protein are the primary remodeling targets identified, and this leads to spontaneous calcium activity due to its interrelationship with the SR proteins. The conclusion of the secondary group meta-analysis aligns perfectly with the AF mechanism provided by Madsen et al. (34). On the other hand, the proposed primary mechanism is the best agglomeration of the mechanisms acquired from individual modifiable/non-modifiable risk factors associated with AF.

Limitations

This review aims to provide a better understanding of the mechanisms involved in the $[Ca^{2+}]_i$ homeostasis within atrial cardiomyocytes and compare their activities among the primary and secondary AF subgroups. Although this review has comprehensively compiled the Ca^{2+} activity from inception to date, it still presents several limitations. The activity of SERCA and PLN appears to be unclear. It is certain that I_{CaL} was reduced in the primary diseased and secondary AF groups. Surprisingly, the SR Ca^{2+} load–leak relationship was unaltered in the primary diseased and secondary AF groups with high heterogeneities. This could be influenced by a variety of non-controllable factors,

such as the variability in the animal and human studies at various stages of AF or diseased states, the type and strength of pharmacological agents applied, and the different experimental settings and methodologies (101, 102).

CONCLUSION

Our study identified that I_{CaL} is reduced in both primary and secondary diseased groups. Furthermore, pRyR-S2808 and NCX1 protein expression are enhanced. The remodeling leads to elevated Ca^{2+} functional activities, such as the frequencies or amplitude of Ca^{2+} spark and Ca^{2+} transient. The main difference identified between primary and secondary diseased groups is the SERCA expression, which is elevated in the primary diseased group and substantially reduced in the secondary paroxysmal AF subgroup. We believe our study will add new evidence to AF mechanisms and treatment targets.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

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AUTHOR CONTRIBUTIONS

SF and SA undertook data extraction, post-processing, and analysis, as well as drafting the manuscript. MG assisted in assessing the quality of the included studies. JZ guided the project and revised the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcvm.2021.662914/full#supplementary-material>

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One-Year Change in the H₂FPEF Score After Catheter Ablation of Atrial Fibrillation in Patients With a Normal Left Ventricular Systolic Function

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Background: It is unclear whether atrial fibrillation (AF) catheter ablation (AFCA) improves the left ventricular (LV) diastolic function. We evaluated the 1-year change in the H₂FPEF score, which reflects the degree of LV diastolic function, after AFCA among patients with a normal LV systolic function.

Methods and Results: We included 1,471 patients (30.7% female, median age 60 years, paroxysmal-type AF 68.6%) who had available H₂FPEF scores at baseline and at 1-year after AFCA to evaluate the 1-year change in the H₂FPEF score (Δ H₂FPEF score_[1-yr]) after AFCA. Baseline high H₂FPEF scores (≥ 6) were independently associated with the female sex, left atrium (LA) diameter, LV mass index, pericardial fat volume, and a low estimated glomerular filtration rate. One year after AFCA, decreased Δ H₂FPEF scores_[1-yr] were associated with baseline H₂FPEF scores of ≥ 6 [OR, 4.19 (95% CI, 2.88–6.11), $p < 0.001$], no diabetes [OR, 0.60 (95% CI, 0.37–0.98), $p = 0.04$], and lower pericardial fat volume [OR, 0.99 (95% CI, 0.99–1.00), $p = 0.003$]. Increased Δ H₂FPEF scores_[1-yr] were associated with a baseline H₂FPEF score of < 6 [OR, 3.54 (95% CI, 2.08–6.04), $p < 0.001$] and sustained AF after a recurrence within 1 year [SustainAF_[1-yr]; OR, 1.89 (95% CI, 1.01–3.54), $p = 0.048$]. Throughout a 56-month median follow-up, an increased Δ H₂FPEF score_[1-yr] resulted in a poorer rhythm outcome of AFCA (at 1 year, log-rank $p = 0.003$; long-term, log-rank $p = 0.010$).

Conclusions: AFCA appears to improve LV diastolic dysfunction. However, SustainAF_[1-yr] may contribute to worsening LV diastolic dysfunction, and it was shown by increased Δ H₂FPEF scores_[1-yr], which was independently associated with higher risk of AF recurrence rate after AFCA.

Clinical Trial Registration: ClinicalTrials.gov Identifier: NCT02138695.

Keywords: atrial fibrillation, catheter ablation, left ventricular diastolic dysfunction, recurrent event, risk score

INTRODUCTION

Atrial fibrillation (AF) and underlying heart failure (HF) have been emerging topics of importance in the field of cardiovascular disease over the past 3 decades and frequently overlap (1, 2). Specifically, AF has been shown to follow HF with preserved ejection fraction (HFpEF) more frequently than HF with reduced ejection fraction (HFrEF) due to the differences in the left ventricular (LV) diastolic dysfunction and the left atrial (LA) remodeling process (2, 3). Prior studies have shown improvements in the LV systolic function (4), performance and quality of life (5), and mortality (6) after AF catheter ablation (AFCA) in HFrEF patients, suggesting that a reduction in AF may be sufficient for a clinical benefit. Nevertheless, there are no specific recommendations for the management of AF in HFpEF patients, and data regarding the efficacy of AFCA in patients with a normal LV systolic function and LV diastolic dysfunction are relatively limited. Although there have been a few studies reporting an improvement in the LV diastolic function after AFCA by maintaining sinus rhythm (7, 8), they adopted conventional approaches that used mainly symptoms and the LV ejection fraction (LVEF) for the diagnosis of HFpEF with various diagnostic accuracies (9). Recently, a novel scoring system has been developed, the H₂FPEF score (10), which can estimate the probability of the underlying HFpEF through six clinical and echocardiographic characteristics, and can be feasibly applied in clinical practice. The aim of this study was to better understand the factors by which LV diastolic function worsens or improves after AF rhythm control by AFCA. In this study, we used the H₂FPEF score at two time points, before and 1 year after the AFCA. We aimed to compare the cardiac structural and functional changes within a year and to evaluate the rhythm outcomes both within a year and over the long-term using the H₂FPEF score.

MATERIALS AND METHODS

Study Subjects

The study protocol adhered to the Declaration of Helsinki and was approved by the institutional review board of the Yonsei University Health system. All patients provided written informed consent for inclusion in the Yonsei AF Ablation Cohort Database (ClinicalTrials.gov Identifier: NCT02138695). From January 2009 to September 2019, 1,471 patients with a diagnosis of AF and a normal LVEF were identified as having clinical and echocardiographic information for the calculation of the H₂FPEF score before AFCA and 1 year after AFCA. All patients underwent AFCA, and the indications for the AFCA complied with the latest guidelines (11). The exclusion criteria for the study were as follows: (1) a reduced LVEF, defined as <50%; (2) a follow-up duration <12 months; and (3) a repeat ablation within a year (Figure 1).

Abbreviations: Δ H₂FPEF score_[1-yr], 1-year change in the H₂FPEF score; SustainAF_[1-yr], sustained AF after a recurrence within a year.

Calculating the H₂FPEF Score at Baseline and 1 Year After the Atrial Fibrillation Catheter Ablation

The H₂FPEF score has six domains based on clinical and echocardiographic values: heaviness (body mass index >30 kg/m², 2 points), hypertension (on two or more antihypertensive medicines, 1 point), atrial fibrillation (paroxysmal or persistent, 3 points), pulmonary hypertension (Doppler echocardiographic estimated pulmonary artery systolic pressure >35 mmHg, 1 point), elderly status (age >60 years, 1 point), and filling pressure (Doppler echocardiographic E/Em > 9, 1 point). The baseline H₂FPEF scores were obtained within 3 months prior to the AFCA, and the 1-year H₂FPEF scores were obtained 1 year after the AFCA with all clinical and echocardiographic values.

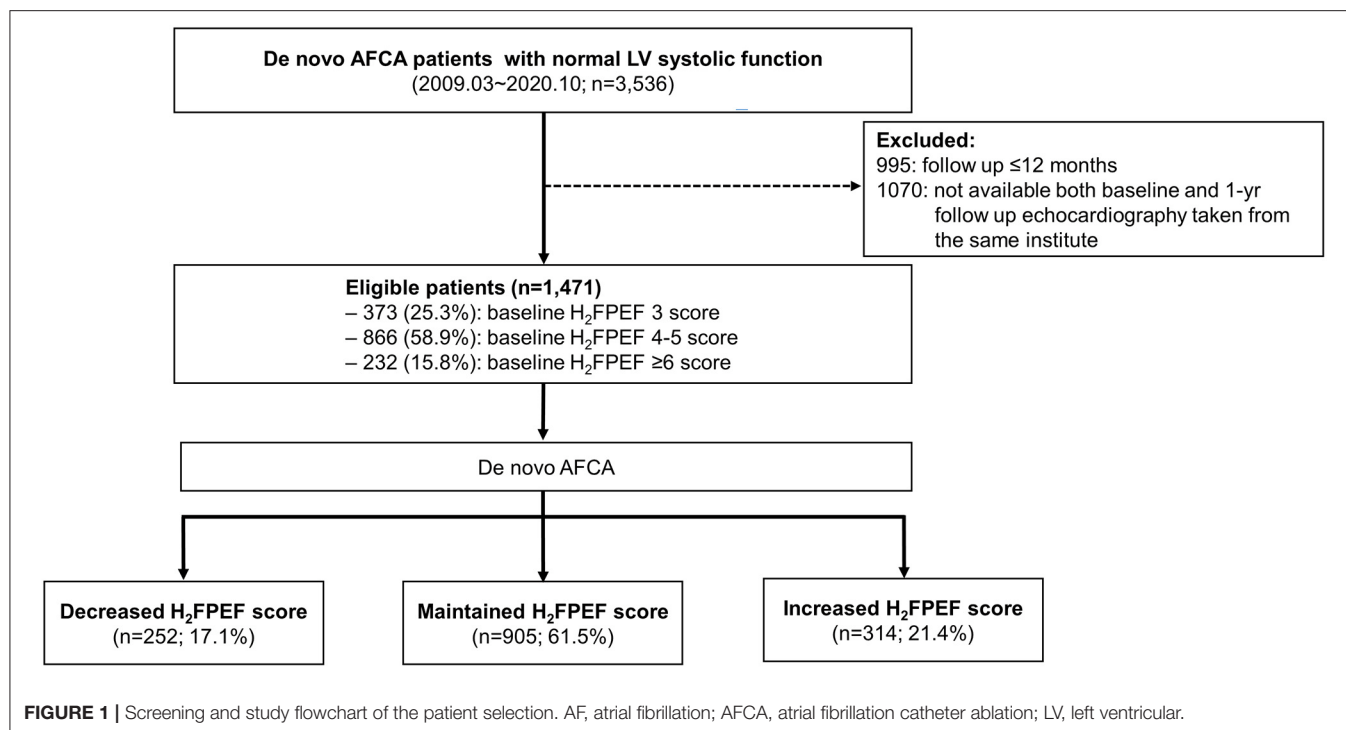
Echocardiographic Measurement and Three-Dimensional Computed Tomography

Transthoracic echocardiography was conducted in all patients using commercially available devices (Vivid 7 or Vivid E9 from GE Healthcare, Chicago, IL, USA, or iE 33 from Philips, Amsterdam, the Netherlands) as recommended by the American Society of Echocardiography (12). Standard images were obtained in the parasternal and apical views through two-dimensional (2D), Doppler, and M-mode imaging, including the LA anteroposterior diameter and the LV end-systolic and end-diastolic dimensions (LVESD and LVEDD). The early Doppler mitral inflow (E) was recorded using pulsed waves from the apical window, with a 1- to 3-mm pulsed Doppler sample volume placed between the tips and mitral leaflets during diastole. The early diastolic mitral annular velocity (Em) was recorded as the peak early diastolic tissue velocity using color Doppler tissue imaging of the septal mitral annulus. The ratio of the early diastolic mitral inflow velocity to the early diastolic mitral annular velocity (E/Em) was calculated. Tricuspid regurgitation (TR) and estimated right atrial (RA) pressure were evaluated using the recommended methods, and the right ventricular systolic pressure (RVSP) was calculated as $4 \times (\text{TR jet})^2 + \text{estimated RA pressure}$ (13). The initial and 1 year after AFCA, the echocardiographies used to estimate the H₂FPEF scores were those performed during an elective visit on stable medication.

Three-dimensional spiral computed tomography (CT) (64-channel, Light Speed Volume CT from GE Healthcare, Chicago, IL, USA, or Brilliance 63 from Philips, Amsterdam, the Netherlands) was performed in all patients, and the scans were analyzed using an imaging-processing workstation (Aquarius; TeraRecon, Inc., Foster City, CA, USA). The LA volume and pericardial fat volume measurements have been described in previous studies (14, 15).

Electrophysiologic Characterization and Radiofrequency Catheter Ablation

Intracardiac electrograms were obtained using the Prucka CardioLab™ Electrophysiology system (GE Healthcare, Chicago,



IL, USA). A 3D electro-anatomical map (Ensite NavX; Abbott Laboratories, Chicago, IL, USA; CARTO3; Johnson & Johnson Inc., USA) was generated using a circumferential pulmonary vein-mapping catheter through a long sheath (Schwartz left 1; Abbott Laboratories, Chicago, IL, USA) and by merging the 3D geometry generated by the electroanatomic mapping system with the corresponding 3D spiral CT images. Left atrium electrogram voltage maps were generated during high right atrial pacing at 500 ms to prevent rate-dependent activation changes and by measuring mean peak-to-peak voltage as previously described (16). All patients underwent a *de novo* procedure with a circumferential pulmonary vein isolation (CPVI). The endpoint of the CPVI was the electric isolation of the PV potentials and bidirectional block of the PVs. We tested whether there was an immediate recurrence of AF within 10 min after cardioversion with an isoproterenol infusion (5–20 μ g/min depending on the β -blocker used with a target sinus heart rate of 120 bpm) to find extra-PV foci triggers, then confirmed successful CPVI 30 min after the initial isolation. Extra-PV foci triggers under an isoproterenol infusion were ablated as much as possible if they were consistent and reproducible. Then, we ended the *de novo* procedure. The detailed procedural techniques and strategies for the AFCA have been presented in our previous studies (17, 18).

Post-ablation Management and Rhythm Follow-Up

The patients were discharged without any antiarrhythmic drugs (AADs) with the exception of those who had symptomatic frequent atrial premature beats, non-sustained atrial tachycardia (AT), early recurrence of AF on telemetry during the admission period, or recurrent extra-PV foci triggers after the AFCA

procedure (13.7%). The clinical and cardiac rhythm information was obtained regularly from an outpatient clinic at 1, 3, 6, and 12 months, and every 6 months thereafter (or whenever symptoms developed). All patients underwent electrocardiogram recordings at every visit, and 24-h Holter monitoring was performed at 3 and 6 months, and every 6 months thereafter, according to the latest guidelines (11). AF recurrence was defined as any episode of AF or AT of at least 30 s in duration. Any ECG documentation of an AF recurrence within the 3-month blanking period was diagnosed as an early recurrence, and an AF recurrence of more than 3 months after the AFCA was diagnosed as a clinical recurrence. We evaluated the time point of the clinical recurrence as follows: within 1 year as a short-term and beyond 1 year as a long-term recurrence. We also estimated the quality of the AF control after the AFCA. We defined patients with sustained AF/AT as those who remained in a sustaining AF/AT rhythm (>30 s) on the final follow-up after the AFCA despite AADs or electrical cardioversion.

Statistical Analysis

The baseline characteristics of the patients were compared using descriptive statistics and presented as median (interquartile interval) values for continuous variables and as numbers (percentages) for categorical variables. To compare the baseline characteristics according to the baseline H₂FPEF and the 1-year change in the H₂FPEF score (Δ H₂FPEF score_[1-yr]) categories, the Mantel-Haenszel chi-squared test was used for categorical variables, and the Kruskal-Wallis H test was used for continuous variables. To identify the factors associated with the baseline H₂FPEF and Δ H₂FPEF score_[1-yr], univariate and multivariable logistic regression analyses were performed. Multivariable Cox

TABLE 1 | Baseline characteristics according to the baseline H₂FPEF score stratification in atrial fibrillation (AF) patients with a normal left ventricular ejection fraction (LVEF).

	Overall (N = 1,471)	3 score (N = 373)	4–5 score (N = 866)	≥6 score (N = 232)	p-value
Age, years	60 (53, 68)	52 (46, 56)	63 (56, 69)	68 (63, 74)	<0.001
Age over 65 years, n (%)	463 (31.5)	0 (0)	316 (36.5)	147 (63.4)	<0.001
Female, n (%)	452 (30.7)	65 (17.4)	282 (32.6)	105 (45.3)	<0.001
FU duration, months	56 (32, 87)	54 (31, 85)	58 (33, 89)	57 (33, 86)	0.232
BMI, kg/m ²	24.6 (23.0, 26.6)	24.5 (23.0, 26.4)	24.4 (22.8, 26.4)	25.4 (23.6, 28.0)	<0.001
Paroxysmal AF, n (%)	1,009 (68.6)	266 (71.3)	597 (68.9)	146 (62.9)	0.092
Smoking, n (%)					0.014
Never	957 (65.1)	232 (62.2)	555 (64.1)	170 (73.3)	
Former/current	514 (34.9)	141 (37.8)	311 (35.9)	62 (26.7)	
Alcohol, n (%)					<0.001
Never	771 (52.4)	159 (42.6)	461 (53.2)	151 (65.1)	
Former/current	700 (47.6)	214 (57.4)	405 (46.8)	81 (34.9)	
CHA ₂ DS ₂ -VASc score	2 (1, 3)	0 (0, 1)	2 (1, 3)	3 (2, 4)	<0.001
Heart failure, n (%)*	96 (6.5)	14 (3.8)	51 (5.9)	31 (13.4)	<0.001
Hypertension, n (%)	714 (48.5)	54 (14.5)	450 (52.0)	210 (90.5)	<0.001
Diabetes, n (%)	222 (15.1)	27 (7.2)	134 (15.5)	61 (26.3)	<0.001
Prior stroke/TIA, n (%)	180 (12.2)	25 (6.7)	113 (13.0)	42 (18.1)	0.004
Vascular disease, n (%)	194 (13.2)	10 (2.7)	135 (15.6)	49 (21.1)	<0.001
Hypertrophic cardiomyopathy, n (%)	36 (2.4)	1 (0.3)	28 (3.2)	7 (3.0)	0.007
Obstructive sleep apnea, n (%)	16 (1.1)	3 (0.8)	11 (1.3)	2 (0.9)	0.720
Chronic obstructive pulmonary disease, n (%)	21 (1.4)	2 (0.5)	14 (1.6)	5 (2.2)	0.202
Thyroid disease, n (%)	108 (7.3)	20 (5.4)	67 (7.7)	21 (9.1)	0.188
Laboratory					
eGFR, ml/min/1.73 m ²	80.3 (69.0, 92.9)	86.1 (75.3, 97.2)	79.5 (68.9, 92.1)	72.0 (59.4, 84.3)	<0.001
hs-CRP, mg/dl	0.7 (0.5, 1.5)	0.7 (0.3, 1.3)	0.8 (0.5, 1.4)	1.0 (0.6, 2.1)	<0.001
Echocardiography					
LA diameter, mm	41 (37, 45)	39 (36, 42)	41 (37, 45)	44 (41, 48)	<0.001
LAVI, ml/m ²	35.0 (28.3, 43.0)	30.3 (25.8, 35.9)	35.6 (28.7, 43.4)	42.0 (35.0, 50.5)	<0.001
LVEF, %	65 (60, 69)	63 (60, 68)	65 (61, 69)	65 (61, 70)	0.009
E/Em	9.2 (7.8, 12.0)	7.0 (6.0, 8.0)	10.0 (8.0, 12.0)	12.9 (11.0, 15.4)	<0.001
E, m/s	0.7 (0.6, 0.8)	0.7 (0.6, 0.8)	0.7 (0.6, 0.8)	0.8 (0.6, 1.0)	<0.001
Em, cm/s	7.0 (6.0, 9.0)	9.5 (8.0, 11.0)	7.0 (5.6, 8.7)	6.0 (5.0, 7.2)	<0.001
TR jet, m/s	2.3 (2.1, 2.5)	2.1 (2.0, 2.3)	2.3 (2.1, 2.5)	2.5 (2.3, 2.8)	<0.001
RVSP, mmHg	26 (22, 30)	24 (21, 27)	26 (23, 30)	30 (26, 37)	<0.001
LVEDD, mm	50 (46, 53)	50 (47, 52)	50 (46, 52)	50 (46, 53)	0.412
LVESD, mm	33 (30, 36)	33 (31, 36)	33 (30, 35)	33 (30, 36)	0.140
LVMI, g/m ²	90.9 (79.4, 102.9)	85.0 (75.8, 94.8)	91.3 (80.1, 104.4)	99.2 (87.9, 112.6)	<0.001
3D-CT, ml					
Pericardial fat volume	101.1 (70.8, 140.9)	91.2 (63.8, 135.3)	101.9 (70.9, 143.3)	113.8 (81.5, 156.1)	<0.001
LA volume	147.3 (120.8, 178.2)	132.3 (113.4, 163.2)	149.0 (122.6, 177.9)	163.9 (140.4, 195.1)	<0.001
Voltage, mV					
LA mean voltage	1.3 (0.8, 1.8)	1.5 (1.0, 2.0)	1.3 (0.8, 1.7)	1.1 (0.7, 1.5)	<0.001
LA pressure, mmHg					
Peak	20 (15, 27)	20 (15, 25)	20 (15, 27)	22 (17, 29)	0.004
Nadir	4 (1, 8)	4 (1, 8)	5 (1, 8)	5 (1, 9)	0.348
Mean	11 (8, 16)	11 (7, 15)	11 (8, 16)	13 (9, 17)	0.006
Medication, n (%)					
ACEI/ARB	493 (33.5)	33 (8.8)	293 (33.9)	167 (72.0)	<0.001
Beta-blocker	487 (33.1)	104 (27.9)	274 (31.7)	109 (47.0)	<0.001

(Continued)

TABLE 1 | Continued

	Overall (N = 1,471)	3 score (N = 373)	4–5 score (N = 866)	≥6 score (N = 232)	p-value
Statin	491 (33.4)	57 (15.3)	322 (37.2)	112 (48.3)	<0.001
AAD [†]	200 (13.7)	40 (10.8)	118 (13.7)	42 (18.1)	0.038
Recurrence after AFCA, n (%)					
Early recurrence	448 (30.5)	111 (29.8)	271 (31.3)	66 (28.4)	0.666
Clinical recurrence					
1-year duration	257 (17.5)	62 (16.6)	152 (17.6)	43 (18.5)	0.830
Total duration	611 (41.5)	145 (38.9)	358 (41.3)	108 (46.6)	0.173
Sustained AF					
1-year duration	56 (3.8)	16 (4.3)	28 (3.2)	12 (5.2)	0.333
Total duration	95 (6.5)	27 (7.2)	51 (5.9)	17 (7.3)	0.568

The data are presented as the number (%) and median (interquartile interval). Non-parametric continuous variables as assessed by the Kolmogorov–Smirnov method were analyzed by the Mann–Whitney U-test. AAD, antiarrhythmic drug; ACEi, angiotensin-converting-enzyme inhibitor; AF, atrial fibrillation; AFCA, atrial fibrillation catheter ablation; ARB, angiotensin receptor blocker; BMI, body mass index; E/Em, ratio of the early diastolic mitral inflow velocity (E) to the early diastolic mitral annular velocity (Em); FU, follow up; eGFR, estimated glomerular filtration rate; hs-CRP, high sensitivity C-reactive protein; LA, left atrium; LVEDD, left ventricular end diastolic dimension; LVEF, left ventricular ejection fraction; LVESD, left ventricular end systolic dimension; LVMI, left ventricular mass index; RVSP, right ventricular systolic pressure; TIA, transient ischemic attack; TR, tricuspid regurgitation. [†]Defined as conventional HFpEF diagnosis criteria: left ventricular ejection fraction ≥50% with exertional dyspnea that was not caused by extracardiac causes. [‡]Including class Ic and III drugs, taken after catheter ablation.

proportional hazard analyses were performed to evaluate the association of the baseline H₂FPEF and ΔH₂FPEF score_[1-yr] with a clinical recurrence in both the short- and long-term periods. A multivariable regression analysis included those variables with significant *p*-values of <0.1 in the univariate analysis. The Cox proportional hazards assumption was tested based on Schoenfeld residuals. Two-sided *p*-values < 0.05 were considered statistically significant. The statistical analyses were conducted using SAS version 9.4 (SAS Institute) and R version 4.0.0 (R Foundation for Statistical Computing) software.

RESULTS

Characteristics of the Patients With High H₂FPEF Scores

Table 1 summarizes patient characteristics depending on the H₂FPEF score. In the 1,471 patients, the median (IQR) age was 60 (53, 68) years, 30.7% were female, and 68.6% had paroxysmal-type AF. The baseline H₂FPEF scores were 3 points in 373 patients (25.3%), 4–5 points in 866 (58.9%), and ≥6 points in 232 (15.8%) patients. Patients with higher H₂FPEF scores were older and had a higher body mass index, hypertension, estimated RVSP, and higher E/Em, as those variables that composed this score. In the higher H₂FPEF score-group, the CHA₂DS₂-VASc score (*p* < 0.001), prevalence of diabetes (*p* < 0.001), a prior stroke (*p* = 0.004), vascular disease (*p* < 0.001), or hypertrophic cardiomyopathy (*p* = 0.007) were higher. The CT-measured LA volume (*p* < 0.001), pericardial fat volume (*p* < 0.001), and LA peak pressure (*p* = 0.004) were higher, and the endocardial bipolar LA voltage (*p* < 0.001) and eGFR (*p* < 0.001) were significantly lower in the higher H₂FPEF score-group. In the multivariate logistic regression analysis (Supplementary Table 1), high baseline H₂FPEF scores (≥6) were independently associated with the female sex [OR, 2.31 (1.35–3.93), *p* = 0.002], higher left atrial (LA) diameter [OR, 1.09

(1.04–1.13), *p* < 0.001], LV mass index [OR 1.02 (1.01–1.03), *p* = 0.002], pericardial fat volume [OR, 1.01 (1.00–1.01), *p* = 0.015], and lower eGFR [OR, 0.98 (0.97–0.99), *p* < 0.001].

ΔH₂FPEF Score_[1-yr]

One year after the AFCA, the H₂FPEF scores decreased in 17.1% of the patients (252), were maintained in 61.5% (905), and increased in 21.4% (314) (Table 2). A reduction in the ΔH₂FPEF score_[1-yr] was more commonly observed in patients with high baseline H₂FPEF scores (Figure 2A) and was independently associated with baseline H₂FPEF scores of ≥6 [OR, 4.19 (2.88–6.11), *p* < 0.001], the absence of diabetes [OR, 0.60 (0.37–0.98), *p* = 0.04], a higher LVEF [OR, 1.03 (1.01–1.06), *p* = 0.011], and a lower pericardial fat volume [OR, 0.99 (0.99–1.00), *p* = 0.003; Table 3]. Increased ΔH₂FPEF scores_[1-yr] were less commonly observed in patients with high H₂FPEF scores (Figure 2B) and were associated with a baseline H₂FPEF score of <6 [OR, 3.54 (2.08–6.04), *p* < 0.001], sustained AF after a recurrence within a year [SustainAF_[1-yr]; OR, 1.89 (1.01–3.54), *p* = 0.048], the LA volume [OR, 1.00 (1.00–1.01), *p* = 0.029], and the pericardial fat volume [OR, 1.00 (1.00–1.01), *p* = 0.032; Table 4].

Rhythm Outcomes After Atrial Fibrillation Catheter Ablation and the H₂FPEF Score

Because we evaluated the H₂FPEF score before and 1 year after the procedure, we compared the 1-year and long-term clinical recurrence rates of AF separately, depending on the baseline H₂FPEF scores and ΔH₂FPEF scores_[1-yr]. In contrast, the baseline H₂FPEF scores did not affect the 1-year rhythm outcome (log rank, *p* = 0.82; Figure 3A), and the clinical recurrence of AF was significantly higher in the patients with an increased ΔH₂FPEF scores_[1-yr] (log rank, *p* = 0.003; Figure 3B). In the multivariate Cox regression analysis, increased ΔH₂FPEF scores_[1-yr] [HR, 2.34 (1.36–4.03), *p* = 0.002] and persistent AF [HR, 1.43 (1.01–2.03), *p* = 0.043]

TABLE 2 | Baseline characteristics according to the change in the H₂FPEF scores in AF patients with a normal LVEF, 1-year after the atrial fibrillation catheter ablation (AFCA).

	Decreased (−2, −1) (N = 252)	Maintained (0) (N = 905)	Increased (+1, +2, +3) (N = 314)	p-value
Age, years*	62 (55, 68)	59 (52, 67)	60 (58, 68)	<0.001
Age over 65 years, n (%)	82 (32.5)	272 (30.1)	109 (34.7)	0.286
v Female, n (%)	78 (31.0)	283 (31.3)	91 (29.0)	0.748
FU duration, months	53 (31, 88)	58 (32, 87)	58 (33, 89)	0.467
BMI, kg/m ²	24.4 (22.8, 26.1)	24.6 (23.0, 26.7)	24.6 (23.2, 26.7)	0.445
Paroxysmal AF, n (%)	183 (72.6)	622 (68.7)	204 (65.0)	0.148
Smoking, n (%)				0.152
Never	157 (62.3)	606 (67.0)	194 (61.8)	
Former/current, n (%)	95 (37.7)	299 (33.0)	120 (38.2)	
Alcohol				0.222
Never	141 (56.0)	477 (52.7)	153 (48.7)	
Former/current	111 (44.0)	428 (47.3)	161 (51.3)	
CHA ₂ DS ₂ -VASc score	2 (1, 3)	1 (0, 3)	2 (1, 3)	0.238
Heart failure, n (%) [†]	20 (7.9)	56 (6.2)	20 (6.4)	0.606
Hypertension, n (%)	133 (52.8)	431 (47.6)	150 (47.8)	0.335
Diabetes, n (%)	29 (11.5)	142 (15.7)	51 (16.2)	0.212
Prior stroke/TIA, n (%)	37 (14.7)	108 (11.9)	35 (11.1)	0.401
Vascular disease, n (%)	33 (13.1)	115 (12.7)	46 (14.6)	0.680
Hypertrophic cardiomyopathy, n (%)	11 (4.4%)	17 (1.9%)	8 (2.5%)	0.077
Obstructive sleep apnea, n (%)	2 (0.8)	12 (1.3)	2 (0.6)	0.529
Chronic obstructive pulmonary disease, n (%)	3 (1.2)	12 (1.3)	6 (1.9)	0.709
Thyroid disease, n (%)	16 (6.3)	68 (7.5)	24 (7.6)	0.800
Laboratory	80.3 (69.8, 91.4)	81.0 (69.4, 93.8)	79.0 (66.7, 90.7)	0.265
eGFR, ml/min/1.73m ²				
hs-CRP, mg/dl	0.8 (0.5, 1.4)	0.7 (0.5, 1.5)	0.8 (0.6, 1.8)	0.170
Echocardiography	41 (38, 45)	41 (37, 45)	41 (37, 46)	0.264
LA diameter, mm				
LAVI, ml/m ²	36.2 (29.4, 44.3)	34.5 (27.7, 42.7)	35.5 (28.4, 43.6)	0.134
LVEF, %	66 (62, 70)	64 (60, 69)	65 (60, 69)	0.005
E/Em,	11.0 (10.0, 13.0)	9.0 (7.0, 12.0)	8.4 (7.3, 9.0)	<0.001
E, m/s	0.8 (0.6, 0.9)	0.7 (0.6, 0.8)	0.7 (0.6, 0.8)	<0.001
Em, cm/s	6.3 (5.0, 8.0)	7.5 (6.0, 9.3)	8.0 (6.0, 9.0)	<0.001
TR jet, m/s	2.4 (2.1, 2.7)	2.2 (2.1, 2.4)	2.3 (2.1, 2.4)	<0.001
RVSP, mmHg	28 (23, 36)	25 (22, 29)	26 (22, 29)	<0.001
LVEDD, mm	50 (46, 53)	50 (47, 53)	49 (46, 52)	0.309
LVESD, mm	33 (30, 36)	33 (30, 36)	33 (31, 35)	0.285
LVMI, g/m ²	92.9 (80.5, 107.1)	90.1 (79.4, 102.3)	91.6 (79.2, 102.3)	0.223
3D-CT, ml	96.8 (63.8, 134.1)	100.9 (71.0, 140.6)	108.4 (78.3, 150.3)	0.012
Pericardial fat volume				
LA volume	144.0 (121.2, 174.2)	146.9 (119.8, 176.6)	152.7 (124.2, 184.1)	0.084
Voltage, mV	1.4 (0.9, 1.8)	1.3 (0.9, 1.8)	1.3 (0.8, 1.7)	0.277
LA mean voltage				
LA pressure, mmHg	20 (15, 27)	20 (15, 27)	21 (16, 27)	0.394
Peak				
Nadir	5 (1, 9)	4 (1, 8)	5 (1, 8)	0.681
Mean	11 (8, 16)	11 (8, 16)	12 (8, 16)	0.333
1-year change in the Echocardiography, Δ	−4 (−6, −1)	−3 (−5, 0)	−2 (−5, 1)	<0.001
Δ LA diameter, mm				
Δ LAVI, ml/m ²	−8.0 (−14.0, −2.3)	−5.2 (−10.7, −0.2)	−3.6 (−9.1, 1.9)	<0.001
Δ LVEF, %	1 (−4, 5)	1 (−3, 6)	1 (−3, 5)	0.459
Δ E/Em	−3.0 (−4.2, −1.4)	0 (−1.1, 1.5)	2.8 (1.0, 4.4)	<0.001
Δ E, m/s	−0.1 (−0.2, 0)	0 (−0.2, 0)	0 (−0.1, 0.1)	<0.001
Δ Em, cm/s	0.9 (−1.0, 2.0)	−0.8 (−2.0, 0.5)	−1.4 (−3.0, 0)	<0.001
Δ TR jet, m/s	−0.1 (−0.4, 0)	0 (−0.2, 0.2)	0.1 (−0.1, 0.4)	<0.001
Δ RVSP, mmHg	−3 (−9, 0)	−1 (−4, 3)	2.2 (−3, 8)	<0.001
Δ LVEDD, mm	−1 (−3, 1)	0 (−2, 2)	0 (−2, 2)	<0.001
Δ LVESD, mm	−1 (−3, 1)	−1 (−3, 1)	0 (−2, 2)	0.007
Δ LVMI, g/m ²	−2.8 (−13.9, 5.6)	1.0 (−9.4, 9.4)	3.3 (−8.8, 13.1)	<0.001

(Continued)

TABLE 2 | Continued

	Decreased (−2, −1) (N = 252)	Maintained (0) (N = 905)	Increased (+1, +2, +3) (N = 314)	p-value
Medication, n (%)	96 (38.1)	298 (33.0)	99 (31.5)	0.218
ACEi/ARB				
Beta-blocker	93 (36.9)	285 (31.5)	109 (34.7)	0.220
Statin	96 (38.1)	289 (32.0)	106 (33.8)	0.188
AAD [‡]	30 (12.0)	118 (13.1)	52 (16.6)	0.204
Recurrence after the AFCA, n (%)	68 (27.0)	281 (31.0)	99 (31.5)	0.416
Early recurrence				
Clinical recurrence	33 (13.1)	151 (16.7)	73 (23.2)	0.004
1-year duration				
Total duration	99 (39.3)	361 (39.9)	151 (48.1)	0.029
Sustained AF	6 (2.4)	32 (3.5)	18 (5.7)	0.093
1-year duration				
Total duration	14 (5.6)	59 (6.5)	22 (7.0)	0.774

The data are presented as the number (%) and median (interquartile interval). Non-parametric continuous variables as assessed by the Kolmogorov–Smirnov method, were analyzed by the Mann–Whitney U-test. AAD, antiarrhythmic drug; ACEi, angiotensin-converting-enzyme inhibitor; AF, atrial fibrillation; AFCA, atrial fibrillation catheter ablation; ARB, angiotensin receptor blocker; BMI, body mass index; EEm, ratio of the early diastolic mitral inflow velocity (E) to the early diastolic mitral annular velocity (Em); FU, follow up; eGFR, estimated glomerular filtration rate; hs-CRP, high sensitivity C-reactive protein; LA, left atrium; LVEDD, left ventricular end diastolic dimension; LVEF, left ventricular ejection fraction; LVESD, left ventricular end systolic dimension; LVMI, left ventricular mass index; RVSP, right ventricular systolic pressure; TIA, transient ischemic attack; TR, tricuspid regurgitation. *Increased vs. maintained, $p < 0.001$; increased vs. decreased, $p = 0.263$; maintained vs. decreased, $p = 0.105$. [‡] Defined as conventional HFpEF diagnosis criteria: left ventricular ejection fraction $\geq 50\%$ with exertional dyspnea that was not caused by extracardiac causes. [‡] Including class Ic and III drugs, taken after catheter ablation.

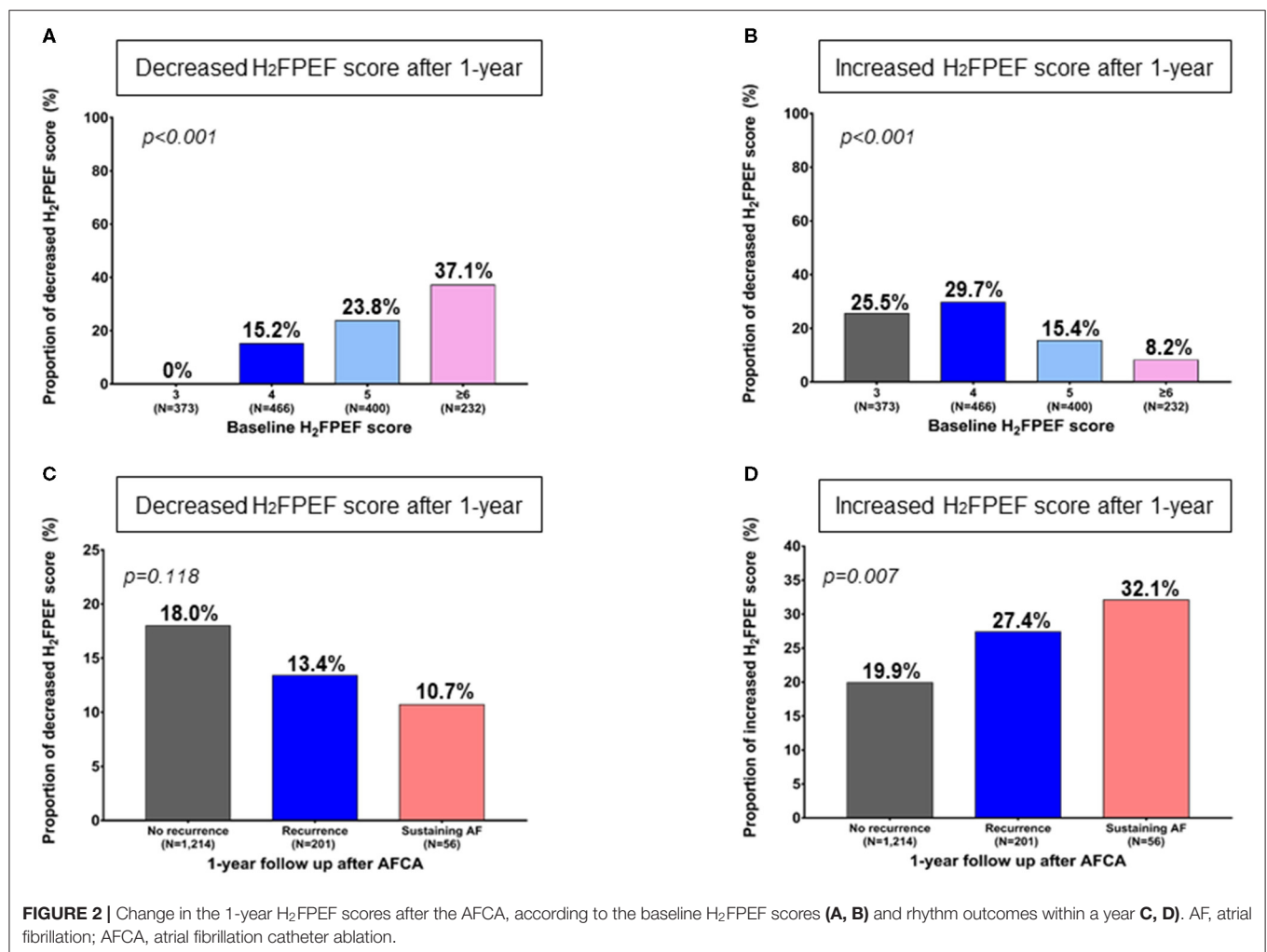


TABLE 3 | Logistic regression analysis for predictors of decreased H₂FPEF scores, 1-year after the AFCA.

	Univariate analysis		Multivariable analysis	
	Unadjusted HR (95% CI)	p-value	Adjusted HR (95% CI)	p-value
Baseline H₂FPEF scores				
<6 score (reference)	1.00 (reference)		1.00 (reference)	
≥6 score	3.81 (2.79–5.21)	<0.001	4.19 (2.88–6.11)	<0.001
Age	1.01 (0.99–1.02)	0.285		
Female	1.01 (0.76–1.36)	0.932		
Paroxysmal AF	1.26 (0.93–1.70)	0.135		
Body mass index	0.98 (0.94–1.03)	0.484		
Smoking	1.16 (0.87–1.53)	0.314		
Alcohol	0.84 (0.64–1.11)	0.217		
Heart failure*	1.30 (0.78–2.16)	0.321		
Hypertension	1.23 (0.94–1.61)	0.139		
Diabetes	0.69 (0.46–1.05)	0.082	0.60 (0.37–0.98)	0.040
Prior stroke/TIA	1.29 (0.88–1.91)	0.194		
Vascular disease	0.99 (0.66–1.48)	0.962		
CHA ₂ DS ₂ -VASc score	1.05 (0.96–1.14)	0.306		
LA diameter	1.00 (0.98–1.03)	0.708		
LVEF	1.04 (1.01–1.06)	0.002	1.03 (1.01–1.06)	0.011
E/Em	1.13 (1.09–1.66)	<0.001		
TR jet velocity	1.16 (0.98–1.36)	0.077		
RVSP	1.09 (1.06–1.11)	<0.001		
LVEDD	1.01 (0.98–1.05)	0.412		
LVESD	0.97 (0.94–1.01)	0.145		
LVMI	1.01 (1.00–1.01)	0.062	1.00 (0.99–1.01)	0.708
eGFR	1.00 (0.99–1.01)	0.745		
hs-CRP	1.00 (0.99–1.00)	0.665		
Pericardial fat volume	1.00 (1.00–1.00)	0.087	0.99 (0.99–1.00)	0.003
LA volume	1.00 (0.99–1.01)	0.540		
LA mean voltage	1.03 (0.81–1.30)	0.825		
LA peak pressure	0.99 (0.97–1.01)	0.166		
Sustained AF[†]				
1-year duration	0.57 (0.24–1.35)	0.199		
Total duration	0.83 (0.46–1.48)	0.522		

AF, atrial fibrillation; AFCA, atrial fibrillation catheter ablation; BMI, body mass index; EEm, ratio of the early diastolic mitral inflow velocity (E) to the early diastolic mitral annular velocity (Em); eGFR, estimated glomerular filtration rate; hs-CRP, high sensitivity C-reactive protein; LA, left atrium; LVEDD, left ventricular end diastolic dimension; LVEF, left ventricular ejection fraction; LVESD, left ventricular end systolic dimension; LVMI, left ventricular mass index; RVSP, right ventricular systolic pressure; TIA, transient ischemic attack; TR, tricuspid regurgitation.

*Defined as conventional HFpEF diagnosis criteria: left ventricular ejection fraction ≥50% with exertional dyspnea that was not caused by extracardiac causes. [†]Defined as patients who remained in a sustained AF rhythm (>30 s) on the final follow-up date despite antiarrhythmic drugs or electrical cardioversion.

were independently associated with an AF recurrence within a year (**Supplementary Table 2**). During the median follow-up of 56 (32, 87) months, increased Δ H₂FPEF scores_[1-yr] [HR, 1.41 (1.01–1.98), $p = 0.045$], the LA diameter [HR, 1.03 (1.01–1.06), $p = 0.002$], and the LA voltage [HR, 0.59 (0.48–0.73), $p < 0.001$] were independently associated with a long-term AF recurrence (**Table 5**). The rhythm outcomes in the overall duration were consistent with the 1-year rhythm outcome depending on the baseline H₂FPEF score (log rank, $p = 0.57$; **Figure 3C**) or Δ H₂FPEF score_[1-yr] (log rank, $p = 0.01$; **Figure 3D**). In the subgroup of patients with baseline H₂FPEF scores of ≥5, the risk of an AF recurrence was significantly higher in the patients with increased Δ H₂FPEF

scores_[1-yr] than in those with reduced Δ H₂FPEF scores_[1-yr] (**Supplementary Figure 1**).

Failed Rhythm Control and the Δ H₂FPEF Score_[1-yr]

Among the 1,471 patients, 257 (17.5%) had an AF recurrence within a year, and 201 (13.7%) had sinus rhythm restored after using antiarrhythmic drugs, but 56 (3.8%) patients had sustained AF under antiarrhythmic drugs even after cardioversion. The proportion of patients with reduced Δ H₂FPEF scores_[1-yr] tended to be higher without a statistical significance among those with no recurrence ($p = 0.118$, **Figure 2C**). However,

TABLE 4 | Logistic regression analysis for predictors of increased H₂FPEF scores, 1-year after the AFCA.

	Univariate analysis		Multivariable analysis	
	Unadjusted OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Baseline H₂FPEF scores				
<6 score	1.14 (0.89–1.46)	0.311	3.54 (2.08–6.04)	<0.001
≥6 score (reference)	1.00 (reference)		1.00 (reference)	
Age	1.03 (1.01–1.04)	<0.001		
Female	0.90 (0.68–1.18)	0.450		
Paroxysmal AF	0.81 (0.62–1.05)	0.114		
Body mass index	1.02 (0.98–1.07)	0.323		
Smoking	1.20 (0.93–1.55)	0.170		
Alcohol	1.21 (0.94–1.55)	0.140		
CHA ₂ DS ₂ -VASc score	1.00 (0.92–1.08)	0.965		
Heart failure*	0.97 (0.58–1.61)	0.899		
Hypertension	0.96 (0.75–1.23)	0.759		
Diabetes	1.12 (0.80–1.57)	0.521		
Prior stroke/TIA	0.88 (0.59–1.30)	0.507		
Vascular disease	1.17 (0.82–1.67)	0.389		
LA diameter	1.02 (0.99–1.04)	0.168		
LVEF	1.00 (0.98–1.02)	0.813		
E/Em	0.89 (0.86–0.93)	<0.001		
TR jet velocity	0.81 (0.56–1.18)	0.276		
RVSP	0.98 (0.96–1.00)	0.071		
LVEDD	0.98 (0.95–1.01)	0.271		
LVESD	0.99 (0.96–1.02)	0.484		
LVMl	1.00 (0.99–1.01)	0.595		
eGFR	1.00 (0.99–1.00)	0.122		
hs-CRP	0.99 (0.97–1.02)	0.435		
Pericardial fat volume	1.00 (1.00–1.01)	0.012	1.00 (1.00–1.01)	0.032
LA volume	1.00 (1.00–1.01)	0.023	1.00 (1.00–1.01)	0.029
LA mean voltage	0.84 (0.67–1.05)	0.126		
LA peak pressure	1.01 (0.99–1.02)	0.405		
Sustained AF[†]				
1-year duration	1.79 (1.01–3.18)	0.047	1.89 (1.01–3.54)	0.048
Total duration	1.12 (0.68–1.83)	0.656		

AF, atrial fibrillation; AFCA, atrial fibrillation catheter ablation; BMI, body mass index; EEm, ratio of the early diastolic mitral inflow velocity (E) to the early diastolic mitral annular velocity (Em); eGFR, estimated glomerular filtration rate; hs-CRP, high sensitivity C-reactive protein; LA, left atrium; LVEDD, left ventricular end diastolic dimension; LVEF, left ventricular ejection fraction; LVESD, left ventricular end systolic dimension; LVMl, left ventricular mass index; RVSP, right ventricular systolic pressure; TIA, transient ischemic attack; TR, tricuspid regurgitation.

*Defined as conventional HFpEF diagnosis criteria: left ventricular ejection fraction ≥50% with exertional dyspnea that was not caused by extracardiac causes. [†]Defined as patients who remained in a sustained AF rhythm (>30 sec) on the final follow-up date despite antiarrhythmic drugs or electrical cardioversion.

the proportion of patients with increased Δ H₂FPEF scores_[1-yr] was significantly higher in the group with sustained AF after a recurrence than in those with sinus rhythm maintained at a year after the AFCA ($p = 0.007$, **Figure 2D**).

DISCUSSION

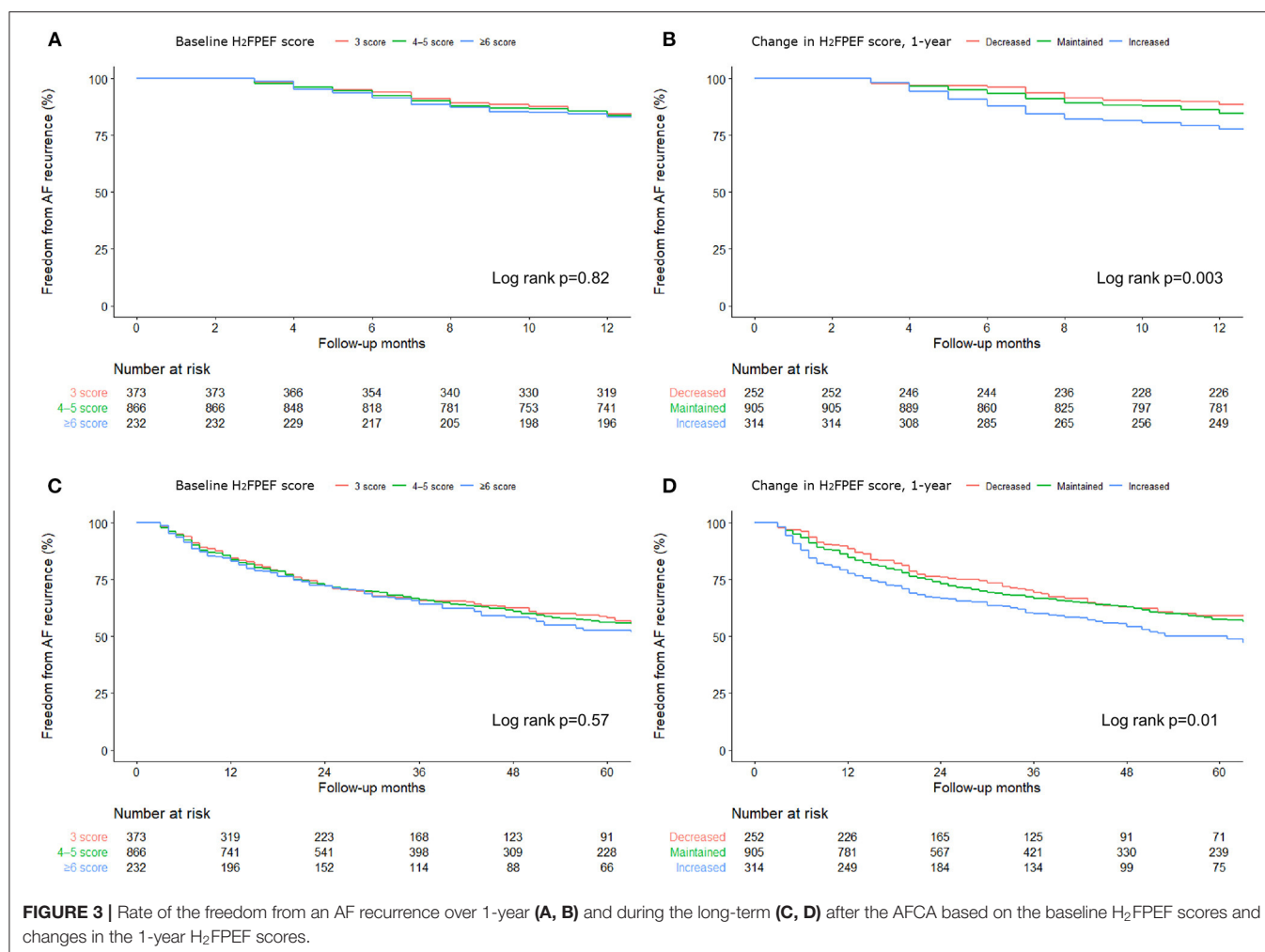
Main Findings

In this study, we observed a change in the H₂FPEF score 1 year after the AFCA in AF patients with a normal LV systolic function. The H₂FPEF score_[1-yr] decreased in 17% of the patients but increased in 21% a year after the AFCA. A high baseline H₂FPEF score, which is related to LV diastolic dysfunction, was independently associated with a reduced Δ H₂FPEF score_[1-yr].

On the other hand, low baseline scores or sustained AF after a recurrence were significantly associated with an increase in the Δ H₂FPEF scores_[1-yr] after the AFCA. Patients with an increased Δ H₂FPEF score_[1-yr] had higher rates of recurrence within a year or longer. Therefore, AFCA improved the H₂FPEF scores_[1-yr] in patients with baseline LV diastolic dysfunction; however, patients with a poor rhythm control and sustained AF despite AFCA had a significant increase in the Δ H₂FPEF scores_[1-yr].

Atrial Fibrillation and the Ventricular Diastolic Function

AF and LV diastolic dysfunction are closely related and have important features in common, such as age, obesity, hypertension, and diabetes (19). LV diastolic dysfunction has



deteriorative effects on the atrial function and structure, which contributes to the development, progression, and maintenance of AF (20). Consequently, AF has an influence on the LV function and LA remodeling (21), and can lead to increasingly sustained AF episodes. Therefore, these two conditions have pathophysiological effects on the occurrence and aggravation of each, and coexistence is associated with a poor prognosis (2, 22, 23). Furthermore, as the AF burden increases chronically, the right ventricular function progressively worsens (24). Reddy et al. (10) proposed a novel risk score, the H₂FPEF score, which includes all of the abovementioned factors, to diagnose HFpEF patients. We adopted this score to evaluate the prognostic utility in AF patients with a normal LVEF after AFCA.

Effects of Atrial Fibrillation Catheter Ablation on the Left Ventricular Diastolic Function

The data to support the clinical benefits of catheter ablation in symptomatic AF patients with HFrEF are strong and compatible with those from previous clinical trials and meta-analyses (6, 25). However, AF is more potently associated with HFpEF

(2) with the prevalence of HFpEF increasing by almost half in AF patients (26). The benefits of AFCA in symptomatic AF patients with HFpEF have been investigated in previous studies (7, 8), but the effects seem less favorable than those in HFrEF patients. Machino-Ohtsuka et al. (7) showed that the longstanding persistent-type AF and a lack of hypertension were factors associated with an improvement in the LV systolic and diastolic indices when sinus rhythm was maintained. Black-Maier et al. (8) showed equivalent rhythm outcomes, all-cause hospitalization, and cardiovascular hospitalization in patients with both HFpEF and HFrEF after AFCA over a median follow-up of 10 months. However, previous studies adopted conventional diagnostic approaches that mainly consisted of symptoms and the LVEF for an HFpEF diagnosis, which show a heterogeneity in terms of the inclusion (9). Thus, we used the H₂FPEF scores that were newly developed for identifying HFpEF patients and which were superior to the previous diagnostic algorithms. Although the baseline H₂FPEF scores do not have prognostic value for rhythm outcomes after AFCA, which is consistent with a recent study (27), increased Δ H₂FPEF scores_[1-yr] were independently associated with the rhythm outcomes in this study. Although the cause–result relationship

TABLE 5 | Cox regression analysis of the predictors of an AF recurrence after the AFCA, during the long-term follow up.

	Univariable analysis		Multivariable analysis	
	Unadjusted HR (95% CI)	p-value	Adjusted HR (95% CI)	p-value
Baseline H ₂ FPEF scores	1.03 (0.96–1.11)	0.421		
Baseline H₂FPEF scores				
3 score (reference)	1.00			
4–5 score	1.03 (0.85–1.25)	0.757		
≥6 score	1.14 (0.89–1.46)	0.311		
Change in H ₂ FPEF scores, 1 year	1.20 (1.07–1.35)	0.002		
Change in H₂FPEF scores, 1-year				
Decreased, <0 (reference)	1.00		1.00	
Maintained, 0	1.06 (0.85–1.32)	0.611	1.06 (0.78–1.44)	0.699
Increased, >0	1.38 (1.07–1.78)	0.012	1.41 (1.01–1.98)	0.045
Age	1.00 (1.00–1.01)	0.460		
Female	1.09 (0.92–1.29)	0.307	1.05 (0.82–1.34)	0.694
Persistent AF	1.71 (1.62–2.24)	<0.001	1.19 (0.93–1.53)	0.163
Body mass index	1.03 (1.00–1.06)	0.038		
Smoking	1.04 (0.86–1.20)	0.821		
Alcohol	0.95 (0.81–1.12)	0.547		
CHA ₂ DS ₂ -VASc score	1.03 (0.98–1.08)	0.221		
Heart failure*	1.43 (1.06–1.93)	0.929		
Hypertension	1.14 (0.89–1.46)	0.019		
Diabetes	1.00 (0.80–1.25)	0.973		
Prior stroke/TIA	1.06 (0.84–1.34)	0.640		
Vascular disease	1.05 (0.84–1.31)	0.667		
LA diameter	1.05 (1.04–1.06)	<0.001	1.03 (1.01–1.06)	0.002
LVEF	0.99 (0.97–1.00)	0.024	0.99 (0.97–1.01)	0.203
E/Em	1.00 (0.99–1.02)	0.629		
TR jet velocity	1.01 (0.94–1.09)	0.751		
RVSP	1.02 (1.01–1.03)	<0.001		
LVEDD	1.01 (1.00–1.03)	0.183		
LVESD	1.02 (1.00–1.04)	0.101		
LVMI	1.00 (1.00–1.01)	0.019	1.00 (0.99–1.01)	0.998
eGFR	1.00 (0.99–1.00)	0.380		
hs-CRP	1.00 (0.99–1.01)	0.769		
Pericardial fat volume	1.00 (1.00–1.00)	0.052	1.00 (1.00–1.00)	0.678
LA volume	1.01 (1.01–1.01)	<0.001		
LA mean voltage	0.56 (0.48–0.66)	<0.001	0.59 (0.48–0.73)	<0.001
LA peak pressure	1.01 (1.00–1.02)	0.010	0.99 (0.98–1.01)	0.304

AF, atrial fibrillation; AFCA, atrial fibrillation catheter ablation; BMI, body mass index; EEm, ratio of the early diastolic mitral inflow velocity (E) to the early diastolic mitral annular velocity (Em); eGFR, estimated glomerular filtration rate; hs-CRP, high sensitivity C-reactive protein; LA, left atrium; LVEDD, left ventricular end diastolic dimension; LVEF, left ventricular ejection fraction; LVESD, left ventricular end systolic dimension; LVMI, left ventricular mass index; RVSP, right ventricular systolic pressure; TIA, transient ischemic attack; TR, tricuspid regurgitation.

*Defined as conventional HFpEF diagnosis criteria: left ventricular ejection fraction ≥50% with exertional dyspnea that was not caused by extracardiac causes.

was unclear, sustained AF despite AFCA had a significant correlation with an increased Δ H₂FPEF score_[1–yr].

Heart Failure With Preserved Ejection Fraction, a Good Candidate for Atrial Fibrillation Catheter Ablation

Among the patients with a normal LV systolic function, which subgroup is the most helpful based on the H₂FPEF scores after AFCA? Those with the most significant decrease in the Δ H₂FPEF scores_[1–yr] were those with a baseline HFpEF score of ≥6, i.e.,

successful rhythm control by AFCA can significantly improve the LV diastolic function in patients with HFpEF. Furthermore, the absence of diabetes, a higher LVEF, and a lower pericardial fat volume were associated with decreased Δ H₂FPEF scores_[1–yr], which suggested that metabolic factors may have influenced the recovery of the LV diastolic function.

Limitations

This study had several limitations. First, since the current study was conducted in a single center and included a relatively

small number of patients, the findings cannot be generalized to all patients with a normal LV systolic function. However, there was also an advantage of this single-center cohort in that the ablation and rhythm follow-up protocols were consistent. Second, although we performed a regular rhythm follow-up in all included patients, the exact AF burden could not be assessed by the Holter monitoring. Third, we excluded patients who did not have both baseline and 1-year follow-up echocardiograms with all parameters taken from the same institute, to calculate appropriate H₂FPEF scores. Fourth, there was some discrepancy between the HF clinically judged by the CH₂A₂DS-VASc and H₂FPEF scores because the clinical HF was classified mainly by the LV systolic function. Finally, because of the limited follow-up duration, we could not determine the long-term changes in the biventricular function and other clinical outcomes in this study. Future prospective and controlled studies are warranted.

CONCLUSION

The H₂FPEF scores decreased in 17% and increased in 21% of the patients with a normal LV function at 1 year after the AFCA. AFCA has shown a tendency to improve the H₂FPEF scores_[1-yr] in the patients with an abnormal diastolic function. However, a poor rhythm control and sustained AF after the AFCA were significantly associated with an increase in the Δ H₂FPEF score_[1-yr]. An increased Δ H₂FPEF score_[1-yr] was an independent prognostic factor for poorer rhythm outcomes after the AFCA.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The institutional review board of the Yonsei University Health system. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

HN-P and MK designed the study, analyzed and interpreted the data, drafted the manuscript, and did the final approval of the manuscript submission. HTY, T-HK, J-SU, BYJ, and M-HL interpreted data and contributed to acquiring patients' clinical data. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcvm.2021.699364/full#supplementary-material>

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Atrial Cardiomyopathy: An Emerging Cause of the Embolic Stroke of Undetermined Source

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Nearly 30% of ischemic strokes have an unknown cause, which are referred to as cryptogenic strokes (CS). Imaging studies suggest that a large proportion of these patients show features that are consistent with embolism, and thus the term embolic stroke of undetermined source (ESUS) was proposed to describe these CS patients. Atrial cardiomyopathy predisposes to thrombus formation and thus embolic stroke even in the absence of atrial fibrillation (AF). This may provide a mechanistic link with ESUS, suggesting that anticoagulant therapy may be more beneficial than antiplatelet therapy in ESUS patients with atrial cardiomyopathy. The present review discusses the concept of atrial cardiomyopathy and ESUS and the relationship between them based on the mechanisms and clinical evidence, suggests that atrial cardiomyopathy may be a potential mechanism of ESUS, and highlights a theoretical basis that supports that anticoagulant therapy may be more applicable to ESUS patients with atrial cardiomyopathy and aims to help us better understand and identify the risk of ESUS, thereby improving the management of these patients in clinical practice.

Keywords: cardiac rhythm abnormalities, risk stratification, atrial cardiomyopathy, structural heart disease, embolic stroke of undetermined source

INTRODUCTION

Several causes can lead to ischemic stroke (IS), including extracranial and intracranial large artery atherosclerosis, cardiogenic embolism, small arterial occlusion, and other uncommonly determined etiology, as detailed in the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) (1). However, 30% of IS have no identifiable causes, which are referred to as cryptogenic stroke (CS). Imaging findings of CS patients demonstrated that up to 60% of CS patients had cortical infarction (2), which would suggest possible embolic origins. Therefore, the term “embolic stroke of undetermined source” (ESUS) was proposed in 2014 to describe non-lacunar IS patients without an identifiable cardioembolic source (Figure 1) (3).

Atrial cardiomyopathy, a pathophysiological concept of the abnormal atrial substrate and function, such as chamber dilation, impaired myocyte function, and fibrosis, is postulated to form a nidus for embolism. Several lines of evidence indicating the potential of atrial cardiomyopathy markers in the ESUS patients support this idea. From a practical viewpoint, anticoagulant therapy may be more beneficial than antiplatelet therapy in ESUS patients with atrial cardiomyopathy. There is an ongoing trial, Atrial Cardiomyopathy and Antithrombotic Drugs in Prevention After

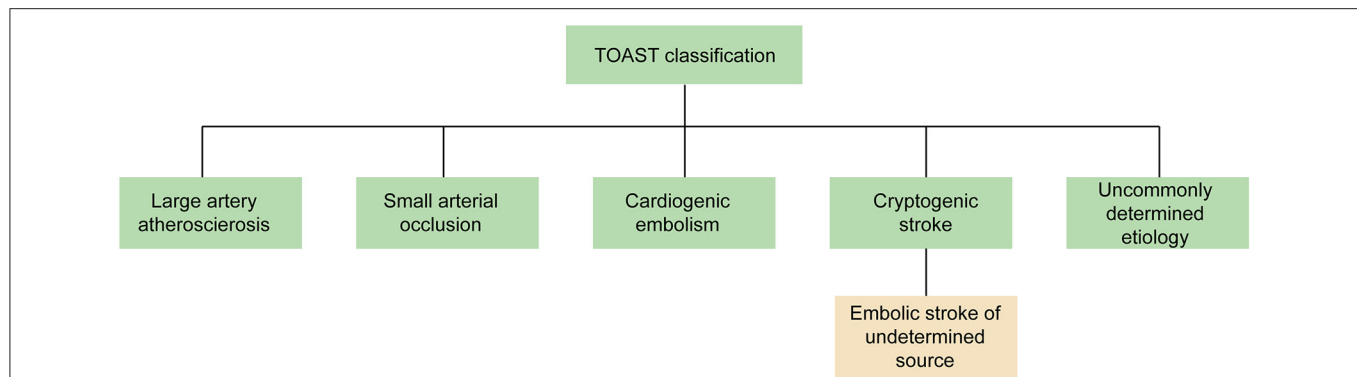


FIGURE 1 | The classification of IS in TOAST. The term “ESUS” is a more specific and clinically useful concept to describe most of CS patients. TOAST, Trial of ORG 10172 in Acute Stroke Treatment; ESUS, embolic stroke of undetermined source; CS, cryptogenic stroke.

Cryptogenic Stroke (ARCADIA) trial, which is testing this hypothesis (4). Therefore, the proposed concept of atrial cardiomyopathy could stimulate us to better understand and identify the risk of ESUS, promoting the management of these patients in clinical practices.

ESUS

Definition and Prevalence

Even though CS accounts for one-third of IS, the definition of CS remains vague. TOAST criteria classify an IS as CS when no evidence can be identified even with sufficient evaluation of etiology. However, CS also consists of stroke with multiple causes and stroke with incomplete survey. Given the vague TOAST criteria and lack of agreement in the community, there has been a slow progress of the prevention in CS patients over the past decades. Most non-lacunar IS are embolic with a major clear source. However, there are many potential embolic sources, including subclinical atrial fibrillation (AF), atrial cardiomyopathy, patent foramen oval (PFO), cancer, non-stenotic artery atherosclerosis, nonatherosclerotic vasculopathies, and left ventricular disease. These causes may overlap in part and interact with each other.

Therefore, the term “embolic stroke of undetermined source” (ESUS) (3) was proposed in 2014 to describe non-lacunar IS patients without an identified cardioembolic source (including AF, valvular heart disease, intracardiac thrombus, cardiac tumors, and infective endocarditis), proximal arterial stenosis $\geq 50\%$ (cervical or intracranial artery supplying the infarct area), and other determined uncommon stroke causes even after a standardized comprehensive evaluation. The term “ESUS” is a more specific concept to describe CS patients in whom embolic source is likely the underlying mechanism (3), which refines the category of CS, facilitating the progress of clinical trials to evaluate the potential of anticoagulant therapy to reduce the stroke recurrence in ESUS patients.

The diagnostic criteria (3) for ESUS include the following: (1) identification of non-lacunar IS by magnetic resonance imaging (MRI) or CT; (2) exclusion of $\geq 50\%$ luminal stenosis

in extracranial or intracranial arteries using MRI/CT-guided vascular imaging; (3) exclusion of major cardioembolic causes with ECG, echocardiography, and Holter monitoring; and (4) exclusion of other uncommonly determined causes of stroke (arteritis, dissection, migraine, and drug misuse). ESUS working group investigators further proposed that this clinic construct is a more clinically useful, definite concept than the vague term of CS (3). This construction uses more specified criteria to distinguish potential embolic sources from other clear sources.

Approximately 17% of IS patients fulfill the ESUS diagnostic criteria (ranging from 9 to 25%), who are typically younger patients (mean age of 65 years) with fewer systemic vascular risk factors, and severity of the strokes is often milder than other types of IS (defines NIHSS of 5) (5). However, the recurrence rate in ESUS averaged 4.5% per year, which is higher than that of non-ESUS IS (5). Given that the causes of ESUS remain unknown, nearly 86% ESUS patients were treated with antiplatelet therapy (5).

Non-vitamin K Antagonist Oral Anticoagulants (NOACs) vs. Aspirin in ESUS

According to the hypothesis that many potential embolic sources result in ESUS, those patients may benefit from the anticoagulation treatment. There are two accomplished randomized trials to evaluate the efficacy of NOACs compared with aspirin in ESUS. The NAVIGATE ESUS trial (6) enrolled 7,213 participants to compare the efficacy and safety of rivaroxaban with aspirin for the prevention of ESUS patients. However, due to the high bleeding rate observed with rivaroxaban (hazard ratio = 2.72; 95% CI, 1.68–4.39; $P < 0.001$), this trial has been prematurely terminated. Disappointingly, the annual rate of primary outcomes (any recurrent stroke) did not significantly differ between the two groups (HR, 1.07; 95% CI, 0.87–1.33; $p = 0.52$). The RE-SPECT ESUS trial (7) compared the efficacy and safety of dabigatran with aspirin for the prevention of ESUS patients. There is a similar rate of first recurrent stroke (HR, 0.84; 95% CI, 0.68–1.03; $p = 0.10$) and

similar safety outcomes (HR, 1.19; 95% CI, 0.85–1.66) in the two groups. In conclusion, although it was postulated that various underlying embolic sources contributing to ESUS would respond to anticoagulation favorably, unfortunately, neither NAVIGATE ESUS nor RE-SPECT ESUS found that oral anticoagulants were better than aspirin for secondary prevention in the ESUS patients.

In order to explore the value of anticoagulant therapy in secondary preventive treatment of ESUS patients, three randomized controlled trials have been conducted, and two of them have obtained results. However, it is disappointing that neither NAVIGATE ESUS trial nor RE-SPECT ESUS test can prove that anticoagulant therapy is more beneficial than antiplatelet therapy for ESUS patients, and the NAVIGATE ESUS trial is even terminated early because of the high incidence of severe bleeding events. The results of the ATTICUS ESUS trial using apixaban have not yet been published; considering that the inclusion criteria require ESUS patients have one of the risk factors of cardiac embolism, the results are highly anticipated.

The heterogeneity of mechanisms responsible for ESUS likely explains these unsatisfactory results of the ESUS trials. There are various underlying embolic mechanisms in ESUS patients; the treatment strategy of ESUS patients should be formulated according to their own different embolic sources. By contrast, there is an overlap of underlying embolic sources in ESUS patients, more than 30% patients have ≥ 3 potential embolic sources, and each patient averagely had two underlying embolic sources (8). The benefit of anticoagulation was likely offset by other causes rarely benefitting from anticoagulation, such as those patients with non-stenosing large-artery atherosclerosis. Thrombi of atrial origin may need anticoagulants, such as subclinical AF, atrial cardiomyopathy, and patent foramen oval (PFO). On the contrary, arterial-origin thrombi in ESUS may benefit from antiplatelet therapy for secondary prevention than anticoagulant therapy, including non-stenotic artery atherosclerosis, nonatherosclerotic vasculopathies, and left ventricular disease. Therefore, finding reliable diagnostic criteria to screen patients with different causes is necessary for individualized treatment, which may be the therapeutic target rather than the entirety concept of ESUS (9).

ATRIAL CARDIOMYOPATHY

Definition and Prevalence

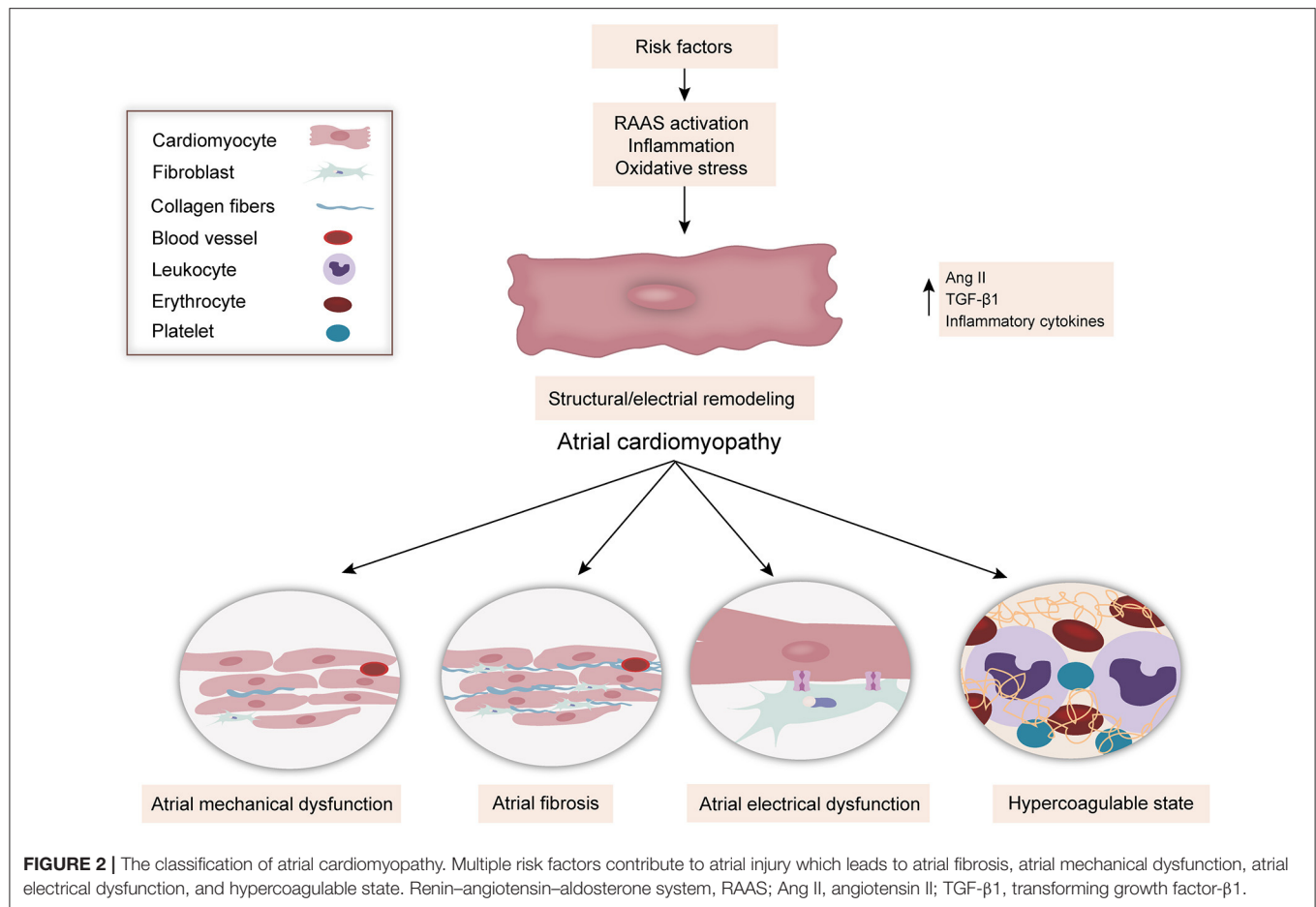
With the progress of relevant studies, atrial cardiomyopathy is considered as a common pathological feature of AF and an independent cause to the risk of stroke. The expert consensus defined atrial cardiomyopathy as “any complex of structural, architectural, contractile, or electrophysiological changes affecting the atria with the potential to produce clinically relevant manifestations” (10). Meanwhile, atrial cardiomyopathy was proposed (11) as a term to describe patients with abnormal atrial substrate and function, including atrial fibrosis, atrial mechanical dysfunction, atrial electrical dysfunction, and hypercoagulable state (**Figure 2**) (12), which can be present even without AF. The term “atrial cardiomyopathy” reframes our understanding of the association between AF and thromboembolism. Over the past decade, there have been

many studies that focus on the underlying abnormal atrial structural and functional changes. AF and atrial cardiomyopathy have bidirectional interactions, with one predisposing to the other, and share common risk factors. This may explain why thromboembolism can be observed even in the absence of AF. Indeed, atrial cardiomyopathy may be the underlying cause of embolic stroke, similar to AF. Although there are no standard diagnostic criteria for atrial cardiomyopathy at present, different markers can be used to indicate atrial cardiomyopathy, for screening and evaluating stroke risk in ESUS patients. These include prolongation of the PR interval (13), abnormal P-wave terminal force in lead V1 (PTFV1) (14), prolonged P-wave durations (15), paroxysmal supraventricular tachycardia (PSVT) (16), left atrial enlargement (LAE) (17), and elevated cardiac biomarkers [e.g., N-terminal pro-brain natriuretic peptide (NT-proBNP) (18), cardiac troponin (cTnT) (18)].

Approximately 63% CS patients have an increased prevalence of markers of atrial cardiomyopathy (defined as NT-proBNP > 250 pg/mL, or PTFV1 > 5,000 $\mu\text{V}\cdot\text{ms}$, or severe LAE) (19), and atrial cardiomyopathy (defined as LA > 38 mm for women and > 40 mm for men or if supraventricular extrasystoles) was present in nearly 45% ESUS patients (8). Recent data showed that atrial cardiomyopathy, defined as PTFV1 > 5,000 $\mu\text{V}\cdot\text{ms}$ or severe LAE, occurred more frequently in ESUS patients than in another non-cardiogenic stroke (26.6 vs. 14.02%; $p < 0.001$) (20). All the studies are supportive of atrial cardiomyopathy as a possible embolic cause for ESUS.

The Relationship Between Atrial Cardiomyopathy and AF

Given recent advances, atrial remodeling caused by several underlying cardiac diseases or systemic conditions is the fundament of the progression of AF. Conversely, AF itself can lead to atrial remodeling. Indeed, AF is a final common pathway of atrial remodeling caused by several cardiac or noncardiac conditions and AF itself can also contribute to atrial remodeling that leads to the progression of AF. Structural remodeling, including changes of atrial tissue, size, cellular ultrastructure, and especially fibrosis, has been believed to be the main cause of AF (21). Atrial fibrosis, the accumulation of fibrillar collagen deposits in the left atrial myocardium, manifests delayed-enhanced MRI (DE-MRI) that provides a noninvasive means to assess the myocardial tissue in AF patients, showing areas of fibrosis in the atria accurately (22, 23). Therefore, DE-MRI has been used to guide physicians to manage AF patients with catheter ablation. A recent study shows that left atrial fibrosis can also be detected by DE-MRI in a general cardiology population, even without structural heart disease or AF (24) and suggests that atrial fibrosis can present to the general population. DE-MRI plays an important role in the evaluation of potential cardiac causes. However, the relationship between atrial fibrosis on DE-MRI and stroke independent of AF remains unclear. Studies focusing on investigating this relationship are needed. Indeed, several risk factors can promote AF to occur, inducing change in atrial endocardial electrograms. The study found that advancing age results in greater abnormalities based on atrial endocardial

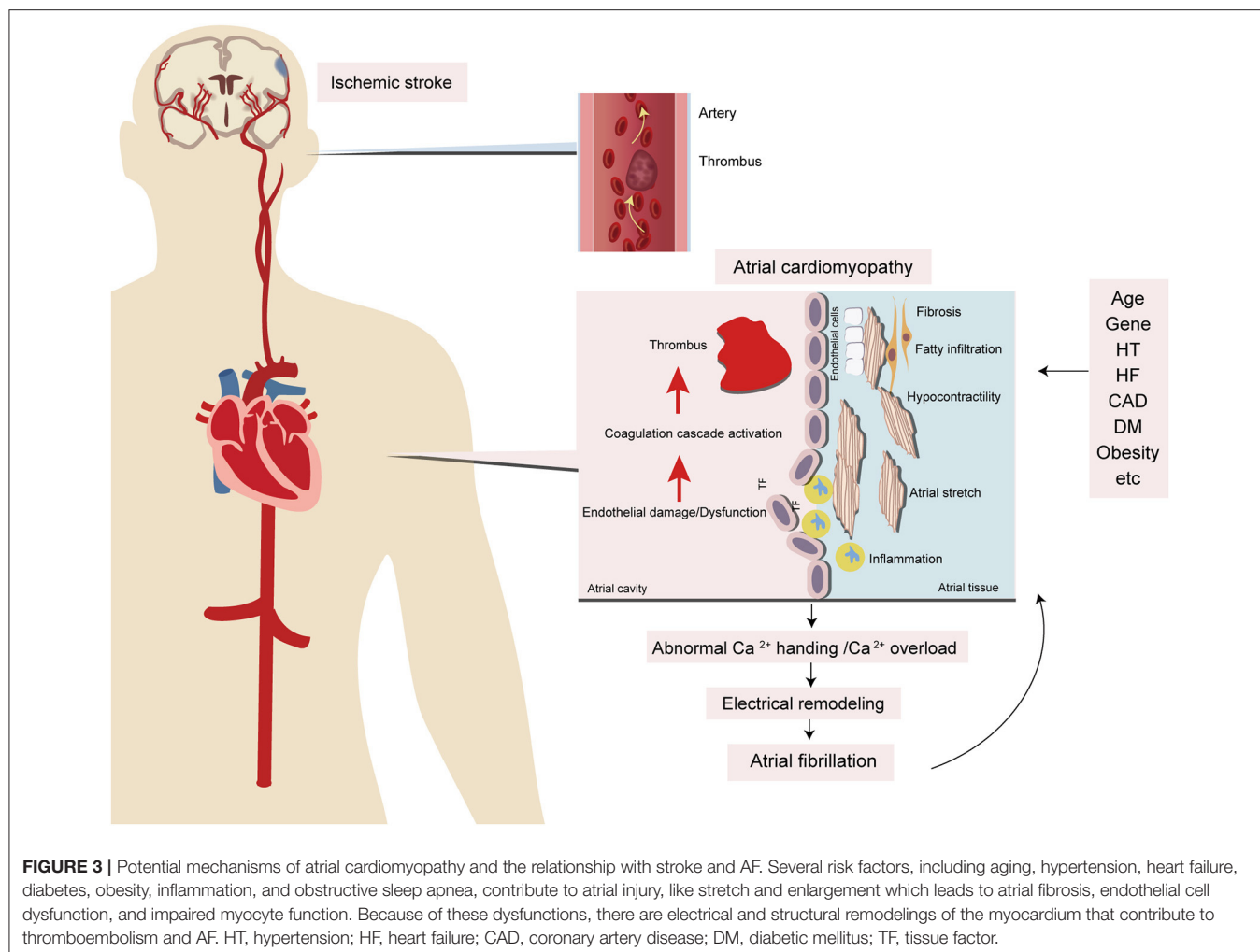


electrograms recorded in patients without AF (25). By contrast, the imaging findings indicated that structural changes in the atria were significantly correlated with the presence and severity of AF (26, 27). The markers of atrial cardiomyopathy can predict the occurrence of AF (28, 29), but atrial cardiomyopathy does not recover even after successful catheter ablation for AF (30). However, management of vascular risk factors in AF patients after catheter ablation can effectively reduce the left atrial size by approximately half (31). Therefore, it is assumed that multiple cardiac and noncardiac conditions contribute to injury of the atrial substrate and cause atrial cardiomyopathy, which can result in AF and drive its progression; conversely, AF can worsen it through atrial remodeling (Figure 3).

Clinical Significance

The relationship between AF and atrial cardiomyopathy reframes our understanding of embolic stroke. Given that AF is the consequence of atrial cardiomyopathy and the markers of atrial cardiomyopathy are strongly associated with stroke independently of AF, it is postulated that atrial cardiomyopathy can cause the embolic stroke even without AF. This may explain several questions about AF-related strokes. First, the ASSERT study (32) showed that only 8% individuals were detected with subclinical AF within the 30 days before stroke, and

the TRENDS trial (33) reported that only 27.5% of patients were diagnosed with AF 30 days prior to the occurrence of cerebrovascular events or systemic emboli. These studies suggested that there is a temporal disassociation between AF and stroke. The CRYSTAL-AF investigation enrolled 441 CS patients to detect the prevalence of AF after CS used long-term monitoring with an insertable cardiac monitor (ICM) (34), suggesting that the result of detecting AF in the ICM group is more effective than the control group. Therefore, the more ambulatory electrocardiogram is done, the longer the duration, and the more likely AF will be detected, leading to improved treatment rates. However, given the limitation of cases in the subgroup, multiple studies are needed to evaluate the relationship between subclinical AF and stroke. Second, in a meta-analysis (35) of 28,836 patients, the rhythm-control therapy had no effect on stroke risk, suggesting that there are other underlying causes of stroke except dysrhythmia. Additional factors, such as atrial cardiomyopathy, may be the major contributors to stroke even without AF. Third, nearly 70% of patients with CS do not have detectable AF after 3 years of continuous heart-rhythm monitoring (34). Fourth, analysis of histological composition of the clots (36) showed that the clots of CS were similar to those of cardioembolic strokes, suggesting that the majority of CS have similar sources such as cardioembolic

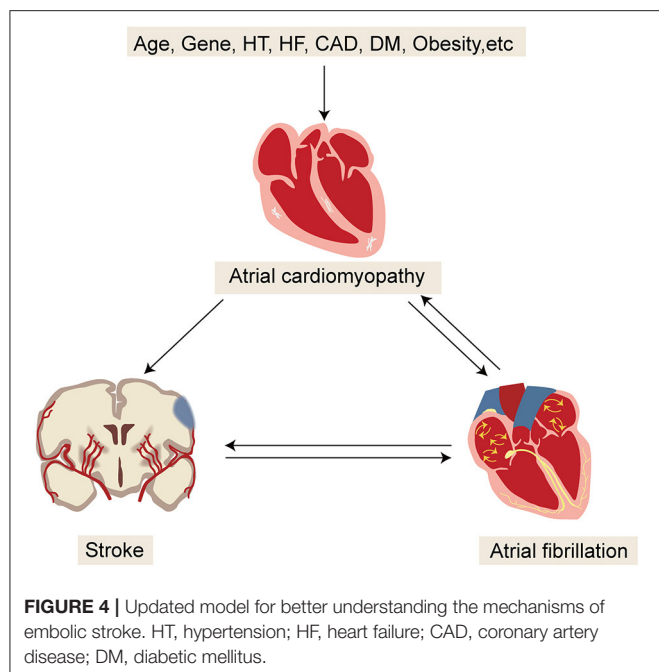


stroke. These lines of evidence indicate that AF is not a necessary factor in the progression of embolic stroke, and atrial cardiomyopathy may be the answer to these unsettled questions. From a practical viewpoint, it is reasonable to conclude that the concept of atrial cardiomyopathy can advance our ability to evaluate the stroke potential cardiogenic risks by markers of atrial cardiomyopathy without AF after prolonged heart-rhythm monitoring. In addition, recent work has shown that left ventricular wall motion abnormalities and changes in left heart function are potential sources of emboli in ESUS patients (37). Several studies also identified that accumulation of epicardial adipose tissue (EAT) around the left atrium is associated with increased risks of stroke (38). Future studies on the relationship between EAT around left atrium and different types of strokes are needed. As for the treatment and prevention, the secondary NAVIGATEESUS Trial analysis suggests that anticoagulant therapy may be more beneficial than antiplatelet therapy in ESUS patients with moderate or severe left atrial enlargement (39). Therefore, anticoagulant therapy could be more beneficial than antiplatelet therapy in ESUS patients with atrial cardiomyopathy.

Mechanisms of Atrial Cardiomyopathy

AF frequently coexists with atrial abnormalities. These pathologically abnormal structures and functions can be induced by the interaction between various factors. These atrial abnormalities may lead to cardiomyocyte and interstitial remodeling, including electrical and structural changes that result in AF and thrombogenesis. By contrast, these atrial abnormalities, or atrial cardiomyopathy, have existed for some period before AF occurs (40). These evidences are supportive of the similar mechanisms underlying AF and atrial abnormalities (Figure 3).

Atrial cardiomyopathy not only occurs with aging but also results from many pathophysiological conditions, including systemic inflammatory conditions and low-grade subclinical inflammatory conditions (hypertension, heart failure, coronary artery disease, and so on) (41–43); these factors interact with each other, leading to activation of the renin–angiotensin–aldosterone system (RAAS) and production of angiotensin II (Ang II) with the potential to induce cardiomyocyte hypertrophy, endothelial abnormalities, and myocardial fibrosis. Ang II produces reactive oxygen species (ROS), leading to abnormal Ca²⁺ handling



and Ca^{2+} overload, which contribute to electrical remodeling. Furthermore, Ang II produces transforming growth factor- $\beta 1$ (TGF- $\beta 1$), one of the key downstream efforts of Ang II that are secreted from cardiomyocytes and fibroblasts, which is a major factor in promoting fibrosis through the TGF- $\beta 1$ /Smad pathway to mediate the downstream gene product and connective tissue growth factor (CTGF), to increase atrial fibrosis and conduction abnormalities, and to promote AF. Eventually, these factors contribute to the electrical and structural remodeling of the myocardium. On the other hand, these inflammatory conditions result in inflammatory cells infiltrating the myocardium. Inflammatory cytokines promote the production of tissue factor (TF) and contribute to thrombogenesis. In addition, these inflammatory conditions can lead to endothelial injury, which promotes TF release from subendocardial tissue, further contributing to the coagulation cascade activation and leading to thrombogenesis (Figures 2, 3).

ATRIAL CARDIOMYOPATHY IS THE CONTRIBUTORY FACTOR OF ESUS

In addition to subclinical AF, other factors may be involved in CS (34). Atrial cardiomyopathy was proposed to be associated with stroke independently of AF because ~65% CS patients have an increased prevalence of markers of atrial cardiomyopathy (11, 19). The results of the Cardiovascular Health Study (CHS) which evaluated the association between atrial cardiomyopathy markers and stroke risk showed that markers of atrial cardiomyopathy were each independently associated with incident stroke (44). Furthermore, atrial cardiomyopathy occurred more frequently in ESUS patients than in those other non-cardiogenic strokes, suggesting that atrial cardiomyopathy may be an underlying mechanism of ESUS (20).

Multiple vascular risk factors, such as aging, hypertension, diabetes, obesity, and obstructive sleep apnea, all of which are associated with a pro-inflammatory milieu, contribute to atrial dysfunction through stretch and enlargement, and eventually to atrial fibrosis, endothelial dysfunction, and impaired myocyte function. Because of these abnormalities, ineffective atrial contractile function can lead to blood stasis and formation of emboli in the atria or left atrial appendage (LAA), contributing to thromboembolism (Figure 3).

Recently, an updated model on the mechanisms of embolic stroke has been made available, which emphasizes the interacting factors among AF, atrial cardiomyopathy, and embolic stroke (Figure 4) (45). Multiple risk factors for stroke, such as aging and vascular risk factors, can undermine the atrial substrate, cause atrial cardiomyopathy, and subsequently increase the risk of AF and thromboembolism. By contrast, AF in turn leads to further worsening of atrial cardiomyopathy and thromboembolism. Once stroke occurs, autonomic changes and inflammation eventually increase the risk of AF. Conceivably, this model can help explain several puzzling observations about AF and stroke.

PROSPECT

There has been increasing interest in recent years directed toward answering several open questions in the field of atrial cardiomyopathy and ESUS. The following unanswered questions merit further studies. First, the exact diagnostic criteria of atrial cardiomyopathy remain undecided, although some investigators hold the view that atrial cardiomyopathy can be diagnosed by the presence of one of the markers such as LAE, PTFV1, NT-proBNP, cTnT, and PSVT; the potential of each biomarker in diagnosing atrial cardiomyopathy remains unclear. By contrast, these markers are indirect in defining the atrial cardiomyopathy; future studies are needed to focus on the relationship between pathological features of atrial cardiomyopathy and ESUS. Second, although the markers of atrial cardiomyopathy are related to the risk of stroke occurrence independent of AF, the temporal relationship of atrial cardiomyopathy and IS remains unclear. Future studies need to investigate the sequence of disease onset. Third, to understand the atrial cardiomyopathy behind AF, we need to advance the ability to predict stroke and AF using a new scoring system. Fourth, the multicenter randomized controlled trials are necessary for comparing the potential of different therapies in patients with atrial cardiomyopathy without AF to reduce the risk of ESUS. The ongoing ARCADIA trial is testing this hypothesis (4).

In conclusion, the theoretical concept of ESUS is based on the fact that most CS is embolic; thus, anticoagulant therapy may be more beneficial for these patients. However, neither the NAVIGATE ESUS trial nor the RE-SPECT ESUS trial has confirmed this hypothesis, thinking that anticoagulant therapy would increase the risk of bleeding. However, considering the variety of potential embolic sources of ESUS, individualized treatments should be carried out according to each cause. Atrial cardiomyopathy may predispose patients to the high risk of ESUS. Several biomarkers can be used to sift patients with atrial cardiomyopathy. For ESUS patients with atrial cardiomyopathy, anticoagulant therapy may be more suitable.

Further investigation of atrial cardiomyopathy as a modifiable stroke risk factor can help us better understand ESUS and choose a suitable option for ESUS patients, promoting the management of these patients in clinical practices.

AUTHOR CONTRIBUTIONS

YN and GLi drafted the manuscript, illustrated, and captioned all figures. YN, GT, GLi, and GLu provided the critical review,

revision of the manuscript, and approved the final draft for publication. All authors contributed to the article and approved the submitted version.

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Association of Night-Time Heart Rate With Ventricular Tachyarrhythmias, Appropriate and Inappropriate Implantable Cardioverter-Defibrillator Shocks

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Background: Night-time heart rate (HR) is expected to reflect more accurately the cardiac autonomic function of modulating cardiovascular activity. Few studies have been conducted on the predictive values of night-time HR in relation to cardioverter-defibrillator therapies.

Aims: To explore the associations of night-time HR with the ventricular tachyarrhythmias (VTAs), appropriate and inappropriate implantable cardioverter-defibrillator (ICD) shocks.

Methods: Patients from the SUMMIT registry receiving ICD or cardiac resynchronization therapy with defibrillator (CRT-D) implantation were retrospectively analyzed using archived home monitoring data. Night-time HR was recorded from 2:00 am to 6:00 am during the first 30 to 60 days after implantation. VTA events and ICD shocks were identified using the intracardiac electrograms by two independent physicians. Restricted cubic splines and smooth curve fitting were conducted to address the non-linear associations between night-time HR and adjusted hazards for clinical outcomes.

Results: Over a mean follow-up duration of 55.8 ± 22.7 months, 187 deaths were observed among 730 patients. VTAs, appropriate and inappropriate ICD shocks were observed in 422 (57.8%), 293 (40.1%), and 72 (10.0%) patients, respectively. Apparent U-shaped non-linear associations of night-time HR with VTAs (P for non-linearity = 0.007), appropriate ICD shocks (P for non-linearity = 0.003) and inappropriate ICD shocks (P for non-linearity = 0.014) were detected. When night-time HR was beyond 60 bpm, every 1 bpm increase in night-time HR could result in 3.2, 3.3, and 4.9% higher risks of VTAs and appropriate and inappropriate ICD shocks, respectively; when night-time HR was lower than 60 bpm, every 1 bpm increase in night-time HR could result in 6.0 and 10.7% lower risks of appropriate and inappropriate ICD shocks. Compared to night-time HR of ≤ 50 or ≥ 70 bpm, night-time HR of 50–70 bpm was associated with

24.9, 30.2, 63.5, and 31.5% reduced incidences of VTA events, appropriate ICD shocks, inappropriate ICD shocks, and all-cause mortality, respectively.

Conclusion: Apparent non-linear associations of night-time HR with VTAs and ICD shocks were detected. An increasing incidence of VTAs and ICD shocks was observed at both low and high levels of night-time HR. Night-time HR of 50–70 bpm might be the optimal therapeutics target for the management of ICD/CRT-D recipients.

Keywords: night-time heart rate, implantable cardioverter-defibrillator, ventricular tachyarrhythmia, cardioverter-defibrillator therapy, cardiac autonomic activity

INTRODUCTION

Life-threatening ventricular tachyarrhythmia (VTA) can be terminated by cardioverter-defibrillator therapies for patients with implantable cardioverter-defibrillator (ICD) implantation (1, 2). Cardioverter-defibrillator therapies have shown survival benefits in the prevention of sudden cardiac death (SCD), but ICD shocks and anti-tachycardia pacing (ATP) therapy remained associated with markedly increased risks of long-term mortality (1, 3–6). The SCD-HeFT study reported that after receiving an appropriate and inappropriate shock, ICD patients had a 5- and 2-fold higher risks of all-cause mortality, respectively (5). Kleemann et al. also observed that the occurrence of ATP therapy was associated with a 2.6 times higher mortality rate (4). Thus, the identification and management of the patients requiring cardioverter-defibrillator therapies are important to improve the prognosis after ICD implantation.

The heart rate (HR) is an individual physical sign as well as a non-invasive and affordable tool that can be easily measured (7). Some epidemiological studies have reported that high resting HR was associated with poor prognosis in cardiovascular events and all-cause mortality in subjects with or without cardiovascular diseases (8–10). Compared with resting HR and 24-h HR, night-time HR is measured during sleep periods and less likely to be affected by diet, environmental factors, and physical and mental activities. Night-time HR is expected to reflect more accurately the cardiac autonomic function of modulating cardiovascular activity (11–13). Furthermore, night-time HR rather than HR measured at other times proved to be a better prognostic mark for accessing cardiovascular risks in Johansen et al. and Palatini et al.'s studies (11, 12). However, few studies have focused on the predictive values of night-time HR in relation to cardioverter-defibrillator therapies.

In this study, the patients who received ICD or cardiac resynchronization therapy with defibrillator (CRT-D) for

the primary or secondary prevention of SCD were analyzed. The night-time HR (02:00–06:00), VTAs and ICD therapy events were obtained from home monitoring recordings, aiming to explore the associations of night-time HR with VTAs requiring cardioverter-defibrillator therapies, appropriate/inappropriate ICD shocks, and all-cause mortality after ICD/CRT-D implantation.

METHODS

Study Design

The Study of Home Monitoring System Safety and Efficacy in Cardiac Implantable Electronic Device-implanted Patients (SUMMIT) registry (Registration No. ChiCTR-ONRC-13003695) is an observational, prospective, and multicenter trial. Patients from the SUMMIT registry were retrospectively analyzed using the archived home monitoring data. The present study adhered to the principles of the Declaration of Helsinki, and it was approved by the ethics committees of Fuwai Hospital (the chief institute) and all other participating organizations. All patients provided written informed consent prior to enrollment.

Study Participants

Patients who received ICD or CRT-D (Biotronik, Germany) implantation between May 2010 and May 2014 were included. ICD/CRT-D devices were implanted according to the guidelines' recommendations and equipped with a continuous home monitoring system. The percentage of average daily ventricular pacing in a single-chamber ICD was <10% or the percentage of average daily atrial and ventricular pacing percentage in a dual-chamber ICD was both <10% during the window period. Patients were excluded if they were <18 years old at implantation, got lost to follow-up, survived for <3 months, diagnosed with a malignant tumor, or scheduled for heart transplantation.

Home Monitoring and Device Programming

After ICD/CRT-D implantation, a continuous home monitoring system was started immediately and transmitted information to the service center every day. Archived home monitoring data included HR, atrial and/or ventricular pacing, supraventricular episodes (atrial fibrillation [AF], atrial flutter [AFL], supraventricular tachycardia [SVT], and sinus tachycardia), VTAs, ATP therapy, ICD shock, etc. Routine follow-ups were also conducted via clinic visits or telephone interviews. If the transmission was interrupted, the research coordinator contacted

Abbreviations: ACEI/ARB, angiotensin-converting enzyme inhibitors or angiotensin receptor blockers; AF, atrial fibrillation; ATP, antitachycardia therapy; BMI, body mass index; CABG, coronary artery bypass grafting; CCBs, calcium channel blockers; CI, confidence interval; HR, heart rate; CRT-D, cardiac resynchronization therapy defibrillators; DCM, hypertrophic cardiomyopathy; DM, diabetes mellitus; HCM, hypertrophic cardiomyopathy; HF, heart failure; HFrEF, heart failure with reduced ejection fraction; ICD, implantable cardioverter-defibrillator; ICM, ischaemic cardiomyopathy; LVEDD, left ventricular end diastolic diameter; LVEF, left ventricular ejection fraction; MI, myocardial infarction; NYHA class, New York Heart Association class; PCI, percutaneous coronary intervention; VTA, ventricular tachyarrhythmia.

the patients or family members to immediately confirm their health conditions.

The basic HR was 40–60 beats per minute (bpm). All patients received ventricular fibrillation (VF) and ventricular tachycardia (VT) monitor zones programmed independently of the device type. VT was detected at rates of ≥ 140 bpm, and VF was detected at rates of ≥ 200 bpm. In addition, ICD/CRT-D devices were equipped with the Biotronik SMART algorithm, which can distinguish VT/VF episodes from SVT episodes after analyzing the waveform and frequency of electrocardiograms (14).

Night-Time and 24-Hour HR Measurement

Night-time HR was obtained from 2:00 am to 6:00 am when patients were at a sleep status. The 24-h HR was obtained from a 24-h period. Both mean values of night-time HR and 24-h HR were calculated as the average daily values during the first 30–60 days after ICD/CRT-D implantation.

Grouping

Based on the cut-off values of night-time HR, acquired using restricted cubic splines and smooth curve fitting, patients were divided into three groups of different night-time HR levels: ≤ 50 bpm night-time HR group ($n = 51$), 50–70 bpm night-time HR group ($n = 558$), and ≥ 70 bpm night-time HR group ($n = 121$).

Study Endpoints

The primary endpoints were the first VTA events and the first appropriate and inappropriate ICD shocks. The first VTA event was defined as the first identified VT/VF episode requiring cardioverter-defibrillator therapies (appropriate ATP therapy or ICD shock) after ICD/CRT-D implantation. Appropriate ICD shock was defined as the ICD shock delivered to the VTAs. Inappropriate ICD shock was defined as the ICD shock delivered for arrhythmias other than ventricular (sinus tachycardia, SVT, atrial tachycardia [AT], AF, AFL, etc.) or for non-arrhythmic events (noise, sensing problems, malfunction, etc.). The VTA events and appropriate and inappropriate ICD shocks were obtained from the intracardiac electrograms of tachycardia events, which were reviewed and further confirmed in a blinded manner by two cardiologists.

The secondary endpoint was all-cause mortality. The cause of death and the date of death were identified based on the death certificates supplied by family members.

Statistical Methods

Continuous variables are presented as mean \pm standard deviation, and categorical variables are presented as frequencies and percentages. One-way analyses of variance were performed to assess the differences between the continuous variables, and the chi-squared tests were used for the categorical variables. The association between night-time HR and 24-h HR was assessed using Pearson's correlation coefficient.

Restricted cubic splines and smooth curve fitting were conducted to explore the non-linear associations between night-time HR and adjusted hazards for clinical outcomes. If a non-linear relationship was detected, the inflection point was adopted as a dichotomizing cutoff value, and a 2-piecewise Cox

proportional hazards model on both sides of the inflection point was constructed for a secondary analysis to further describe their non-linear associations. Multivariate Cox proportional hazard models were adjusted for independent, considerable variables with a $P \leq 0.05$ in the univariate analysis.

The corresponding values of night-time HR, with lower limits of 95% confidence intervals (CIs) and $\ln \text{HR} = 0$, were obtained from the smooth curving fitting. Based on the adopted cut-off values, the patients were divided into three different groups of night-time HR levels. Kaplan–Meier survival curves with log-rank tests and univariate/multivariate Cox regression models were constructed to evaluate the predictive values of different night-time HR levels for different clinical outcomes, to explore the appropriate therapeutic targets for the management of ICD/CRT-D recipients.

Hazard ratios and 95% CIs were calculated to determine the impact. Statistical significance was set at $P < 0.05$, and all tests were two-sided. Statistical analyses were conducted using SPSS Statistics version 23.0 (IBM Corp., Armonk, USA) and R version 4.0.3 (Bunny–Wunnies Freak Out, The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Baseline Characteristics

In this retrospective cohort study, 730 out of 1,015 consecutive patients receiving ICD or CRT-D implantation for primary or secondary prevention of SCD were included. Patients were excluded due to incomplete or unavailable home monitoring data ($n = 227$), $>10\%$ of average daily atrial or ventricular pacing percentage ($n = 50$), age at implantation <18 years old ($n = 3$), survival period of <3 months after device implantation ($n = 3$), and lost to follow-up ($n = 2$).

The mean age at implantation was 60.4 ± 13.9 years old, and male was dominant in this cohort (74.8%). ICD implantation was indicated in 537 patients (73.6%), with a mean left ventricular ejection fraction (LVEF) of $42.8 \pm 14.9\%$ and a mean left ventricular end-diastolic diameter (LVEDD) of 58.7 ± 13.2 mm. A total of 392 patients (53.7%) were diagnosed with heart failure (HF) before implantation, and 296 cases (40.5%) had HF reduced ejection fraction (HFrEF). A total of 425 patients (58.2%) received the device implantation for ICD secondary prevention. Among these patients, 120 (28.2%) had documented VF and resuscitated SCD, 230 (54.1%) had a history of documented sustained VT, and 75 (17.6%) had a history of unexplained syncope and could be induced to VT or VF during the electrophysiological study.

Table 1 illustrates the difference in baseline characteristics between the different groups of night-time HR levels. Patients with higher night-time HR levels were older at implantation ($P = 0.006$), and more patients in the higher night-time HR groups were implanted with CRT-D implantation ($P < 0.001$), ICD primary prevention ICD indication ($P < 0.001$), NYHA class III–IV ($P < 0.001$), lower LVEF ($P < 0.001$), larger LVEDD ($P = 0.001$), more comorbidities including HF ($P < 0.001$), HFrEF ($P < 0.001$), ischemic cardiomyopathy (ICM) ($P = 0.039$), percutaneous coronary intervention (PCI) ($P = 0.013$), prior AF

TABLE 1 | Baseline characteristics.

	Total (<i>N</i> = 730)	≤50 bpm night-time HR group (<i>n</i> = 51)	50–70 bpm night-time HR group (<i>n</i> = 558)	≥70 bpm night-time HR group (<i>n</i> = 121)	<i>P</i> -value
HR monitoring data					
Nighttime HR (bpm)	61.3 ± 9.1	47.9 ± 2.4	59.1 ± 5.0	77.0 ± 6.1	–
24-h HR	70.9 ± 9.8	57.1 ± 4.9	69.1 ± 6.8	84.8 ± 8.2	–
Demographic characteristics					
Age at implantation (years)	60.4 ± 13.9	54.7 ± 14.9	60.6 ± 14.0	62.0 ± 12.7	0.006
Sex, male (<i>n</i> , %)	546 (74.8%)		182 (74.9%)	176 (72.1%)	0.412
BMI (Kg/m ²)	23.6 ± 3.0	23.8 ± 2.6	23.6 ± 3.1	23.2 ± 2.9	0.258
ICD implantation (<i>n</i> , %)	537 (73.6%)	50 (98.0%)	426 (76.3%)	61 (50.4%)	<0.001
Primary prevention (<i>n</i> , %)	305 (41.8%)	12 (23.5%)	236 (42.3%)	57 (47.1%)	<0.001
Echocardiographic characteristics					
LVEF (%)	42.8 ± 14.9	50.9 ± 13.6	43.6 ± 14.9	35.8 ± 12.7	<0.001
LVEDD (mm)	58.7 ± 13.2	52.7 ± 9.7	58.7 ± 13.3	61.3 ± 13.4	0.001
Comorbidities					
HF (<i>n</i> , %)	392 (53.7%)	11 (21.6%)	288 (51.6%)	93 (76.9%)	<0.001
HFrEF (<i>n</i> , %)	296 (40.5%)	7 (13.7)	206 (36.9%)	83 (68.6%)	<0.001
NYHA class III-IV (<i>n</i> , %)	362 (49.6%)	11 (21.6%)	264 (47.3%)	87 (71.9%)	<0.001
Hypertension (<i>n</i> , %)	228 (31.2%)	10 (19.6%)	178 (31.9%)	40 (33.1%)	0.173
DM (<i>n</i> , %)	76 (10.4%)	5 (9.8%)	57 (10.2%)	14 (11.6%)	0.897
Stroke (<i>n</i> , %)	16 (2.2%)	0 (0.0%)	16 (2.9%)	0 (0.0%)	0.080
DCM (<i>n</i> , %)	171 (23.4%)	9 (17.6%)	129 (23.1%)	33 (27.3%)	0.372
HCM (<i>n</i> , %)	29 (4.0%)	4 (7.8%)	24 (4.3%)	1 (0.8%)	0.071
ICM (<i>n</i> , %)	247 (33.8%)	9 (17.6%)	197 (35.3%)	41 (33.9%)	0.039
Prior MI (<i>n</i> , %)	102 (14.0%)	3 (5.9%)	79 (14.2%)	20 (16.5%)	0.178
PCI (<i>n</i> , %)	65 (8.9%)	1 (2.0%)	46 (8.2%)	18 (14.9%)	0.013
CABG (<i>n</i> , %)	8 (1.1%)	0 (0.0%)	7 (1.3%)	1 (0.8%)	0.679
Valve disease (<i>n</i> , %)	16 (2.2%)	0 (0.0%)	13 (2.3%)	3 (2.5%)	0.538
Prior AF (<i>n</i> , %)	83 (11.4%)	3 (5.9%)	59 (10.6%)	21 (17.4%)	0.046
Preimplant presyncope (<i>n</i> , %)	41 (5.6%)	4 (7.8%)	32 (5.7%)	5 (4.1%)	0.608
Preimplant syncope (<i>n</i> , %)	151 (20.7%)	17 (33.3%)	117 (21.0%)	17 (14.0%)	0.016
Medication					
Betablockers (<i>n</i> , %)	413 (56.6%)	32 (62.7%)	313 (56.1%)	68 (56.2%)	0.654
ACEI/ARBs (<i>n</i> , %)	257 (35.2%)	16 (31.4%)	189 (33.9%)	52 (43.0%)	0.138
Aldosterone antagonists (<i>n</i> , %)	260 (35.6%)	12 (23.5%)	198 (35.5%)	50 (41.3%)	0.083
CCBs (<i>n</i> , %)	61 (8.4%)	4 (7.8%)	44 (7.9%)	13 (10.7%)	0.583
Statins (<i>n</i> , %)	169 (23.2%)	11 (21.6%)	128 (22.9%)	30 (24.8%)	0.874
Loop-diuretics (<i>n</i> , %)	189 (25.9%)	9 (17.6%)	132 (23.7%)	48 (39.7%)	<0.001
Digoxin (<i>n</i> , %)	141 (19.3%)	7 (13.7%)	99 (17.7%)	35 (28.9%)	0.011
Amiodarone (<i>n</i> , %)	212 (29.0%)	23 (45.1%)	160 (28.7%)	29 (24.0%)	0.019
Antiplatelets (<i>n</i> , %)	152 (20.8%)	10 (19.6%)	116 (20.8%)	26 (21.5%)	0.962

ACEIs, angiotensin-converting enzyme inhibitors; AF, atrial fibrillation; ARBs, angiotensin receptor blockers; BMI, body mass index; bpm, beats per minute; CABG, coronary artery bypass grafting; CCB, calcium channel blockers; CRT-D, cardiac resynchronization therapy defibrillator; DCM, dilated cardiomyopathy; DM, diabetes mellitus; HCM, hypertrophic cardiomyopathy; HF, heart failure; HFrEF, heart failure with reduced ejection fraction; HR, heart rate; ICM, ischemic cardiomyopathy; LVEDD, left ventricular end-diastolic dimension; LVEF, left ventricular ejection fraction; MI, myocardial infarction; PCI, percutaneous coronary intervention.

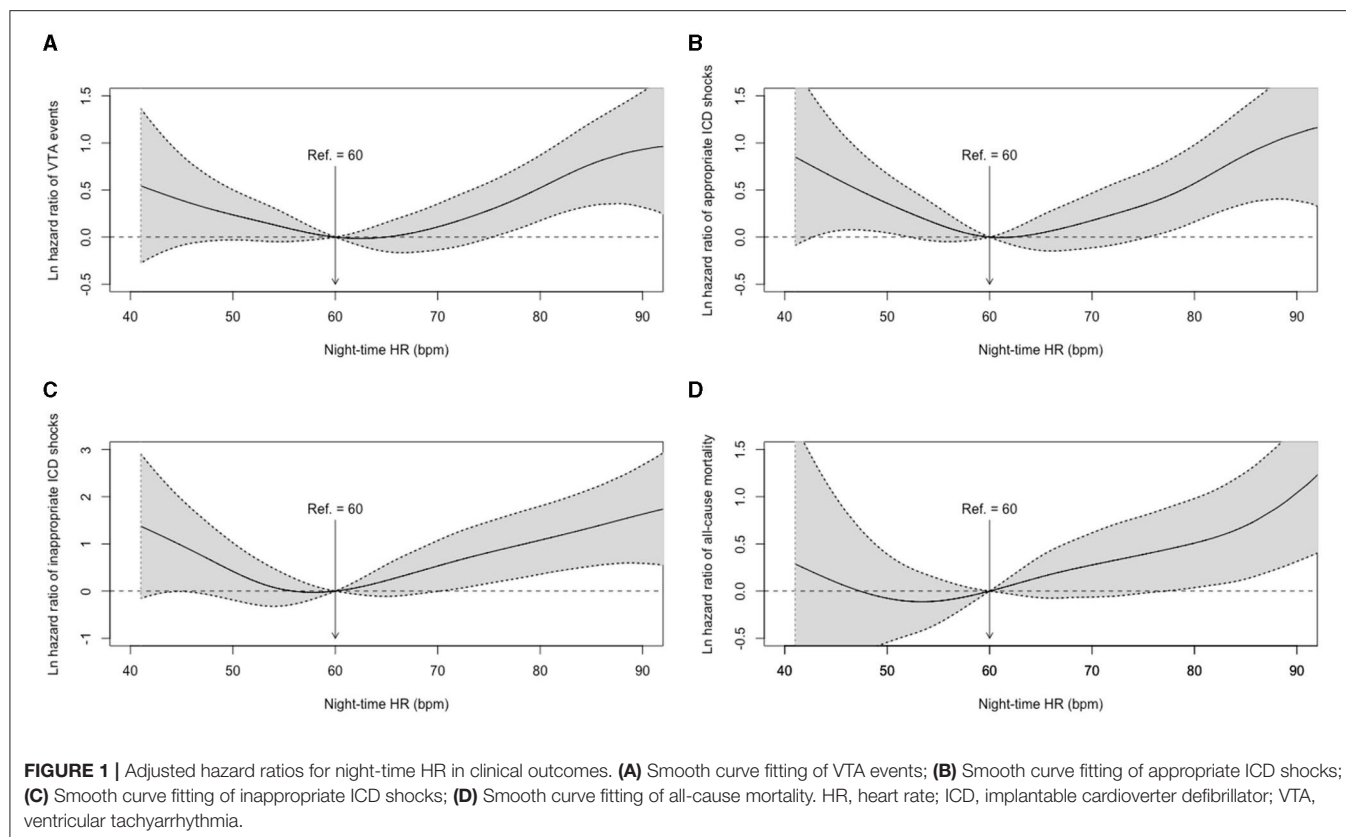
($P = 0.046$), more use of loop-diuretics ($P < 0.001$), digoxin ($P = 0.011$), but less preimplant syncope ($P = 0.016$), and amiodarone use ($P = 0.019$).

HR and Clinical Outcomes

The mean values of night-time HR and 24-h HR were 61.3 ± 9.1 and 70.9 ± 9.8 bpm, respectively. Night-time HR was

closely correlated with 24-h HR, detected by Pearson correlation coefficient (Pearson's $r = 0.868$, $P < 0.001$).

During a mean follow-up period of 55.8 ± 22.7 months, the VTA events requiring appropriate cardioverter-defibrillator therapies were observed in 422 patients (57.8%). A total of 293 patients (40.1%) experienced appropriate ICD shocks, and 72 patients (10.0%) experienced inappropriate ICD shocks.



Inappropriate ICD shocks were triggered by AF/AFL/AT/SVT with rapid ventricular conduction ($n = 58$), sinus tachycardia ($n = 1$), T-wave oversensing ($n = 2$), and interruption ($n = 11$). Regarding mortality, 187 deaths (25.6%) occurred among 730 ICD/CRT-D recipients. The most common cause of death was progressive HF (41.2%).

The incidences of VTAs, appropriate and inappropriate ICD shocks, and all-cause mortality were compared across the three groups of different night-time HR levels. Compared to those in ≤ 50 bpm or ≥ 70 bpm night-time HR groups, the incidences of VTA events (62.7 vs. 56.6 vs. 61.2%, $P = 0.501$) and appropriate ICD shocks (49.0 vs. 38.4 vs. 44.6%, $P = 0.180$) in the 50–70 bpm night-time HR group were lower but not significant. Patients in the 50–70 bpm night-time HR group had a significantly lower incidence of inappropriate ICD shocks (19.6 vs. 7.7 vs. 16.5%, $P = 0.001$). However, the incidence of death from any cause increased continuously from 15.7 to 24.0 to 37.2% in the ≤ 50 , 50–70, and ≥ 70 bpm night-time HR groups, respectively ($P = 0.003$).

Non-linear Association of Night-Time HR With Clinical Outcomes

Restricted cubic spline regression analysis was conducted to detect the non-linear associations between night-time HR and adjusted hazard ratios for VTA events, appropriate and inappropriate ICD shocks, and all-cause mortality. The results of

the smooth curve fitting are shown in **Figure 1**. Apparent non-linear associations of night-time HR with the adjusted hazards for the first VTA events (P non-linearity = 0.007), the first appropriate ICD shock (P for non-linearity = 0.003), and the first inappropriate ICD shock (P for non-linearity = 0.014) were detected with a typical U-shaped curve when adjusted for valuable variables, including age at implantation, sex, LVEF, LVEF, prior AF and preimplant syncope. However, a non-linear relationship between night-time HR and all-cause mortality was not detected (P for non-linearity = 0.352).

Based on the U-shaped smooth curve fitting, the inflection points for VTA events, appropriate and inappropriate ICD shocks were detected at 62.0, 61.0, and 58.0 bpm of night-time HR, respectively. Therefore, 60 bpm was adopted as a dichotomizing cutoff value to further describe the non-linear associations in a secondary analysis (**Table 2**).

In patients with night-time HR < 60 bpm, night-time HR was shown to be a protective factor in appropriate ICD shock (hazard ratio = 0.960, 95% CI: 0.923–0.999, $P = 0.045$) and inappropriate ICD shock (hazard ratio = 0.901, 95% CI: 0.827–0.981, $P = 0.017$) in univariate Cox regression model. However, no significant difference was observed in VTA events (hazard ratio = 0.977, 95% CI: 0.944–1.010, $P = 0.172$). In multivariate Cox regression models, adjusted for age at implantation, sex, LVEF, LVEDD, prior AF, and preimplant syncope, the results remained consistent. Every 1 bpm increase in night-time HR could result in 6.0 and 10.7% decreased risks of appropriate ICD

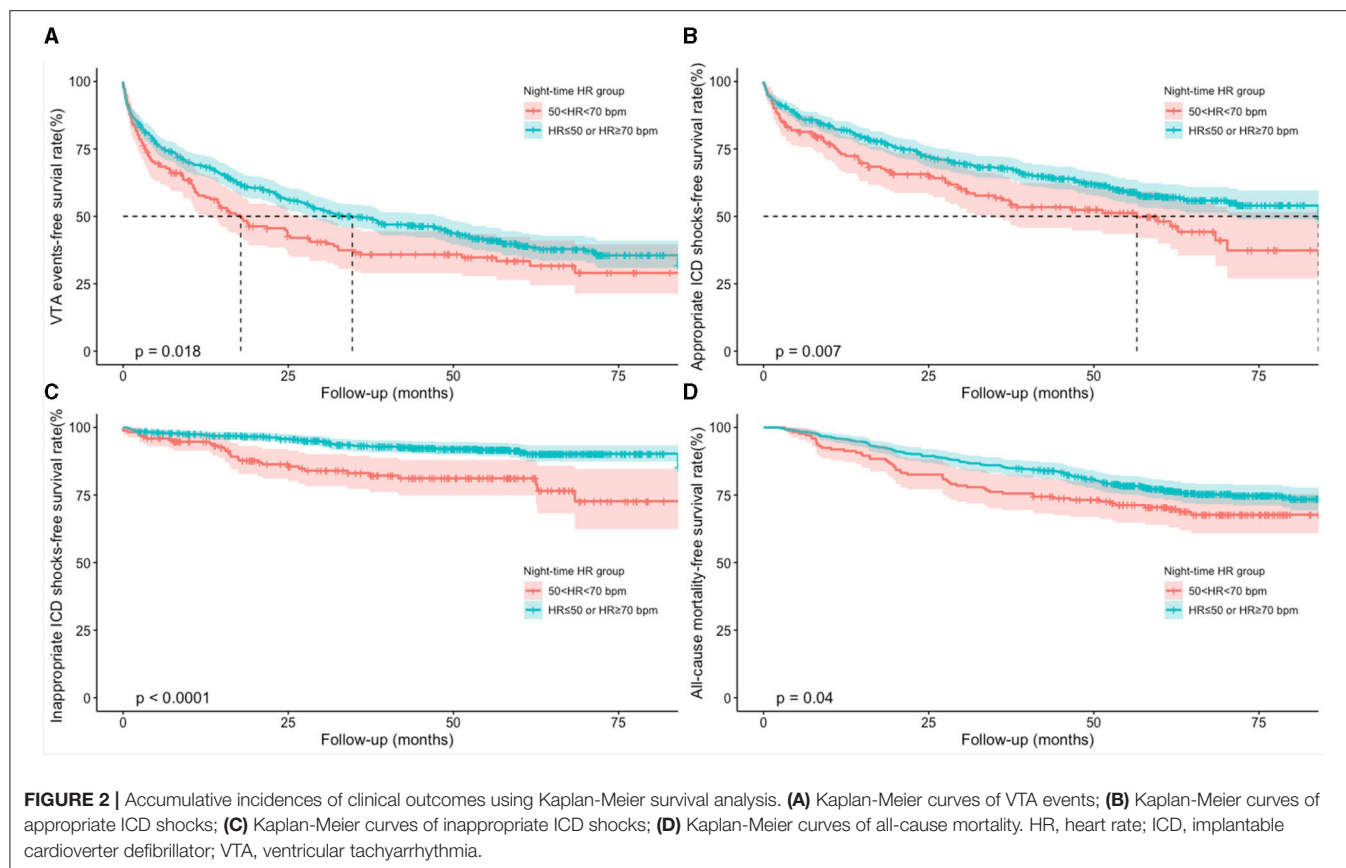


TABLE 2 | Night-time HR's effects on VTAs and ICD shocks.

Night-time HR (per 1 bpm/increase)*	Univariate Cox regression analysis		Multivariate Cox regression analysis	
	Hazard ratio (95%CI)*	P-value	Hazard ratio (95%CI)*	P-value
Night-time HR < 60 bpm (n = 364)				
VTA events	0.977 (0.944–1.010)	0.172	0.967 (0.933–1.001)	0.060
Appropriate ICD shock	0.960 (0.923–0.999)	0.045	0.940 (0.901–0.980)	0.004
Inappropriate ICD shock	0.901 (0.827–0.981)	0.017	0.893 (0.817–0.976)	0.013
Night-time HR ≥ 60 bpm (n = 366)				
VTA events	1.034 (1.016–1.053)	<0.001	1.032 (1.013–1.052)	0.001
Appropriate ICD shock	1.035 (1.013–1.057)	0.002	1.033 (1.011–1.056)	0.004
Inappropriate ICD shock	1.049 (1.014–1.085)	0.006	1.049 (1.012–1.087)	0.009

Multivariate Cox regression analysis was adjusted for age at implantation, sex, LVEF, LVEDD, prior AF, and preimplant syncope. AF, atrial fibrillation; CI, confidence interval; HR, heart rate; LVEDD, left ventricular end-diastolic dimension; LVEF, left ventricular ejection fraction; VTA, ventricular tachyarrhythmia.

*per 1 bpm increase in night-time HR.

shock (hazard ratio = 0.940, 95% CI: 0.901–0.980, $P = 0.004$) and inappropriate ICD shock (hazard ratio = 0.893, 95% CI: 0.817–0.976, $P = 0.013$), respectively.

In patients with night-time HR ≥ 60 bpm, night-time HR was shown to be an independent risk factor for VTA events (hazard ratio = 1.034, 95% CI: 1.016–1.053, $P < 0.001$), appropriate ICD shock (hazard ratio = 1.035, 95% CI: 1.013–1.057, $P = 0.002$), and inappropriate ICD shock (hazard ratio

= 1.049, 95% CI: 1.014–1.085, $P = 0.006$). After adjusting for considerable variables in the multivariate models, the results remained consistent. Each additional 1 bpm increase in night-time HR could result in 3.2, 3.3, and 4.9% higher risks of VTA events (hazard ratio = 1.032, 95% CI: 1.013–1.052, $P = 0.001$), appropriate ICD shocks (hazard ratio = 1.033, 95% CI: 1.011–1.056, $P = 0.004$), and inappropriate ICD shocks (hazard ratio = 1.049, 95% CI: 1.012–1.087, $P = 0.009$), respectively.

TABLE 3 | Predictive values of 50–70 bpm of night-time HR for clinical outcomes.

Night-time HR (50–70 bp vs. ≤ 50 or ≥ 70 bpm)	Univariate Cox regression analysis		Multivariate Cox regression analysis	
	Hazard ratio (95%CI)	P-value	Hazard ratio (95%CI)	P-value
VTA events	0.768 (0.616–0.957)	0.019	0.751 (0.600–0.941)	0.013
Appropriate ICD shock	0.703 (0.542–0.910)	0.007	0.698 (0.536–0.909)	0.008
Inappropriate ICD shock	0.370 (0.232–0.591)	<0.001	0.365 (0.225–0.592)	<0.001
All-cause mortality	0.718 (0.522–0.987)	0.041	0.685 (0.492–0.953)	0.025

Multivariate Cox regression analysis was adjusted for age at implantation, sex, ICD indication, ICD primary prevention, LVEF, LVEDD, DM, ICM, MI, prior AF, preimplant syncope, usages of ACEI/ARBs, aldosterone antagonists, loop-diuretics, and digoxin. ACEIs, angiotensin-converting enzyme inhibitors; AF, atrial fibrillation; ARBs, angiotensin receptor blockers; CI, confidence interval; DM, diabetes mellitus; HR, heart rate; ICM, ischemic cardiomyopathy; LVEDD, left ventricular end-diastolic dimension; LVEF, left ventricular ejection fraction; MI, myocardial infarction; VTA, ventricular tachyarrhythmia.

Predictive Values of Night-Time HR of 50–70 bpm

The corresponding values of night-time HR were 50.0 and 75.3 bpm when the lower limits of 95% CI with Ln HR for the VTA events were zero; the cut-off values for appropriate ICD shocks were 52.2 and 75.4 bpm; and the cut-off values of inappropriate shocks were 45.0 and 70.2 bpm. After considering the obtained cut-off values comprehensively, night-time HR of 50–70 bpm was adopted to evaluate its predictive values in the following Kaplan-Meier survival analysis and Cox regression analysis.

The accumulative incidences of VTAs, appropriate and inappropriate ICD shocks, and death from any cause were compared using Kaplan-Meier survival analysis (**Figure 2**). Compared to those with ≤ 50 bpm or ≥ 70 bpm night-time HR, patients with 50–70 bpm night-time HR group had significantly lower accumulative incidence rates of VTA events (log rank, $P = 0.018$), appropriate ICD shocks (log rank, $P = 0.007$), inappropriate ICD shock (log rank, $P < 0.001$), and all-cause mortality (log rank, $P = 0.040$).

Univariate and multivariate Cox regression models were used to explore the predictive values of night-time HR of 50–70 bpm in clinical outcomes (**Table 3**). Univariate Cox regression analysis demonstrated that patients with 50–70 bpm night-time HR had significantly lower incidences in VTA events (hazard ratio = 0.768, 95% CI: 0.616–0.957, $P = 0.019$), appropriate ICD shocks (hazard ratio = 0.703, 95% CI: 0.542–0.910, $P = 0.007$), inappropriate ICD shocks (hazard ratio = 0.370, 95% CI: 0.232–0.591, $P < 0.001$), and all-cause mortality (hazard ratio=0.718 95% CI: 0.522–0.987, $P = 0.041$), compared to those with night-time HR of ≤ 50 or ≥ 70 bpm. After adjusted for age at implantation, sex, ICD primary prevention, ICD indication, LVEF, LVEDD, diabetes mellitus (DM), ischemic cardiomyopathy (ICM), myocardial infarction (MI), prior AF, preimplant syncope, use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers (ACEI/ARBs), aldosterone antagonists, loop-diuretics, and digoxin, the results remained consistent. Night-time HR of 50–70 bpm was associated with 24.9, 30.2, 63.5, and 31.5% reduced incidences of VTA events, appropriate ICD shocks, inappropriate ICD shocks, and all-cause mortality,

respectively, compared to those with night-time HR of ≤ 50 or ≥ 70 bpm.

DISCUSSION

In this cohort, the associations of night-time HR with VTA events, appropriate and inappropriate ICD shocks, and all-cause mortality were explored using restricted cubic splines and smooth curve fitting in 730 ICD/CRT-D recipients. First, the apparent non-linear associations of night-time HR with VTAs, appropriate and inappropriate ICD shocks were detected with a typical U-shaped curve. Second, when night-time HR was beyond 60 bpm, every 1 bpm increase in night-time HR could significantly contribute to 3.2, 3.3, and 4.9% higher risks of VTA events, appropriate and inappropriate ICD shocks; and when night-time HR was below 60 bpm, every 1 bpm increase in night-time HR was associated with 6.0 and 10.7% reduced risks of appropriate and inappropriate ICD shocks, respectively. Third, night-time HR of 50–70 bpm could result in 24.9, 30.2, 63.5, and 31.5% lower incidences of VTA events, appropriate ICD shocks, inappropriate ICD shocks, and all-cause mortality, respectively, compared to those with night-time HR of ≤ 50 or ≥ 70 bpm.

In the present study, night-time HR was obtained in ICD/CRT-D patients. The home monitoring system was able to supply continuous recordings of HR, and night-time HR was acquired from 2:00 am to 6:00 am during sleeping periods. Night-time HR may not be influenced by diet, physical activity, or mental stress, etc. (11, 12). It could reflect the circadian rhythm of HR better than resting HR or 24-h HR (11, 12). However, compared to night-time HR, resting HR has been discussed more widely in previous studies. Resting HR could predict longevity and cardiovascular diseases in healthy individuals or patients with cardiovascular conditions (8–10). High resting HR has been associated with the development of different clinical events, including HF, hospitalizations for HF, MI, cardiovascular death, and all-cause mortality (7, 15–17). An analysis from ONTARGET and TRANSCEND demonstrated that whether in diabetic or non-diabetic individuals, the risks of cardiovascular death, MI, hospitalizations for HF and all-cause mortality rose when resting HR was above 75–80 bpm (16). The Melbourne Collaborative Cohort Study discovered that the individuals with temporal

increases in resting HR over a decade had higher risks of death in the general population (17). In patients with HFrEF, the SHIFT trial showed that every 5-bpm increase in HR resulted in 16% higher risk of HF hospitalizations and cardiovascular death, and the risks were decreased after ivabradine treatment (18). The Swedish Heart Failure Registry reported that β -blocker use was associated with reduced mortality in HFrEF (19). Regarding VTA events, Beinart et al. observed that resting HR >63 bpm (prior to ICD/CRT-D implantation) could lead to 81 and 76% increased risks of VT/VF events and appropriate ICD therapy, respectively, compared to resting HR ≤ 63 bpm, but did not discuss appropriate and inappropriate ICD shock events (20). Most previous studies paid attention on the negative effects of elevated resting HR. Fewer studies obtained the HR value during night-time hours and evaluate its predictive values using restricted cubic splines and smooth curve fitting.

Patients with higher night-time HR were generally older and had poor physical conditions, more comorbidities and medication. Additionally, night-time HR showed a positive correlation with all-cause mortality, which was consistent with the previous findings (16, 17). The underlying mechanisms of negative effects might be that elevated resting HR could reflect or lead to progressed or accelerated oxidative stress, vascular stiffness, left ventricular dysfunction, endothelial dysfunction, systemic inflammation, plaque rupture, myocardial oxygen consumption, and cardiac autonomic dysfunction (7, 21). In contrast to previous studies, the associations of night-time HR with VTAs, appropriate and inappropriate ICD shocks were detected in ICD/CRT-D recipients using restricted cubic splines and smooth curve fitting. The apparent non-linear associations demonstrated an increased incidence of VTA events, appropriate and inappropriate ICD shock at both low and high levels of night-time HR, with risks rising progressively when night-time HR was beyond 60 bpm. The current evidence supports that high night-time HR could result in increased risks of VTAs requiring ICD therapies, which remained consistent in MADIT-RIT trial (20). High night-time HR in sinus rhythm were related to excessive sympathetic activation and depressed parasympathetic activity, probably contributing to the development of VTAs and subsequent appropriate ICD therapies (13, 21). Inappropriate ICD shocks might be triggered by atrial tachyarrhythmia with rapid ventricular conduction, which could be directly influenced by cardiac autonomic activity (5).

This study also discovered that the patients with low night-time HR (≤ 50 bpm) had increased incidences of VTAs, ICD shocks and mortality. Such typical J-shaped non-linear relationships between resting HR and cardiovascular outcomes were previously reported in HF patients (20, 21). A meta-analysis from three prospective cohorts (Cardiovascular Health Study, Health ABC study, and Kuopio Ischemic Heart Disease Study) (21) and an analysis from ONTARGET and TRANSCEND (16) demonstrated increased risks of cardiovascular outcomes at low levels of resting HR < 60 bpm. One possible explanation for this result might be that more patients with low resting HR were combined with sinus node or conduction diseases (21). Furthermore, the patients in the low night-time HR group (≤ 50 bpm) had more amiodarone use but less use of ACEI/ARBs, aldosterone antagonists, loop-diuretics, and

digoxin, compared to the moderate and high night-time HR groups. Another possible mechanism might be attributed to the complex medication, which could influence the night-time HR through the autonomic nervous system.

ICD/CRT-D devices were able to supply continuous night-time HR monitoring data. The present study emphasized the importance of night-time HR monitoring after ICD/CRT-D implantation (17). Night-time HR could assist in identifying the individuals with increasing risks of VTAs, appropriate ICD shock, as well as inappropriate ICD shock, which was beneficial to improve the longevity. Regarding the optimal night-time HR management targets, the smooth curve fitting showed the lowest incidences of VTAs, ICD shocks and mortality when night-time HR was maintained at ~ 60 bpm. However, it was impractical to maintain the night-time HR at 60 bpm in real-world clinical applications. Then, the cut-off values of night-time HR were adopted based on the smooth curve fitting, and their predictive values were explored further. Multivariate Cox regression analysis showed that night-time HR of 50–70 bpm was associated with 25–64% reduced incidences of VTA events and ICD shocks. Appropriate night-time HR of 50–70 bpm might be the optimal therapeutic targets for the management of ICD/CRT-D recipients in clinical practice.

LIMITATION

There are some limitations in this study. First, a prospective study is required to explore the therapeutic target values of the night-time HR after ICD/CRT-D implantation. Second, more studies are required to further explain the underlying mechanisms, especially for the patients with a relatively high incidence of VTAs, appropriate and inappropriate ICD shocks in the low night-time HR (≤ 50 bpm) group. Third, although the multivariate analyses were adjusted for potential confounding variables to minimize their imbalance, ICD indications were not balanced across the three groups. Caution should be exercised while generalizing the results to populations other than ICD/CRT-D recipients.

CONCLUSION

Apparent non-linear associations of night-time HR with VTAs and ICD shocks were detected. Increasing incidences of VTAs, appropriate and inappropriate ICD shocks were observed at both low and high levels of night-time HR in ICD/CRT-D recipients. Night-time HR of 50–70 bpm might be the optimal therapeutic targets for the management of ICD/CRT-D recipients.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The present study, which conformed to the Declaration of Helsinki, was approved by the ethics committee of Fuwai Hospital (the chief institute) and all other participating organizations (Zhongshan Hospital Fudan University, Nanjing Drum Tower Hospital, Shanghai First People's Hospital et al.). The patients/participants provided their written informed consent to participate in this study. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XS, BZ, and SZhao performed the conception or design of the work. XS, BZ, KC, WH, YS, WX, FW, XF, HN, YD,

ZL, and SZhang contributed to the acquisition, analysis, and interpretation of data for the work. XS and BZ drafted the manuscript. SZhao and SZhang critically revised the manuscript. All authors gave final approval and agreed to be accountable for all aspects of work ensuring integrity and accuracy.

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Prognostic Impact of the Symptom of New-Onset Atrial Fibrillation in Acute Myocardial Infarction: Insights From the NOAFCAMI-SH Registry

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Background: New-onset atrial fibrillation (NOAF) is a common complication during acute myocardial infarction (AMI) and sometimes can be completely asymptomatic, but the clinical implications of these asymptomatic episodes require further characterization. The objective of this study was to investigate the short- and long-term prognostic impact of post-MI NOAF based on the presence of AF-related symptoms.

Methods: The New-Onset Atrial Fibrillation Complicating Acute Myocardial Infarction in Shanghai (NOAFCAMI-SH) registry was a retrospective cohort including participants with AMI without a documented history of AF. Patients with NOAF were divided into two groups according to the AF-related symptoms. The primary endpoint was all-cause mortality.

Results: Of 2,399 patients included, 278 (11.6%) developed NOAF of whom 145 (6.0%) with asymptomatic episodes and 133 (5.5%) with symptomatic ones. During hospitalization, 148 patients died [106, 10, and 32 in the sinus rhythm (SR), asymptomatic, and symptomatic NOAF groups, respectively]. After multivariable adjustment, only symptomatic NOAF was associated with in-hospital mortality [odds ratio (OR): 2.32, 95% confidence interval (CI): 1.36–3.94] compared with SR. Over a median follow-up of 2.7 years, all-cause mortality was 3.2, 12.4, and 11.8% per year in the SR, asymptomatic, and symptomatic NOAF groups, respectively. After adjustment for confounders, it was the asymptomatic NOAF [hazard ratio (HR): 1.61, 95% CI: 1.09–2.37] rather than the symptomatic one (HR: 1.37, 95% CI: 0.88–2.12) that was significantly related to mortality. Similar results were also observed for cardiovascular mortality [HRs and 95% CI were 1.71 (1.10–2.67) and 1.25 (0.74–2.11) for asymptomatic and symptomatic NOAF, respectively]. Both asymptomatic and symptomatic NOAF episodes were associated with heart failure, whereas only those with symptomatic NOAF were at heightened risk of ischemic stroke. Our exploratory analysis further identified patients with asymptomatic high-burden NOAF as the highest-risk population (mortality: 19.6% per year).

Conclusion: Among patients with AMI, symptomatic NOAF is related to in-hospital mortality and asymptomatic NOAF is associated with poor long-term survival.

Registration: URL: <https://clinicaltrials.gov/>; Unique identifier: NCT03533543.

Keywords: acute myocardial infarction, atrial fibrillation, symptom, mortality, heart failure, ischemic stroke

INTRODUCTION

Atrial fibrillation (AF) is one of the most common arrhythmias worldwide, with a growing public burden due to the aging of the population. Atrial fibrillation is often intermittent and asymptomatic; sometimes it can only be detected during the diagnostic evaluation of patients presenting with cryptogenic stroke (1, 2). Debates over the screening modality, prognostic impact, and management of these asymptomatic AF episodes still exist (3–10).

Among patients with acute myocardial infarction (AMI), nearly 5–20% of whom will develop new-onset atrial fibrillation (NOAF), which is generally accompanied by increased risks of subsequent death and ischemic stroke (11, 12). Similar to the condition in the general population, NOAF during AMI can also be completely asymptomatic (13). Given the potential adverse impact of asymptomatic AF, researches focusing on the prevalence, clinical profiles, as well as prognostic implications of asymptomatic NOAF complicating AMI are of great clinical importance in helping the decision-making for out-patient ECG monitoring strategy as well as stroke and decompensated heart failure (HF) prophylaxis (14). However, until now, only in the sensitivity analysis of an observational AMI registry had researchers explored the impact of asymptomatic AF on prognosis (15).

Accordingly, using data from the New-Onset Atrial Fibrillation Complicating Acute Myocardial Infarction in ShangHai (NOAFCAMI-SH) registry, we aimed to perform a retrospective analysis to describe the clinical features and to investigate the impact of asymptomatic and symptomatic NOAF during AMI on in-hospital and long-term survival.

METHODS

Study Population

The design of the NOAFCAMI-SH registry has been previously described (16, 17). In brief, this is a retrospective cohort study from a tertiary academic medical center, which included patients who experienced an AMI, did not have a medical history of AF, and received continuous electronic monitoring (CEM) during hospitalization between February 2014 and March 2018. For the present analysis, all NOAFCAMI-SH participants were included, while event-free survival was only analyzed among individuals who were discharged alive with morbidity follow-up available. **Supplementary Figure 1** illustrates a CONSORT diagram of the study population. This study was conducted according to the Declaration of Helsinki, and the protocol of the NOAFCAMI-SH registry had been approved by the ethics committee of the Shanghai Tenth People's Hospital. Informed

consent was not required as all data were deidentified during the analytic stages.

Asymptomatic and Symptomatic NOAF Ascertainment

The occurrence of AF episodes was identified according to the individuals' CEM data. AF was diagnosed based on the consensus guidelines as follows: absolutely irregular RR intervals, no distinct P waves, and lasted for at least 30 s (14). NOAF was defined as patients without a history of AF who developed the first documented AF during the index AMI hospitalization. Patients would be systematically interviewed for their symptoms whenever an AF episode presented. Symptomatic NOAF was determined if the occurrence of NOAF event was simultaneously accompanied by any discomfort (e.g., chest tightness, palpitation, shortness of breath, etc.) or the need for emergent cardioversion. Asymptomatic NOAF was determined as any asymptomatic events of NOAF (13). The analyzed population was divided into three groups: sinus rhythm (SR), asymptomatic NOAF, and symptomatic NOAF.

Baseline Covariates

Baseline covariates consisted of patient demographics, medical history, in-hospital examination, and medications, which were ascertained by a detailed review of electronic medical records during or before the index hospitalization. Demographics included age, sex, smoking status, and body mass index. Medical history included hypertension, diabetes, hyperlipidemia, chronic kidney disease (CKD), HF, MI, percutaneous coronary intervention (PCI), peripheral artery disease (PAD), and stroke/transient ischemic attack (TIA). The in-hospital examination included creatinine, peak-TnT, peak NT-pro BNP, and angiographic and echocardiographic data. Medications included the use of antiplatelet agents, oral anticoagulants, ACE inhibitor/angiotensin receptor blocker (ACEI/ARB), β -blocker, diuretic, and amiodarone.

Outcome Measures

The primary outcome was all-cause death. Secondary outcomes included cardiovascular death, HF hospitalization, and ischemic stroke. All deaths without a definite non-cardiovascular cause (e.g., severe pneumonia, malignant tumors, end-stage renal disease, traffic accidents, etc.) would be treated as cardiovascular deaths. HF hospitalization was defined as any admission with a primary diagnosis of HF at discharge requiring intravenous diuretics. Ischemic stroke was defined as the occurrence of a new focal neurologic deficit considered to be ischemic in origin, with signs or symptoms lasting over 24 h. Patients were followed from the index discharge to the date of the presence of an outcome

of interest, death, or last follow-up (April 2019), whichever came first. Clinical outcomes were evaluated by a comprehensive review of the patient's medical records.

Statistical Analysis

Categorical variables were presented as frequencies and proportions and were compared with the χ^2 or Fisher's exact test, as appropriate. Continuous variables were presented as

means or medians and were compared with the one-way analysis of variance or Kruskal–Wallis test, as appropriate.

Treating SR as the reference, multivariable logistic regression models were established to investigate the association of asymptomatic and symptomatic NOAF with in-hospital death. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using three multivariable logistic regression models. In model 1, we adjusted for age and sex. In model 2, we

TABLE 1 | Patient characteristics.

	Sinus rhythm (N = 2,121)	Asymptomatic NOAF (N = 145)	Symptomatic NOAF (N = 133)	P-value
Demography and medical history				
Age (years), mean \pm SD	64.7 \pm 12.2* [‡]	73.8 \pm 11.2	74.8 \pm 9.7	<0.001
Men	1,651 (77.8)* [‡]	96 (66.2)	90 (67.7)	<0.001
Body mass index (kg/m ²), mean \pm SD	24.6 \pm 3.3	24.4 \pm 3.4	24.2 \pm 3.7	0.570
Current smoker	959 (45.2)* [‡]	46 (31.7)	38 (28.6)	<0.001
Hypertension	1,353 (63.8)	100 (69.0)	96 (72.2)	0.076
Diabetes mellitus	804 (37.9)	57 (39.3)	58 (43.6)	0.409
Hyperlipidemia	578 (27.3)	29 (20.0)	29 (21.8)	0.072
Chronic kidney disease	185 (8.7)	20 (13.8)	14 (10.5)	0.103
History of heart failure	105 (5.0)* [‡]	20 (13.8)	16 (12.0)	<0.001
Peripheral artery disease	75 (3.5)*	11 (7.6)	9 (6.8)	0.012
Prior AMI	139 (6.6)	11 (7.6)	15 (11.3)	0.106
Prior PCI	184 (8.7)	18 (12.4)	17 (12.8)	0.103
Prior stroke/TIA	237 (11.2)* [‡]	22 (15.2)	28 (21.1)	0.001
Initial presentation				
Out-of-hospital cardiac arrest	39 (1.8)	6 (4.1)	6 (4.5)	0.026
STEMI	1,299 (61.2)	93 (64.1)	81 (60.9)	0.781
On admission Killip > I	291 (13.7)* [‡]	41 (28.3)	45 (33.8)	<0.001
SBP (mmHg), median (IQR)	137 (120–154)* [‡]	136 (120–156)	130 (111–151)	0.031
HR (bpm), median (IQR)	79 (69–90)* [‡]	80 (68–93) [‡]	89 (72–104)	<0.001
GRACE risk score, mean \pm SD	118.8 \pm 28.0* [‡]	141.1 \pm 28.9	149.2 \pm 27.6	<0.001
CHA ₂ DS ₂ -VASc score, mean \pm SD	2.5 \pm 1.8* [‡]	3.6 \pm 1.8	4.0 \pm 1.8	<0.001
In-hospital examination and outcomes				
Creatinine (mg/dl)	0.88 (0.75–1.04)* [‡]	0.95 (0.81–1.29)	1.04 (0.85–1.37)	<0.001
Peak troponin-T (ng/ml)	2.87 (0.77–7.76)* [‡]	4.67 (0.80–10.00)	4.24 (1.12–9.93)	0.006
Log peak NT-pro BNP (pg/ml)	3.14 (2.83–3.50)* [‡]	3.57 (3.24–3.96)	3.82 (3.49–4.18)	<0.001
PCI with stent	1,764 (83.2)* [‡]	108 (74.5)	91 (68.4)	<0.001
Pre-PCI TIMI flow 2 or 3	1,006 (52.3) [‡]	52 (42.6)	43 (38.7)	0.003
Post-PCI TIMI flow 2 or 3	1,886 (98.0) [‡]	119 (97.5)	102 (91.9)	<0.001
Left atrial diameter (mm)	38 (35–41)* [‡]	40 (37–43)	41 (36–43)	<0.001
LVEF (%)	53 (43–60)* [‡]	50 (38–58)	45 (34–55)	<0.001
Total CEM duration (hours)	144.6 (109.0–198.3)* [‡]	185.5 (141.6–277.0)	211.4 (151.7–307.3)	<0.001
Total AF duration (hours)	–	13.4 (5.0–70.7)	14.4 (3.8–64.0)	0.518
AF burden (%)	–	8.41 (2.79–37.63)	8.41 (1.56–35.52)	0.445
Longest AF episode duration (hours)	–	11.0 (4.7–57.1)	8.9 (2.7–42.8)	0.175
AF from admission duration (hours)	–	28.9 (6.5–71.9)	29.6 (4.0–56.9)	0.187
Maximum HR in AF rhythm (bpm)	–	90 (74–114) [‡]	142 (128–156)	<0.001
In-hospital death	106 (5.0) [‡]	10 (6.9) [‡]	32 (24.1)	<0.001
Length of hospitalization (days)	7 (5–9)* [‡]	8 (6–12)	9 (7–13)	<0.001

Values presented as mean \pm SD, median (IQR), or n (%). The level of statistical significance was $p < 0.017$ for *sinus rhythm vs. asymptomatic NOAF; [‡]asymptomatic NOAF vs. symptomatic NOAF; and [‡]sinus rhythm vs. symptomatic NOAF, after multiple comparisons. AMI, acute myocardial infarction; CEM, continuous electronic monitoring; HR, heart rate; LVEF, left ventricular ejection fraction; GRACE, Global Registry of Acute Coronary Events; PCI, percutaneous coronary intervention; SBP, systolic blood pressure; TIA, transient ischemic attack; TIMI, thrombolysis in myocardial infarction.

further adjusted for cardiovascular risk factors (current smoker, hypertension, diabetes, CKD, previous MI, previous stroke/TIA, and PAD). In model 3, we further adjusted for admission characteristics [systolic blood pressure (SBP), heart rate, initial Killip class, out-of-hospital cardiac arrest, and left ventricular ejection fraction (LVEF)] and in-hospital PCI. For long-term survival analyses, event-free survival curves were estimated with the Kaplan–Meier method and compared using log-rank tests. We calculated hazard ratios (HRs) with 95% CIs by multivariable Cox proportional hazards analyses, and the candidate covariates included the following: (i) the Global Registry Acute Coronary Events (GRACE) risk score as a whole and (ii) variables in model 3. Of note, for the ischemic stroke evaluation, the individual components (age, sex, a history of HF, hypertension, diabetes, stroke/TIA, and vascular disease) in the CHA₂DS₂-VASc score were adjusted. The assumption of proportional hazards was verified by a visual examination of the log (minus log) curves.

The propensity score method was also used to compare the SR with either asymptomatic or symptomatic NOAF. Binary logistic regression analysis was used to calculate propensity scores to balance baseline characteristics (covariates listed in **Supplementary Material**). Two sets of propensity scores were calculated, one for comparing SR with asymptomatic NOAF and the other to compare SR with symptomatic NOAF. Matching was performed with a 1:3 matching protocol without replacement, using a caliper width equal to 0.10 of the SD of the propensity score.

Subgroup and Sensitivity Analyses

The associations between asymptomatic and symptomatic NOAF and mortality were explored in subgroups as follows: age (≥ 75 vs. < 75 years), gender (male vs. female), AMI type (STEMI vs. NSTEMI), and whether the patient underwent PCI (yes vs. no). Additionally, several sensitivity analyses were

TABLE 2 | Medications during hospitalization and at discharge.

	Sinus rhythm (N = 2,121)	Asymptomatic NOAF (N = 145)	Symptomatic NOAF (N = 133)	P-value
Medications during hospitalization				
Aspirin	2,021 (95.3)	140 (96.6)	122 (91.7)	0.130
P2Y ₁₂ receptor inhibitor	2,086 (98.3)	140 (96.6)	132 (99.2)	0.185
GP II _b /III _a inhibitor	1,803 (85.0)	123 (84.8)	107 (80.5)	0.366
Vasoactive agent	524 (24.7) [‡]	56 (38.6) [†]	75 (56.4)	<0.001
Oral anticoagulant	2 (0.1) [‡]	2 (1.4)	4 (3.0)	<0.001
ACEI/ARB	1,326 (62.5)	1,326 (62.5)	86 (64.7)	0.876
β -blocker	1,626 (76.7)	100 (69.0)	103 (77.4)	0.103
Statin	2,073 (97.7)	139 (95.9)	129 (97.0)	0.328
Diuretic	665 (31.4) [‡]	95 (65.5) [†]	110 (82.7)	<0.001
Amiodarone	237 (11.2) [‡]	67 (46.2) [†]	118 (88.7)	<0.001
Medications at discharge				
Aspirin	1,858 (92.2)	119 (88.1)	88 (87.1)	0.057
P2Y ₁₂ receptor inhibitor	1,941 (96.3) [*]	122 (90.4)	98 (97.0)	0.002
Oral anticoagulant	2 (0.1) [‡]	6 (4.4)	4 (4.0)	<0.001
ACEI/ARB	1,205 (59.8)	73 (54.1)	54 (53.5)	0.208
β -blocker	1,488 (73.8) [*]	77 (57.0)	64 (63.4)	<0.001
Statin	1,939 (96.2) [*]	123 (91.1)	96 (95.0)	0.014
Diuretic	258 (12.8) [‡]	37 (27.4)	31 (30.7)	<0.001
Amiodarone	25 (1.2) [‡]	11 (8.1) [†]	21 (20.8)	<0.001

Values presented as n (%). The level of statistical significance was $p < 0.017$ for *sinus rhythm vs. asymptomatic NOAF; [†]asymptomatic NOAF vs. symptomatic NOAF; and [‡]sinus rhythm vs. symptomatic NOAF, after multiple comparisons. ACEI/ARB, angiotensin-converting enzyme inhibitors/angiotensin receptor blocker.

TABLE 3 | Unadjusted and multivariable-adjusted logistic models for in-hospital mortality.

	Unadjusted	P-value	Model 1	P-value	Model 2	P-value	Model 3	P-value
	OR (95% CI)		OR (95% CI)		OR (95% CI)		OR (95% CI)	
Sinus rhythm	Reference	–	Reference	–	Reference	–	Reference	–
Asymptomatic NOAF	1.41 (0.72–2.76)	0.318	0.79 (0.39–1.57)	0.500	0.85 (0.42–1.70)	0.642	0.53 (0.24–1.17)	0.117
Symptomatic NOAF	6.02 (3.87–9.38)	<0.001	3.59 (2.26–5.70)	<0.001	3.59 (2.24–5.74)	<0.001	2.32 (1.36–3.94)	0.002

Model 1 is adjusted for age and sex. Model 2 is adjusted for model 1 + cardiovascular risk factors (current smoker, hypertension, diabetes, prior stroke, prior PAD, and prior MI). Model 3 is adjusted for model 2 + admission characteristics (Killip class, heart rate, SBP, out-of-hospital cardiac arrest, LVEF, and creatinine) and in-hospital PCI. CI, confidence interval; OR, odds ratio; PAD, peripheral artery disease. Other variables refer to **Table 1**.

also conducted. First, adjustment for differences in baseline characteristics was performed using stabilized inverse probability of treatment weighting (IPTW) models. Second, we further adjusted for medication usage (aspirin, ACEI/ARB, β -blocker, statin, and oral anticoagulant). Third, we censored patients who died within 1 month after discharge. Fourth, to minimize the potential misclassification of NOAF, we repeated the analysis by excluding those with prior stroke/TIA who were at high risk of asymptomatic AF (1, 2). Moreover, we performed an exploratory analysis in which patients with or without AF symptoms were further grouped according to the NOAF burden of 10.87% to investigate its interaction effects with AF symptoms, given the prognostic importance of the burden of post-MI NOAF (17). All analyses were performed with Stata v14.0 and R v3.6.3. A two-sided $P < 0.05$ was thought to be statistically significant.

RESULTS

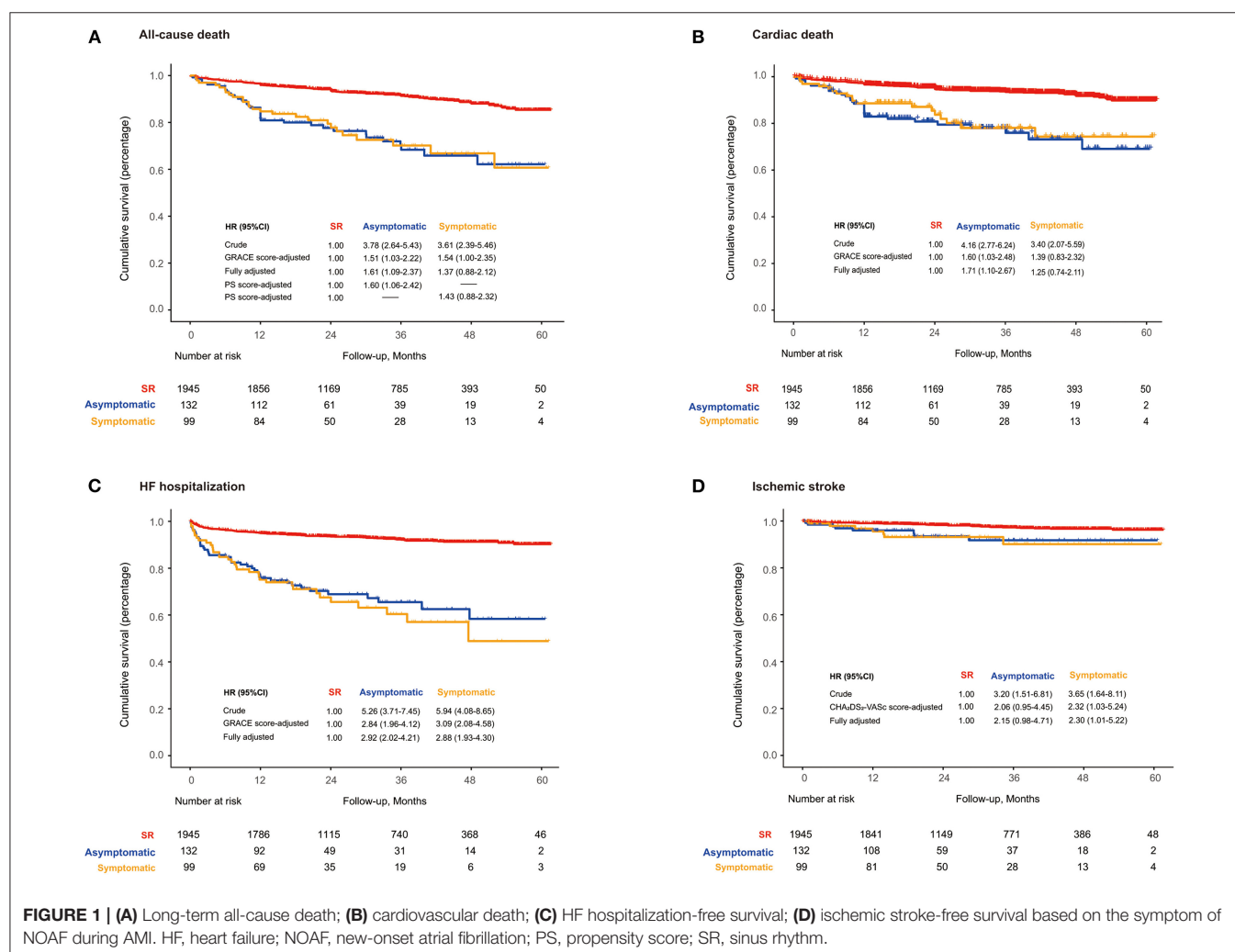
Study Population

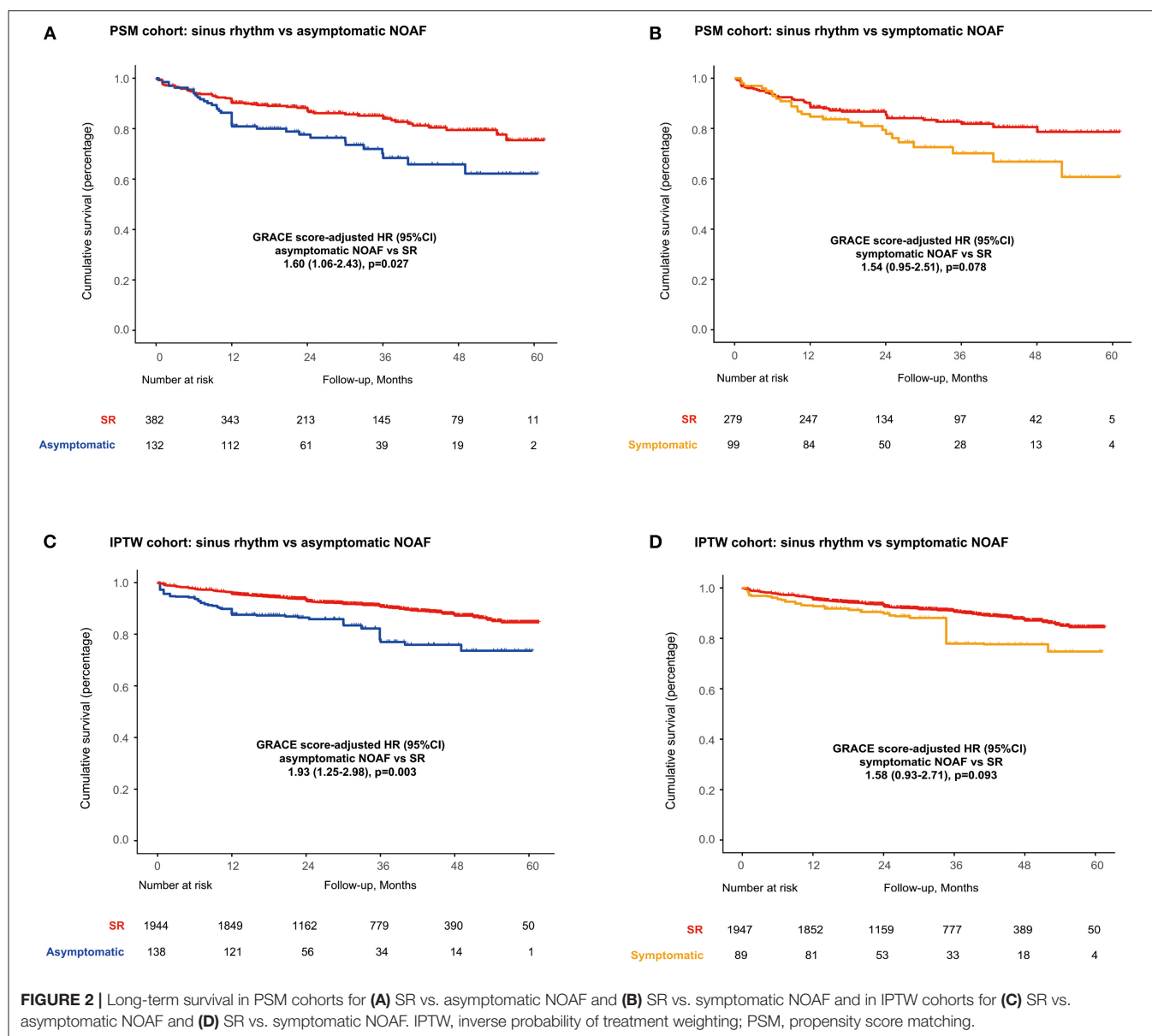
Among the 2,399 participants included in the NOAFCAMI-SH registry, 278 (11.6%) developed NOAF during their hospital

stay. Among those, 145 (6.0%) experienced asymptomatic NOAF and 133 (5.5%) had symptomatic NOAF. Baseline characteristics are shown in **Table 1**. Patients in the NOAF group were older, mainly female, more likely to have a history of HF, with a higher GRACE score and CHA₂DS₂-VASC score, with a lower LVEF value, and less likely to undergo PCI for reperfusion when compared with those in the SR group. No significant difference was observed between asymptomatic and symptomatic NOAF except admission heart rate. **Table 2** demonstrates the use of medications. Patients with symptomatic NOAF were more likely to be prescribed vasoactive agents, diuretics, and amiodarone when compared with the other two groups.

In-hospital Mortality

A total of 148 (6.2%) patients died during hospitalization, of whom 106 (5.0%), 10 (6.9%), and 32 (24.1%) were in the SR, asymptomatic NOAF, and symptomatic NOAF groups, respectively. As shown in **Table 3**, when treating the SR as the reference, the fully adjusted ORs and 95% CIs were 0.60 (0.28–1.29) and 2.35 (1.38–3.98) for the asymptomatic and symptomatic NOAF, respectively.



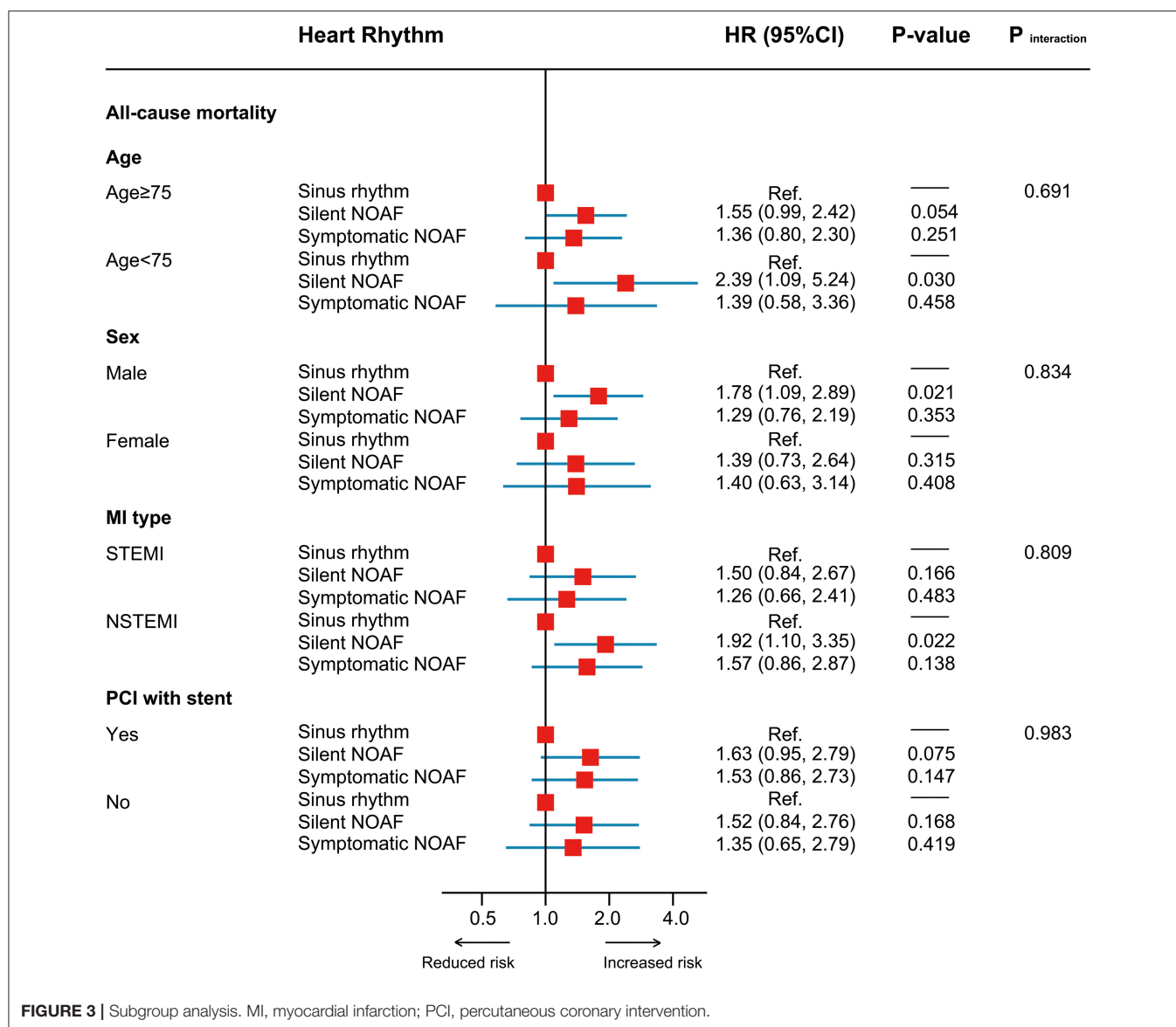


Long-Term Outcomes

Over a median follow-up of 2.7 years (IQR: 1.6–3.9), all-cause mortality was 3.2% (2.7–3.7%) for the SR, 12.4 (8.9–17.2%) for asymptomatic NOAF, and 11.8% (8.0–17.3%) for symptomatic NOAF. When compared with SR, the HRs and 95% CIs were 1.51 (1.03–2.22) for asymptomatic NOAF and 1.54 (1.00–2.35) for symptomatic NOAF after accounting for GRACE score and 1.61 (1.09–2.37) and 1.37 (0.88–2.12), respectively, after full adjustment. After adjustment for the propensity scores, the HR for asymptomatic NOAF was 1.60 (1.06–2.42) and 1.43 (0.88–2.32) for symptomatic NOAF, compared with the SR (Figure 1A). Moreover, it was the asymptomatic NOAF (HR: 1.71, 95% CI: 1.10–2.67) rather than the symptomatic one (HR: 1.25, 95% CI: 0.74–2.11) that was significantly associated with elevated cardiovascular mortality (Figure 1B). Both asymptomatic (HR:

2.92, 95% CI: 2.02–4.21) and symptomatic NOAF (HR: 2.88, 95% CI: 1.93–4.30) episodes were significantly associated with increased risk of HF hospitalization (Figure 1C). Only patients with symptomatic NOAF were at heightened long-term risk of ischemic stroke compared to those with SR (HR: 2.30, 95% CI: 1.01–5.22; Figure 1D).

As illustrated in **Supplementary Figure 2**, patient's characteristics were well-balanced in the propensity score-matched (PSM) cohorts. In the matched cohorts, long-term mortality was 6.3% (4.9–8.1%) for the SR and 12.4% (8.9–17.2%) for asymptomatic NOAF (asymptomatic NOAF vs. SR, HR: 1.60, 95% CI: 1.06–2.43; $P=0.027$) and 7.0% (5.2–9.4%) for the SR and 11.8% (8.0–17.3%) for symptomatic NOAF (symptomatic NOAF vs. SR, HR: 1.58, 95% CI: 0.93–2.71; $P=0.093$) (Figures 2A,B). IPTW analyses demonstrated similar results (Figures 2C,D).



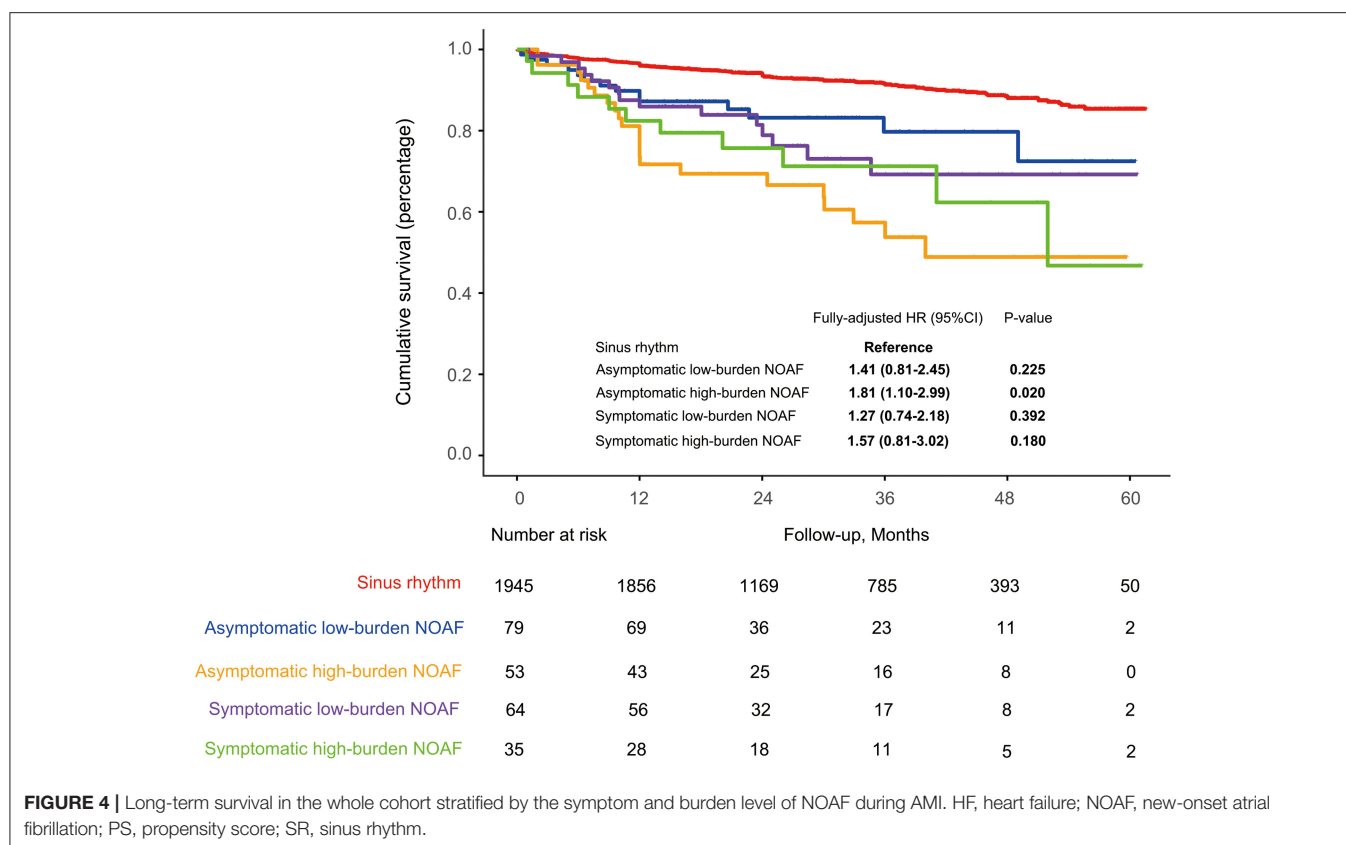
No significant heterogeneity in HR was observed across all subgroups (**Figure 3**). In the sensitivity analysis, results remained robust after adjustment for the medication usage, censoring patients who died within 1 month after discharge or excluding those with a prior stroke/TIA (**Supplementary Figure 3**). In the exploratory analysis, patients with asymptomatic high-burden NOAF were identified as the highest-risk population with all-cause mortality of 19.6% per year [fully adjusted HR (treating SR as the reference): 1.81, 95% CI: 1.10–2.99, $P = 0.020$; **Figure 4**].

DISCUSSION

The present analysis found that the incidence rate of post-MI asymptomatic NOAF was 6.0%. Symptomatic NOAF episodes were significantly associated with increased in-hospital mortality, whereas only asymptomatic episodes were related to poor long-term survival. Similar results

were observed for the risk evaluation of cardiovascular death. Notably, patients with high-burden asymptomatic NOAF represented the highest-risk population for all-cause death. In addition, when compared to the SR, both asymptomatic and symptomatic NOAF were associated with a heightened risk of HF hospitalization, but only symptomatic NOAF was challenged by a higher risk of ischemic stroke.

AF represents the most common arrhythmia in daily clinical practice, but its incidence is still thought to be underestimated since AF sometimes can be completely asymptomatic. Nowadays, technical advances in cardiac implantable electronic devices allow for the early detection of asymptomatic AF, and the prevalence of asymptomatic AF is varied across different settings (18), but data about asymptomatic AF in the AMI population remain limited. In our study, the incidence rate of in-hospital asymptomatic NOAF was $\approx 6.0\%$, which is lower than that



reported in a French registry where nearly 16.0% of the AMI individuals presented with asymptomatic AF (13). We postulated the difference might be explained as follows: the former study included patients with pre-existing AF who might have received β -blockers for rate control, thus alleviating the symptoms of AF. Besides, the high usage rate of amiodarone for cardioversion purposes reflects our concerns on the detrimental impact of post-MI NOAF, and this could also contribute to the low rate of asymptomatic AF (11, 12).

An important finding of our study was that the impacts of asymptomatic and symptomatic NOAF episodes on short- and long-term survival were divergent. After adjustment for conventional cardiovascular confounders, we showed that symptomatic NOAF was associated with 2-fold increased mortality during hospitalization; in contrast, only the asymptomatic NOAF was significantly related to poor long-term survival (HR: 1.72, 95% CI: 1.17–2.53, $P = 0.005$). The robustness of our results was further validated in the PSM and IPTW cohorts. Preceding studies had identified several pivotal risk factors of in-hospital death, such as age, heart rate, SBP, Killip class, etc. (19, 20). Given the fact that the majority of aforementioned risk factors presented in the symptomatic NOAF group (Table 1), it was not hard to understand such increased in-hospital mortality.

Debates concerning the prognostic implications of asymptomatic and symptomatic AF still exist in various settings (4, 6, 9). In a subanalysis of the Atrial Fibrillation

Follow-up Investigation of Rhythm Management (AFFIRM) trial, although the crude mortality was higher in the symptomatic AF group compared with that in the asymptomatic group (27 vs. 19%), statistical significance was not achieved after adjusting for a history of coronary artery disease, HF, and LVEF (HR: 1.07, 95% CI: 0.79–1.46, $P = 0.67$) (7). By contrast, Boriani et al. demonstrated that asymptomatic AF was significantly associated with increased 1-year mortality as compared with symptomatic AF (9.4 vs. 4.2%, $P < 0.0001$) (3). This time, we demonstrated that the asymptomatic NOAF was significantly associated with poor long-term survival. Similarly, Stamboul et al. had also reported that when treating patients with SR as the reference, those with asymptomatic AF during AMI were at higher risk of 1-year cardiovascular events even after multivariate adjustment (OR: 2.24, 95% CI: 1.02–4.93, $P = 0.046$) (15). Although the exact mechanism was still unclear, we considered this could partially be ascribed to the insufficient clinical concerns for patients without AF symptoms, thus leading to the inappropriate or delayed use of optimal management (4). Besides, Guenancia et al. showed that AF recurrences were more frequent in patients with symptomatic AF during AMI than in those with asymptomatic AF, which would make the asymptomatic NOAF even more difficult to be detected and treated (21). Our exploratory analysis in which patients with asymptomatic high-burden AF episodes (AF burden >10.87%) were recognized as the highest-risk population underscored the clinical importance of strengthened ECG monitoring and AF burden control, since the

asymptomatic AF had been determined by Potpara et al. as more likely to progress into a permanent pattern when compared to the symptomatic one (HR: 1.6, 95% CI: 1.1–2.2, $P = 0.009$) (8). Technical advances with respect to AF detection, for example, the use of smart device-based photoplethysmography technology (22), may be useful in patients with NOAF during AMI for the long-term AF screening, AF burden evaluation, as well as further clinical decision-making.

In line with prior studies, our results with respect to HF hospitalization further corroborated the fact that NOAF was an important risk factor of HF after AMI (17, 23), which was independent of AF symptoms. Interestingly, we found that only the symptomatic NOAF during AMI was significantly associated with an increased risk of ischemic stroke, which was different from previous reports that patients with asymptomatic AF were at high risk of ischemic stroke due to suboptimal anticoagulation (8, 24). We assumed it might be explained by the low usage rate of oral anticoagulant (OAC) among post-MI NOAF individuals ($\approx 7.4\%$); therefore, OAC treatment would have little impact on the analyzed population. In fact, as reported in the Chinese Acute Myocardial Infarction (CAMI) registry, only 5.1% of AMI patients concomitant with AF had been prescribed warfarin, and the rate of the combined use of warfarin and dual antiplatelet was even lower ($\approx 1.7\%$) (25). Such a striking gap may be due to the careful prescription of OAC after AMI given the risk of intracranial hemorrhage is higher in the Asian population (26). Accordingly, based on the present study, we postulated that patients' clinical profiles could be the dominant factor for the elevated risk of ischemic stroke, as patients with symptomatic NOAF had a relatively higher CHA₂DS₂-VASc score compared to those with asymptomatic NOAF (3.6 ± 1.8 vs. 4.0 ± 1.8 ; **Table 1**).

Limitations

The present analysis is retrospective in nature and thus subject to limitations about the uniformity of data collection. However, we performed a manual review of all admission records, rather than rely on coded information to both adjudicate the diagnosis of AMI as well as NOAF ascertainment. Although patients with a documented history of AF had been excluded, we cannot eliminate the possibility of NOAF misclassification as patients with an undiagnosed AF may be included. Because of lacking data on the specific causes of death (e.g., due to HF, stroke, bleeding, etc.), we cannot evaluate the association of the NOAF symptoms with cause-specific mortality. Also, the low rate of oral anticoagulant usage may limit the generalization

of our results to other cohorts. Finally, the number of patients who developed NOAF in this study is limited, and further studies with a larger sample size are highly desirable to confirm our findings.

CONCLUSIONS

Our results indicated that patients with post-MI symptomatic NOAF were the high-risk population of in-hospital death, and those with asymptomatic NOAF, especially concomitant with a high AF burden, had poor long-term survival. These findings highlight the importance of strengthened management for symptomatic NOAF episodes during the acute phase of AMI and the usefulness of extensive ECG monitoring among patients with asymptomatic NOAF to facilitate AF detection as well as timely initiation of treatment.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The ethics committee of the Shanghai Tenth People's Hospital. The ethics committee waived the requirement of written informed consent for participation.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcvm.2021.677695/full#supplementary-material>

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Prognostic Potential of Heart Rate and Hypertension in Multiple Myeloma Patients

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Background: The prognosis of patients with multiple myeloma (MM) is variable and partly depends on their cardiovascular status. The presence of arrhythmias can lead to worse outcomes. Therefore, this study aimed to evaluate the potential of heart rate (HR) and hypertension in predicating the outcomes of MM patients.

Methods: This study retrospectively enrolled patients with MM between January 1, 2010, and December 31, 2018, at the First Affiliated Hospital of Xi'an Jiaotong University. The endpoint was all-cause mortality. The Pearson's chi-square test was used to assess the association between hypertension and outcomes. Univariate and multivariate Cox proportional hazards models were developed to evaluate the relationship between HR and all-cause mortality.

Results: A total of 386 patients were included. The mean HR was 83.8 ± 23.1 beats per minute (bpm). Patients with HR > 100 bpm had a higher all-cause mortality (79.4%, 50/63) than those with $60 \leq \text{HR} \leq 100$ bpm (39.9%, 110/276) and < 60 bpm (19.1%, 9/47) ($p < 0.001$). Subgroup analysis based on the International Staging System and sex revealed similar relationships ($p < 0.01$). When stratified by age, patients with HR > 100 bpm had higher all-cause mortality than those with a lower HR when age was < 65 years or 65–75 years ($p < 0.001$) but not > 75 years. The proportion of patients with hypertension was 54.7% (211/386). However, hypertension was not associated with all-cause mortality in MM patients ($\chi^2 = 1.729$, $p > 0.05$). MM patients with HR > 100 bpm had the highest all-cause mortality.

Conclusions: The prognostic potential of HR may be useful in aiding risk stratification and promoting the management of these patients.

Keywords: multiple myeloma, risk stratification, heart rate, hypertension, cardiac rhythm abnormalities

INTRODUCTION

Multiple myeloma (MM) is a malignant neoplasm characterized by the clonal proliferation of malignant plasma cells in the bone marrow and monoclonal protein in the blood or urine and is associated with organ dysfunction (1). The median age at diagnosis is 70 years, and approximately 62% of patients with MM are ≥ 65 years at the time of diagnosis (1, 2). Fortunately, the survival

of patients with MM has improved dramatically over the past few decades because of therapeutic advancements (3, 4). Consequently, patients with increased age often have a high baseline incidence of coexisting cardiovascular diseases, including arrhythmias, hypertension, heart failure, and myocardial ischemia. In addition, MM is often associated with other chronic diseases, such as chronic kidney disease, which in turn leads to a high risk of cardiac events (5). Furthermore, chemotherapeutic agents for MM can give rise to treatment-related cardiotoxicities (6).

Heart rate (HR) is closely related to the development of cardiovascular morbidity and mortality in various diseases (7–9). Our recent work showed that some ECG parameters were related to the outcomes of MM (10), however, the association between HR, hypertension, and their prognostic potential in MM is rarely reported. Therefore, this study aims to assess the potential of HR and hypertension in predicating the outcomes of patients with MM.

METHODS

Ethnic and Consent

This study was performed according to the Declaration of Helsinki with informed consent obtained from the patients or their family members for research purposes. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University (Approval No. XJTU1AF2020LSK-179).

Participants and Groups

Consecutive MM patients were enrolled between January 2010 and December 2018 at the First Affiliated Hospital of Xi'an Jiaotong University. Multiple myeloma was diagnosed based on the results of testing and bone marrow pathology and confirmed by a hematologist. The treatment of MM patients was performed according to the new National Comprehensive Cancer Network (NCCN) guidelines (11). Based on HR, all patients were divided into three groups (HR >100 beats per minute (bpm), $60 \leq \text{HR} \leq 100$ bpm, and HR <60 bpm). In this study, increased HR was generally defined as an HR over 100 bpm. All patients were divided into two groups according to baseline peripheral blood pressure (BP): hypertension (defined as having a systolic BP ≥ 140 mm Hg and/or a diastolic BP ≥ 90 mm Hg at baseline) and non-hypertension (12). Patients with missing information, a history of myocardial infarction, paced rhythm, or active infection (25 patients) and those who were lost to follow-up were excluded.

Clinical Data Information

The baseline characteristics and clinical information and HR were collected from electronic medical records. HR was measured with a 12-lead electrocardiogram within 2 months of diagnosis for one patient and was obtained after at least 5 min of rest in the supine position during hospitalization; BP was measured at rest at least twice. Patients were

stratified by the International Staging System (ISS) (13). All-cause mortality follow-up data were obtained from the data of survey participation until October 2019. The follow-up was conducted by outpatient visits, telephone calls, or other electronic media.

TABLE 1 | Baseline characteristics of all patients.

Patient demographics	N = 386
Age (years)	61.7 \pm 9.7
Male, n (%)	227 (58.8)
Follow-up duration (months)	18.8 (9.5–36.8)
Systolic BP (mm Hg)	140.3 \pm 19.1
Diastolic BP (mm Hg)	77.5 \pm 12.6
Mean arterial pressure (mm Hg)	98.5 \pm 13.3
Hypertension, n (%)	211 (54.7)
Log NT-proBNP (pg/mL)	3.06 \pm 0.79
Troponin T (ng/mL)	0.280 (0.100–0.386)
Log lactate dehydrogenase (U/L)	2.38 \pm 0.22
Serum albumin (g/L)	31.65 \pm 6.05
Blood urea nitrogen (mmol/L)	6.92 (5.00–11.75)
Creatinine (mg/dL)	1.00 (0.69–2.57)
eGFR (mL/min/1.73 m ²)	73.41 (24.74–99.32)
Calcium (mmol/L)	2.33 \pm 0.39
Serum potassium (mmol/L)	3.55 \pm 0.50
Serum sodium (mmol/L)	134.6 \pm 5.8
Complete blood count	
White blood cell ($\times 10^9$ /L)	4.7 \pm 2.5
Hemoglobin (g/L)	84.5 \pm 23.5
Platelet ($\times 10^9$ /L)	157.0 \pm 85.9
Immune type of myeloma, n (%)	
IgG/IgA/IgD	276 (71.5)
Light chain κ/λ	99 (25.6)
None secreted	11 (2.9)
ISS Stage, n (%)	
I	55 (14.3)
II	161 (41.7)
III	170 (44.0)
HR (bpm)	
<60, n (%)	83.8 \pm 23.1 47 (12.2)
60–100, n (%)	276 (71.5)
>100, n (%)	63 (16.3)
Treatment, n (%)	
ASCT	38 (9.8)
No ASCT	348 (90.2)
Bortezomib	142 (36.8)
Lenalidomide	34 (8.8)
Both bortezomib and lenalidomide	19 (4.9)
Conventional chemotherapy	186 (48.2)
Best supportive care	28 (7.3)

Median (interquartile range), mean \pm SD; eGFR, estimated glomerular filtration rate; ISS, International Staging System; ASCT, autologous stem cell transplantation; HR, heart rate; bpm, beats per minute; BP, blood pressure.

TABLE 2 | Univariate and multivariate analyses for overall mortality.

	Univariate analysis			Multivariate analysis		
	Hazard ratio	95%CI	p-value	Hazard ratio	95%CI	p-value
Age (years)	1.000	0.984–1.016	0.999			
Male sex	1.273	0.928–1.745	0.134			
Log NT-proBNP (pg/mL)	1.812	1.487–2.207	<0.001	1.163	0.911–1.485	0.224
Troponin T (ng/mL)	17.701	4.209–74.437	<0.001	3.112	0.527–18.361	0.210
Log lactate dehydrogenase (U/L)	3.220	1.827–5.673	<0.001	1.868	1.012–3.450	0.046
Serum albumin < 35 g/L	1.195	0.847–1.686	0.309			
Blood urea nitrogen (mmol/L)	1.028	1.009–1.047	0.003	0.972	0.933–1.013	0.181
Creatinine (mg/dL)	1.105	1.057–1.156	<0.001	1.062	0.964–1.169	0.223
eGFR (mL/min/1.73 m ²)	0.992	0.988–0.996	<0.001	0.999	0.992–1.006	0.759
Calcium (mmol/L)	1.087	0.751–1.574	0.658			
Serum potassium (mmol/L)	1.053	0.761–1.456	0.757			
Serum sodium (mmol/L)	0.944	0.918–0.971	<0.001	0.999	0.967–1.031	0.929
Complete blood count						
White blood cell ($\times 10^9/L$)	1.098	1.041–1.158	0.001	1.075	1.018–1.135	0.009
Hemoglobin (g/L)	0.981	0.975–0.987	<0.001	0.988	0.980–0.997	0.007
Platelet ($\times 10^9/L$)	0.999	0.997–1.001	0.477			
ISS Stage						
I	1			1		
II	1.956	1.053–3.632	0.034	1.324	0.685–2.561	0.404
III	3.385	1.854–6.179	<0.001	1.441	0.685–3.029	0.335
HR (bpm)						
<60	1			1		
60–100	3.436	1.737–6.799	<0.001	2.548	1.271–5.108	0.008
>100	8.087	3.961–16.510	<0.001	4.330	2.042–9.181	<0.001

eGFR, estimated glomerular filtration rate; ISS, International Staging System; HR, heart rate; bpm, beats per minute.

Statistical Analysis

Continuous data were presented as mean \pm standard deviation (SD) or median (interquartile range), and categorical variables were expressed as counts and percentages. The Pearson's chi-square test or Fisher's exact test was utilized for mortality to evaluate correlations between different groups. Survival curves were plotted with Kaplan–Meier analysis, and differences in survival were tested by the log-rank test. Univariate and multivariate Cox proportional hazards models were developed to identify the relationship between HR and all-cause mortality. The proportional hazards assumption was assessed. The software package SPSS version 24 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. A $p < 0.05$ was considered statistically significant.

RESULTS

Baseline Characteristics

A total of 386 patients were enrolled in this study. The mean age of the patients was 61.7 ± 9.7 years, and 58.8% (227/386) were male. The median follow-up duration of 18.8 (9.5–36.8) months (Table 1), 169 patients (43.8%) died. The proportions of patients with HR >100 bpm, $60 \leq \text{HR} \leq 100$ bpm and HR <60 bpm were 16.3% (63/386), 71.5% (276/386), and 12.2% (47/386),

respectively. Sixty-three patients, including 17 patients with paroxysmal atrial fibrillation and four patients with paroxysmal supraventricular tachycardia, had increased HR. The proportion of patients with hypertension was 54.7% (211/386). One patient (0.3%, 1/386) was diagnosed with pulmonary embolism according to a CT pulmonary angiogram and received five cycles of a BCD regimen consisting of bortezomib, cyclophosphamide, and dexamethasone. The patient achieved complete remission and was alive through the end of follow-up. Nine patients (2.3%, 9/386) had heart failure, and 20 patients (5.2%, 20/386) had paroxysmal atrial fibrillation. Heart rate measurement data were obtained during sinus rhythm though they had the history of paroxysmal atrial fibrillation and supraventricular tachycardia in this study.

Prognostic Values of Heart Rate

The association between HR and all-cause mortality was evaluated. The mean HR of all patients was 83.8 ± 23.1 bpm. After several methods including the log-log survival curves, time-dependent Cox regression model and Schoenfeld residuals were used to test proportional hazards assumption for survival analysis, Cox model was identified to be suitable for the evaluation in our work, and the hazards were proportional. HR was independently associated with all-cause mortality ($p < 0.01$)

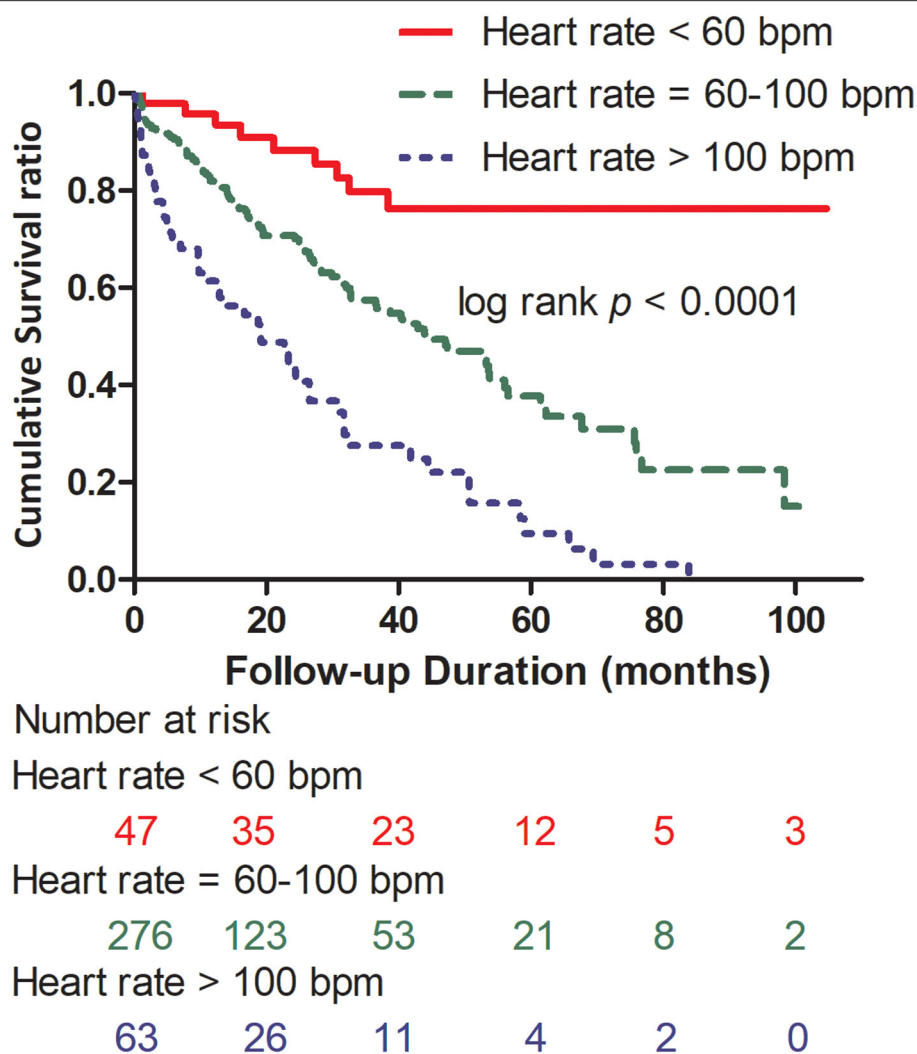


FIGURE 1 | Kaplan–Meier survival curves according to the presence of heart rate >100 bpm, 60 ≤ heart rate ≤ 100 bpm, and heart rate <60 bpm, log-rank $p < 0.0001$. HR, heart rate; bpm, beats per minute.

(Table 2). Patients with HR > 100 bpm had a higher all-cause mortality (79.4%, 50/63) than those with 60 ≤ HR ≤ 100 bpm (39.9%, 110/276) and < 60 bpm (19.1%, 9/47) ($p < 0.001$). HR showed a close association with all-cause mortality in the three groups (Figure 1).

Prognostic Value of Heart Rate Stratified by Sex

The mean age of male patients was 61.4 ± 10.0 years. Of the 227 male patients, the proportions of patients with HR >100 bpm was 16.3% (37/227), 60 ≤ HR ≤ 100 bpm was 71.4% (162/227), and HR <60 bpm was 12.3% (28/227). Patients with HR >100 bpm had higher all-cause mortality (78.4%, 29/37) than patients with 60 ≤ HR ≤ 100 bpm (45.1%, 73/162) and HR <60 bpm (25.0%, 7/28) ($p < 0.001$) in the male group. The mean age of female patients was 62.1 ± 9.4 years. Of the 159 female patients, the proportions of patients with

HR > 100 bpm, 60 ≤ HR ≤ 100 bpm and HR <60 bpm were 16.4% (26/159), 71.7% (114/159), and 11.9% (19/159), respectively. Patients with HR > 100 bpm had higher all-cause mortality (80.8%, 21/26) than patients with 60 ≤ HR ≤ 100 bpm (32.5%, 37/114) and HR < 60 bpm (10.5%, 2/19) ($p < 0.00001$) in the female group. In conclusion, HR showed prognostic potential in both the male and female subgroups (Figure 2).

Prognostic Value of Heart Rate Stratified by ISS Stage

The mean ages of patients were 59.5 ± 11.2 , 61.7 ± 9.6 , and 62.4 ± 9.3 years in Stages I, II, and III, respectively. Patients with Stage I, Stage II, and Stage III accounted for 14.3% (55/386), 41.7% (161/386), and 44.0% (170/386) of all patients. All-cause mortality of patients in Stage I, II, and III was 21.8% (12/55), 38.5% (62/161), and 55.9% (95/170), respectively. All-cause

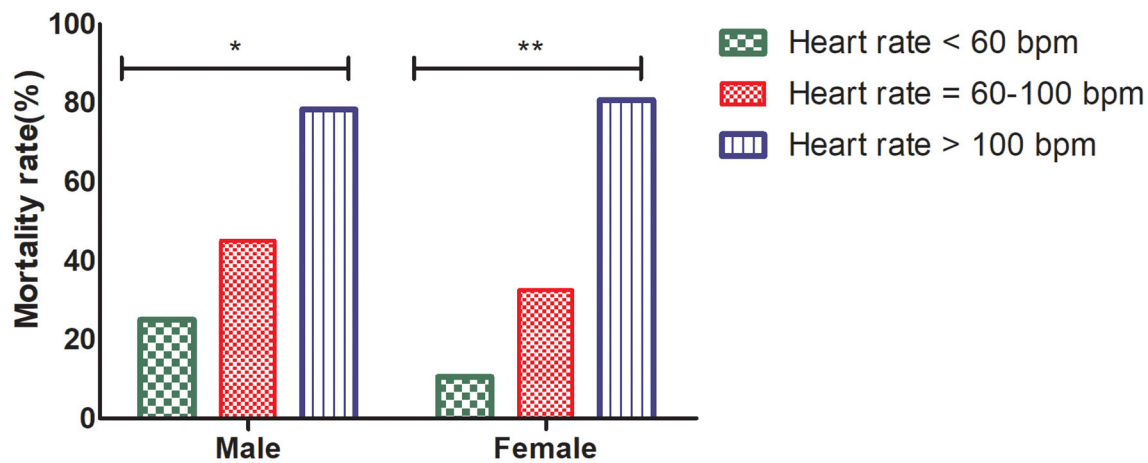


FIGURE 2 | All-cause mortality of HR > 100 bpm, $60 \leq \text{HR} \leq 100$ bpm, and HR < 60 bpm in the male and female groups, Pearson's chi-square test, * $p < 0.001$, ** $p < 0.00001$. HR, heart rate; bpm, beats per minute.

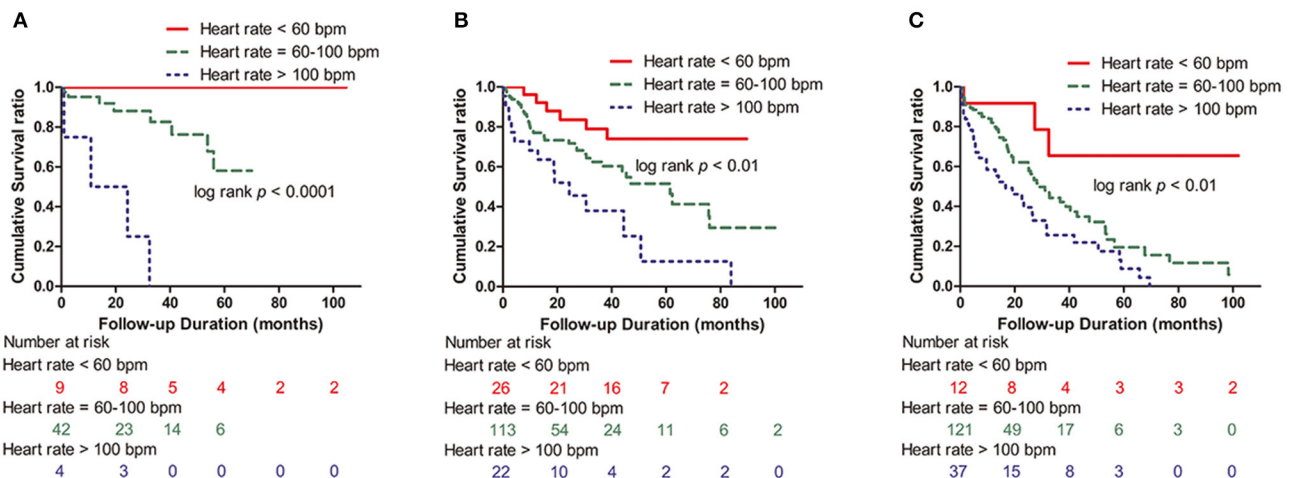


FIGURE 3 | Kaplan-Meier survival curves according to the presence of HR > 100 bpm, $60 \leq \text{HR} \leq 100$ bpm, and HR < 60 bpm in the ISS groups. (A) Stage I group (log-rank $p < 0.0001$). (B) Stage II group (log-rank $p < 0.01$). (C) Stage III group (log-rank $p < 0.01$); ISS, International Staging System; HR, heart rate; bpm: beats per minute.

mortality of patients with HR > 100 bpm, $60 \leq \text{HR} \leq 100$ bpm and HR < 60 bpm was 100.0% (4/4), 19.0% (8/42), and 0% (0/9) in Stage I; 68.2% (15/22), 36.3% (41/113), and 23.1% (6/26) in Stage II; and 83.8% (31/37), 50.4% (61/121), and 25.0% (3/12) in Stage III, respectively. In conclusion, patients with HR > 100 bpm had higher all-cause mortality than patients with $60 \leq \text{HR} \leq 100$ bpm and HR < 60 bpm in all three ISS stages ($p < 0.01$) (Figure 3).

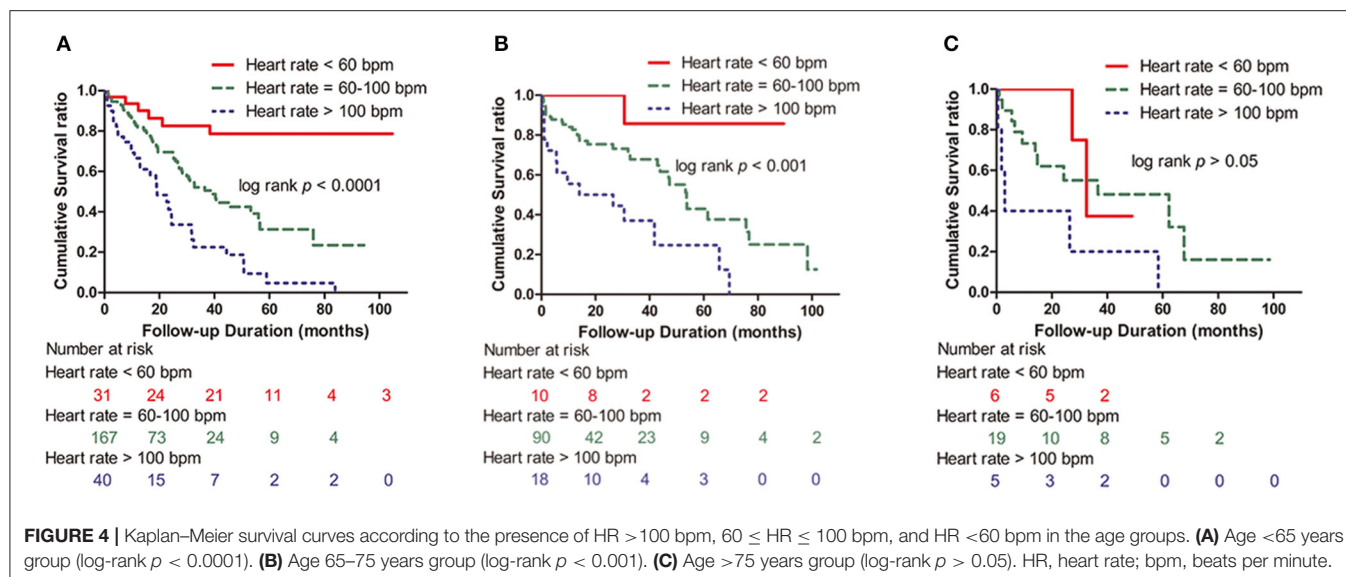
Prognostic Value of Heart Rate Stratified by Age

In the age subgroup analysis, patients were divided into the following age groups: <65, 65–75, and >75. Patients <65 years old accounted for 61.7% (238/386), those 65–75 years old accounted for 30.5% (118/386), and those >75 years old

accounted for 7.8% (30/386) of all patients. All-cause mortality in the age groups <65 years, age 65–75 years and age >75 years was 42.9% (102/238), 41.5% (49/118), and 60.0% (18/30), respectively. In the <65 year and 65–75 year groups, there was a significant correlation between all-cause mortality and HR ($p < 0.0001$, $p < 0.001$) (Figures 4A,B). In those aged >75 years, however, there was no statistical significance in all-cause mortality among the three groups of patients with HR > 100 bpm, $60 \leq \text{HR} \leq 100$ bpm, and HR < 60 bpm (log-rank $p > 0.05$) (Figure 4C).

Prognostic Value of Hypertension

The mean arterial pressure was 98.5 ± 13.3 mm Hg. The proportion of patients with hypertension was 54.7% (211/386) in our study. The relationship between hypertension and all-cause



mortality was evaluated. All-cause mortality in the hypertension and nonhypertension group was 40.8% (86/211) and 48.6% (85/175). However, hypertension was not associated with all-cause mortality in patients with MM ($\chi^2=1.729$, $p > 0.05$).

DISCUSSION

The prognostic potential of HR was evaluated in 386 patients with MM in our study. The findings indicated that HR was independently related to all-cause mortality in MM patients.

The prognosis of MM commonly depends on the identification of disease biology markers, patient factors, and cytogenetic classification (14–16). Accurate risk stratification and advancements in the management of MM have significantly increased survival and quality of life; however, many more cardiovascular complications are emerging (17). Drugs to treat MM show various cardiotoxicity profiles in MM patients. In addition, MM itself and related chronic diseases predispose patients to a high risk of cardiac events (18).

Cardiac arrhythmias are encountered frequently in patients with MM in clinical practice (19). Early identification of cardiac arrhythmias and burden is important for improving the prognosis of patients with MM. Previous studies have shown that fast HR is prospectively related to the development of cardiovascular morbidity and mortality and is an independent risk factor for atherosclerosis (7–9). Some important advantages of HR measurement are that it is less costly, noninvasive, and quick. This prompted us to perform this study with the main findings that fast HR was independently related to all-cause mortality in MM patients.

Arrhythmias are also the most common cardiac complications in patients with MM receiving chemotherapy, and the incidence of arrhythmias is considerably higher than the expected incidence for a given age. In a previous work, a total of 35,486 patients with MM were identified, of whom 31.2% had cardiac arrhythmias (19). MM patients have a heavy arrhythmia burden with a

high risk of atrial fibrillation, which contributes to considerable hospitalization costs. Tachycardia was identified as a significant predictor of overall mortality (20). A total of 622 patients with cancer, including lung cancer, leukemia, lymphoma, or MM, were assessed for mortality adjusting for age and other factors that were significantly different between patients with an HR ≥ 100 bpm and those with an HR < 100 bpm. In addition, cancer patients with experience tachycardia within 1 year after cancer diagnosis may have higher mortality rates up to 10 years after the diagnosis of tachycardia. Bradyarrhythmias and sinus node dysfunction have also been described in patients with MM undergoing chemotherapy (21, 22). A retrospective study indicated that MM patients using thalidomide had an HR of <60 bpm during follow-up, and 19% of thalidomide patients developed symptom-related bradycardia (21). A likely explanation is that thalidomide inhibited TNF- α expression and activity and led to over activity of the parasympathetic system. Sixty-three (16.3%) patients had HR >100 bpm in our study. Increased HR is a manifestation of impaired cardiac function. The specific mechanism of increased HR in patients with MM is not known but could be a complex matter (17, 18, 23). First, it probably involves both endothelial dysfunction and atrial changes, such as atrial dilatation, sinoatrial node disease, dysfunction of the conduction system, and disruption of normal atrial musculature, which contribute to the development of atrial arrhythmias. In addition, increased adrenergic and increased preload might also lead to atrial arrhythmias induced by electrical remodeling and shortening of the atrial effective refractory period.

Autologous stem cell transplantation (ASCT) is one of the recommended and reasonable choices for MM patients, especially for patients at first relapse and high-risk patients. The incidence of supraventricular arrhythmias during ASCT was approximately 9%. Patients with older age, electrocardiographic abnormalities, and a prior history of arrhythmias had a higher risk of developing supraventricular arrhythmias (24).

Atrial fibrillation was one of the most frequent cardiovascular complications in patients with MM receiving ASCT (27%) (24, 25). The percentage of transplantation (9.8%, 38/386) seemed low in our study, although it was affected by many factors, such as physical condition, age, economic inequality, and medical reimbursement policy. Treatment philosophies of physicians were associated with patient choice. Patients in underdeveloped regions had less access to transplantation. All-cause mortality of the patients who received ASCT with HR >100 bpm, $60 \leq \text{HR} \leq 100$ bpm and HR <60 bpm was 66.7% (4/6), 8.0% (2/25) and 0% (0/7), respectively. Similarly, HR showed a close association with all-cause mortality in the three groups. In addition, anemia reflected the higher ISS stages and could lead to arrhythmia. Increased HR was one of the most common symptoms of anemia. The multivariate Cox regression analysis showed that hemoglobin was also an independent risk factor (HR = 0.988, 95% CI: 0.980–0.997). A significant negative correlation between hemoglobin and HR was observed from linear regression analysis ($p < 0.001$). Interestingly, the patients without anemia (hemoglobin >120 g/L) still had an elevated HR in our study. HR was independently associated with all-cause mortality in patients without anemia ($p < 0.0001$, **Supplementary Figure 1**).

Amyloidosis is a disease in which abnormal immunoglobulin protein deposits aggregates in tissues or organs, such as the kidney, heart, liver, gastrointestinal tract and so on. The incidence of MM-associated light chain amyloidosis is approximately 12–15%, and approximately 38% of newly diagnosed MM patients have clinically occult light chain amyloidosis, which is often overlooked (26). Amyloid deposits can thicken the walls of the heart in myocardial amyloidosis. Restrictive cardiomyopathy is one of the most typical types. Furthermore, the conduction system of the heart is affected, causing arrhythmias and heart block. Endomyocardial biopsy is invasive and difficult to perform. One patient was suspected to have myocardial amyloidosis with typical echocardiography features; however, the patient did not undergo endomyocardial biopsy in the study. In addition, seven patients (1.8%, 7/386) were diagnosed with renal amyloidosis by kidney biopsy in our study.

Previous evidence indicated that sex was one of the important factors of 2-year survival (27). Therefore, the patients were divided into two groups for further analysis of the sex subgroup (10). There was a worse prognosis in the male and female groups with higher HR than in those with a low HR in our study. In addition, the mortality in the female group was higher than that in the male group when the HR was faster than 100 bpm, but no significant effect was observed ($p > 0.05$).

There are several staging systems representing the severity of MM, such as the Durie-Salmon Staging system (DSS), ISS, and Revised International Staging System (R-ISS), at different periods. The DSS classifies patients with MM based on immunoglobulin, hemoglobin, and calcium levels and the number of bone lesions to predict tumor burden and estimate prognosis. ISS, based on serum β -2 microglobulin and albumin, constitutes a potent and powerful MM staging system. Although

DSS and ISS are controversial in predicting overall mortality, ISS tends to have a better predictive ability than DSS. The R-ISS staging system, which was published in 2015, affected outcomes in the new medicine era (14). As a simple and convenient tool, ISS is widely accepted as prognostic staging system for this condition (13, 28) in clinical practice. The R-ISS was based on ISS stages, chromosomal abnormalities, and serum lactate dehydrogenase and is more comprehensive and robust than the ISS. However, new testing technology and the high cost of detection techniques have affected its wide use and acceptance, and not all patients undergo interphase fluorescent *in situ* hybridization (iFISH) detection. This retrospective study was conducted between January 1, 2010, and December 31, 2018. In the study, 76.2% (294/386) of the patients underwent only FISH, 51.6% (199/386) underwent only karyotype analysis, and 49.5% (191/386) underwent both tests. Although there was a significant positive correlation between ISS and HR in linear regression analysis ($p < 0.001$), no difference in all-cause mortality among the three ISS subgroups of patients with HR >100 bpm was observed ($p > 0.05$). In the ISS stage subgroup analysis, patients with fast HR had higher all-cause mortality than those with lower HR in the three ISS groups. Similarly, Markus et al. demonstrated that resting HR was an independent predictor of fatal outcomes in patients with advanced cancer (8).

Advanced age was associated with worse outcomes and probably identified a subpopulation of patients with a higher prevalence of cardiovascular comorbidities. Given the median age at diagnosis of MM, the incorporation of geriatric assessments into treatment decision-making has recently become the focus of investigations (15, 29).

In the subgroup analysis of age, there was a significant correlation of all-cause mortality and HR with age <75 years ($p < 0.001$) but not with age >75 years. This could be because age-related cardiovascular comorbidities attenuate the influence of MM itself on all-cause mortality.

Hypertension is commonly reported in MM patients in clinical trials and may be associated with older age, disease-related complications, and consequences of MM treatments (30–32). A retrospective cohort study reported that hypertension is a risk factor for the development of malignant hypertension in MM patients (33). In our study, however, hypertension was not an independent risk factor for all-cause mortality in MM patients, in part due to the small sample size or various types of BP measurements.

We acknowledge the limitations. First, given the retrospective nature of the study, not all clinical information, such as prescription details of chemotherapy drugs, antibiotics, therapeutic regimen, and strategies and disease state, was available. Second, crossing of survival curves was presented in **Figure 4C**. It was caused by a variety of reasons, such as the small number of patients (30 cases). In addition, the small study cohort limits our ability to systematically analyze the different antiarrhythmic and antihypertensive therapies prescribed, which might have influenced the results.

CONCLUSION

Fast HR was independently associated with high all-cause mortality in MM patients, especially in the group aged <75 years. Our work shows that HR may help to formulate the risk stratification in patients with MM, promoting the management of these high-risk patients with MM and improving their prognosis by reducing cardiac event.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University. The patients/participants provided their written informed consent to participate in this study.

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AUTHOR CONTRIBUTIONS

YY and CS contributed to the conception and design of the work and critically revised the manuscript. MT, LC, and JS contributed to acquisition, analysis, or interpretation of data. JW drafted the manuscript. YLo, MW, CS, and YLi critically revised the manuscript. All authors gave final approval and agreed to be accountable for all aspects of the work ensuring integrity and accuracy.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcvm.2021.681484/full#supplementary-material>

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Abrogation of CC Chemokine Receptor 9 Ameliorates Ventricular Electrical Remodeling in Mice After Myocardial Infarction

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Introduction: Myocardial infarction (MI) triggers structural and electrical remodeling. CC chemokine receptor 9 (CCR9) mediates chemotaxis of inflammatory cells in MI. In our previous study, CCR9 knockout has been found to improve structural remodeling after MI. Here, we further investigate the potential influence of CCR9 on electrical remodeling following MI in order to explore potential new measures to improve the prognosis of MI.

Methods and Results: Mice was used and divided into four groups: CCR9^{+/+}/Sham, CCR9^{-/-}/Sham, CCR9^{+/+}/MI, CCR9^{-/-}/MI. Animals were used at 1 week after MI surgery. Cardiomyocytes in the infarcted border zone were acutely dissociated and the whole-cell patch clamp was used to record action potential duration (APD), L-type calcium current ($I_{Ca,L}$) and transient outward potassium current (I_{to}). Calcium transient and sarcoplasmic reticulum (SR) calcium content under stimulation of Caffeine were measured in isolated cardiomyocytes by confocal microscopy. Multielectrode array (MEA) was used to measure the conduction of the left ventricle. The western-blot was performed for the expression level of connexin 43. We observed prolonged APD₉₀, increased $I_{Ca,L}$ and decreased I_{to} following MI, while CCR9 knockout attenuated these changes (APD₉₀: 50.57 ± 6.51 ms in CCR9^{-/-}/MI vs. 76.53 ± 5.98 ms in CCR9^{+/+}/MI, $p < 0.05$; $I_{Ca,L}$: -13.15 ± 0.86 pA/pF in CCR9^{-/-}/MI group vs. -17.05 ± 1.11 pA/pF in CCR9^{+/+}/MI, $p < 0.05$; I_{to} : 4.01 ± 0.17 pA/pF in CCR9^{-/-}/MI group vs. 2.71 ± 0.16 pA/pF in CCR9^{+/+}/MI, $p < 0.05$). The confocal microscopy results revealed CCR9 knockout reversed the calcium transient and calcium content reduction in sarcoplasmic reticulum following MI. MEA measurements showed improved conduction velocity in CCR9^{-/-}/MI mice (290.1 ± 34.47 cm/s in CCR9^{-/-}/MI group vs. 113.2 ± 14.4 cm/s in CCR9^{+/+}/MI group, $p < 0.05$). Western-blot results suggested connexin 43 expression was lowered after MI while CCR9 knockout improved its expression.

Conclusion: This study shows CCR9 knockout prevents the electrical remodeling by normalizing ion currents, the calcium homeostasis, and the gap junction to maintain APD and the conduction function. It suggests CCR9 is a promising therapeutic target for MI-induced arrhythmia, which warrants further investigation.

Keywords: myocardial infarction, chemokine receptor 9, action potential, ion channel, calcium transient, cardiac conduction, connexin 43

INTRODUCTION

Myocardial infarction (MI) is a disease of continuous ischemia and necrosis of cardiomyocytes caused by thrombus formation due to coronary atherosclerosis and rupture of vascular plaques, resulting in acute vascular occlusion (1). It brings a huge burden to the national economy and seriously affects the quality of life of patients. It is estimated that globally, ischemic heart disease affects around 126 million individuals (1,655 per 100,000), which is approximately 1.72% of the world's population. Nine million deaths were caused by IHD globally. By the year 2030, the prevalence rate is expected to exceed 1,845 per 100,000 population. The cost of MI is expected to account for 1–1.5% of the gross domestic product in countries like the United States (2). Cardiac remodeling including structural and electrical remodeling occurs after MI, which lead to hypertrophy, heart failure, arrhythmias as well as sudden cardiac death (SCD) (3). And ventricular arrhythmia is the leading cause responsible for SCD after MI (4). Electrical remodeling, including changes in ion channel currents and action potentials (APs) by myocardial conduction disorder, is pathological basis of arrhythmias following MI. The changes of repolarization currents, such as reduction in potassium current, including the transient outward current (I_{to}), the inward rectifier potassium current (I_{K1}), as well as the slow delayed rectifier potassium current (I_{Ks}), could lead to the prolongation of ventricular action potential duration (APD), and increased dispersion of ventricular repolarization, which are potential mechanisms for ventricular arrhythmias and SCD (5). As far, targeted drugs and various interventional techniques are the main clinical treatments to improve patient outcomes following MI. However, potential adverse reactions and poor prognosis cannot be totally resolved (6–8). Therefore, noninvasive, nonpharmacological, and clinically applicable technical approaches are being explored, such as stem and progenitor cell therapy or genetic therapy, since these promising strategies are specifically targeted and without drug toxicity compared with traditional means (5, 9, 10).

Chemokine receptors are mainly expressed on inflammatory cells like neutrophils, monocytes and macrophages. They also exist on other cells including endothelial cells and tumor cells (11). Chemokines as well as chemokine receptors play an important role in cardiovascular diseases: chemokine receptor 2 (CCR2)/chemokine ligand 2 (CCL2) participate in atherosclerosis and pathophysiology in MI. Inhibition of CCL2/CCR2 by using inhibitors or genetic technology can reduce inflammation and inhibit detrimental ventricular remodeling (12). Chemokine ligand 21 (CCL21)/chemokine receptor 7

(CCR7) play an important role in MI, neutralization antibody of CCL21 can improve ventricular remodeling by reducing infarct size and suppressing collagen content in myocardium after acute MI (13). Development of atherosclerosis needs the chemokine receptor 1 (CCR1)/chemokine receptor 5 (CCR5) to recruit monocytes (14). Thymus-expressed chemokine (CCL25) is the only known ligand for CC chemokine receptor 9 (CCR9). Like other chemokine receptors, CCR9 is also detected on lymphocytes, monocytes, macrophages and dendritic cells (DCs) (15). CCR9 was found to be involved in inflammatory diseases like inflammatory bowel disease, rheumatoid arthritis, and cancer (16–20). In cardiovascular system, only 2 literatures reported the relationship between CCR9 and heart diseases (21, 22). In the previous study, we found CCR9 abrogation could improve ventricular structural remodeling by reducing infarct size, inhibiting inflammation and fibrosis after MI, thus first reported CCR9 plays a crucial role in MI (21). In the other study, CCR9 was found to be related to pathological myocardial hypertrophy (22). Our present study is aimed to use CCR9 gene knockout mice to explore the effect of CCR9 in ventricular electrical remodeling following MI, and to discover novel methods that can prevent deteriorating outcomes after MI.

MATERIALS AND METHODS

Experimental Mice

Global CCR9 knockout mice (CCR9-KO, C57BL/6J background) were purchased from the European Mouse Mutant Archive (EM:02293. B6;129-Ccr9tm1Dgen/H). The mice were bred and kept in our animal house with specific pathogen-free environment in Renmin hospital of Wuhan university. Male mice with an age of 6–8 weeks (body weight 24–27g) were used in our study. All the protocols in our experiment were authorized by the Animal Care and Use Committee of Renmin Hospital of Wuhan University. The pain and suffering of animals were reduced to minimize during the experiments.

Left Coronary Artery Ligation

The surgery was conducted as described before (21). In short, mice were anesthetized with sodium pentobarbital (intraperitoneal injection, 50 mg/kg). After getting anesthesia, small animal ventilator was used to maintain normal breathing. For subjects in the MI groups, the chest was opened, the heart was fully exposed, and the left anterior descending coronary was ligated. The mice in sham groups underwent the pericardium opening as well but without ligation. The mice were divided into four group: wild type sham group (CCR9^{+/+}/Sham), CCR9

knockout sham group (CCR9^{-/-}/Sham), wild type MI group (CCR9^{+/+}/MI), and CCR9 knockout MI group (CCR9^{-/-}/MI). The animals were used for experiments at 1 week following MI surgery.

Isolation of Single Ventricular Cardiomyocytes

After the mice were anesthetized by sodium pentobarbital, the heart was quickly removed as soon as the chest was opened, then soaked in the iced Ca²⁺-free Tyrode's solution and appendages were pruned. The ascending aorta cannulation was quickly performed and perfused the heart with Ca²⁺-free Tyrode's solution for around 5 mins, followed by enzymatic solution for 6–7 mins until the full digestion was achieved. The heart was then transferred to Ca²⁺-free Tyrode's solution containing BSA (1 mg/ml) to stop digestion. Then, the left ventricle was cut out and the infarct border zone was reserved and dispersed into single cell with a polished Pasteur pipette. The cells were kept in the BSA solution at room temperature after the cell suspension was filtered.

Patch Clamp Recording

Since APD change in the perfused heart was observed in both CCR9^{+/+}/MI mice and CCR9^{-/-}/MI mice in our previous study, we wondered whether there were cardiac ionic currents alternation in single cardiomyocytes. Patch-clamp analyses were performed using acutely isolated ventricular myocytes from the infarcted border zone. Action potential duration (APD), transient outward potassium current (I_{to}) and L-type calcium current ($I_{Ca,L}$) were recorded by whole-cell patch-clamp. The glass microelectrodes were pulled and the microelectrode resistance ranges from 1 to 3 M Ω . A gentle negative pressure was given to rupture the cell membrane. The pipette solution for AP recording was (mmol/L): NaCl 5; KCl 15; K-glutamate 130; MgCl₂ 21; MgATP 5; CaCl₂ 1; HEPES 10; EGTA 5, and Tyrode's solution was used as the extracellular solution. $I_{Ca,L}$ was measured with bath solution (mmol/L: CsCl 20; TEA-Cl 135; MgCl₂ 0.5; HEPES 5; CaCl₂ 1.8) and pipette solution (mmol/L: CsOH 110; CsCl 20; TEACl 10; Aspartate 90; HEPES 10; EGTA 10; MgATP 5; creatine phosphate sodium 5; GTP 0.4; leupeptin 0.1). For $I_{Ca,L}$ recording, a slow voltage ramp with holding potential ranging from -70 to -45 mV was applied to inactivate sodium and T-type calcium current, and then voltage was increased to +65 mV step by step in 10 mV increments. The bath solution for I_{to} was (mmol/L): NaCl 138; MgCl₂ 1.0; KCl 5.4; CaCl₂ 1.8; HEPES 10; Glucose 10; nifedipine 0.02 and pipette solution (mmol/L) was: KOH 130; KCl 15; MgATP 5; CaCl₂ 1; L-glutamic acid 130; MgCl₂ 1; NaCl 5; EGTA 5; HEPES 10. I_{to} was measured with a holding potential -80 mV and using a depolarizing voltage step to +70 mV from a 40 ms pre-pulse of -40 mV to inactivate the sodium channel.

Calcium Image

Isolated ventricular myocytes from infarct border zone were centrifuged (1,200 rpm, 3 min). The supernatant was removed, and the precipitation was resuspended in Tyrode's solution in a 1.5 ml centrifuge tube. Then 10 μ M Fluo 4-AM was used to incubate the cells without light for 1 h at 37°C. After incubation,

the cells were washed by using Tyrode's solution for three times. Then, cells were kept on the ice and calcium transients were measured using a dual-beam excitation fluorescence photometry setup (Leica, Wetzlar, Germany). The emission wavelength and receiving wavelength for recording was 460–480 nm and 500–550 nm, respectively. A home-made cell groove with two platinum wires at one end was used to pass the electrical stimulation to the cells. The cells were given a field stimulation, of which the stimulus frequency was 0.5 and 1 Hz. The stimulation was repeated six times. After recording Ca²⁺ transient in both stimulation frequency, we switched the perfusion to normal Tyrode's solution with 10 mM caffeine to empty Ca²⁺ storage in sarcoplasmic reticulum (SR) to record the SR calcium content.

Multielectrode Array (MEA)

MEA measurements were made *in vitro* mouse heart 1 week after MI. The preparation of the isolated perfused heart was as above. The isolated heart was perfused with 37°C Tyrode's solution. After recovery of the spontaneous rhythms, the MEA dish containing 32 monopolar electrodes (32 Map) with interelectrode distance of 500 μ m to cover a 3 \times 3 mm square was placed on left ventricle. Electrical stimulation with the stimulating voltage ranging from 1V to 7V and a duration of 1 ms was applied. Cardio2D+ software (Multi Channel Systems) was used to acquire the data sampled at 10 kHz. Activation maps were produced and the conduction velocity (CV) was calculated by analyzing the whole data.

Western Blot

Structural remodeling occurs after MI, which can lead to changes in gap junctions among cardiac myocytes. In the previous study, we observed CCR9 knockout could improve these serious structural remodeling, so we wondered whether CCR9 knockout could affect these changes in gap junction. Here, western blot was applied to detect the expression level of connexin 43 (Cx43) in ventricular tissue from infarcted border zone. The mice were sacrificed to dissect the LV infarct border zone. The dissected tissues were grinded to extracted proteins and quantified protein concentrations by BCA assay kit. Forty microgram protein sample was loaded and proteins were separated by 10% sodium dodecylsulphate (SDS)-polyacrylamide gel electrophoresis (PAGE), then transferred to a polyvinylidene difluoride (PVDF) membrane. After the transferring, the PVDF membrane was removed and transferred into sealing solution for mild vibration at 4°C overnight with anti-Cx43 primary antibody (1:1,000, abcam, ab235585) followed by rabbit IgG (1:2,000, abcam, ab6721) for 1 h at room temperature. Development and photographic recording were performed with Bio-RDA gel imaging system.

Statistical Analysis

All data were presented as the means \pm SEM. Unpaired student's *t*-test or one-way analysis of variance (ANOVA) was used to make comparisons between two groups by using graphPad Prism version 5. Chi-Square Test with Fisher's exact test was used for the categorical variables. $P \leq 0.05$ was considered significant statistically.

RESULTS

CCR9 Knock Down Inhibits APD Prolongation Following MI

AP was recorded in isolated left ventricular cardiomyocytes around the infarction area using the whole cell patch clamp. MI markedly prolonged the APD₉₀ (76.53 ± 5.98 ms in CCR9^{+/+}/MI, $n = 6$ cells from 3 hearts, vs. 36.91 ± 4.64 ms in CCR9^{+/+}/Sham, $n = 11$ cells from 3 hearts, $p < 0.05$). Loss of CCR9 inhibited the prolongation of APD induced by MI (50.57 ± 6.51 ms in CCR9^{-/-}/MI, $n = 6$ cells from 3 hearts vs. 76.53 ± 5.98 ms in CCR9^{+/+}/MI, $n = 6$ cells from 3 hearts, $p < 0.05$; **Figure 1**).

CCR9 Knockout Attenuates MI-Induced Calcium Current Increase and Reduction of Transient Outward Potassium Current After MI

The original $I_{Ca,L}$ traces for isolated ventricular myocytes from the infarcted border zone among different groups were shown in **Figure 2A**. MI markedly increased the amplitude of $I_{Ca,L}$, which can be reduced by CCR9 knockout. The current density-voltage (I - V) correlations for the $I_{Ca,L}$ of each group were shown in **Figure 2B**, which showed that MI significantly increased the peak current when compared with the WT groups (-17.05 ± 1.11 pA/pF in CCR9^{+/+}/MI group, $n = 8$ cells from 4 hearts vs. -10.02 ± 0.83 pA/pF in CCR9^{+/+}/Sham, $n = 9$ cells from 3 hearts, $p < 0.01$; -13.15 ± 0.86 pA/pF in CCR9^{-/-}/MI group, $n = 10$ cells from 4 hearts vs. -8.76 ± 0.49 pA/pF in CCR9^{-/-}/Sham, $n = 9$ cells from 3 hearts, $p < 0.01$), while CCR9 knockout reduced the current density compared with CCR9^{+/+}/MI (-13.15 ± 0.86 pA/pF in CCR9^{-/-}/MI group, $n = 10$ cells from 4 hearts vs. -17.05 ± 1.11 pA/pF in CCR9^{+/+}/MI, $n = 8$ cells from 4 hearts, $p < 0.05$). Besides, we also recorded I_{to} , the results showed the peak I_{to} was significantly reduced in MI mice (+60 mV: 2.71 ± 0.16 pA/pF in CCR9^{+/+}/MI group, $n = 8$ cells from 4 hearts vs. 7.95 ± 0.31 pA/pF in CCR9^{+/+}/Sham, $n = 8$ cells from 3 hearts, $p < 0.05$; 4.01 ± 0.17 pA/pF in CCR9^{-/-}/MI group, $n = 8$ cells from 4 hearts vs. 8.03 ± 0.35 pA/pF in CCR9^{-/-}/Sham, $n = 6$ cells from 3 hearts, $p < 0.05$), while CCR9 deficiency notably attenuated MI-induced I_{to} reduction (4.01 ± 0.17 pA/pF in CCR9^{-/-}/MI group, $n = 8$ cells from 4 hearts vs. 2.71 ± 0.16 pA/pF in CCR9^{+/+}/MI, $n = 8$ cells from 4 hearts, $p < 0.05$, **Figures 2C,D**). These data showed the MI induced $I_{Ca,L}$ increasing and I_{to} decreasing, while absence of CCR9 can inhibit these ionic currents changing, therefore prevented APD prolongation following MI.

CCR9 Deficiency Restores Calcium Handling After MI

Arrhythmia following MI is associated with calcium homeostasis disorder. We further carried out the calcium image experiment to observe calcium handling in the ventricular cardiomyocytes following MI. As shown in **Figures 3A,B**, MI induced the Ca^{2+} transient amplitudes decreased in both 0.5 and 1 Hz,

but was only statistically significant in 1 Hz (0.53 ± 0.09 in CCR9^{+/+}/MI group, $n = 7$ cells from 4 hearts, vs. 0.95 ± 0.05 in CCR9^{+/+}/Sham group, $n = 9$ cells from 3 hearts, $p < 0.05$; 0.86 ± 0.08 in CCR9^{-/-}/MI group, $n = 13$ cells from 4 hearts vs. 1.14 ± 0.11 in CCR9^{-/-}/Sham group, $n = 12$ cells from 3 hearts, $p > 0.05$). Loss of CCR9 could restore calcium transient amplitude in 1 Hz (0.86 ± 0.08 in CCR9^{-/-}/MI group, $n = 13$ cells from 4 hearts vs. 0.53 ± 0.09 in CCR9^{+/+}/MI group, $n = 7$ cells from 4 hearts, $p < 0.05$). No difference was observed in Ca^{2+} transient amplitudes between 0.5 and 1 Hz. The SR calcium content experiment revealed decreased SR Ca^{2+} content after MI in CCR9^{+/+}/MI mice but not the CCR9^{-/-}/MI mice (0.90 ± 0.11 in CCR9^{+/+}/MI group, $n = 8$ cells from 4 hearts vs. 1.23 ± 0.10 in CCR9^{+/+}/Sham group, $n = 13$ cells from 3 hearts, $p = 0.05$; 1.24 ± 0.09 in CCR9^{-/-}/MI group, $n = 11$ cells from 4 hearts vs. 1.37 ± 0.12 in CCR9^{-/-}/Sham group, $n = 11$ cells from 3 hearts, $p > 0.05$). CCR9 deficiency restored SR calcium content (1.24 ± 0.09 in CCR9^{-/-}/MI group, $n = 11$ cells from 4 hearts vs. 0.90 ± 0.11 in CCR9^{+/+}/MI group, $n = 8$ cells from 4 hearts, $p < 0.05$, **Figure 3C**). On the whole, these results indicate that CCR9 deficiency restores abnormal SR Ca^{2+} storage and Ca^{2+} release caused by MI.

CCR9 Knockout Improves the Conduction Function After MI

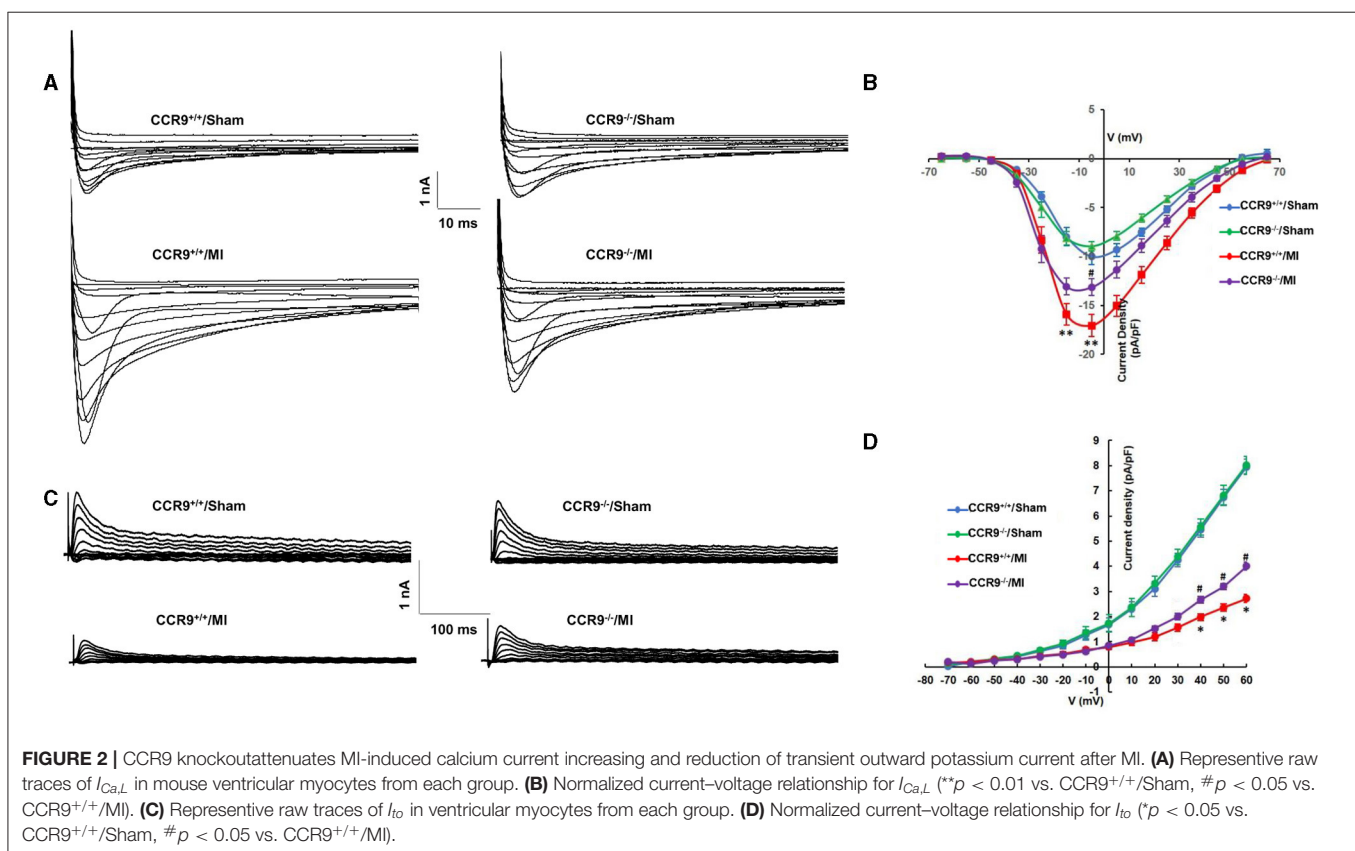
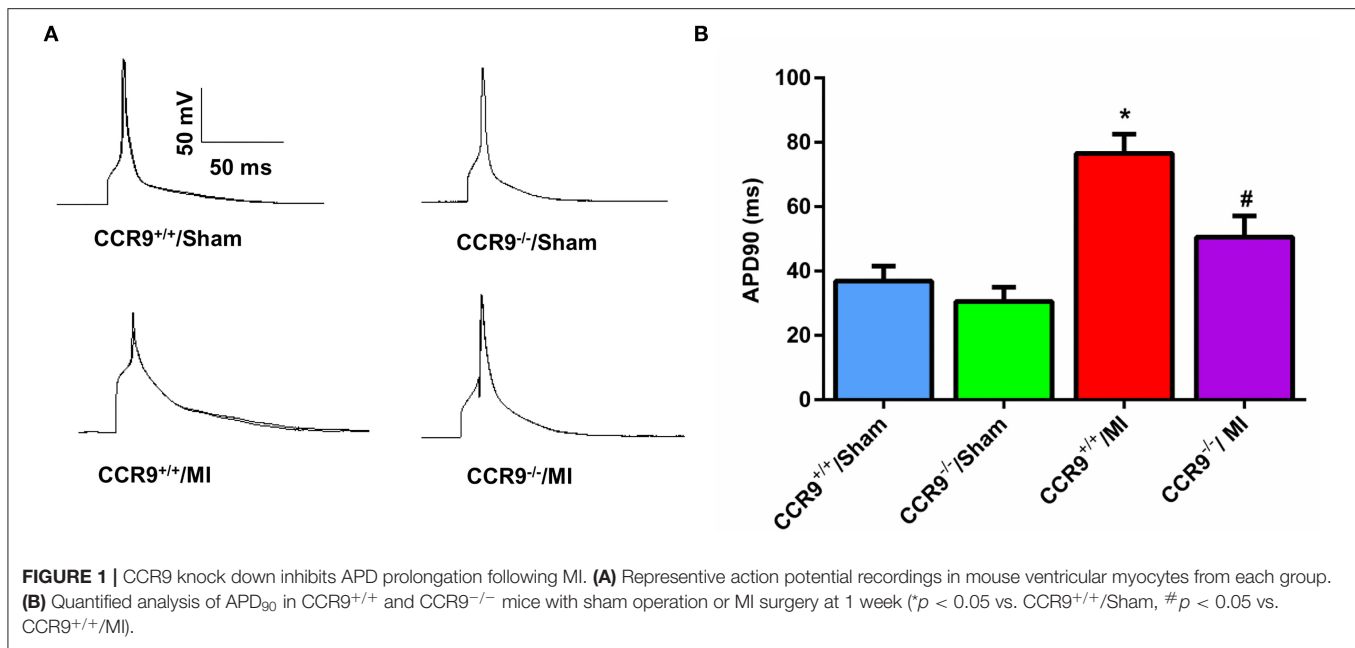
Conduction activation maps (**Figure 4A**) as well as CV maps (**Figure 4B**) on anterior wall of left ventricle surface displayed more crowded isochrones and a slower CV in the MI group than that in the sham group (113.2 ± 14.4 cm/s in CCR9^{+/+}/MI group, $n = 5$ hearts vs. 636.2 ± 112.1 cm/s in CCR9^{+/+}/sham group, $n = 5$ hearts, $p < 0.01$; 290.1 ± 34.47 cm/s in CCR9^{-/-}/MI group, $n = 6$ hearts vs. 568.6 ± 56.4 cm/s in CCR9^{-/-}/Sham group, $n = 4$ hearts, $p < 0.01$). What's more, left ventricle CV was faster and exhibited better homogeneity in the CCR9^{-/-}/MI group (290.1 ± 34.47 cm/s in CCR9^{-/-}/MI group, $n = 6$ hearts vs. 113.2 ± 14.4 cm/s in CCR9^{+/+}/MI group, $n = 5$ hearts, $p < 0.05$; **Figure 4C**).

CCR9 Knock Out Preserves the Expression of Cx43

The westernblot result revealed that the expression level of Cx43 was decreased following MI (Protein/GAPDH, 0.16 ± 0.02 in CCR9^{+/+}/MI group, $n = 4$ hearts vs. 0.74 ± 0.02 in CCR9^{+/+}/sham group, $n = 4$ hearts, $p < 0.01$; 0.42 ± 0.04 in CCR9^{-/-}/MI group, $n = 4$ hearts vs. 0.72 ± 0.03 in CCR9^{-/-}/Sham group, $n = 4$ hearts, $p < 0.01$), while loss of CCR9 could preserve the expression of Cx43 (Protein/GAPDH, 0.42 ± 0.04 in CCR9^{-/-}/MI group, $n = 4$ hearts vs. 0.16 ± 0.02 in CCR9^{+/+}/MI group, $n = 4$ hearts, $p < 0.01$, **Figures 5A,B**).

DISCUSSION

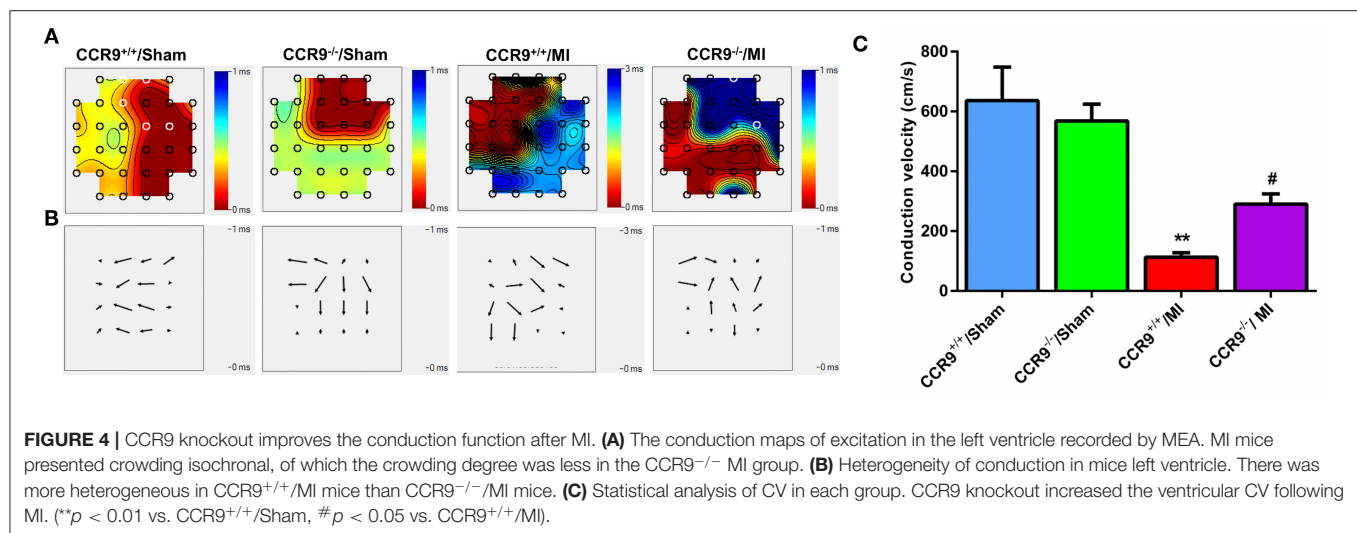
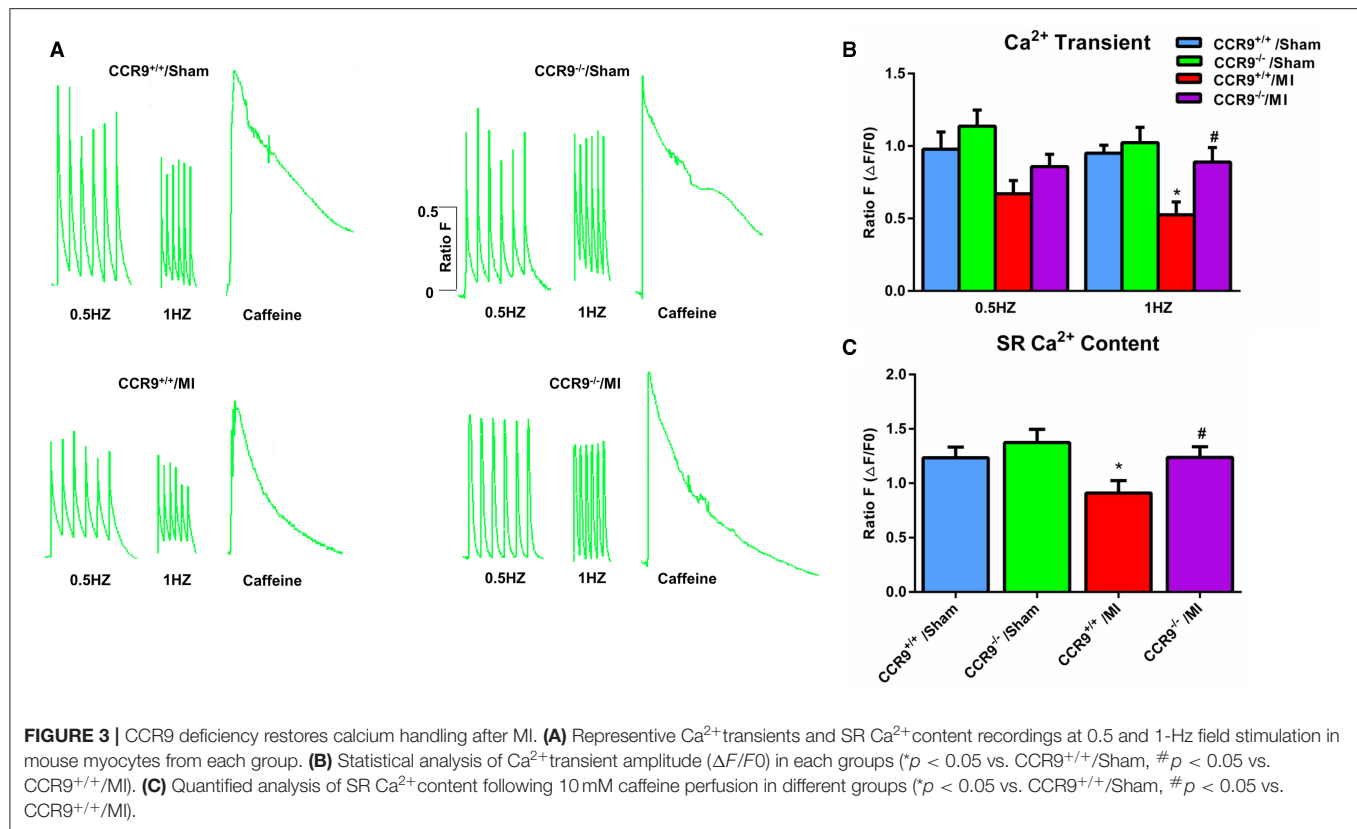
The major findings in this study include: (1) The APD was prolonged, $I_{Ca,L}$ was increased and I_{to} was decreased following MI in mice, while CCR9 knockout attenuated these changes. (2) CCR9 knockout reversed the calcium transient and calcium



content reduction in sarcoplasmic reticulum following MI. (3) The conduction function was preserved in CCR9^{-/-}/MI mice due to the preserved Cx43 expression.

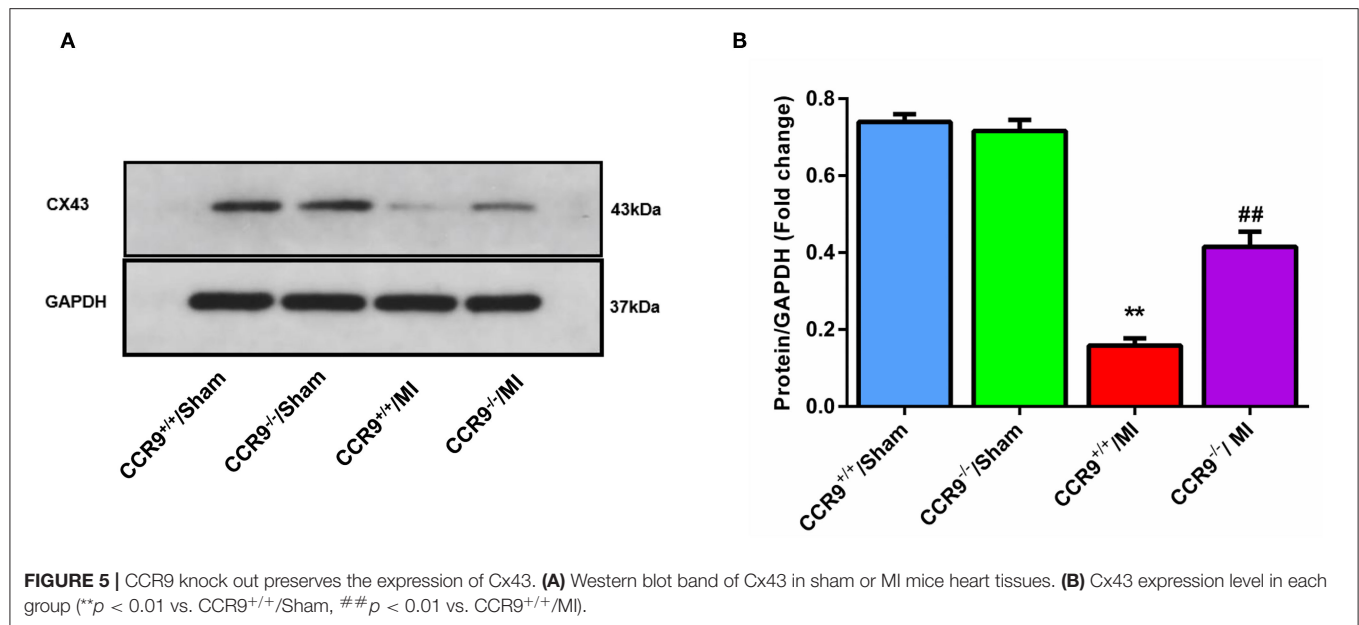
SCD is the main cause of death in patients with acute MI. Arrhythmia usually occurs in the early phase of acute MI, and

ventricular arrhythmia is the most common cause of death in MI patients (4). The main factors of arrhythmia after MI include: first, the heterogeneity of scar repair is the risk matrix for arrhythmia after MI, and the more severe myocardial fibrosis, the higher the incidence of arrhythmia (23–25). After MI, collagen



fibers were deposited in the surrounding area of infarction, and the surviving myocardial cells and collagen fibers interacted with each other. Due to the electrical heterogeneity between myocardial cells and fibroblasts, reentry produced and electrical conductivity became abnormal, which induce arrhythmia after MI (26–28). Studies have shown that when myocardial cells were co-cultured with fibroblasts, the higher the proportion of

fibroblasts, the slower the conduction speed, and the higher incidence of abnormal electrical activity (29). Through *in vivo* study, Michael and colleagues reported that Cx43 expression at myofibroblast-cardiomyocyte junctions was much less than that in remote region, which may result in low electrical conductivity in myofibroblast-cardiomyocyte junction area (30). Secondly, inflammatory response after MI can promote the occurrence of



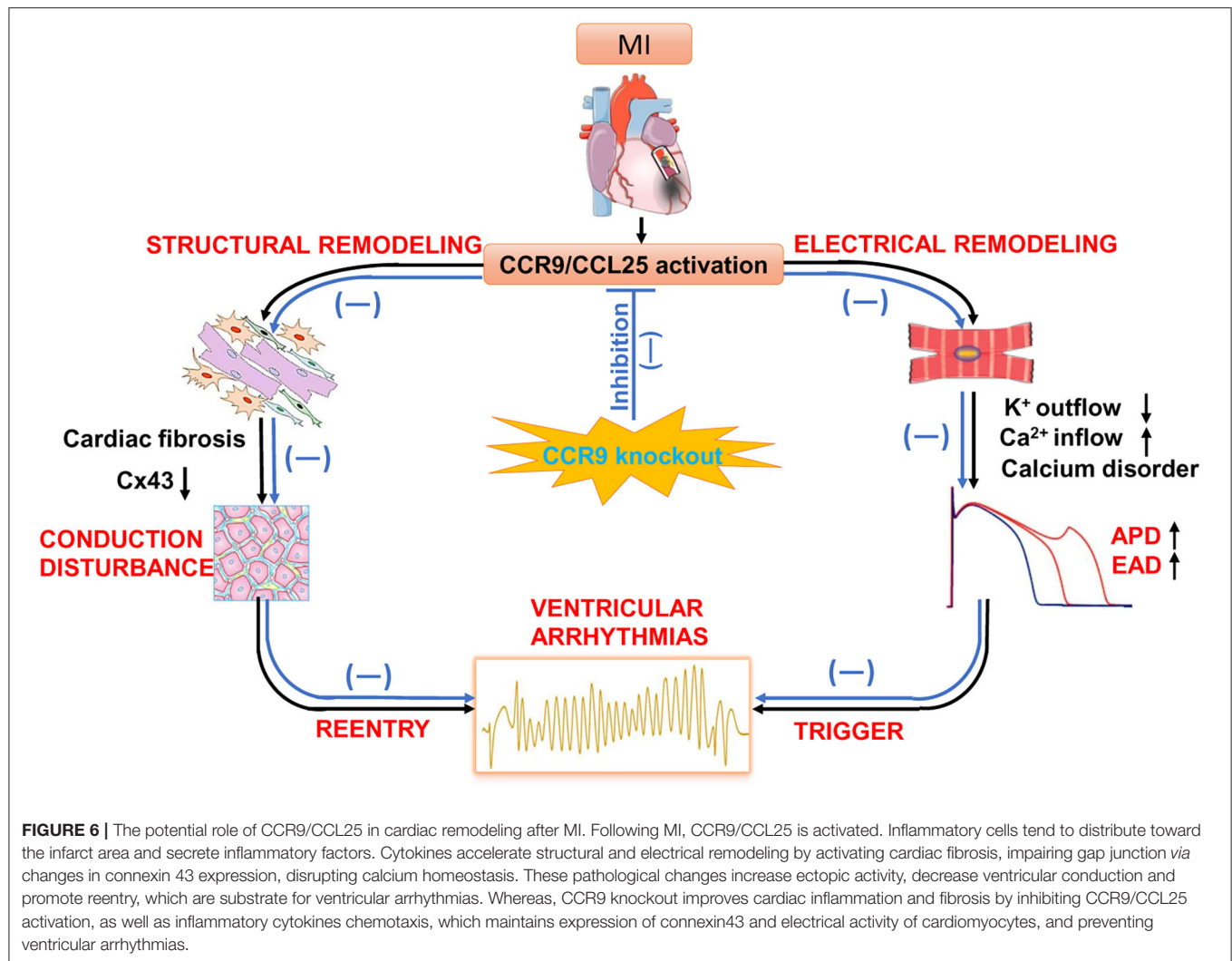
arrhythmia. Inflammatory response can cause both structural and electrical remodeling. A negative inflammatory factor can activate fibroblasts to differentiate into fibroblasts, increase collagen synthesis and fibrosis, and thus induce arrhythmia. On the other hand, cytokines and other inflammatory factors can directly affect the function of ion channels, affect proteins that regulate intracellular calcium homeostasis and intercellular gap junction (31–34).

Our previous investigation found that severe inflammatory response and myocardial fibrosis occurred after myocardial infarction, and CCR9 gene knockout can alleviate inflammation and myocardial fibrosis. Therefore, we attempted to further explore whether CCR9 gene knockout also has an impact on electrical remodeling after MI. In this part of the study, CCR9 gene knockout mice were studied to establish an acute MI model, and the cell electrophysiological properties were explored. We found that after MI, the APD₉₀ of the ventricular myocytes in the remote area was prolonged, which can be explained by the increased $I_{Ca,L}$ and the decreased I_{to} . These results were accordant to other studies on electrophysiological consequences following MI (35). However, for CCR9^{-/-}/MI mice, the APD₉₀ was shortened compared with wild type mice after MI. Consistent with APD₉₀ alternation, the $I_{Ca,L}$ was decreased and I_{to} was also increased in CCR9^{-/-}/MI mice when compared with wild type MI mice. This finding is novel. CCR9 knockout improves cardiac inflammation and fibrosis by inhibiting CCR9/CCL25 activation as well as inflammatory cytokines chemotaxis, which maintains electrical activity of cardiomyocytes and reduces the APD prolongation as well as the ion current disorder (Figure 6).

Also, we showed that the electrical conductivity was slowed and gap junction protein expression of Cx43 was decreased obviously after MI, which was consistent with the results

reported in literatures (36, 37), which have shown that Cx43 knockout mice under myocardial ischemia stress is prone to occur ventricular tachycardia. Cardiac conduction block and anisotropy happened in conditional Cx43 knockout mice, and ventricular arrhythmia susceptibility increase (38–40). Cx43 is the main protein that constitutes the gap junction between myocardial cells. Gap junction mediate movement of ions from cell to cell and is vital for impulse conduction through the Purkinje fibers and ventricular myocardium (41). After MI, the expression of Cx43 is reduced, and the uncoupling between myocardial cells can cause the electrical conduction block between myocardial cells, and subsequently induce malignant arrhythmia. Thus, inflammation, fibrosis, abnormal ion channel function as well as decreased Cx43 expression after MI triggering conduction abnormality, contribute to the reentry. Meanwhile, calcium handling disorder accelerates the occurrence of triggered activity. Triggering and reentry are the pathological basis of arrhythmia and sudden cardiac death (Figure 6).

Our further study previously showed that CCR9 gene knockout can reduce inflammation and improve the structural remodeling after MI. The results of our current study indicated that CCR9 gene knockout can shorten the prolonged APD after MI through influencing the calcium current and potassium current, as well as the calcium handling, which indicated that inhibiting CCR9 can improve the electrical remodeling and reduce the occurrence of malignant arrhythmia after MI. Moreover, CCR9 gene knockout reserves the conduction function and the expression of Cx43 in myocardial tissue after MI, suggesting that CCR9 knockout diminishes the inflammatory response and fibrosis, which can consequently save some Cx43 expression from MI induced degradation, and thus alleviated the electrical uncoupling and conduction



abnormalities among myocardial cells. At the same time, CCR9 gene knockout reduces inflammatory response and fibrosis, and these structural remodeling also play an important role in improving electrical remodeling. Therefore, early inhibition of CCR9 expression or chemotaxis of CCR9 positive cells in acute MI is expected to prevent malignant arrhythmia after MI.

LIMITATIONS

There are limitations to be addressed for the present study. First, this study is based on mice, which has different pathophysiological procedures from human beings. So, further exploration on larger animals as well as patients is warranted. Secondly, only CCR9 knockout mice are used here. It would be optimal if transgenic CCR9 mice could also be applied as another study group. Additionally, we need to perform more study to explore the underlying mechanisms and pathway in the future.

CONCLUSION

To address arrhythmias following MI, drugs and implantable cardioverter defibrillators (ICDs) as well as radiofrequency ablation were applied, while the mortality is still high and there are side effects for drugs and life quality is reduced due to devices implanted. Therefore, novel treatment strategies including gene therapy are in urgent need. In this study, CCR9 abrogation was suggested to ameliorate MI-induced electrical remodeling by affecting ion current, AP, calcium homeostasis and cardiac conduction as well as gap junction, suggesting it may be a novel pharmaceutical target for the treatment of MI-induced arrhythmia.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by the Animal Care and Use Committee of Renmin Hospital of Wuhan University.

AUTHOR CONTRIBUTIONS

YH, DH, C-XH, and H-SD, conceived and designed the study and drafted the manuscript. YH, DH, H-SD, TS, TW, Y-HT, and Y-TC performed the experiments. YH, DH, H-SD, Y-TC,

and HB-M analyzed the experiment data. YH, C-XH, and DH wrote the manuscript. All co-authors participate in editing of the manuscript.

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Predicting All-Cause Mortality Risk in Atrial Fibrillation Patients: A Novel LASSO-Cox Model Generated From a Prospective Dataset

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Background: Although mortality remains high in patients with atrial fibrillation (AF), there have been limited studies exploring machine learning (ML) models on mortality risk prediction in patients with AF.

Objectives: This study sought to develop an ML model that captures important variables in order to predict all-cause mortality in AF patients.

Methods: In this single center prospective study, an ML-based mortality prediction model was developed and validated using a dataset of 2,012 patients who experienced AF from November 2018 to February 2020 at the First Affiliated Hospital of Shantou University Medical College. The dataset was randomly divided into a training set (70%, $n = 1,223$) and a validation set (30%, $n = 552$). A total of 122 features were collected for variable selection. Least absolute shrinkage and selection operator (LASSO) and random forest (RF) algorithms were used for variable selection. Ten ML models were developed using variables selected by LASSO or RF. The best model was selected and compared with conventional risk scores. A nomogram and user-friendly online tool were developed to facilitate the mortality predictions and management recommendations.

Results: Thirteen features were selected by the LASSO regression algorithm. The LASSO-Cox model achieved an area under the curve (AUC) of 0.842 in the training dataset, and 0.854 in the validation dataset. A nomogram based on eight independent features was developed for the prediction of survival at 30, 180, and 365 days following discharge. Both the time dependent receiver operating characteristic (ROC) and decision curve analysis (DCA) showed better performances of the nomogram compared to the CHA₂DS₂-VASC and HAS-BLED models.

Conclusions: The LASSO-Cox mortality predictive model shows potential benefits in death risk evaluation for AF patients over the 365-day period following discharge. This novel ML approach may also provide physicians with personalized management recommendations.

Keywords: atrial fibrillation, machine learning, prediction model, mortality, risk factors

INTRODUCTION

AF is one of the most common chronic cardiovascular health problems globally (1–3). In Europe and the USA, 2–3 % of the population suffers from AF (4), and it is estimated that AF will affect 6–12 million people in the USA by 2,050 and 17.9 million people in Europe by 2,060 (5, 6). The incidence of AF is not high among young people but increases with age, reaching more than 10 % in those >80 years of age (7). The inevitable global aging of the population, combined with a cumulative increase in chronic cardiovascular diseases, will lead to considerable growth in the number of AF patients in the next few decades. AF is associated with a nearly five-fold increased risk of ischemic stroke (8, 9), and provokes significant increases in all-cause mortality along with important financial burden (10, 11). Consequently, higher risk of all-cause mortality associated with AF has become a significant public health issue (1, 11–13).

Several classic risk scores, including CHA₂DS₂-VASc and HAS-BLED scores, predict clinical outcomes, such as for stroke, bleeding and mortality (14–17). Machine learning can learn to identify the underlying pattern and classes from multidimensional data by utilizing computational algorithms (18). Based on novel ML algorithms, more accurate and intelligent models, such as the Global Anticoagulant Registry in the Field (GARFIELD)-AF risk model and the Multilayer Neural Network artificial intelligence model, have been developed (19–21). In contrast to the high awareness regarding clinical outcomes of AF in Europe and the USA, there is limited knowledge for East Asia. In addition, few ML models have used multi-dimensional features to predict future mortality of AF patients.

Advances in supervised ML allow the recognition and translation of multi-dimensional data into valuable models (21, 22). The use of machine learning for predicting clinical outcomes may enable physicians to improve efficiency, reliability, and accuracy of management decisions. In the present study, we used multiple ML approaches that included LASSO feature selection and the Cox proportional hazards regression model to predict all-cause mortality outcome over the 30–365-day period after discharge in patients with AF.

METHODS

Study Cohort

For machine learning model construction, a prospective observational study was undertaken using data from patients who were hospitalized for evaluation and treatment of AF between November 2018 and February 2020 at the First Affiliated Hospital of Shantou University Medical College. Inclusion criteria were a diagnosis of AF and availability of complete data concerning clinical indicators for evaluating AF and follow-up. The diagnosis of AF required recording the heart rhythm by electrocardiogram (ECG). Three diagnostic criteria shown by ECG are: (1) absolutely irregular RR intervals, (2) no discernible, distinct P waves, and (3) an episode lasting at least 30 s. Many individuals with AF have both symptomatic and asymptomatic episodes. The exclusion criteria were pregnant women, age ≤ 18, or patients who refused follow-up.

Data Collection

A systemic clinical evaluation for AF was conducted during the hospitalization when patients were enrolled. Overall, 122 variables were initially used for the selection of key features (**Supplementary Table 1**), which included medical histories, physical examinations, laboratory examination results, medications, comorbidities, ultrasonic cardiogram, CHA₂DS₂-VASc score, and HAS-BLED score. Follow-up by outpatient follow-up and/or telephone interview was carried out at 30, 180, and 365 days after discharge. The main outcome of the AF cohort was all-cause death.

This study complied with the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the First Affiliated Hospital of Shantou University Medical College. All participants provided written informed consent to participate in this study. All procedures were performed in conformity with the European Society of Cardiology guidelines (23).

Variable Selection and Model Development

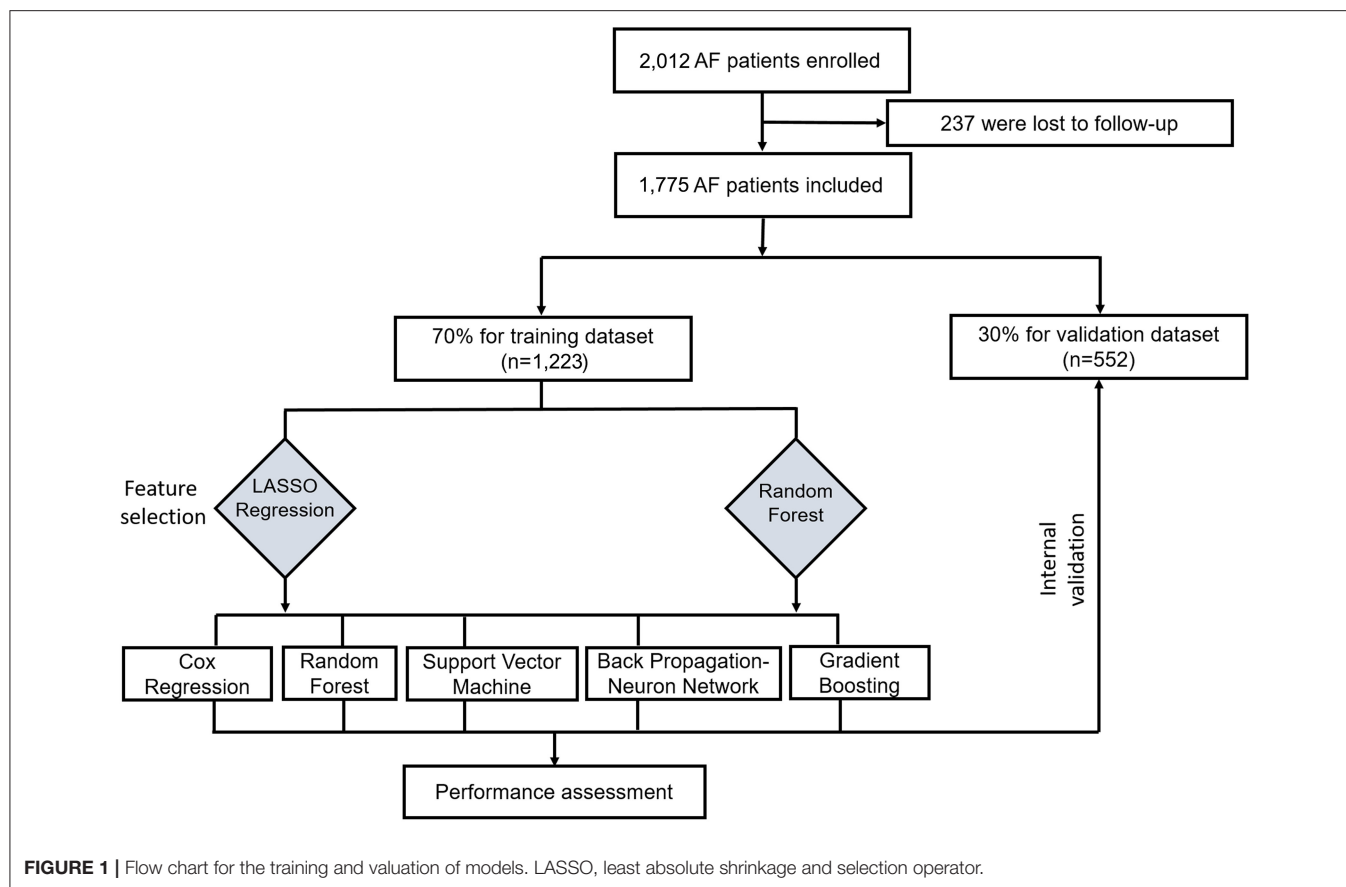
Due to the 122 variables present in the dataset, conducting variable selection was necessary and could lead to improved prediction performance. Both the LASSO algorithm (24) and RF (25) were used to select the features for model training. The top 20 predictor variables were chosen using RF based on relative variable importance (26).

We used five algorithms, including Cox regression, RF, support vector machines (SVM) (27), backpropagation neural networks (BP-NN) (28), and gradient boosting (GB) (29), to train models using the variables that were selected by LASSO and RF. Ultimately, 10 models, including LASSO-Cox, LASSO-RF, LASSO-SVM, LASSO-BP-NN, LASSO-GB, RF-Cox, RF-RF, RF-SVM, RF-BP-NN, and RF-GB, were established.

Statistical Analyses and Model Performance Measures

Statistical analyses were performed using SPSS 23.0 (Inc., Chicago, Illinois, USA), X-tile 3.6.1 (30), and R (version 4.0.2; R Foundation for Statistical Computing, Vienna, Austria) software. Continuous variables are presented as the mean ± standard derivation. We used multiple imputation to account for missing data on continuous variables if missing data was <30% (31). Missing values were imputed using the “mice” package. Categorical variables are presented as numbers and percentages. Statistical differences of continuous variables were examined by two-tailed *t*-tests or Mann-Whitney *U* tests. Categorical variables were analyzed by the chi-square test or Fisher exact test. Various R packages were used to conduct this study. The glmnet package was used for logistic regression with LASSO regularization (32). Random forest, e1071, neural net, and gbm packages were used for the RF, SVM, BP-NN, and GB models, respectively (29, 33).

The predictive accuracy of the LASSO-Cox model was compared with the performances of CHA₂DS₂-VASc and HAS-BLED scores. The performances of the models were assessed by the AUC derived from receiver operating characteristics curves. A nomogram for predicting the 30-, 180- or 360-day survival was established using the LASSO-Cox regression model, and the cut-off value for mortality risk stratification was calculated.



The nomogram and calibration plots were generated with the rms package. The pROC package was used to plot ROC curves. Kaplan-Meier curves were produced using the survival package. $P < 0.05$ was considered to indicate statistical significance.

RESULTS

Patient Baseline Demographics

This study was conducted according to the flow chart shown in **Figure 1**. Eligible study participants consisted of 1,775 AF patients. A total of 1,223 AF patients were randomly assigned in the training dataset and 552 patients in the validation dataset. Baseline characteristics of the study cohort are shown in **Table 1**. The mean age was 69.22 years (SD = 12.05 years) for the training dataset and 69.02 years (SD = 11.65 years) for the validation dataset. The mean CHA₂DS₂-VASc was 3.37 (SD = 1.18) in the training set and 3.19 (SD = 1.80) in the validation set. There were no significant differences in diabetes, atherosclerosis, prior stroke, heart failure, cerebral hemorrhage, cancer, renal insufficiency, bleeding, current smoker status, statin medication, and urine ketone bodies in the training set compared with the validation set. An all-cause mortality end point event occurred for 194 of the 1,775 patients (10.9%, 111 males and 83 females), 143 in the training set (11.7%) and 51 in the validation set

(9.2%). There was no significant difference in all-cause death rate between the training and validation set.

Feature Selection and Model Performance Comparison

LASSO coefficient profiles of the 122 variables and ten-fold cross-validation for tuning parameter selection in the LASSO model are shown in **Figure 2**. Thirteen variables were selected by the LASSO regression algorithm, including CHA₂DS₂-VASc, stroke, cancer, red cell volume distribution width-coefficient of variation (RDW-CV), statin medication use, lymphocyte ratio, neutrophil-to-lymphocyte ratio, basophilic granulocyte number, urine ketone body (KET), blood glucose (GLU), blood urea nitrogen (BUN), cholinesterase (CHE), and monoamine oxidase (MAO). In addition, the top-20 variables were selected by the RF algorithm (**Supplementary Table 2**). Next, we built 10 models using these two sets of selected features, and their prediction performances were described using AUC, sensitivity, and specificity (**Figure 3**). The key performance of machine learning was evaluated by AUC.

Among the 10 models, LASSO-BP-NN had the highest AUC (0.910, 95% CI: 0.875–0.944) in the training dataset, but a relatively low AUC (0.685, 95% CI: 0.613–0.756) in the validation dataset. The LASSO-Cox model, over the 1-year follow-up,

TABLE 1 | Baseline characteristics of the AF prospective cohort.

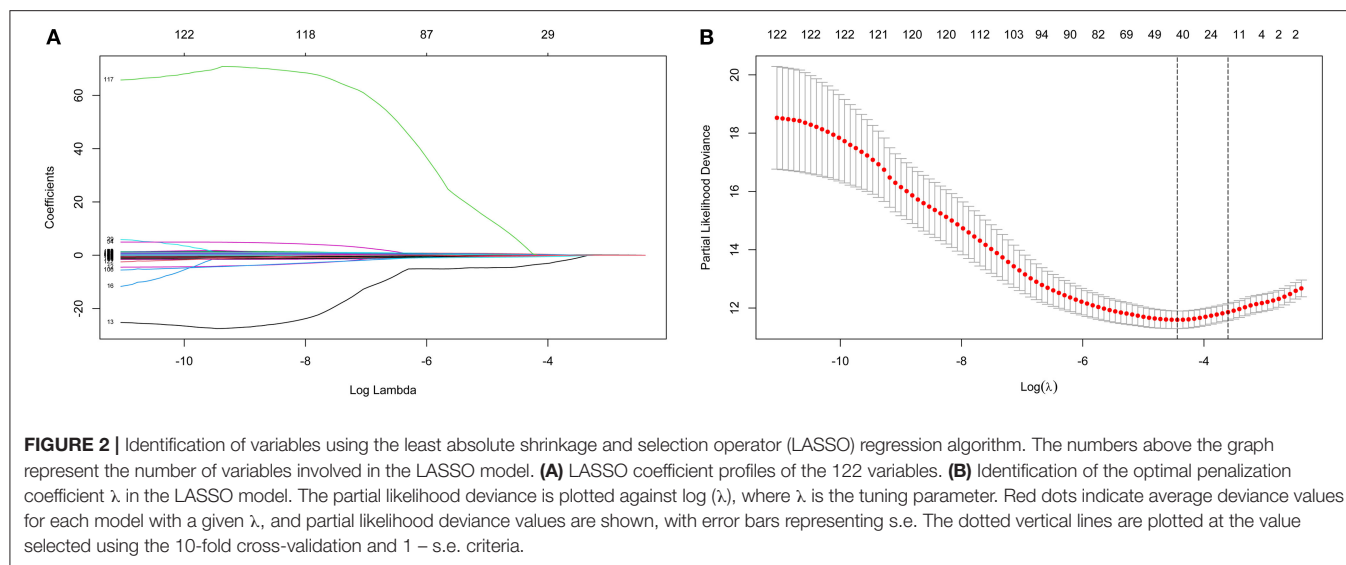
Variable	Training set (n = 1,223)	Validation set (n = 552)	P-value
Age (years)	69.22 ± 12.05	69.02 ± 11.65	0.749
Gender: male	682 (55.8)	334 (60.5)	0.062
Diabetes mellitus	324 (26.5)	163 (29.5)	0.184
Atherosclerosis	446 (36.5)	202 (36.6)	0.959
Hypertension	759 (62.1)	297 (53.8)	<0.001
Prior stroke	319 (26.1)	127 (23.0)	0.167
Cerebral hemorrhage	36 (2.9)	9 (1.6)	0.103
Heart failure	319 (26.1)	119 (21.6)	0.041
Cancer	80 (6.5)	30 (5.4)	0.371
Renal insufficiency	154 (12.6)	67 (12.1)	0.788
Bleeding	47 (3.8)	22 (4.0)	0.886
Current smoker status	347 (28.4)	150 (27.2)	0.603
Alcohol	87 (7.1)	31 (5.6)	0.241
Anticoagulant treatment	696 (56.9)	299 (54.2)	0.281
β-Blocker treatment	546 (44.6)	237 (42.9)	0.502
Digoxin treatment	398 (32.5)	192 (34.8)	0.354
Statin medication	486 (39.7)	230 (41.7)	0.443
WBC (10 ⁹ /L)	8.43 ± 3.71	8.30 ± 3.29	0.47
KET			0.463
0	1,093 (89.4)	497 (90.0)	
1	124 (10.1)	52 (9.4)	
2	6 (0.5)	2 (0.4)	
3	0 (0.0)	1 (0.2)	
Lymphocyte ratio	20.20 ± 10.65	20.59 ± 10.93	0.48
Creatinine (mmol/L)	119.05 ± 78.09	117.84 ± 73.22	0.758
RDW-CV (%)	14.53 ± 2.01	14.36 ± 1.79	0.962
Platelet (10 ⁹ /L)	205.18 ± 69.61	202.30 ± 73.27	0.426
Platelet distribution width (%)	15.62 ± 1.98	15.67 ± 1.93	0.648
GLU (mmol/L)	7.00 ± 3.65	6.76 ± 3.28	0.191
BUN (mmol/L)	7.77 ± 4.75	7.92 ± 5.17	0.542
CHE (U/L)	6.42 ± 1.96	6.41 ± 1.96	0.927
MAO (U/L)	4.89 ± 2.35	4.91 ± 2.68	0.862
Neutrophils/lymphocytes (%)	5.85 ± 6.61	5.96 ± 7.19	0.748
CHA ₂ DS ₂ -VASc score			0.51
0	59 (4.8)	33 (6.0)	
1	139 (11.4)	76 (13.8)	
2	198 (16.2)	99 (17.9)	
3	256 (20.9)	103 (18.7)	
4	250 (20.4)	110 (19.9)	
5	169 (13.8)	68 (12.3)	
6	100 (8.2)	46 (8.3)	
7	42 (3.4)	12 (2.2)	
8	10 (0.8)	5 (0.9)	

Data are represented as mean ± standard deviation or frequency (percentage). WBC, White blood cell; KET, Urine ketone body; RDW-CV, Red cell volume distribution width - coefficient of variation; GLU, Blood glucose; BUN, Blood urea nitrogen; CHE, Cholinesterase; MAO, Monoamine oxidase.

achieved an AUC of 0.842 (95% CI: 0.809–0.875) in the training dataset, and an AUC of 0.854 (95% CI: 0.807–0.901) in the validation dataset. Due to the very good performances in both the training set and validation set, the LASSO-Cox regression was chosen as the best model.

Nomogram Construction

Based on the Cox proportional hazards regression analysis, we identified eight independent risk factors in the training cohort. CHA₂DS₂-VASc (hazard ratio, HR = 1.188, *P* = 0.002), stroke (HR = 1.717, *P* = 0.008), cancer (HR = 2.208, *P* = 0.002),



statin medication use (HR = 0.341, $P < 0.001$), KET (HR = 1.730, $P = 0.006$), BUN (HR = 1.037, $P = 0.003$), CHE (HR = 0.889, $P = 0.032$), and MAO (HR = 1.133, $P < 0.001$) were all significantly associated with mortality in AF patients (**Supplementary Table 3**).

A nomogram based on the eight independent features from the training cohort was developed for the prediction of the 30-, 180-, and 365-day survival (**Figure 4**). The nomogram demonstrated that MAO contributes the most to survival, followed by CHE, KET, BUN, CHA₂DS₂-VASc, stroke, statin use, and cancer. The total score, obtained by adding the scores for each of the eight features, helped in estimating the 30-, 180-, and 365-day survival rate for each individual patient.

Validation and Calibration of the Nomogram

ROC curves were used to evaluate the predictive ability for 30-, 180-, and 365-day survival in both the training and validation sets. Our Cox model demonstrated good discriminative ability in both the training (30-day AUC: 0.848, 180-day AUC: 0.826, 365-day AUC: 0.762) and validation (30-day AUC: 0.834, 180-day AUC: 0.788, 365-day AUC: 0.841) datasets for the 30-, 180-, and 365-day survival rates (**Supplementary Figure 1**). The calibration plots of our nomogram also showed optimal agreement between the actual observations and the predicted outcomes both in the training set and validation set (**Supplementary Figure 2**) for all time points. Thus, the above nomogram-based results displayed good accuracy for predicting the 30-, 180-, and 365-day survival of AF patients.

Comparison of the Nomogram With CHA₂DS₂-VASc and HAS-BLED Models for Predictive Performance

The time-dependent ROCs of the training and validation sets (**Figure 5**) based on the nomogram were higher than those based

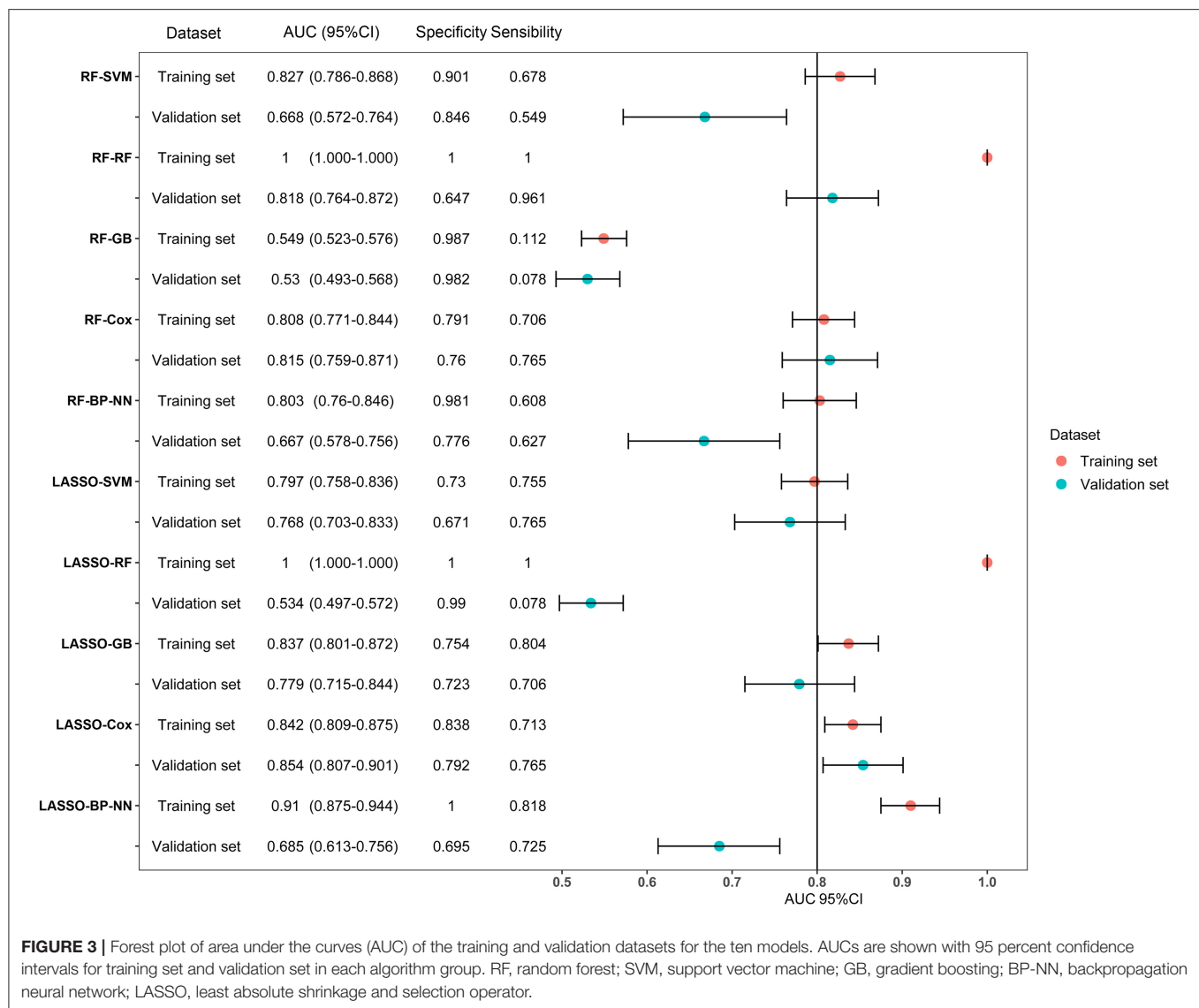
on the traditional CHA₂DS₂-VASc and HAS-BLED models. These results indicate that our nomogram has greater potential for accurately predicting prognosis compared to the traditional models. DCA was performed to compare the net benefit of the nomogram with that of the traditional CHA₂DS₂-VASc and HAS-BLED scores. Compared to the CHA₂DS₂-VASc and HAS-BLED scores, the curve of our nomogram showed larger net benefit (**Figure 6**). We further converted the nomogram to a web calculator for the clinician's convenience (<https://afnom.shinyapps.io/DynNomapp/>).

In addition, the optimal cut-off point was determined using the X-tile program to accomplish risk stratification. As shown in **Supplementary Figure 3**, the optimal cut-off point was 0.8. Thus, we stratified the AF patients into a low-risk group (≤ 0.8) and high-risk group (> 0.8). Kaplan–Meier curves showed that the high-risk group exhibited poorer survival than the low-risk group in both the training and validation sets (**Supplementary Figure 4**).

DISCUSSION

This study investigated a novel LASSO-Cox model for the prediction of all-cause mortality in patients with AF to identify AF patients at high risk and to provide personalized treatment using a data-driven approach. Several important findings were identified. First, eight independent risk factors predicted all-cause mortality, including CHA₂DS₂-VASc score, CHE, KET, BUN, MAO, stroke, statin medication use, and cancer. Second, a LASSO-Cox model for 30-, 180-, and 365-day risk prediction was established and validated. Third, the use of the nomogram and risk stratification enables the prediction of mortality for AF patients.

Machine learning can identify non-linear associations and identify interactions in complex and multidimensional variables. The use of the LASSO ML algorithm for variable selection is a well-established method that has been previously utilized for



cancer, heart failure, and AF populations (34–36). The advantages of the LASSO algorithm are high accuracy and stability. Cox proportional hazards regression is a traditional model, that is mainly used to analyze the prognosis of cancer and other chronic diseases. Indeed, our LASSO-Cox model was robust and displayed good discriminatory power in predicting all-cause mortality both in the training and the validation dataset.

There is growing evidence that AF significantly worsens the mortality rate (37–39). Furthermore, AF is an independent risk factor for higher risk of mortality (11). While worse outcomes among AF patients have been confirmed in various studies from Europe and North America, data from East Asia is limited.

Traditional guidelines in AF have focused on identifying patients with different risks of stroke and major bleeding. Several studies have developed and examined prediction models or risk scores in AF patients for stroke, major bleeding,

or composite outcomes, although not exclusively for death outcomes (19, 23, 40). Recently, a death risk score based on age, biomarkers, and clinical history (ABC) was developed and performed well in two large independent clinical trial cohorts (41). However, the detection of novel biomarkers such as GDF-15 are not easily performed in developing countries and regions.

In this LASSO-Cox model, not taking statins is an independent risk factor for AF-associated death. As recently reported, the levels of total cholesterol (TC) are non-linearly associated with all-cause mortality, as well as cancer and cardiovascular disease mortality, in the American population (42). Thus, it is necessary to maintain TC in a moderate range by statin medication. The GARFIELD-AF and ROCKET AF studies have shown that heart failure and sudden cardiac death are the major reasons for death of AF patients taking oral anticoagulant

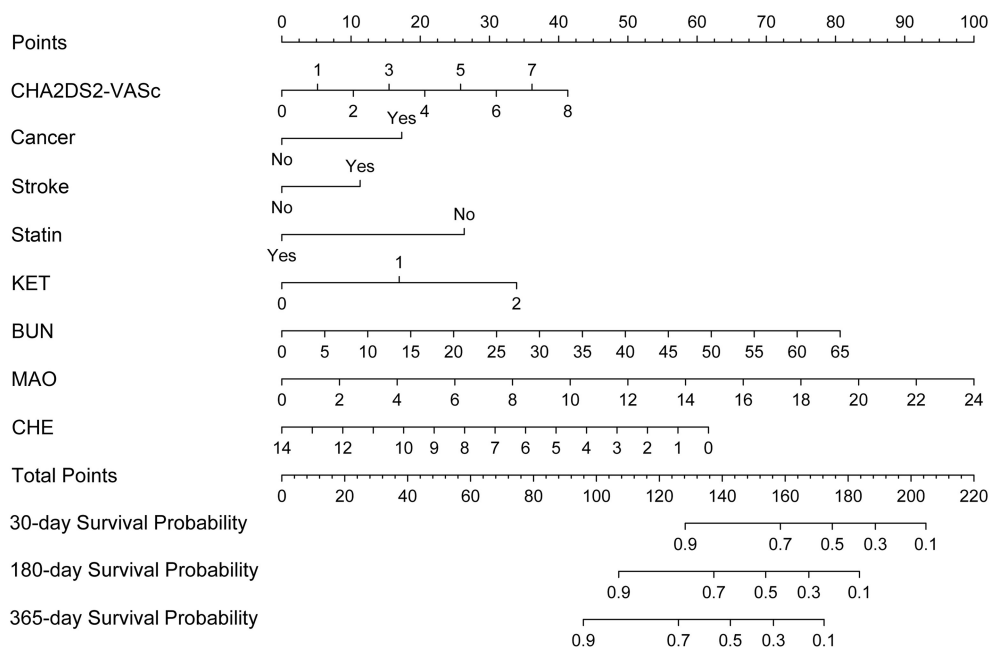


FIGURE 4 | Nomogram for predicting 30-, 180-, and 365-day survival probabilities for AF patients. To calculate patient survival probabilities, obtain points for each covariate value by dropping a vertical line from the points axis to the value of each covariate, calculate the total points obtained from all eight covariate values, and then drop a vertical line from the total points axis to locate the associated 30-, 180-, or 365-day survival probability. KET, urine ketone body; BUN, blood urea nitrogen; CHE, cholinesterase; MAO, monoamine oxidase.

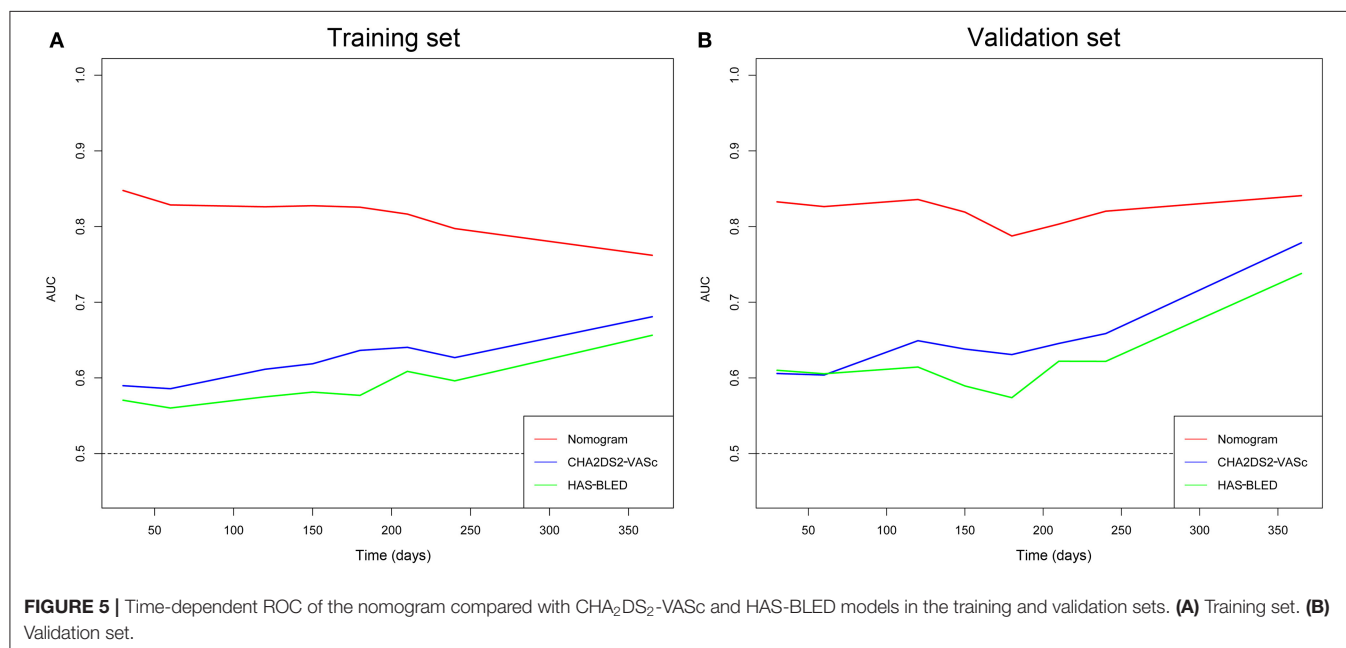


FIGURE 5 | Time-dependent ROC of the nomogram compared with CHA₂DS₂-VASc and HAS-BLED models in the training and validation sets. **(A)** Training set. **(B)** Validation set.

medication (38, 43). Death risk prediction in these patients may give rise to more intense management of risk factors, such as valvular heart disease, myocardial dysfunction, and coronary heart disease.

Among the independent risk factors of death, the four common laboratory examination indicators, including MAO, BUN, CHE, and KET, are strongly associated with mortality. Contemporary AF trials show that cardiac-related deaths account

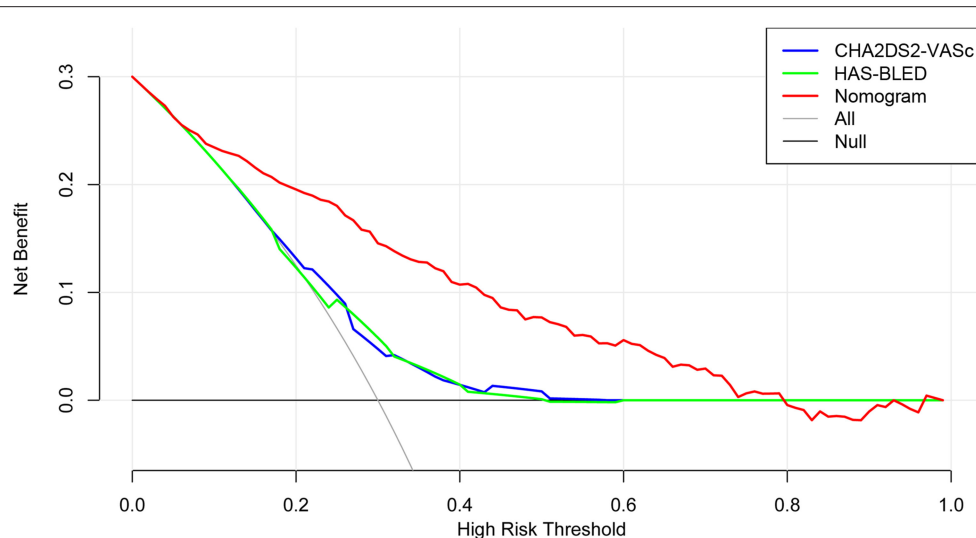


FIGURE 6 | Decision curve analysis of the nomogram, CHA₂DS₂-VASc score and HAS-BLED score. The y-axis represents the net benefit and the x-axis represents the threshold probability. The null plot represents the assumption that no patients survive, while the all plot represents the assumption that all patients survive at a specific threshold probability.

for the vast majority of all deaths, whereas stroke and bleeding represent a small fraction (44). In our study, MAO is recognized as the most important mortality risk factor in AF patients. Elevated MAO is known to be associated with liver cirrhosis and chronic congestive heart failure. Recent studies show that MAO is a major source of deleterious reactive oxygen species (ROS), regulating cardiomyocyte aging or death (45, 46). Myocardial ROS are involved in the pathophysiology of cardiovascular diseases such as hypertension and heart failure (47, 48), and are important markers of atrial fibrillation in patients after cardiac surgery (49). Thus, MAO inhibition therapy is protective in several settings of cardiac stresses such as pressure overload heart failure, diabetic cardiomyopathy and chronic ischemic heart disease (47). Further studies exploring the potential relationship between AF and ROS are needed.

Increased BUN levels are mainly triggered by impaired renal function, which might be highly related to the occurrence of ischemic stroke in AF patients despite adequate therapeutic warfarin anticoagulation (50). A Swedish study showed that neoplastic disease and renal failure contribute to the increased risk of all-cause mortality in AF patients, which is consistent with our result (11). Declination of cholinesterase is associated with the advanced liver cirrhosis, hepatic failure, and myocardial infarction. Inhibition of CHE has been reported to directly affect the intrinsic cardiac nervous system (51). In addition, increased levels of KET reflects the severity of diabetes, and AF patients with diabetes mellitus have a higher mortality rate (52–54). Collectively, the above risk factors suggest a renewed emphasis on the management of comorbidities such as liver cirrhosis, renal dysfunction, heart failure, and diabetes mellitus, is essential to improve the overall survival and quality of life in AF patients.

The nomogram could provide clinicians with the opportunity to assess risk of all-cause mortality by using a data-driven approach. An additional strength of the LASSO-Cox model is that the eight predictive factors in this nomogram are widely and easily available internationally. In order to facilitate medical use, the clinical implementation of the LASSO-Cox model can either be based on the nomogram, or preferably an online tool.

Limitations

Several limitations of this LASSO-Cox model should be considered. First, validation of this model was performed using a dataset generated from a single center. The performance of our LASSO-Cox model in external datasets needs to be tested by data from other institutions. Second, the LASSO-Cox model did not include information about biomarkers, such as NT-proBNP and hs-cTnT. However, considering that these biomarkers often require additional examination, thus increasing the difficulty of acquisition, our model has good accuracy and ease of application. Third, multiple imputation for the missing values is a potential source of bias. Nevertheless, multiple-imputation is a commonly used rigorous technique for imputation (55).

CONCLUSION

A new LASSO-Cox model for predicting risk of all-cause mortality in patients with AF was successfully developed, and internally validated. The LASSO-Cox model using CHA₂DS₂-VASc score, statin medication, medical history (stroke, cancer), and four clinical examination parameters (KET, BUN, MAO, and CHE), performed well and

may assist physicians in decision-making when treating AF patients.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

This study complied with the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the First Affiliated Hospital of Shantou University Medical College. All participants provided written informed consent to participate in this study. All procedures were performed in conformity with European society of cardiology guidelines. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

AUTHOR CONTRIBUTIONS

SW, YQC, and XT: concept and design, data analysis and interpretation, critical revision of article, and approval. YC, MW, ZX, XN, and BW: statistics, data analysis, and drafting of article. YC, SW, JY, CC, YQC, and RL: data collection, data analysis, critical revision of article, and approval. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcvm.2021.730453/full#supplementary-material>

Supplementary Figure 1 | Receiver operating characteristic (ROC) curves for 30-, 180-, and 365-day survival rates. **(A)** Training set. **(B)** Validation set.

Supplementary Figure 2 | Calibration curves for the nomogram in the training and validation sets. Y-axis represents the actual survival rate, while the X-axis represents the nomogram-predicted survival rate. The blue dotted line indicates perfect prediction by an ideal model. **(A–C)** 30-, 180-, and 365-day survival rates in the training set. **(D–F)** 30-, 180-, and 365-day survival rates in the validation set.

Supplementary Figure 3 | Determination of the cut-off score, for the mortality risk stratification, using the X-tile program. A cut-off score ≤ 0.8 indicates low-risk, and > 0.8 indicates high-risk.

Supplementary Figure 4 | Kaplan-Meier curves for the high-risk group and low-risk group in the training and validation sets. **(A)** Training set. **(B)** Validation set.

Supplementary Table 1 | The 122 variables and the missing rates collected in the dataset.

Supplementary Table 2 | Top-20 variables selected by the RF algorithm.

Supplementary Table 3 | Hazard ratios and 95% confidence intervals of 8 variables in the Cox proportional hazards model.

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Effectiveness and Safety of Cryoablation in Patients With Atrial Fibrillation Episodes of <24 h Duration: A Propensity-Matched Analysis

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Background: Paroxysmal atrial fibrillation (AF) is closely related to pathophysiologic processes and clinical outcomes. However, it is uncertain whether cryoablation of pulmonary veins isolation is effective and safe for patients with symptomatic and drug refractory AF episodes of <24-h duration.

Methods: The patients were designed into Group A (253 patients with paroxysmal AF episodes of <24-h duration) and Group B (253 patients with paroxysmal AF lasting for 24 h or longer) on a 1:1 basis by identical propensity scores. Mortality, stroke/transient ischemic attack (TIA), and complications relevant to the cryoablation procedure were compared, and recurrence of atrial tachyarrhythmia was analyzed for clinical independent predictors.

Results: The rate of atrial tachyarrhythmia recurrence was 21.74% in Group A and 30.04% in Group B, respectively ($P = 0.042$). At 12-month follow-up from the procedure, lower incidences of stroke/TIA endpoint of the patients were observed in Group A compared with Group B by Kaplan–Meier analysis [HR 0.34 (0.13–0.87), $P = 0.025$]. No significant differences in mortality and complications relevant to the cryoablation procedure were observed between Group A and Group B. Moreover, adjusted multivariable Cox regression analysis showed that < 24-h paroxysmal AF type (HR 0.644, 95% CI: 0.455–0.913, $P = 0.014$) and left atrium diameter (LAD) (>40 mm) (HR 1.696, 95% CI: 1.046–2.750, $P = 0.032$) were independently associated with the incidence of recurrence of atrial tachyarrhythmia in the study.

Conclusion: Our findings indicated that < 24-h paroxysmal AF type was obviously associated with an increased success rate of cryoablation and reduced incidence of stroke/TIA during the follow-up period. Therefore, there is superior effectiveness and similar safety in patients with AF episodes of <24-h duration compared with patients with longer paroxysmal AF duration.

Keywords: cryoablation, paroxysmal atrial fibrillation, pulmonary veins isolation, atrial fibrillation episodes of <24-h duration, recurrence of atrial tachyarrhythmia

INTRODUCTION

Atrial fibrillation (AF), the most common sustained arrhythmia in adults, is associated with an increased risk of morbidity and mortality in the general population (1). A very promising and successful interventional therapy for symptomatic and drug refractory AF is cryoablation of the left atrium (LA), which isolates pulmonary veins (PVs) targeting the initiating triggers inside the PVs in patients with no or minimal structural heart disease (2). Paroxysmal AF, which is defined as episode duration <7 days recommend by contemporary North American and European guidelines, may not reflect pathophysiologic processes or clinical outcomes after catheter ablation (3–5). AF episodes limited to <24 continuous hours are closely related to pathophysiologic processes and clinical outcomes (6).

However, no data to date are available about the effectiveness and safety of cryoablation in patients with AF episodes of <24-h duration. Therefore, the aims of the present study were to evaluate the effectiveness and safety of cryoablation in patients with AF episodes of <24-h duration after adjusting for the confounding variables by propensity-matched analysis.

METHODS

Study Population

A total of 1,265 consecutive patients presenting with successful cryoballoon-based ablation for symptomatic and drug refractory paroxysmal AF on admission were enrolled in this retrospective and observational study between January 2016 and December 2018 in the Department of Cardiology of Shanghai Tenth People's Hospital. Patients were excluded subsequently with cardiomyopathy, sick sinus syndrome or atrioventricular conduction disturbance, and valvulopathy. Similarly, patients were excluded with age <18 or >80 years, advanced malignancies, severe systemic infections, renal insufficiency, severely reduced liver function, rheumatic and immune disease, thyroid disease, and hematological disorders. Baseline characteristics of all patients were analyzed. Patients were split into the following groups: Group A (patients with paroxysmal AF episodes of <24-h duration) and Group B (patients with paroxysmal AF lasting for 24 h or longer). According to the ethical principles of the Declaration of Helsinki and contemporary North American and European guidelines, written informed consents were obtained from all the subjects (4, 5, 7). This study was granted an exemption from requiring ethics approval by the Ethics Committee of Shanghai Tenth People's Hospital because it was a retrospective observational study.

Cryoablation Procedure

In accordance with the North American and European guidelines for the management of patients with AF, transesophageal echocardiography was performed on all candidates to exclude the presence of thrombi before the cryoablation procedure (4, 5).

Cryoablation procedures were conducted in the patients following the attainment of consents. A steerable 12-F inner diameter sheath was advanced into the LA through interatrial

septum *via* once successful transseptal puncture from the right femoral vein. PV-to-cryoballoon occlusion was assessed with the help of a 50% diluted contrast medium injected into the PV and confirmed by retrograde radiopaque contrast agent retention using fluoroscopic guidance to position the second generation 28-mm cryoballoon catheter. PVs isolation was performed by freezing with a “single-shot” delivery of liquid refrigerant to the balloon, resulting in circumferential and transmural lesions around each pulmonary-vein antrum, and verified by the Achieve mapping catheter during and after each freezing procedure (8). Cryoablation time was 180 s for the isolation of the PVs with the LA (9, 10). In addition, time to isolation was observed by the loss of PV potentials and conduction block during the freezing procedure. In all patients, 30 min after the initial isolation, sustained PVs isolation was demonstrated successfully by the absence of any PV potentials or dissociated PV activity, which failed to induce >30 s rapid atrial arrhythmia by PV stimulation. Just in patients with positive atrial arrhythmia inducibility monitored by the Achieve catheter, additional freezing ablation time of a freezing cycle of 180 s was employed to eliminate residual potentials with a bidirectional conduction block as the endpoint. An activated clotting time of at least 300 s was measured every 30 min, and systemic anticoagulation was maintained by intravenous heparin routinely throughout the cryoablation procedure (11).

Oral antiarrhythmic drugs (AADs) and anticoagulation were routinely administrated and lasted for at least to 3 months according to the guidelines. Subsequently, administration of anticoagulation continued in patients with a ≥ 2 CHA₂DS₂-VASc score on the risk stratification of stroke. Medications were kept based on clinical status of the patients, including beta-blockers, angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs), diuretics, and statins.

Clinical Outcomes and Follow Up

All patients were examined at 1, 3, 6, and 12 months in the outpatient clinic after cryoballoon-based ablation procedure during the follow-up period. ECG recordings and 24-h Holter monitoring were done during every follow-up visit to detect arrhythmias.

The primary composite endpoints of this study after a blanking period of 90 days were as follows: (1) first recurrence of symptomatic or asymptomatic atrial tachyarrhythmia (AF, atrial flutter, or atrial tachycardia) lasting for 30 s or longer by 12-lead ECG or 24-h Holter monitoring in the absence of AADs therapy; (2) appropriate treatment relevant to recurrence of atrial tachyarrhythmia, of AADs prescription (class I or III), or repeat ablation procedure. The major adverse cardiovascular events (MACEs) were defined as secondary endpoints were as follows: all-cause death, cardiac death, stroke, or transient ischemic attack (TIA) from any cause, and complications related to the therapeutic intervention, including phrenic nerve injury (PNI), pericardial tamponade, PV stenosis (PVS), pulmonary embolism (PE), and deep vein thrombosis (DVT).

Statistical Analysis

A non-parsimonious logistic regression model, namely propensity scores matching, had its confounders adjusted for the likelihood of demographic and clinical variables of each patient enrolled, including age, sex, body mass index, smoking, hypertension, diabetes, previous coronary artery disease (CAD), previous heart failure, history of coronary revascularization, the use of ACEIs, anticoagulants and beta-blockers, left atrium diameter (LAD), left ventricular ejection fraction (LVEF), and CHA₂DS₂-VASc score. The patients, from groups of paroxysmal AF episodes of <24-h duration and lasting for 24 h or longer, were matched on a 1:1 basis by using identical propensity scores with a 0.01 standardized caliper width.

All data were analyzed statistically by use of SPSS 26.0. Continuous variables were compared by Student's *t*-test or the Mann-Whitney *U* test appropriately. The differences between categorical values were assessed by the Chi-squared test as appropriate. The survival curves of composite end events were plotted using the Kaplan-Meier method, and significances were explored statistically using the log-rank test. A Cox regression analysis was done to identify the factors associated with the composite endpoint. A *p*-value of <0.05 was considered significant statistically.

RESULTS

Baseline Characteristics for Subjects

A total of 1,265 consecutive patients were recruited in the study, including 255 patients with paroxysmal AF episodes of <24-h duration (Group A) and 1,010 patients with paroxysmal AF lasting for 24 h or longer (Group B). Crude baseline clinical characteristics are shown in **Table 1**. All patients received cryoablation in the study. Among them, patients with paroxysmal AF episodes of <24-h duration were more likely to have younger age (57.50 ± 10.17 vs. 60.39 ± 9.44 years; $P < 0.001$), lower BMI (25.48 ± 3.13 vs. 26.05 ± 2.86 kg/m²; $P = 0.002$), a smaller LAD (36.21 ± 4.29 vs. 37.72 ± 4.27 mm; $P < 0.001$), a larger LVEF (55.45 ± 2.22 vs. 54.81 ± 3.25 %; $P = 0.003$), and receiving medication of ACEI/ARB (37.25 vs. 26.53%; $P = 0.001$) as compared with patients with paroxysmal AF lasting for 24 h or longer. Overall, 697/1,265 (55.10%) non-propensity-matched patients were on beta-blocker drugs: 122/265 (47.84%) patients of Group A compared to 575/1,100 (56.93%) patients of Group B ($P = 0.01$). Of the whole population, 253/255 (99.22%) cryoablation patients of Group A were matched with 253/1,100 (23.00%) individuals in Group B on a 1:1 basis using identical propensity scores. The baseline characteristics for propensity-matched patients with paroxysmal AF are shown in **Table 2** after an adjustment for 15 confounding factors.

Follow-Up Analysis

Major Adverse Cardiovascular Events

In **Table 3**, MACEs in patients of Group A are compared with those of propensity-matched patients of Group B during the follow-up period. In the Group A and Group B, the total mortality rates were 0.4 and 0.4% ($P > 0.99$), and cardiovascular

TABLE 1 | Baseline characteristics for non-propensity-matched patients with paroxysmal AF.

Characteristics	Group A (<i>n</i> = 255)	Group B (<i>n</i> = 1,010)	<i>P</i>
Age, years	57.50 ± 10.17	60.39 ± 9.44	<0.001
Male, <i>n</i> (%)	129 (50.59)	463 (45.84)	0.18
BMI, kg/m ²	25.48 ± 3.13	26.05 ± 2.86	0.002
Smoking, <i>n</i> (%)	109 (42.75)	484 (47.92)	0.14
High blood pressure, <i>n</i> (%)	190 (74.51)	718 (71.09)	0.31
Diabetes, <i>n</i> (%)	60 (23.53)	212 (20.99)	0.39
Prior CAD, <i>n</i> (%)	74 (29.02)	308 (30.50)	0.65
Previous heart failure, <i>n</i> (%)	15 (5.88)	89 (8.81)	0.16
History of coronary revascularization, <i>n</i> (%)	43 (16.86)	199 (19.70)	0.33
LAD, mm	36.21 ± 4.29	37.72 ± 4.27	<0.001
LVEF, %	55.45 ± 2.22	54.81 ± 3.25	0.003
Medications			
ACEIs or ARBs, <i>n</i> (%)	95 (37.25)	268 (26.53)	0.001
Beta-blockers, <i>n</i> (%)	122 (47.84)	575 (56.93)	0.01
Anticoagulants, <i>n</i> (%)	111 (43.53)	474 (46.93)	0.36
Mean CHA ₂ DS ₂ -VASc score	2.10 ± 1.04	2.19 ± 1.05	0.24

BMI, body mass index; CAD, coronary artery disease; LAD, left atrium diameter; LVEF, left ventricular ejection fraction; ACEIs, angiotensin converting enzyme inhibitors; ARBs, angiotensin receptor blockers.

TABLE 2 | Baseline characteristics for propensity-matched patients with paroxysmal AF.

Characteristics	Group A (<i>n</i> = 253)	Group B (<i>n</i> = 253)	<i>P</i>
Age, years	57.57 ± 10.18	57.47 ± 10.10	0.91
Male, <i>n</i> (%)	127 (50.20)	124 (49.01)	0.86
BMI, kg/m ²	25.51 ± 3.12	25.48 ± 3.17	0.92
Smoking, <i>n</i> (%)	109 (43.08)	106 (41.90)	0.86
High blood pressure, <i>n</i> (%)	188 (74.30)	186 (73.52)	0.92
Diabetes, <i>n</i> (%)	58 (22.92)	56 (22.13)	0.92
Prior CAD, <i>n</i> (%)	73 (28.85)	67 (26.48)	0.62
Previous heart failure, <i>n</i> (%)	15 (5.93)	18 (7.11)	0.72
History of coronary revascularization, <i>n</i> (%)	43 (17.00)	39 (15.42)	0.72
LAD, mm	36.24 ± 4.29	36.33 ± 4.30	0.82
LVEF, %	55.43 ± 2.22	55.40 ± 2.30	0.84
Medications			
ACEIs or ARBs, <i>n</i> (%)	95 (37.55)	97 (38.33)	0.93
Beta-blockers, <i>n</i> (%)	120 (47.43)	119 (47.04)	>0.99
Anticoagulants, <i>n</i> (%)	110 (43.48)	106 (41.90)	0.79
Mean CHA ₂ DS ₂ -VASc score	2.10 ± 1.04	2.07 ± 1.01	0.76

BMI, body mass index; CAD, coronary artery disease; LAD, left atrium diameter; LVEF, left ventricular ejection fraction; ACEIs, angiotensin converting enzyme inhibitors; ARBs, angiotensin receptor blockers.

death rates were 0.4% and 0 ($P > 0.99$), respectively. Kaplan-Meier survival analysis indicated that the Group A patients had no significant difference of a cumulative incidence of total

mortality compared with the Group B patients [HR 0.99 (0.06–15.83), $P > 0.99$] (**Figure 1A**).

After a follow-up period, stroke/TIA was observed in 1.58% of patients of Group A and 5.14% of Group B ($P = 0.045$) (**Table 3**).

TABLE 3 | Mortality and adverse events in propensity-matched patients during the follow-up.

Parameters	Group A (n = 253)	Group B (n = 253)	P
Mortality			
Cardiovascular death, n (%)	1 (0.40%)	0	>0.99
All-cause death, n (%)	1 (0.40%)	1 (0.40%)	>0.99
Stroke or TIA, n (%)	4 (1.58%)	13 (5.14%)	0.045
Complications related to cryoablation procedure	9 (3.56%)	13 (5.14%)	0.51
PNI, n (%)	7 (2.77%)	9 (3.56%)	0.80
Pericardial tamponade, n (%)	1 (0.40%)	2 (0.79%)	>0.99
PVS, n (%)	1 (0.40%)	0	>0.99
PE, n (%)	0	1 (0.40%)	>0.99
DVT, n (%)	0	1 (0.40%)	>0.99

TIA, transient ischemic attack; PNI, phrenic nerve injury; PVS, pulmonary vein stenosis; PE, pulmonary embolism; DVT, deep vein thrombosis.

In **Figure 1B**, Kaplan–Meier curves show lower incidences of the stroke/TIA endpoint of the patients in Group A than Group B [HR 0.34 (0.13–0.87), $P = 0.025$].

All patients received systemic screening for complications related to the therapeutic intervention, which was subsequently observed in nine patients of Group A and 13 patients of Group B (3.56 vs. 5.14%; $P = 0.51$) (**Table 3**). No significant differences about procedure-related complications, including PNI, pericardial tamponade, PVS, PE, and DVT, were noted between patients from Group A and Group B receiving cryoablation in the Kaplan–Meier survival analysis [HR 0.66 (0.27–1.59), $P = 0.36$] (**Figure 1C**). PNI occurred in seven (2.77%) patients of Group A and nine (3.56%) patients of Group B in the study population ($P = 0.80$) (**Table 3**). After a 2-month follow-up, the sniff test showed a total recovery of diaphragmatic palsy in six out of seven (85.71%) patients of Group A and seven out of nine (77.78%) patients of Group B, respectively, and only still mild dyspnea in residual three patients.

Recurrence of Atrial Tachyarrhythmia

The recurrence of atrial tachyarrhythmia data was documented from all patients. Kaplan–Meier analysis reveals that lower recurrences of atrial tachyarrhythmia were observed in Group A

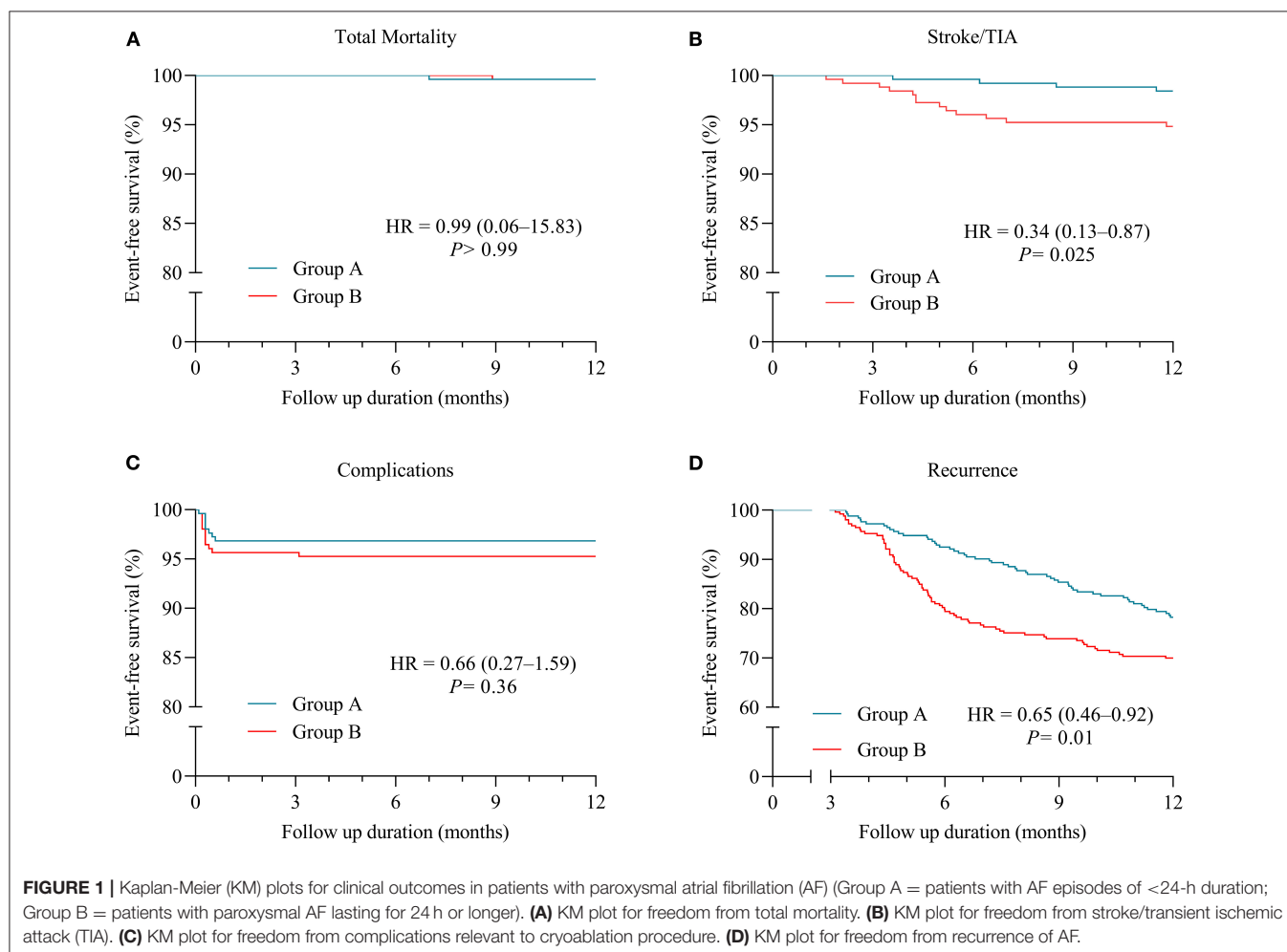


TABLE 4 | Recurrence of atrial tachyarrhythmia in propensity-matched patients during the follow-up.

Parameters	Group A (n = 253)	Group B (n = 253)	P
Atrial tachyarrhythmia, n (%)	55 (21.74)	76 (30.04)	0.042
Atrial tachycardia, n (%)	19 (7.51)	35 (13.83)	0.03
Atrial flutter, n (%)	18 (7.11)	20 (7.91)	0.87
AF, n (%)	18 (7.11)	21 (8.30)	0.74
Early recurrence (4–9 months)	43 (17.00)	71 (28.06)	0.003
Late recurrence (10–12 months)	12 (4.74)	5 (1.98)	0.08
Appropriate treatments			
AADs prescription, n (%)	11 (4.35)	18 (7.11)	0.25
Direct-current cardioversion, n (%)	8 (3.16)	11 (4.35)	0.64
Repeat ablation, n (%)	35 (13.83)	47 (18.58)	0.18

AF, atrial fibrillation; AADs, antiarrhythmic drugs.

when compared with Group B [HR 0.65 (0.46–0.92), $P = 0.01$] (Figure 1D). In Table 4, 55 (21.74%) patients in Group A and 76 (30.04%) patients in Group B present with total recurrence of atrial tachyarrhythmia ($P = 0.042$).

Subgroup analysis is summarized in Table 4 based on atrial tachyarrhythmia types. Ablation patients with <24 h AF had markedly reduced risk of atrial tachyarrhythmia recurrence compared with those with paroxysmal AF episodes sustained more than 24 h (7.51 vs. 13.83%; $P = 0.03$). In patients with AF, the rate of recurrence showed no significant difference between Group A and Group B (7.11 vs. 7.91%; $P = 0.87$, 7.11 vs. 8.30; $P = 0.74$). Lower incidence of early AF recurrence in patients was documented in Group A than Group B (17.00 vs. 28.06%; $P = 0.003$), whereas a similar effect on late AF recurrence for patients was observed from Group A and Group B (4.74 vs. 1.98%; $P = 0.08$). Similarly, appropriate treatments for atrial tachyarrhythmia, including AADs prescription, direct-current cardioversion, and repeat ablation, were statistically similar between the two groups.

Predictors of Recurrence of Atrial Tachyarrhythmia

As seen in Table 5, the univariate analysis of the propensity-matched patients shows the incidence of recurrence of atrial tachyarrhythmia during the follow-up period. However, multivariable Cox regression analysis indicated that <24-h paroxysmal AF type (HR 0.644, 95% CI: 0.455–0.913, $P = 0.014$) and LA diameter (>40 mm) (HR 1.696, 95% CI: 1.046–2.750, $P = 0.032$) were independently related to the incidence of recurrence of atrial tachyarrhythmia in the study patients after controlling for confounding factors, including a history of diabetes, prior CAD, and LVEF.

DISCUSSION

Main Findings

The main findings of the present study are listed in the following: (1) patients with AF episodes of <24-h duration had lower risks of stroke/TIA and recurrence of atrial tachyarrhythmia than those who had paroxysmal AF lasting for 24 h or longer

after a blanking period of 3 months following cryoablation; (2) no significant differences, including total death rate from any cause and complications related to cryoablation procedure, were observed between patients with AF episodes limited to <24 h and those with paroxysmal AF episodes sustained more than 24 h; (3) both <24-h paroxysmal AF type and LA diameter (>40 mm) were independently related to the incidence of recurrence of atrial tachyarrhythmia. The propensity matching analysis was carried out to adjust for confounders of demographic and clinical variables in the evaluation of outcomes of patients. Taken together, these findings confirm the superior effectiveness of cryoablation for patients with AF episodes of <24-h duration compared with patients with longer paroxysmal AF duration *via* a propensity-matched analysis. Furthermore, the safety of cryoablation is similar between the groups.

Prior Studies

The classification of paroxysmal AF, with episode duration <7 days (3), may not reflect the pathophysiologic process underlying AF or perioperative complications with AF (6). A prior observational and randomized study reported that class IC AADs, such as propafenone and flecainide, were more efficient for the acute conversion of paroxysmal AF or flutter episodes of <24-h duration to sinus rhythm (12). On a secondary analysis of a randomized clinical trial, patients with AF episodes limited to <24 continuous hours had a significantly lower incidence of arrhythmia recurrence following cryoballoon or irrigated radiofrequency catheter ablation than those with AF lasting for 24 h or longer (6). Electrical and structural remodeling changes of the atrium, which occurred in 24 h and achieved a steady-state as early as 48 h after the onset of an AF episode, played an important role in increasing atrial vulnerability to AF induction and duration (13–15). This parallels the observational findings of substantial decreases in the conversion of AF to sinus rhythm after 24 continuous hours of AF onset.

Recurrence of Atrial Tachyarrhythmia

As previously reported, AADs therapy for patients with AF had obviously high symptomatic AF recurrence compared with cryoablation (2). Cryoablation of AF, aiming for circumferential lesions and durable electrical isolation of the PVs, was non-inferior to radiofrequency-based ablation in terms of efficacy and safety for the treatment of patients with symptomatic paroxysmal AF, for whom at least one antiarrhythmic drug had failed (16, 17). However, in the FIRE and ICE study, a significant proportion of patients underwent first-generation cryoballoon ablation. Additionally, the second-generation cryoballoon ablation, facilitated shorter time to PVs isolation procedure and enhanced higher rates of freedom from AF, was considered as a reasonable and promising choice for patients suffering from paroxysmal AF (9, 18). The STOP AF trial demonstrated that cryoablation with the second-generation cryoballoon is an effective alternative to antiarrhythmic treatment of patients with symptomatic and drug-refractory AF (2). Likewise, a large, prospective, randomized, and controlled study showed comparable efficacy of open irrigated radiofrequency and cryoballoon catheter ablation for PVs isolation in patients with

TABLE 5 | Cox regression analysis for predictors of recurrence of atrial tachyarrhythmia in propensity-matched patients.

Variables	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	P	Hazard ratio	95% CI	P
AF type (<24 h)	0.656	0.463–0.928	0.017	0.644	0.455–0.913	0.014
Diabetes	0.697	0.445–1.094	0.117	0.846	0.507–1.408	0.518
Prior CAD	0.689	0.454–1.047	0.081	0.735	0.459–1.177	0.200
LAD (>40 mm)	1.956	1.265–3.025	0.003	1.696	1.046–2.750	0.032
LVEF (\leq 45%)	3.725	1.523–9.108	0.004	2.554	0.951–6.859	0.063

AF, atrial fibrillation; CAD, coronary artery disease; LAD, left atrium diameter; LVEF, left ventricular ejection fraction.

paroxysmal AF (19). As in our study, the ablation technique was used with the second-generation cryoballoon.

Structural remodeling of the atrium due to myolysis, cardiomyocyte apoptosis, and the activation of fibrotic pathways *via* fibroblasts, is closely related to the pathophysiologic process underlying atrial arrhythmia (20). Also, AF-related electrical and structural remodeling began as early as 24 h after the occurrence of AF (13–15). Evolving evidence showed that earlier catheter ablation may be a useful strategy in AF rhythm-reversion and maintenance of sinus rhythm, thus improving the prognosis of AF (21). The CIRCA-DOSE study about the early ablation of AF, with lesser recurrences of AF and lower post-ablation AF burdens compared with ablation of AF longer than 24 h, led to the notion that early cryoablation of AF was a beneficial tool in preventing AF development (6). However, these previous studies had not adequately assessed the effects of AF episodes of <24-h duration on the recurrence of atrial tachyarrhythmia after cryoablation. In our study, only a 24-h time point was selected to assess the impact of AF episodes duration on clinical outcomes and prognosis after cryoablation. Two different ablation types, including RF ablation and cryoablation, mixed together to perform secondary analysis of the CIRCA-DOSE study, may lead to information bias and selection bias (6).

In the current study, we confirmed that cryoballoon catheter ablation, an ablation technique for PVs isolation in patients with paroxysmal AF, had lower AF recurrences after a blanking period following cryoablation in consistent with prior studies. Furthermore, the present study showed evidence that cryoablation for paroxysmal AF lasting for 24 h or longer conferred a significantly greater recurrence of atrial tachyarrhythmia after ablation as compared with cryoablation for <24-h AF, especially in 4–9 months follow-up period. Early AF recurrence was negatively correlated with extensive PV reconnections after the blanking period (22). This observation is in consistent with the idea that early intervention, including radiofrequency and cryoballoon catheter ablation for PVs isolation, for paroxysmal AF episodes of <24-h duration, is acknowledged as a cutoff time point of the onset of the progressive anatomic and electrical changes related with atrial arrhythmia, may improve clinical prognosis.

Adverse Clinical Events

AF cryoablation offers several potential advantages over AADs therapy for AF, such as arrhythmia freedom and safety

outcomes (2). The FIRE and ICE trial demonstrated that there was no significant difference between the cryoballoon and radiofrequency ablation for paroxysmal AF with regard to overall safety (17). Cryoballoon ablation seems to be associated with a significantly lower risk of a serious complication in comparison to RF ablation, such as pericardial effusion and tamponade (23). Successful catheter ablation of AF reduced the risk of the total vascular events in patients with AF with CHA₂DS₂-VASc score \geq 1, including stroke/TIA (24). A previous study showed that phrenic nerve palsies were more frequent in patients with AF by second-generation cryoballoon ablation than patients with AF by RF ablation, and recovered in all patients during the follow-up period (18). The overall incidence of atrial esophageal fistula remains rare in both RF and cryoballoon ablation procedures (23).

The CIRCA-DOSE study had not assessed the effects of AF recurrence after ablation on the clinical outcome of stroke/TIA (6). Higher incidence of stroke/TIA was investigated in patients with paroxysmal AF lasting for 24 h or longer compared with patients with AF episodes of <24-h duration in our study after cryoablation. AF recurrence is an independent risk factor for stroke/TIA. The possible explanations may be AF recurrence and delayed oral anticoagulant drugs administration after cryoablation in patients with paroxysmal AF lasting for 24 h or longer. Successful cryoablation reduced the risk of stroke/TIA in patients with AF episodes of <24-h duration. Likewise, in a previous study, the risk of ischemic stroke has been observed to increase substantially among patients with AF lasting for 24 h or longer (25, 26). High incidence of stroke/TIA was investigated in patients with AF lasting for 24 h or longer, which led to the notion that AF with >24 h was a highly relevant threshold for oral anticoagulation initiation.

The safety of early cryoablation was not evaluated in the CIRCA-DOSE study previously (6). In the current study, no significant difference in peri-procedural complications was observed between the groups. A procedural complication, PNI, was induced and maintained due to freezing-induced large axonal loss of phrenic nerve during the cryoablation step. However, the incidence rate of PNI was low in the whole patient, mostly recovered from diaphragmatic palsy during the follow-up period. Moreover, the risk for MACE, including all-cause death and cardiac death, was comparative in patients with AF episodes of <24-h duration with that in patients with paroxysmal AF lasting for 24 h or longer during the follow-up period. In general,

our study revealed that cryoablation for treating paroxysmal AF episodes of <24-h duration is a safe tool for the first-line treatment of symptomatic AF.

LIMITATIONS

There were several potential limitations to this study. First, it was a single-center and retrospective study, which was not designed in advance before the cardiac events occurred. Further prospective and randomized trials are required to confirm whether cryoablation for treating AF episodes of <24-h duration is effective and safe as compared with paroxysmal AF lasting for 24 h or longer. Second, only 253 enrolled patients were matched on a 1:1 basis by using identical propensity scores in this study. Therefore, there may be some information bias. Third, as the death rate is low in these cryoablation patients of our study, a further study with much larger sample size and long-term follow-up is necessary to get more exact data information about the mortal difference of patients with AF. Fourth, asymptomatic or short-lasting AF episodes may occur unnoticed by symptoms of the patients, ECG, and scheduled 24-h Holter monitoring. Therefore, there may be some overestimated effectiveness of the results. Finally, a potential mechanism of the occurrence and maintenance of AF, fibrosis of atrial myocytes, was not analyzed among the whole population in this study.

CONCLUSION

In conclusion, patients with <24-h AF episodes are at significantly reduced risks of stroke/TIA and recurrence of atrial

tachyarrhythmia when compared to patients with paroxysmal AF lasting for 24 h or longer. In this study, <24-h paroxysmal AF type and >40 mm LA diameter were independently related to the incidence of recurrence of atrial tachyarrhythmia.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

This study was granted an exemption from requiring ethics approval by Ethics Committee of Shanghai Tenth People's Hospital because it was a retrospective observational study. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CJ conceived and designed this study, performed cryoablation procedures, analyzed the data, and wrote the manuscript. DZ and KT carried out subjects recruitment and data interpretation and performed cryoablation procedures. YW, XL, and PJ contributed to data collection and manuscript revision. YX and BH contributed to the supervision of this study. All authors contributed to the article and approved the submitted version.

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Fragmented QRS Is Independently Predictive of Long-Term Adverse Clinical Outcomes in Asian Patients Hospitalized for Heart Failure: A Retrospective Cohort Study

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Background: Fragmented QRS (fQRS) results from myocardial scarring and predicts cardiovascular mortality and ventricular arrhythmia (VA). We evaluated the prevalence and prognostic value of fQRS in Asian patients hospitalized for heart failure.

Methods and Results: This was a retrospective cohort study of adult patients hospitalized for heart failure between 1st January 2010 and 31st December 2016 at a tertiary center in Hong Kong. The baseline ECG was analyzed. QRS complexes (<120 ms) with fragmented morphology in ≥ 2 contiguous leads were defined as fQRS. The primary outcome was a composite of cardiovascular mortality, VA, and sudden cardiac death (SCD). The secondary outcomes were the components of the primary outcome, myocardial infarction, and new-onset atrial fibrillation. In total, 2,182 patients were included, of whom 179 (8.20%) had fQRS. The follow-up duration was 5.63 ± 4.09 years. fQRS in any leads was associated with a higher risk of the primary outcome (adjusted hazard ratio (HR) 1.428 [1.097, 1.859], $p = 0.001$), but not myocardial infarction or new-onset atrial fibrillation. fQRS in > 2 contiguous leads was an independent predictor of SCD (HR 2.679 [1.252, 5.729], $p = 0.011$). In patients without ischaemic heart disease ($N = 1,396$), fQRS in any leads remained predictive of VA and SCD (adjusted HR 3.526 [1.399, 8.887], $p = 0.008$, and 1.873 [1.103, 3.181], $p = 0.020$, respectively), but not cardiovascular mortality (adjusted HR 1.064 [0.671, 1.686], $p = 0.792$).

Conclusion: fQRS is an independent predictor of cardiovascular mortality, VA, and SCD. Higher fQRS burden increased SCD risk. The implications of fQRS in heart failure patients without ischaemic heart disease require further studies.

Keywords: fragmented QRS, heart failure, Asian, ventricular arrhythmia, sudden cardiac death, myocardial fibrosis

INTRODUCTION

First described by Boineau and Cox in 1973, fragmented QRS (fQRS) is the manifestation of myocardial scarring on 12-lead surface electrocardiogram (ECG) (1, 2). Though initially described in the context of ischaemic heart disease (IHD), fQRS has been observed in other conditions where myocardial scarring or fibrosis is present, such as hypertrophic cardiomyopathy and cardiac sarcoidosis (2, 3). The presence of fQRS has been shown to be predictive of all-cause mortality and ventricular arrhythmia (VA) (4), and several morphological criteria and classification systems have been set forth by a number of research groups, including those by Das et al. and Haukilahti et al. (2, 5). While fQRS has been shown to be predictive of adverse cardiovascular outcomes in patients with heart failure (4), most studies have focused on either acute outcomes of hospitalized patients, or long-term outcomes of ambulatory patients (6, 7). With the rising prevalence of heart failure in Asia (8, 9), there is an ever greater need for good prognostic markers in Asian patients with heart failure. As data on the prevalence and long-term prognostic power of fQRS in Asian patients hospitalized with heart failure are lacking, we aimed to bridge this gap in evidence with the current study.

MATERIALS AND METHODS

This was a retrospective cohort study approved by The Joint Chinese University of Hong Kong—New Territories East Cluster Clinical Research Ethics Committee. All patients aged ≥ 18 years old who were hospitalized for heart failure, as identified using relevant ICD-9 codes, between 1st January 2010 and 31st December 2016 at a single tertiary center in Hong Kong were included. Patients who had wide QRS (≥ 120 ms) on the first ECG recorded during index hospitalization, missing primary outcome data, and those who did not have any ECG done during the index hospitalization were excluded. The patients were identified using the Clinical Data Analysis and Reporting System (CDARS), a territory-wide database that centralizes patient information from local hospitals and ambulatory and outpatient facilities. Mortality data were obtained from the Hong Kong Death Registry, a government registry with the registered death records of all Hong Kong citizens linked to CDARS.

Patient demographics, prior comorbidities (as identified by ICD-9 codes [Supplementary Table 1; anemia was additionally identified by a baseline hemoglobin level <13 g/dL (males) or <12 g/dL (females)], and baseline medication usage were extracted. The baseline Charlson comorbidity index was calculated to reflect comorbid statuses. The baseline ECG obtained on the first heart failure admission was selected for automated analysis. The paper speed of all ECG performed in public institutions in Hong Kong has been standardized to 25 mm/s, which therefore applies to all ECG analyzed in this study. Automated measurements were performed on digital ECG tracings using the Philips ECGVue program (Standard Edition), with the ECG waveform data captured at a sample rate of 4 MHz and reduced to 500 samples per second with 5 μ V resolution. We defined fQRS using the criteria set forth by Das et al., where fQRS

was defined as the presence of an additional R wave (R'), notching in the nadir of the S wave, or presence of more than two R' waves in at least two contiguous leads within any myocardial territory (anterior, inferior, or lateral) with QRS duration of <120 ms (5). QRS complexes with fragmented morphology in a single lead were not classified as fQRS.

The primary outcome was a composite of cardiovascular mortality, VA, and SCD. The secondary outcomes were the individual components of the primary composite outcome, myocardial infarction (MI), and new-onset atrial fibrillation (AF). All patients were followed up till 31st December 2019. All event occurrences were identified using ICD-9 codes.

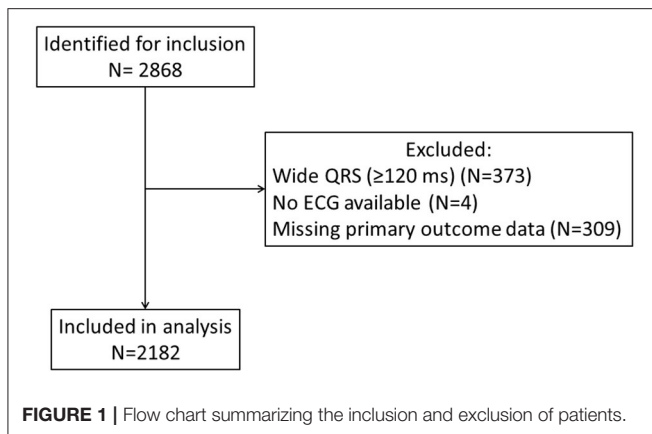
All continuous variables were expressed as mean \pm standard deviation and compared using Student's *t*-test. Dichotomous variables were compared using Fisher's exact test. All outcomes were analyzed by univariate and multivariate Cox regression adjusting for baseline comorbidities that significantly predicted the outcomes. Hazard ratios (HR) were used as the summary statistic. Subgroup analyses were done to assess the prognostic power of fQRS in patients with and without ischaemic heart disease, respectively. The impact of fQRS burden in patients with fQRS was also explored by comparing those who only have two leads with fQRS morphology against those who have more than two such leads. All *p*-values were two-sided, and *p* < 0.05 was considered significant. All statistical analyses were performed using SPSS software version 25.0 (IBM Corp, New York, USA). The raw data supporting the conclusions of this article will be made available on reasonable request to any of the corresponding authors, without undue reservation.

RESULTS

In total, 2,868 patients fulfilled the inclusion criteria. After excluding patients with missing ECG (*N* = 4), missing primary outcome data (*N* = 309), and wide QRS complex (*N* = 373), 2,182 patients were included in the analysis (Figure 1). We identified fQRS in 179 patients (8.20%), more of whom were males than those without fQRS [98 of 179 (54.7%) vs. 934 of 2003 (46.6%), *p* = 0.042], but were not significantly different in age (75.4 ± 14.8 vs. 74.4 ± 13.5 years, *p* = 0.403) or Charlson comorbidity index (4.91 ± 2.29 vs. 5.14 ± 2.39 , *p* = 0.211); 1,588 (72.8%) had known heart failure prior to the index hospitalization. Patients with fQRS had significantly higher rates of chronic renal diseases (*p* = 0.011) but lower rates of anemia (*p* = 0.020). Other baseline characteristics were not significantly different between the two cohorts (Table 1). Follow-up durations were similar between cohorts (5.53 ± 4.21 vs. 5.64 ± 4.08 years, *p* = 0.733), without any loss to follow-up. The primary composite outcome was met in 566 (25.9%) patients, while the secondary outcome of cardiovascular mortality was met in 419 (19.2%) patients, VA in 56 (2.6%) patients, SCD in 212 (9.7%) patients, MI in 353 (16.2%) patients, and new-onset AF in 829 (38.0%) patients.

Prognostic Value of the Presence of fQRS

Cox regression (Table 2) showed that the presence of fQRS predicted the primary composite outcome in univariate analysis



(HR 1.507 [1.160, 1.959], $p = 0.002$), which remained significant after multivariable adjustments (adjusted HR 1.428 [1.097, 1.859], $p = 0.008$; **Figure 2A**). Both univariate and multivariate analyses demonstrated that fQRS was strongly predictive of cardiovascular mortality, VA, and SCD. Importantly, the prognostic power of fQRS for the primary composite outcome, VA, and SCD were independent of prior history of VA and SCD.

Subgroup Analysis by IHD Status

Subgroup analysis was performed on patients with [786 (36.0%)] and without [1,396 (64.0%)] IHD (**Supplementary Table 2**). fQRS was present in 75 (9.5%) patients with IHD, and 104 (7.4%) patients without IHD. Patients with IHD were followed up for slightly longer periods (5.82 ± 4.13 vs. 5.29 ± 4.00 years, $p = 0.003$). Among patients with IHD, the primary composite outcome was met in 228 (29.0%) patients, while the outcome of cardiovascular mortality was met in 171 (21.8%) patients, VA in 28 (3.6%) patients, SCD in 83 (10.6%) patients, MI in 204 (26.0%) patients, and new-onset AF in 249 (31.7%) patients. Among patients without IHD, these were met in 338 (24.2%), 248 (17.8%), 28 (2.0%), 129 (9.2%), 149 (10.7%), and 580 (41.5%) patients, respectively. Cox regression showed that in patients with IHD, fQRS predicted the primary composite outcome (adjusted HR 1.511 [1.030, 2.215], $p = 0.035$), cardiovascular mortality (adjusted HR 1.615 [1.053, 2.479], $p = 0.028$), VA (adjusted HR 2.880 [1.160, 7.154], $p = 0.023$), and SCD (adjusted HR 1.881 [1.014, 3.481], $p = 0.045$). However, fQRS did not predict the primary composite outcome in patients without IHD (adjusted HR 1.342 [0.929, 1.938], $p = 0.117$), despite remaining strongly predictive of VA (adjusted HR 3.336 [1.343, 8.282], $p = 0.009$) and SCD (adjusted HR 1.928 [1.135, 3.278], $p = 0.015$).

Prognostic Value of fQRS Burden

Patients with fQRS were stratified into those with two contiguous leads with fQRS (112 patients; 5.13% of all patients; 62.6% of patients with fQRS), and those with more than two such leads (67 patients; 3.07% of all patients; 37.4% of patients with fQRS), as summarized in **Supplementary Table 3**. The two subgroups were not significantly different in any baseline characteristics. Cox regression demonstrated significantly higher risk of the primary

TABLE 1 | Baseline characteristics and follow-up durations of included patients.

	No fQRS (<i>N</i> = 2,003)	fQRS present (<i>N</i> = 179)	<i>P</i> -value
Follow-up duration, years	5.64 ± 4.08	5.53 ± 4.21	0.733
Demographics			
Male, <i>N</i> (%)	934 (46.6)	98 (54.7)	0.042
Age, years	74.4 ± 13.5	75.4 ± 14.8	0.403
Comorbidities			
Charlson comorbidity index	5.14 ± 2.39	4.91 ± 2.29	0.211
Anemia, <i>N</i> (%)	785 (39.2)	54 (30.2)	0.020
Known heart failure prior to index admission, <i>N</i> (%)	1,469 (73.3)	119 (66.5)	0.054
Diabetes mellitus, <i>N</i> (%)	616 (30.8)	50 (27.9)	0.447
Chronic renal diseases, <i>N</i> (%)	277 (13.8)	13 (7.26)	0.011
Hypertension, <i>N</i> (%)	918 (45.8)	69 (38.5)	0.071
Ischaemic heart disease, <i>N</i> (%)	711 (35.5)	75 (41.9)	0.089
Myocardial infarction, <i>N</i> (%)	185 (9.2)	19 (10.6)	0.505
Prior ventricular arrhythmia, <i>N</i> (%)	21 (1.0)	5 (2.8)	0.056
Prior sudden cardiac death, <i>N</i> (%)	22 (1.1)	4 (2.2)	0.159
Atrial fibrillation, <i>N</i> (%)	370 (18.5)	41 (22.9)	0.162
Stroke/TIA, <i>N</i> (%)	264 (13.2)	18 (10.1)	0.294
Medications			
ACEI/ARB, <i>N</i> (%)	1,016 (50.7)	95 (53.1)	0.585
Beta-blockers, <i>N</i> (%)	964 (48.1)	92 (51.4)	0.435
Diuretics, <i>N</i> (%)	755 (37.7)	69 (38.5)	0.810
Non-dihydropyridine calcium channel blockers, <i>N</i> (%)	749 (37.4)	55 (30.7)	0.089
Nitrates, <i>N</i> (%)	518 (25.8)	57 (31.8)	0.092
Statins and fibrates, <i>N</i> (%)	621 (31.0)	51 (28.5)	0.554
Anticoagulants, <i>N</i> (%)	320 (16.0)	33 (18.4)	0.397
Antiplatelets, <i>N</i> (%)	798 (39.8)	74 (41.3)	0.691
Sodium channel-blocking antiarrhythmics, <i>N</i> (%)	6 (0.300)	1 (0.562)	0.451
Potassium channel-blocking antiarrhythmics, <i>N</i> (%)	56 (2.80)	3 (1.68)	0.478
Dihydropyridine calcium channel blockers, <i>N</i> (%)	174 (8.69)	16 (8.94)	0.890
Digoxin, <i>N</i> (%)	174 (8.69)	21 (11.7)	0.172

ACEI, angiotensin-converting-enzyme inhibitors; ARB, angiotensin II receptor blockers; fQRS, fragmented QRS.

Statistically significant results were highlighted in bold.

composite outcome in the latter (HR 1.841 [1.119, 3.029], $p = 0.016$; **Figure 2B**), driven by a significantly higher risk of SCD (HR 2.866 [1.350, 6.081], $p = 0.006$) which remained significant even after adjustment for clinical history of VA and SCD (HR 2.679 [1.252, 5.729], $p = 0.011$). All other outcomes were not significantly different in risk between the two subgroups.

DISCUSSION

In this retrospective cohort study of heart failure patients, fQRS is strongly and independently predictive of cardiovascular

TABLE 2 | Cox regression analysis of all 2,182 patients.

Outcome		Univariable		Multivariable model	
		HR [95% CI]	P-value	HR [95% CI]	P-value
Composite primary outcome ^a		1.507 [1.160, 1.959]	0.002	1.428 [1.097, 1.859]^b	0.008
Secondary outcomes	CV mortality	1.373 [1.008, 1.871]	0.044	1.405 [1.030, 1.917]^c	0.032
	VA	3.231 [1.707, 6.119]	0.0003	2.017 [1.039, 3.917]^d	0.038
	SCD	1.832 [1.231, 2.727]	0.003	1.638 [1.097, 2.446]^e	0.016
	MI	1.023 [0.700, 1.496]	0.905	0.890 [0.607, 1.304] ^f	0.890
	New-onset AF	1.111 [0.874, 1.413]	0.391	1.123 [0.882, 1.431] ^g	0.347

Hazard ratios were referenced against patients without fragmented QRS.

^aA composite of cardiovascular mortality, ventricular arrhythmia, and sudden cardiac death.

^bAdjusted for age, Charlson comorbidity index, anemia, chronic renal diseases, ischaemic heart disease, prior VA, prior SCD, prior MI, and diabetes mellitus.

^cAdjusted for age, sex, anemia, Charlson comorbidity index, chronic renal disease, ischaemic heart disease, prior MI, and diabetes mellitus.

^dAdjusted for age, sex, Charlson comorbidity index, anemia, ischaemic heart disease, prior MI, prior VA, and prior SCD.

^eAdjusted for age, sex, Charlson comorbidity index, prior VA, prior SCD, and diabetes mellitus.

^fAdjusted for age, sex, Charlson comorbidity index, known heart failure prior to index hospitalization, prior MI, ischaemic heart disease, diabetes mellitus, prior AF, and anemia.

^gAdjusted for age, sex, Charlson comorbidity index, known heart failure prior to index hospitalization, prior MI, ischaemic heart disease, diabetes mellitus, and prior VA.

AF, atrial fibrillation; CI, confidence interval; CV, cardiovascular; HR, hazard ratio; MI, myocardial infarction; SCD, sudden cardiac death; VA, ventricular arrhythmia.

Statistically significant results were highlighted in bold.

mortality, VA, and SCD, with the presence of fQRS in more than two contiguous leads being associated with significantly higher risk of SCD. Additionally, we showed that while fQRS was strongly predictive of VA and SCD regardless of IHD status, though fQRS was only predictive of cardiovascular mortality in patients with IHD.

Previous studies have shown that fQRS is predictive of mortality and arrhythmic events in patients with heart failure (4). To the authors' best knowledge, the present study is the first study focusing on Asian patients hospitalized with heart failure that reported long-term clinical outcomes, with a mean follow-up duration exceeding 5 years. We confirmed that the elevated risk of adverse outcomes in patients with fQRS persists in the long term, independent of prior history of VA. The magnitudes of elevated risk we observed were also comparable to previous studies (4). Previous studies on Asian patients with heart failure have found fQRS in 21.5–49% of patients (6, 10, 11). This wide range of prevalence was in spite of all these studies, including ours, using the same criteria set forth by Das et al. (5) It is thus likely that such variations were driven by the different inclusion criteria and, in turn, the severity and etiology of heart failure in included patients. For instance, Igarashi et al. reported the highest prevalence (49%) in a cohort of patients with ischaemic cardiomyopathy undergoing cardiac resynchronization therapy, contrasting the 21.5% reported by Pei et al. who analyzed patients with either dilated or ischaemic cardiomyopathy on optimal medical therapy (6, 10). In contrast, we identified fQRS in 8.20% of included patients. The lower rate was likely due to the less selective inclusion criteria of the current study, including patients regardless of previous heart failure diagnosis, admissions, and severity. Although this might introduce some heterogeneity, our study population closely reflect heart failure patients that clinicians see and manage on a daily basis. This strength was

further reinforced by our use of a territory-wide database as data source. Overall, our results are useful for guiding clinicians in their care of patients with heart failure.

Additionally, we found that a higher burden of fQRS was associated with further increased risk of SCD. A similar concept has been shown by Debonnaire et al. in patients with hypertrophic cardiomyopathy (12). This finding may be explained mechanistically by higher fQRS burden reflecting more extensive myocardial scarring (13). By choosing a cutoff of two contiguous leads with fQRS, our results meant that the presence of additional leads with QRS complexes of fragmented morphology beyond the classification criteria would be additionally predictive of SCD. This allowed for a straightforward and intuitive interpretation, potentially facilitating clinical applications. The concept of fQRS burden was also echoed by Roudijk et al., who recently quantified fQRS burden by the total number of deflections in the QRS complex in all leads of a 12-lead ECG (14). Although the resultant index (quantitative fQRS, i.e., Q-fQRS) was not significantly prognostic in their studied population of arrhythmogenic cardiomyopathy, Q-fQRS may objectively provide more granularity in terms of fQRS burden than simpler, morphologically dichotomous approaches as used in the current study. The value of Q-fQRS deserves further studies and investigation in broader populations.

Importantly and interestingly, we showed that fQRS was predictive of SCD but not cardiovascular mortality in patients without IHD. It must be noted that non-ischaemic cardiomyopathies are a heterogeneous entity with various etiologies, and the pathophysiological changes leading to the presence of fQRS might vary accordingly. It is thus possible that fQRS has varying significance in these patients depending on the etiology of cardiomyopathy. Further studies are warranted in this area.

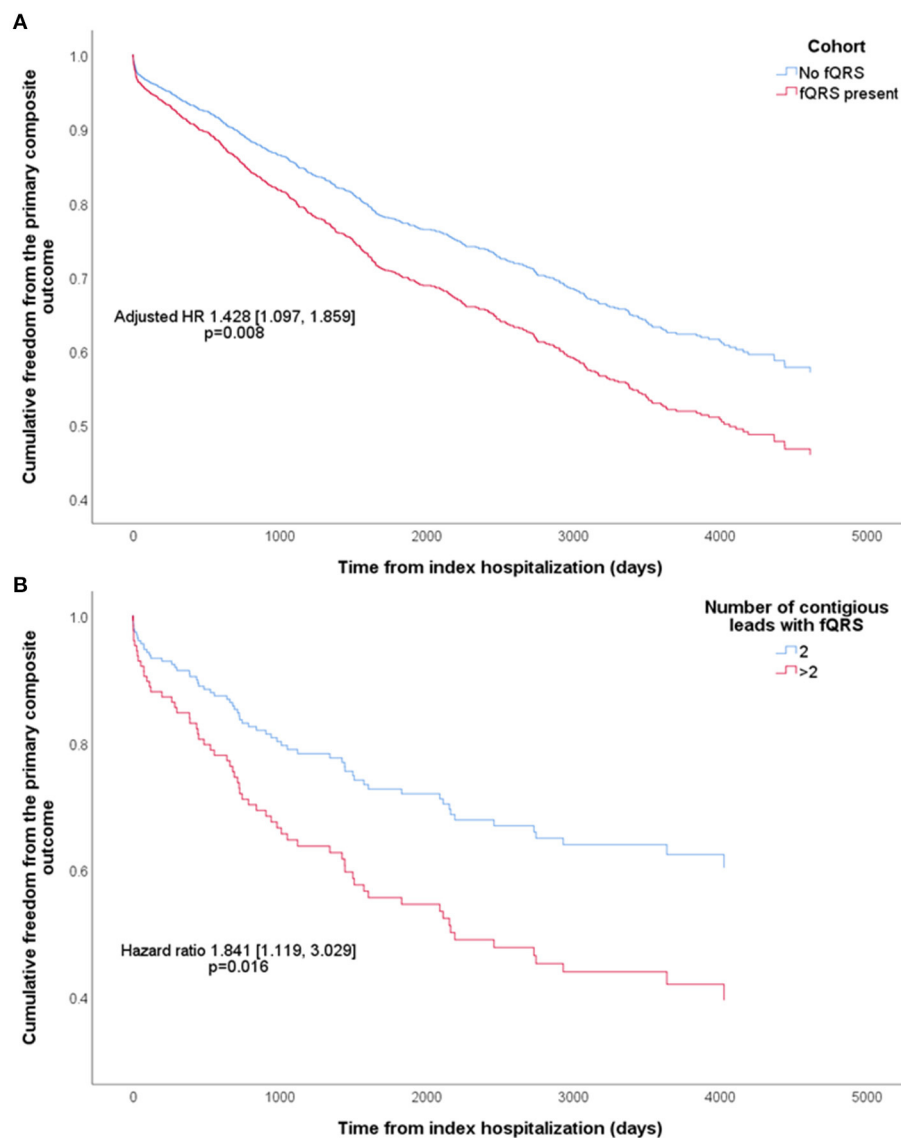


FIGURE 2 | Kaplan-Meier curves of cumulative freedom from the primary composite outcome of **(A)** all patients, stratified by the presence of fragmented QRS (fQRS), and **(B)** patients with fQRS, stratified by fQRS burden. The hazard ratio (HR) shown was adjusted for baseline characteristics that predicted the primary composite outcome.

Clinical Relevance

fQRS may serve as a simple yet powerful predictor of adverse outcomes in patients hospitalized for heart failure, facilitating risk stratification and management of such patients. For instance, the presence of fQRS may alert clinicians to arrange more intensive follow-ups and monitoring, especially for arrhythmia. A higher fQRS burden should also alert clinicians to a higher risk of VA and SCD. Further research of the applicability of fQRS in different patient population have the potential to influence decision pathways for primary and secondary prevention of arrhythmic events and SCD. Further investigation of the interactions between fQRS and other important echocardiographic and functional factors [e.g., frailty

(15, 16)] may further impact the management of heart failure in general.

LIMITATIONS

This study has several limitations. First, due to the nature of CDARS, we had no accompanying echocardiographic or functional data. We recognize that echocardiographic data, including left ventricular ejection fraction and other measures of systolic and diastolic function, is critical for prognosticating and classifying heart failure (17, 18). While the lack of these parameters limits interpretation, our findings remain clinically useful as ECG measurements are more readily available than

echocardiographic measurements. We have also included the Charlson comorbidity index to better capture the patients' overall comorbid status. Second, the included patients with heart failure were heterogeneous in etiology and phenotype. As shown by the subgroup analysis on patients with or without IHD, such differences may affect the prognostic value of fQRS. The lack of information about non-ischaemic etiologies of heart failure in our cohort also limits the results' generalizability. Third, our analysis did not account for differences in the morphologies of fQRS, which may have prognostic implications (2). In addition to affecting the classification of fQRS, morphological considerations may also have implications in the quantification of fQRS burden, as discussed above. Although more detailed quantification of fQRS burden deserves further investigation (14), our results remain valid and clinically relevant given the strong prognostic values of fQRS burden as defined in this study. Fourth, data obtained from CDARS could not be adjudicated. However, all data entry were done by clinicians not involved in this study, and CDARS has been used extensively in other peer-reviewed publications (19, 20).

CONCLUSION

The presence of fQRS was independently predictive of cardiovascular mortality, VA, and SCD in Asian patients hospitalized for heart failure. Having fQRS in more than two contiguous leads independently predicted further increased risk of SCD. However, fQRS did not predict cardiovascular mortality in patients without IHD, warranting further studies.

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DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Joint Chinese University of Hong Kong—New Territories East Cluster Clinical Research Ethics Committee. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

JC and GT conceptualized this study. JZ, SL, AL, MT, and KL organized the database. JC performed the statistical analyses and wrote the first draft of the manuscript. GT wrote sections of the manuscript. KJ, TL, LR, YL, and QZ provided critical input. All authors contributed to manuscript revision, read, and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcvm.2021.738417/full#supplementary-material>

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Incidence of Arrhythmias and Their Prognostic Value in Patients With Multiple Myeloma

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Background: Arrhythmias are common cardiovascular complications in multiple myeloma (MM) patients and are related to a poor prognosis.

Objective: This study aimed to assess the burden of arrhythmias and their prognostic value in patients with MM.

Methods: This was a retrospective study of patients with MM between January 2015 and April 2020 at the First Affiliated Hospital of Xi'an Jiaotong University. The incidence of arrhythmia and associated risk factors were evaluated. The relationship between the type of arrhythmia and survival was analyzed.

Results: A total of 319 patients with MM were identified, and 48.0% (153/319) had arrhythmias. The most common type of arrhythmia was sinus tachycardia (ST) (15.0%, 48/319), followed by sinus bradycardia (SB) (14.4%, 46/319), premature atrial contractions (PACs) (6.3%, 20/319), conduction disorders (CDs) (6.0%, 19/319), atrial fibrillation (AF) (6.0%, 19/319), premature ventricular contractions (PVCs) (4.4%, 14/319) and paroxysmal supraventricular tachycardia (PSVT) (0.6%, 2/319). The patients with arrhythmias had higher levels of log NT-proBNP and creatinine, greater bortezomib use, and a higher incidence of diabetes than those without arrhythmias ($P < 0.05$). The all-cause mortality rates of patients without arrhythmias and those with AF, ST, PACs, CDs, SB, and PVCs were 50.6% (84/166), 73.7% (14/19), 60.4% (29/48), 60.0% (12/20), 52.6% (10/19), 34.8% (16/46), and 28.6% (4/14), respectively. In a subgroup analysis of patients experiencing different types of arrhythmias, patients with SB had lower all-cause mortality than patients with AF ($P < 0.01$). Univariate and multivariate Cox analyses showed that there was a positive statistically significant association between SB and survival (HR: 0.592 [0.352–0.998], $P = 0.049$) in a subgroup analysis of different arrhythmias.

Conclusions: Patients with MM had a heavy arrhythmia burden, and in this study, approximately half of MM patients had arrhythmias. MM patients with SB were associated with lower all-cause mortality than those with AF. SB might be an independent positive factor for prognosis.

Keywords: risk stratification, arrhythmias, multiple myeloma, prognosis, cardiovascular complication

INTRODUCTION

Multiple myeloma (MM) is one of the most common malignant hematologic tumors, accounting for 10% of hematopoietic tissue tumors and 1% of all cancers (1). MM is a clonal plasma-cell neoplasm, and its features include hypercalcemia, renal dysfunction, anemia, bone lesions, and other organ damage (2). With advancements in biologically targeted treatment in recent years, the survival of MM patients has improved significantly (3).

Cardiac complications such as arrhythmias are common in patients with MM (4, 5). One study including ~20% of all United States community hospitals used a nationwide inpatient sample dataset and reported the burden of cardiac arrhythmias in patients with MM (6). The percentage of patients with arrhythmias among MM patients (20.0%, 18,064/88,507) was found to be greater than that among the general population (13.8%). Many factors have been reported to contribute to the increased incidence of cardiac arrhythmias in patients with MM, such as older age, comorbid cardiovascular conditions, chemotherapeutic agents, and the treatment of coexisting cardiac disease, cardiac amyloidosis, autologous stem cell transplantation (ASCT), and electrolyte abnormalities (6–11). In our previous work, the QTc interval and heart rate were independently associated with all-cause mortality in patients with MM (12, 13). However, the burden of different arrhythmias and the relation between these arrhythmias and prognostic survival in MM patients remain unclear in real-world studies. Therefore, this study aimed to retrospectively investigate the prevalence of different arrhythmias and evaluate their prognostic potential to guide the management of these patients.

METHODS

Study Population

This retrospective study was approved by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University (Approval No. XJTU1AF2020LSK-179) and was conducted in accordance with the guidelines of the Declaration of Helsinki. Informed consent was obtained from all participants. Patients diagnosed with MM and hospitalized between January 2015 and April 2020 at the First Affiliated Hospital of Xi'an Jiaotong University were included. The exclusion criteria were as follows: (1) age younger than 18 years; (2) patients who were not newly diagnosed; (3) patients with missing data on electrocardiograms (ECGs) during diagnosis or induction treatment; (4) lack of clinical data; (5) patients with a paced rhythm; and (6) patients who were lost to follow-up. The flowchart of the study population is shown in **Figure 1**. Thus, 319 patients were ultimately enrolled in the analysis.

Data Collection

All anonymized data including clinical information, personal history, and laboratory examination results were collected from the electronic medical record system of the hospital and analyzed. The diagnosis of MM was confirmed by a hematologist. Induction therapy was identified according

to the European Society for Medical Oncology (ESMO) Clinical Practice Guidelines and National Comprehensive Cancer Network (NCCN) guidelines (14, 15). The International Staging System (ISS) was used for patient stratification (16). Pulmonary hypertension (PH) was defined as an estimated right ventricular systolic pressure >40 mm Hg on transthoracic echocardiogram (17).

Arrhythmias include ventricular and supraventricular arrhythmias which are defined by American College of Cardiology/American Heart Association/Heart Rhythm Society (ACC/AHA/HRS) Guideline (18–20). A 12-lead ECG was used to detect arrhythmias in the study. The type of heart rhythm from ECGs was extracted, including atrial fibrillation (AF), atrial flutter, paroxysmal supraventricular tachycardia (PSVT), premature atrial contractions (PACs), premature ventricular contractions (PVCs), sinus tachycardia (ST), sinus bradycardia (SB) and conduction disorders (CDs) (right bundle branch block, left bundle branch block and atrioventricular block) and normal sinus rhythm (without arrhythmias). Patients were categorized into the group with arrhythmias and the group without arrhythmias according to the ECG results.

Outcome Variables

The study endpoint was all-cause mortality. All participants were followed up until death or November 2020. Follow-up measures included outpatient visits, telephone calls or other electronic communications due to social quarantine measures during the coronavirus 2019 (COVID-19) pandemic.

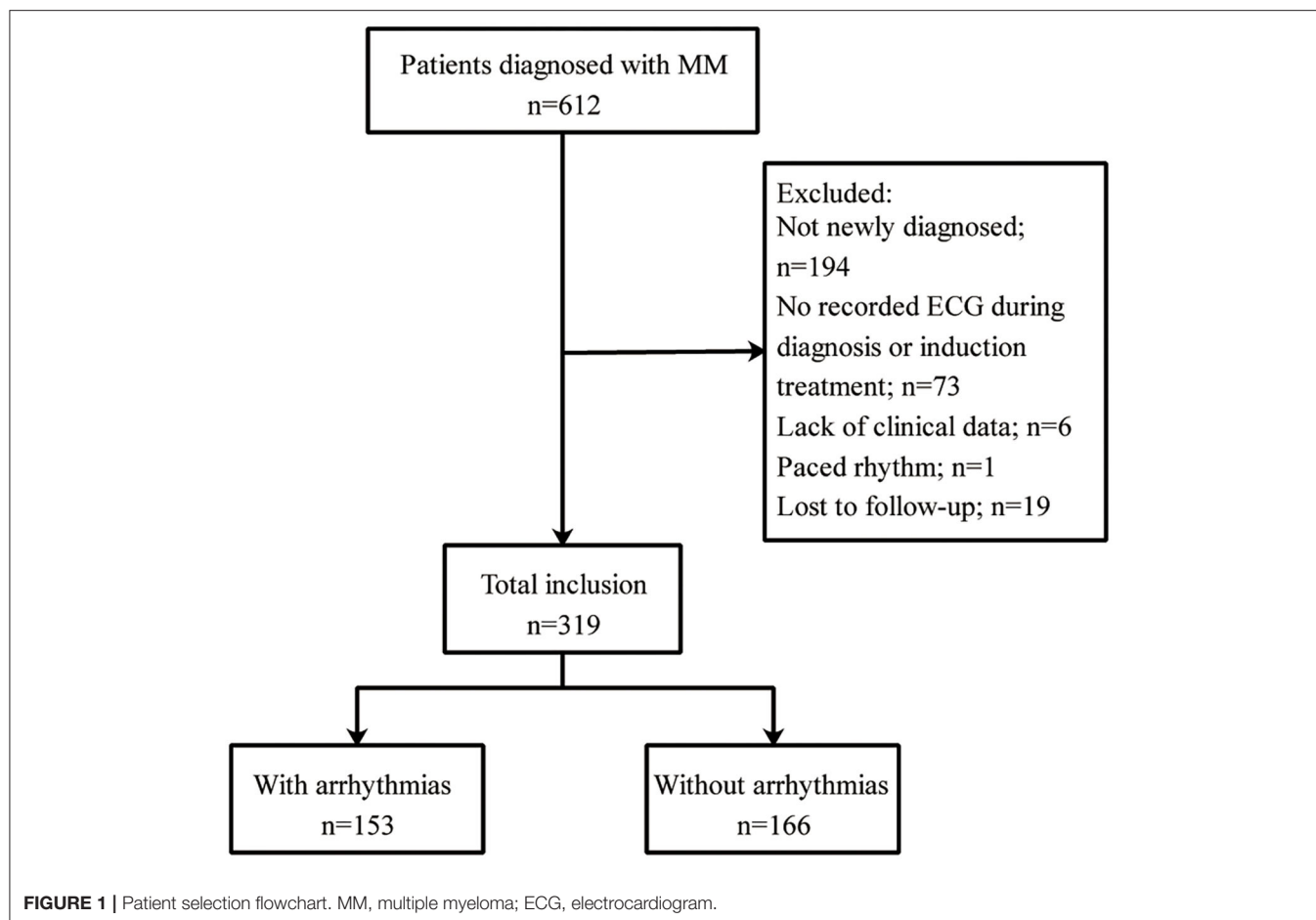
Statistical Analysis

All statistical analyses were conducted using SPSS version 24. All continuous variables were presented as the means \pm standard deviations or medians (interquartile ranges) and were compared using Student's *t*-test. The Kruskal-Wallis test was used when the data were not normally distributed. Categorical variables were expressed as percentages and compared using Pearson's chi-square test or Fisher's exact test to explore differences between groups in terms of all-cause mortality. Univariable logistic regression models were used to determine the risk of arrhythmia. Survival curves were plotted by the Kaplan-Meier method to depict differences in survival, and the log-rank test was utilized to assess statistical significance. Univariate and multivariate Cox proportional hazards models were used to identify independent predictors for the type of arrhythmia. The hazards were proportional. A $P < 0.05$ was considered statistically significant.

RESULTS

Baseline Clinical Characteristics

A total of 319 patients (mean age: 61.7 ± 9.8 years) were included, and 60.2% (192/319) were male. Among these patients, 153 patients (48.0%) had arrhythmias (**Figure 1**). There were no significant differences between the groups in terms of age, sex or laboratory findings, but differences in log N-terminal pro-brain natriuretic peptide (NT-proBNP)



and creatinine levels, bortezomib use, supportive care measures and diabetes incidence were observed (Table 1). The patients with arrhythmias had higher levels of log NT-proBNP (3.08 ± 0.80 vs. 2.89 ± 0.78 pg/mL, $P = 0.028$) and creatinine [1.01 (0.70 – 2.53) vs. 0.85 (0.65 – 1.54) mg/dL, $P = 0.038$]. Chemotherapy agents including bortezomib, lenalidomide, thalidomide, anthracyclines, cyclophosphamide and supportive care measures were compared between the two groups. The proportion of patients who received bortezomib (54.2 vs. 40.4%, $P = 0.013$) was higher in patients with arrhythmias than in those without arrhythmias. However, the group with arrhythmias had a lower rate of receiving supportive care measures (6.5 vs. 13.3%, $P = 0.046$). For comorbidities, the percentage of patients with diabetes was higher in the group with arrhythmias than in the group without arrhythmias (16.3 vs. 8.4%, $P = 0.031$). Logistic regression analysis demonstrated that the log NT-proBNP level, bortezomib use and diabetes were independent predictors of arrhythmias (Supplementary Table 1). In terms of the transthoracic echocardiographic parameters, left ventricular ejection fraction, left ventricular end-systolic diameter, left ventricular ejection fraction and PH were evaluated. No significant difference was found between the two groups.

Characteristics and Incidence of Arrhythmias

Overall, 153 (48.0%) of 319 patients had arrhythmias. The types of abnormal rhythms observed included AF, PSVT, PACs, PVCs, ST, SB, and CDs (right bundle branch block, left bundle branch block and first-degree and Mobitz I atrioventricular block). The most common arrhythmia was ST (15.0%, 48/319), followed by SB (14.4%, 46/319), PACs (6.3%, 20/319), CDs (6.0%, 19/319), AF (6.0%, 19/319), PVCs (4.4%, 14/319), and PSVT (0.6%, 2/319) (Table 2). There was only one patient with atrial flutter, and this patient was included in the AF group. In addition, there were 15 patients with more than one type of arrhythmia (Supplementary Table 2). Among these patients, it is important to note that there was one patient who had three types of arrhythmias: ST and right and left bundle branch block. The all-cause mortality was 40.0% (6/15) among patients with more than one type of arrhythmia. There was no significant difference in prognosis between the patients with one arrhythmia and those with more than one arrhythmia ($P > 0.05$).

Prognostic Value of Arrhythmias

The median follow-up duration was 18.3 (9.9–29.2) months. There was no significant difference in all-cause mortality between the group with arrhythmias and the group without arrhythmias

TABLE 1 | Baseline characteristics of all patients.

Patient demographics	Total N = 319	Arrhythmias		P value
		With N = 153	Without N = 166	
Age (years)	61.7 ± 9.8	62.2 ± 9.9	61.3 ± 9.6	0.389
Male, n (%)	192 (60.2)	98 (64.1)	94 (56.6)	0.176
Log NT-proBNP (pg/mL)	2.98 ± 0.80	3.08 ± 0.80	2.89 ± 0.78	0.028
LDH (U/L)	224.77 ± 153.35	239.73 ± 198.05	210.98 ± 93.76	0.094
Serum albumin (g/L)	32.35 ± 6.40	32.58 ± 6.57	32.14 ± 6.24	0.548
BUN (mmol/L)	6.66 (5.17–10.31)	6.87 (5.26–10.68)	6.43 (4.99–9.85)	0.188
Creatinine (mg/dL)	0.92 (0.67–2.13)	1.01 (0.70–2.53)	0.85 (0.65–1.54)	0.038
eGFR (mL/min/1.73 m ²)	77.0 (29.1–119.9)	71.3 (26.1–111.9)	82.2 (40.3–127.4)	0.051
Serum calcium (mmol/L)	2.27 ± 0.38	2.29 ± 0.40	2.25 ± 0.35	0.314
Serum potassium (mmol/L)	3.94 ± 0.60	3.94 ± 0.56	3.94 ± 0.63	0.980
Serum sodium (mmol/L)	139.4 ± 4.7	139.4 ± 4.8	139.4 ± 4.7	0.926
Complete blood count				
White blood cell (× 10 ⁹ /L)	5.34 ± 2.45	5.16 ± 2.20	5.51 ± 2.65	0.210
Hemoglobin (g/L)	85.2 ± 22.2	84.7 ± 23.2	85.7 ± 21.4	0.688
Platelet (× 10 ⁹ /L)	164.4 ± 80.6	162.0 ± 88.4	166.6 ± 72.9	0.610
Immune type of myeloma, n (%)				
IgG/IgA/IgD	240 (75.2)	114 (74.5)	126 (75.9)	0.650
Light chain κ/λ	73 (22.9)	35 (22.9)	38 (22.9)	
None secreted	6 (1.9)	4 (2.6)	2 (1.2)	
ISS stage, n (%)				
I	45 (14.1)	20 (13.1)	25 (15.1)	0.813
II	145 (45.5)	72 (47.0)	73 (44.0)	
III	129 (40.4)	61 (39.9)	68 (40.9)	
Treatment*, n (%)				
Bortezomib	150 (47.0)	83 (54.2)	67 (40.4)	0.013
Lenalidomide	30 (9.4)	16 (10.5)	14 (8.4)	0.536
bortezomib and lenalidomide	21 (6.6)	13 (8.5)	8 (4.8)	0.186
Thalidomide	71 (22.3)	36 (23.5)	35 (21.1)	0.600
Anthracyclines [#]	134 (42.0)	68 (44.4)	66 (39.8)	0.397
Liposomal daunorubicin	45 (14.1)	26 (17.0)	19 (11.4)	0.155
Cyclophosphamide [§]	135 (42.3)	66 (43.1)	69 (41.6)	0.777
Supportive care	32 (10.0)	10 (6.5)	22 (13.3)	0.046
Comorbidities, n (%)				
CAD	19 (6.0)	12 (7.8)	7 (4.2)	0.172
Hypertension	96 (30.1)	53 (34.6)	43 (25.9)	0.089
Diabetes	39 (12.2)	25 (16.3)	14 (8.4)	0.031
Echocardiographic parameters, n = 199				
LVEDD, mm	50.1 ± 5.1	50.6 ± 4.7	49.6 ± 5.5	0.175
LVESD, mm	31.3 ± 4.5	31.8 ± 4.8	30.7 ± 4.1	0.077
IVS, mm	8.5 ± 1.3	8.4 ± 1.2	8.6 ± 1.4	0.557
LVEF (%)	66.7 ± 6.5	66.2 ± 7.0	67.3 ± 5.9	0.228
PH, n (%)	20 (10.1)	12 (12.1)	8 (8.0)	0.334

Median (interquartile range), mean ± SD; NT-proBNP, N-terminal pro-brain natriuretic peptide; LDH, lactate dehydrogenase; eGFR, estimated glomerular filtration rate; ISS, International Staging System; CAD, coronary artery disease; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; IVS, interventricular septum; LVEF, left ventricular ejection fraction; PH, pulmonary hypertension.

*The patients had taken the agents.

[#]Anthracyclines included epirubicin and pirubicin.

[§]Included cyclophosphamide and ifosfamide.

TABLE 2 | Characteristics of arrhythmias in MM patients.

Type of arrhythmia	Number of patients	Incidence among the MM cohort (%)	Distribution of arrhythmias (%)
AF ^{&}	19	6.0	12.4
PSVT	2	0.6	1.3
PBs [§]	33	10.3	21.6
PACs	20	6.3	13.1
PVCs	14	4.4	9.2
ST	48	15.0	31.4
SB	46	14.4	30.1
CDs [%]	19	6.0	12.4
RBBB	10	3.1	6.5
LBBB	3	0.9	2.0
First-degree and Mobitz I AV block	7	2.2	4.6
Total [®]	153	48.0	

MM, multiple myeloma; AF, atrial fibrillation; PSVT, paroxysmal supraventricular tachycardia; PBs, premature beats; PACs, premature atrial contractions; PVCs, premature ventricular contractions; ST, sinus tachycardia; SB, sinus bradycardia; CDs, conduction disorders; RBBB, right bundle branch block; LBBB, left bundle branch block; AV, atrioventricular.

[&] There was one patient with atrial flutter included in the atrial fibrillation group.

[§] There was one patient with both premature atrial and ventricular contractions.

[%] There was one patient with both right and left bundle branch block.

[®] Fifteen patients had more than one type of arrhythmia.

($P > 0.05$). The prognostic value of different types of arrhythmias was also evaluated. The all-cause mortality rates of patients with ST, SB, AF, PACs, PVCs, and CDs and in patients without arrhythmias were 60.4% (29/48), 34.8% (16/46), 73.7% (14/19), 60.0% (12/20), 28.6% (4/14), 52.6% (10/19), and 50.6% (84/166), respectively (**Figure 2**). The patients with SB had lower all-cause mortality than those with AF ($P < 0.01$).

Furthermore, in the subgroup analysis of patients with different arrhythmias, univariate Cox analysis showed that there was a significant positive correlation between SB and survival [hazard ratio (HR): 0.500 [0.298–0.837], $P = 0.008$]. In addition, SB was also independently negatively associated with all-cause mortality (HR: 0.592 [0.352–0.998], $P = 0.049$) (**Table 3**).

DISCUSSION

Approximately half of the patients (48.0%) had arrhythmias in our study, including ST (15.0%), SB (14.4%), PACs (6.3%), CDs (6.0%), AF (6.0%), PVCs (4.4%), and PSVT (0.6%). Patients with SB had lower all-cause mortality than those with AF, which might be an independent positive factor for prognosis.

Advances in the treatment and management of MM patients have significantly prolonged survival; however, the number of patients with cardiovascular complications is increasing. In previous studies, arrhythmias have been shown to be common in MM patients and to cause undesirable results. In a retrospective cohort study of 32,193 patients, nearly 60% of patients experienced cardiac events. The incidence of cardiac arrhythmias was 14% for newly diagnosed patients and 17% for

relapsed patients, and the incidence of CDs was 2% for newly diagnosed and 2% for relapsed patients (21). In a large national database of 88,507 patients, 20% were found to have arrhythmias, with the most common type being AF (67.7%), followed by paroxysmal ventricular tachycardia (5.0%), atrial flutter (3.9%), and paroxysmal atrial tachycardia (2.2%) (6). The incidence of arrhythmias was high in our study, with ST (15.0%) and SB (14.4%) occurring more frequently than AF (6.0%). Many factors, including comorbidities, cardiac function, organ dysfunction and various cardiotoxic chemotherapeutics, can increase the incidence of arrhythmia.

The distribution of diabetes was found to be similar between patients with and without arrhythmias in the MM population in a previous study (6); however, there is increasing evidence that the presence of diabetes impacts the incidence of arrhythmia. Diabetes is closely associated with cardiac arrhythmias, especially in patients with chronic kidney disease, hypoglycemia and hyperglycemia, and the most common arrhythmias are AF, conduction abnormalities, and ventricular arrhythmias (22–26). Patients with MM often experience renal dysfunction, electrolyte abnormalities, anemia and hyperglycemia, which are factors for arrhythmia. The percentage of patients with diabetes was higher in the group with arrhythmias than in the group without arrhythmias, and diabetes might aggravate the risk of cardiac arrhythmias among MM patients in our study.

Left ventricular ejection fraction was evaluated and compared, but no significant differences were observed between the group with arrhythmias and the group without arrhythmias. NT-proBNP is another important measure of cardiac function; with high levels of NT-proBNP, cardiac function may be affected (27), and heart failure can even develop. Given the retrospective nature of our study, the incidence of the exact type of heart failure could not be determined, but NT-proBNP was found to be an independent predictor of arrhythmias after adjusting for creatinine.

The rate of arrhythmias in relapsed MM patients was higher than that in newly diagnosed patients (21). Although a large difference was observed between relapsed patients and newly diagnosed patients, to some extent, this finding indicated that the tumor burden in MM patients might increase the incidence of arrhythmias. In our study, tumor burden was assessed by ISS stage and specific clinical features, such as hypercalcemia, renal insufficiency, and anemia. Renal damage has been linked to higher rates of arrhythmias and sudden death (28), and anemia has been shown to increase the risk of arrhythmias and hypertension (29, 30). However, there was no difference in these indicators between the group with arrhythmias and the group without arrhythmias in our study.

Arrhythmias were also the most common cardiovascular complications during anti-myeloma therapy treatment with proteasome inhibitors, immunomodulators, corticosteroids, alkylating agents, anthracyclines, etc. (15). A large retrospective study reported a low incidence of cardiac arrhythmias with bortezomib-based treatment (31). In our study, approximately half of the patients received bortezomib and the proportion of patients who received bortezomib (54.2 vs. 40.4%, $P = 0.013$) was higher in patients with arrhythmias than in those

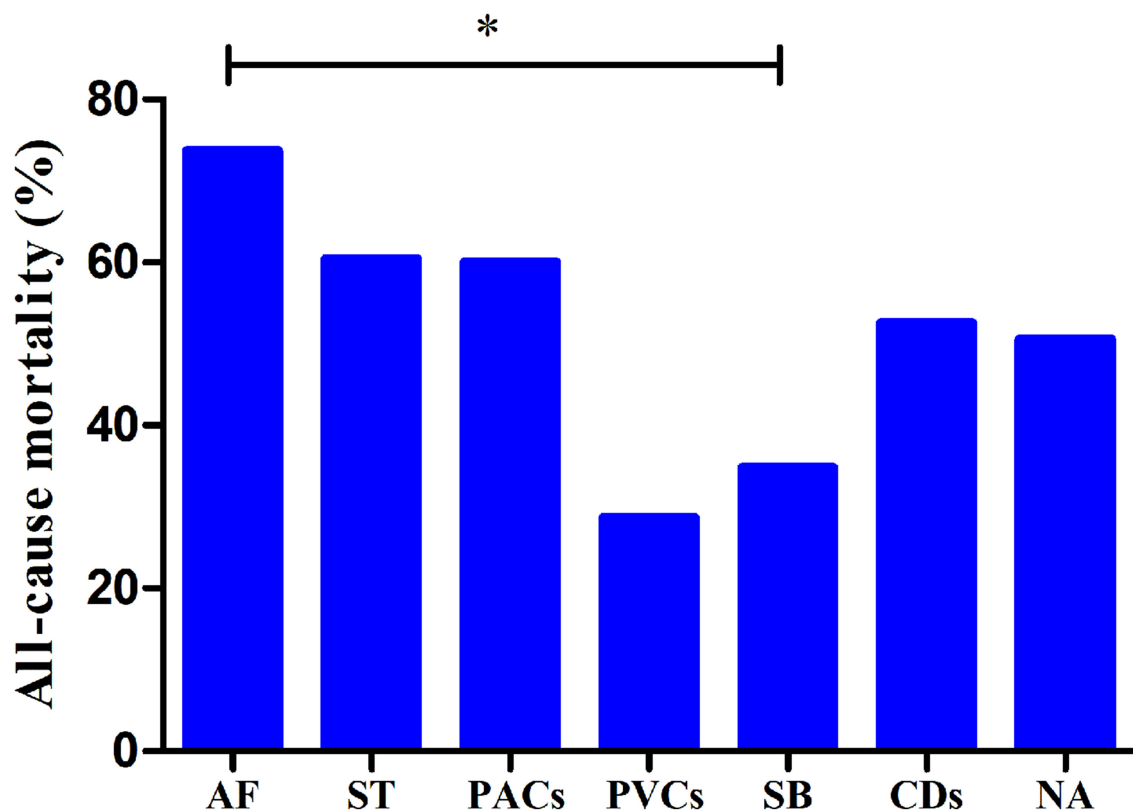


FIGURE 2 | All-cause mortality rates in patients with different types of arrhythmias. * $P < 0.01$; AF, atrial fibrillation; ST, sinus tachycardia; PACs, premature atrial contractions; PVCs, premature ventricular contractions; SB, sinus bradycardia; CDs, conduction disorders; WOs, without arrhythmias.

without arrhythmias for the following reasons. First, prescription bortezomib is the most recommended medication, especially among patients with cardiovascular risk factors or comorbidities. Second, lenalidomide use is limited in patients with renal dysfunction and is cost prohibitive. Third, carfilzomib is not easy to obtain. Based on the above factors, a high rate of bortezomib use was accepted in the arrhythmia group.

The distribution and incidence rates of SB were 30.1% (46/153) and 14.4% (46/319), respectively. The rates of patients who received thalidomide, cyclophosphamide and anthracycline were 22.3, 42.3, and 42.0%, respectively. Chemotherapy agents associated with bradycardia in patients with MM have been described in previous studies. Thalidomide and lenalidomide have been associated with the development of bradyarrhythmias and AF, and the incidence of SB in patients is 1–10% (10, 32). Other studies showed that SB was observed in ~26–53% of patients who received thalidomide as initial therapy for early-stage myeloma (33) and was found in more than 10% of patients who received cyclophosphamide (34). Apart from thalidomide- or lenalidomide-induced arrhythmias, most anthracycline-induced arrhythmias are benign; however, critical bradycardia and AF have been reported (35, 36). Limited by the small cohort and the single-center retrospective design, it was difficult to identify the relationship between chemotherapy agents and different types of arrhythmias.

Cardiac amyloidosis might lead to bradycardia, low voltage and atrioventricular block, and these novel agents and their neurotoxicity exacerbate bradycardia (37). Although endocardial biopsy and speckle tracking echocardiography technology are conducive to the early detection and diagnosis of cardiac amyloidosis, they are not widely used at our center, and the proportion of patients with cardiac amyloidosis is unclear.

Sex differences are also an important factor and should be considered due to their impact on diagnosis, cardiovascular complications, and therapeutic interventions. Different levels of hormones may affect the incidence and prevalence of arrhythmias between men and women. Little is known about sex differences in arrhythmias among MM populations. In this study, the incidence rates of arrhythmias in women and men were 17.2 and 30.7%, and no significant difference was found between women and men ($P > 0.05$). For example, the percentage of AF in women was higher than that in men, and the all-cause morbidity of AF in women was lower than that in men. These findings are similar to the previous studies (38, 39); however, no significant differences in the incidence of AF and all-cause morbidity between males and females were found in our study, which may be due to the small size of cases and other factors.

SB was found to be a positive prognostic factor for survival in MM patients in this study; this finding was consistent

TABLE 3 | Univariate and multivariate Cox regression analysis for all-cause mortality.

	Univariate analysis			Multivariate analysis		
	HR	95% CI	P	HR	95% CI	P
Age (years)	1.008	0.992–1.023	0.327			
Male	1.275	0.929–1.752	0.133			
Log NT-proBNP (pg/mL)	1.914	1.565–2.341	<0.0001	1.627	1.312–2.018	<0.0001
LDH (U/L)	1.002	1.001–1.002	<0.0001	1.002	1.001–1.002	<0.0001
eGFR (mL/min/1.73 m ²)	0.992	0.989–0.996	<0.0001	1.000	0.995–1.004	0.853
Serum albumin (g/L)	0.986	0.963–1.010	0.253			
Serum potassium (mmol/L)	0.971	0.729–1.295	0.843			
Hemoglobin (g/L)	0.985	0.978–0.992	<0.0001	0.993	0.985–1.001	0.105
CAD	1.724	0.995–2.989	0.052			
Hypertension	1.099	0.791–1.527	0.575			
Diabetes	1.521	0.986–2.347	0.058			
ISS Stage, n (%)						
I	1			1		
II	1.746	0.996–3.062	0.052	1.425	0.794–2.555	0.235
III	3.196	1.837–5.563	<0.0001	2.004	1.051–3.824	0.035
AF	1.664	0.961–2.880	0.069			
PACs	1.705	0.945–3.076	0.076			
PVCs	0.494	0.183–1.335	0.164			
ST	1.485	0.992–2.223	0.054			
SB	0.500	0.298–0.837	0.008	0.592	0.352–0.998	0.049
CDs	1.099	0.580–2.086	0.772			
Types of arrhythmias						
0	1					
1	1.138	0.834–1.555	0.415			
>1	0.943	0.411–2.163	0.890			

HR, hazard ratio; NT-proBNP, N-terminal pro-brain natriuretic peptide; LDH, lactate dehydrogenase; eGFR, estimated glomerular filtration rate; CAD, coronary artery disease; ISS, International Staging System; AF, atrial fibrillation; PACs, premature atrial contractions; PVCs, premature ventricular contractions; ST, sinus tachycardia; SB, sinus bradycardia; CDs, conduction disorders.

with the results of previous studies (40–42). One retrospective cohort study indicated that bradycardia was associated with good neurologic outcomes during therapeutic hypothermia for comatose survivors of out-of-hospital cardiac arrest and should not be aggressively treated during this period (40). Patients with relative bradycardia were found to have lower mortality, even after adjusting for the confounding factor of septic shock disease (41). Similarly, Kyriazopoulou et al. noted that cardiac arrest patients who exhibited SB during targeted temperature management had a lower 180-day mortality and better final neurological prognosis (42). However, it is challenging to determine the value of bradycardia. Moreover, bradycardia was not associated with incident cardiovascular disease or mortality in a community-based cohort (43). Bradycardia is usually considered an adverse prognostic indicator; severe or prolonged bradycardia might cause heart failure, hypotension, and syncope. The prognosis of patients with symptomatic bradycardia was found to be poor if they were not treated with a pacemaker. Temporary bradycardia in patients with COVID-19 was determined to be associated with a high rate of short-term morbidity and poor outcomes (44). The common causes

of bradycardia are structural heart disease, CDs, or other cardiac conditions. In terms of pathology, dysfunction of the sinus node, atrioventricular nodal tissue, and the specialized His-Purkinje conduction system might predispose patients to bradycardia. SB was a main kind of bradycardia in our study, with a percentage of 97.8% (45/46), and only one patient had conduction disorder. The positive effects of SB on mortality might be because a lower heart rate could reflect depressed sympathetic activation. However, the exact mechanisms underlying this relationship await further study.

The present study had several potential limitations. First, this was a study with small size. Second, our study had a single-center retrospective design; therefore, clinical data related to the treatment of coexisting cardiac disease, antiarrhythmic drugs, dynamic ECG, the atropine test and patient response, chemotherapeutic regimens, and the out-of-hospital use of thalidomide were not available. Third, speckle tracking echocardiography technology and imaging technology were not widely used in our center, so the proportion of patients with cardiac amyloidosis was unclear.

CONCLUSIONS

Patients with MM had a heavy arrhythmia burden, and in this study, approximately half of MM patients had arrhythmias. MM patients with SB were associated with a lower all-cause mortality rate than those with AF. SB might be an independent positive factor for prognosis. Arrhythmias should be considered in all patients and could help to guide cardiovascular risk assessment.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiao Tong University (Approval No. XJTU1AF2020LSK-179). The patients/participants provided their written informed consent to participate in this study.

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AUTHOR CONTRIBUTIONS

CS and JW contributed to the conception and design of the work and critically revised the manuscript. MT critically revised the manuscript. MT, LZ, SW, JS, and HL contributed to the acquisition, analysis, and interpretation of data. YL drafted the manuscript. All authors approved the manuscript and agreed to be accountable for all aspects of the work, ensuring its integrity and accuracy.

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Differential Risk of Dementia Between Patients With Atrial Flutter and Atrial Fibrillation: A National Cohort Study

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Objectives: Atrial fibrillation (AF) is linked to an increased risk of stroke and dementia. Atrial flutter (AFL) is also linked to an increased risk of stroke but at a different level of risk as compared to AF. Little is known about the difference in the risk of dementia between AF and AFL. This study aims to investigate whether the risk of dementia is different between AF and AFL.

Methods: Patients with newly diagnosed AF and AFL during 2001–2013 were retrieved from Taiwan's National Health Insurance Research Database. Patients with incomplete demographic data, aged <20 years, history of valvular surgery, rheumatic heart disease, hyperthyroidism, and history of dementia were excluded. The incidence of new-onset dementia was set as the primary outcome and analyzed in patients with AF and AFL after propensity score matching (PSM).

Results: A total of 232,425 and 7,569 patients with AF and AFL, respectively, were eligible for analysis. After 4:1 PSM, we included 30,276 and 7,569 patients with AF and AFL, respectively, for analysis. Additionally, patients with AF ($n = 29,187$) and AFL ($n = 451$) who received oral anticoagulants were enrolled for comparison. The risk of dementia was higher in patients with AF compared with patients with AFL (subdistribution hazard ratio (SHR) = 1.52, 95% CI 1.39–1.66; $p < 0.0001$) before PSM and remained higher in patients with AF (SHR = 1.14, 95% CI 1.04–1.25; $p = 0.0064$) after PSM. The risk of dementia was higher in patients with AF without previous history of stroke after PSM but the risk did not differ between patients with AF and AFL with previous history of stroke. Among patients who received oral anticoagulants, the cumulative incidences of dementia were significantly higher in patients with AF than in patients with AFL before and after PSM (all $P < 0.05$).

Conclusions: This study found that, among patients without history of stroke, the risk of dementia was higher in patients with AF than in patients with AFL, and CHA₂DS₂-VASC score might be useful for risk stratification of dementia between patients with AF and AFL.

Keywords: atrial fibrillation, atrial flutter, dementia, stroke, CHA₂DS₂-VASC score

INTRODUCTION

Atrial fibrillation (AF) had been reported to be linked to an increased risk of ischemic stroke, thromboembolism, heart failure, myocardial infarction, and death (1, 2). Moreover, there is increasing evidence that AF is also a risk factor for cognitive decline and dementia, which might be independent of ischemic stroke (3–6). Atrial flutter (AFL) and AF share the common risk factors and therefore, should contribute to similar clinical events. Accordingly, clinical guidelines recommend AFL should be treated as AF in terms of anticoagulation to prevent stroke and systemic thromboembolism (7). However, our previous study showed that the incidence of ischemic stroke was significantly higher in the AF cohort than in the AFL cohort at a CHA₂DS₂-VASc score [Heart failure, Hypertension, Age ≥ 75 (doubled), Diabetes, Stroke (doubled), Vascular disease, Age 65–74, and Sex category (female)] ≥ 1 (8). Therefore, differential risk of ischemic stroke exists between AF and AFL. There is no study to examine the risk of dementia between patients with AFL and AF. We hypothesized that patients with AF had a higher risk of dementia than patients with AFL independent of the previous history of stroke. Accordingly, we conducted this large population-based national cohort study to evaluate the incidence of dementia in patients with AF and AFL.

MATERIALS AND METHODS

Data Source

Taiwan's National Health Insurance started in 1995 and covers 99.5% (23 million) of the residents in Taiwan. The National Health Insurance Research Database (NHIRD) provides the data of all inpatient and outpatient services, diagnoses, emergency room visits, prescriptions, examinations, operations, and expenditures and are updated biannually. The diseases were diagnosed using the International Classification of Diseases, ninth revision, Clinical Modification (ICD-9-CM) and version 2001 codes. This study was approved by the Institutional Review Board of Chang Gung Memorial Hospital (202100865B1). The dataset used in this study was held by the Taiwan Ministry of Health and Welfare (MOHW). Any researcher interested in accessing this dataset can submit an application form to the MOHW requesting access. (Email: stcarol-wu@mohw.gov.tw).

Study Patients

Electronic medical records from the NHIRD between January 1, 2001 and December 31, 2013 were retrieved for patients with a discharge diagnosis or at least two consecutive outpatient clinic diagnoses of AF (ICD-9-CM: 427.31) and AFL (ICD-9-CM: 427.32). The time when AF or AFL was first diagnosed was assigned as the index date. The coverage period of our database was from 1997 to 2013; therefore, we excluded patients who were diagnosed with both AF and AFL between 1997 and 2000. Furthermore, we excluded patients who had incomplete demographic data ($<0.1\%$), aged <20 years, history of valvular surgery, rheumatic heart disease, hyperthyroidism, and history of dementia (**Figure 1**). The remaining patients were categorized into two groups as newly diagnosed AF or newly diagnosed AFL

without history of dementia. Furthermore, we excluded those patients who received radiofrequency ablation because these factors might also affect the risk of stroke and dementia.

Study Design

The primary outcome of dementia was analyzed after propensity score matching (PSM) between AF and AFL population in three different cohorts. In cohort 1, patients with AF ($n = 232,425$) and AFL ($n = 7,569$) were eligible population after serial excluding criteria. Cohort 2 [patients with AF ($n = 200,646$) and AFL ($n = 6,683$)] enrolled those patients without any history of ischemic stroke in cohort 1, while cohort 3 [patients with AF ($n = 31,779$) and AFL ($n = 886$)] enrolled those patients with a history of ischemic stroke in cohort 1 (**Figure 1**). The PSM was performed with 4:1 ratio under adjusting covariates including age, sex, comorbidities, and medications between AF and AFL cohorts. Furthermore, the primary outcome was also analyzed by stratifying the groups by CHA₂DS₂-VASc score in cohort 1 (**Figure 1**).

Study Outcome, Covariates, and Follow-Up

The primary outcome was dementia, which was defined when the diagnosis was made during hospitalization or ≥ 2 consecutive clinic visits. The diagnostic code of dementia was validated in previous NHIRD studies (9, 10). The first date when dementia was coded during follow-up was assigned as the date of the endpoint. Each patient in the three cohorts was followed up until the date of dementia occurrence, death, or December 31, 2013, whichever occurred first.

The covariates included comorbidities, namely hypertension, diabetes mellitus, dyslipidemia, ischemic heart disease, peripheral arterial disease, chronic obstructive pulmonary disease, renal function status, abnormal liver function, gout, systemic thromboembolism, myocardial infarction, stroke, and heart failure, and medications.

These comorbidities were ascertained according to the ICD-9-CM codes combined with medication use. The patients with systemic thromboembolism, myocardial infarction, stroke, and heart failure were defined as having any inpatient diagnosis before the index date. Most of these diagnostic codes were validated in previous NHIRD studies (11–15). Similarly, data on medication usage were retrieved on claim-based data of the previous year.

Ascertainment of AF/AFL and CHA₂DS₂-VASc Score

All patients who had AF or AFL diagnosis defined according to the diagnosis made at least once during hospitalization or ≥ 2 consecutive clinic visits. The accuracy of AF or AFL diagnosis using ICD-9-CM code in the NHIRD has been confirmed in previous studies (16, 17).

Because CHA₂DS₂-VASc score has been reported to be useful for risk stratification regarding ischemic stroke and dementia among patients with AF and AFL (18–21), we stratified patients using CHA₂DS₂-VASc score to compare the risk of dementia between patients with AF and AFL before and after PSM. Comorbidities in CHA₂DS₂-VASc score were ascertained

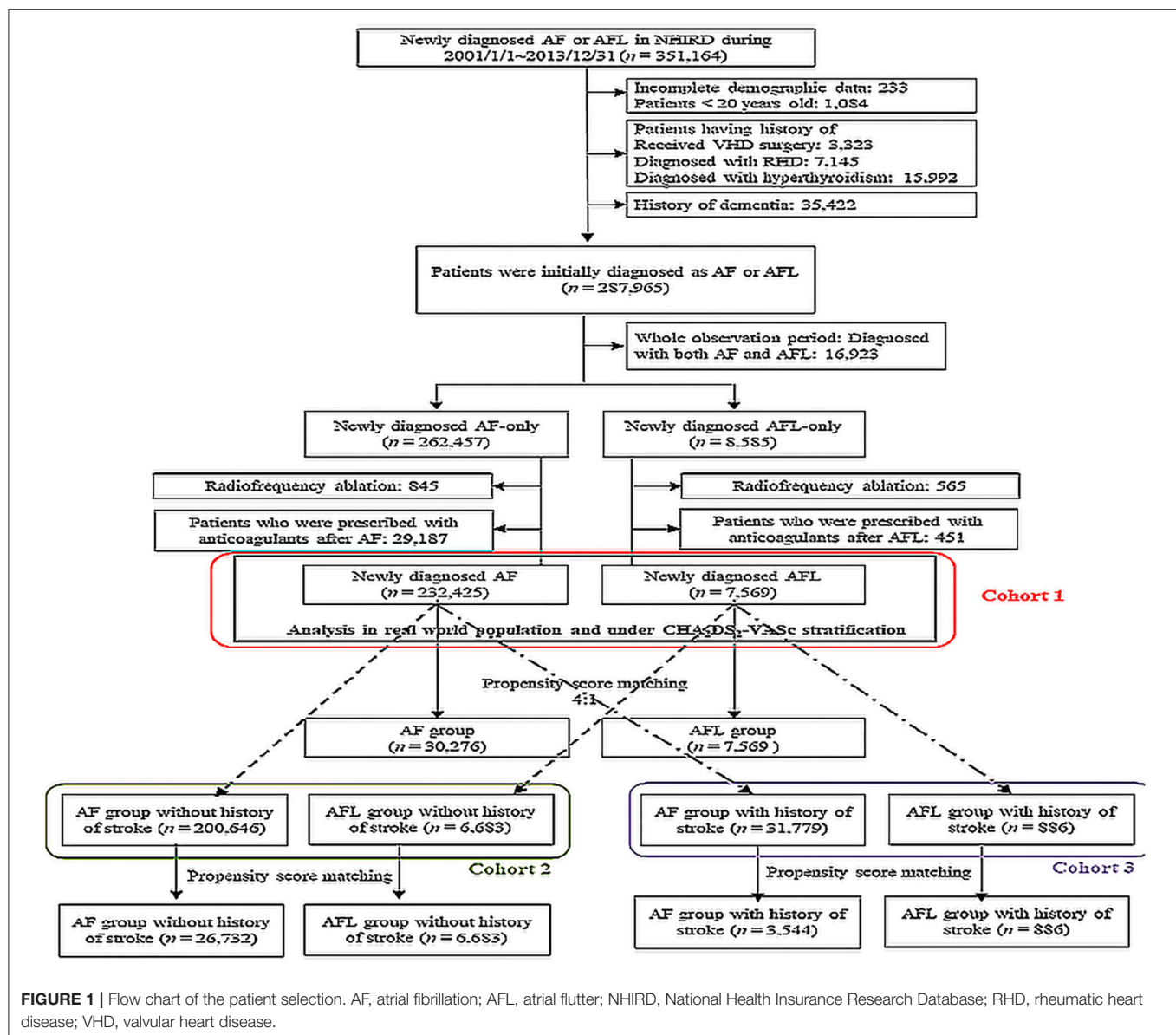


FIGURE 1 | Flow chart of the patient selection. AF, atrial fibrillation; AFL, atrial flutter; NHIRD, National Health Insurance Research Database; RHD, rheumatic heart disease; VHD, valvular heart disease.

according to the ICD-9-CM codes combined with medication use and the diagnostic codes were validated in previous NHIRD studies (12–15).

Statistical Analysis

The propensity score matching was performed with 4:1 ratio in patients with AF and AFL. The covariates in the propensity score calculation were the age, gender, 12 comorbidities, 10 types of medication, and index date. The matching was processed using a greedy nearest neighbor algorithm with a caliper of 0.2 times the SD of the logit of the propensity score. The quality of matching was assessed using the standardized mean difference (SMD) between the two groups after matching, where a value <0.1 indicated a negligible difference (Table 1). The risk of dementia was compared between patients with AF and AFL

before and after PSM. The risk of dementia between patients with AF and AFL was also compared after stratification by CHA₂DS₂-VASc score and previous history of stroke. A $P < 0.05$ was considered statistically significant. No adjustment for multiple testing (multiplicity) was performed in this study. All statistical analyses were performed using commercial software [Statistical Analysis System (SAS) V.9.4], including the “*psmatch*” procedure for PSM, “*phreg*” procedure for survival analysis, and “%*cif*” macro for generating a cumulative incidence function through Fine and Gray’s method.

Patient and Public Involvement

Since this study is a retrospective cohort study based on a national insurance database, in no part or stage of the research were the patients/public involved.

TABLE 1 | Baseline characteristics of the atrial fibrillation and atrial flutter groups before and after propensity score matching.

Variables	All patients			Propensity score matched	
	AFL (n = 7,569)	AF (n = 232,425)	P-value	AF (n = 30,276)	P-value
Age (years; mean \pm SD)	67.41 \pm 6.0	72.21 \pm 3.3	<0.0001	67.31 \pm 5.7	0.6889
Age group			<0.0001		0.725
< 65 years	2,841 (37.5%)	59,556 (25.6%)		11,449 (37.8%)	
65~74 years	1,883 (24.9%)	59,783 (25.7%)		7,399 (24.4%)	
\geq 75 years	2,845 (37.6%)	113,086 (48.7%)		11,428 (37.7%)	
Gender			<0.0001		0.6485
Male	4,689 (62.0%)	131,832 (56.7%)		18,842 (62.2%)	
Female	2,880 (38.0%)	100,593 (43.3%)		11,434 (37.8%)	
Comorbidities					
Hypertension	4,199 (55.5%)	139,437 (60.0%)	<0.0001	16,672 (55.1%)	0.5216
Diabetes Mellitus	1,463 (19.3%)	44,672 (19.2%)	0.813	5,834 (19.3%)	0.9066
Ischemic heart disease	2,546 (33.6%)	86,362 (37.2%)	<0.0001	10,111 (33.4%)	0.6909
Heart failure	901 (11.9%)	29,841 (12.8%)	0.0166	3,539 (11.7%)	0.6037
Dyslipidemia	1,086 (14.3%)	30,650 (13.2%)	0.0033	4,189 (13.8%)	0.25
Gout	753 (9.9%)	24,095 (10.4%)	0.2398	3,037 (10.0%)	0.8305
Chronic obstructive pulmonary disease	1,360 (18.0%)	45,954 (19.8%)	0.0001	5,419 (17.9%)	0.8881
Peripheral arterial disease	321 (4.2%)	11,007 (4.7%)	0.0458	1,242 (4.1%)	0.5875
Renal status			0.0137		0.717
Non-chronic kidney disease	6,384 (84.3%)	198,371 (85.3%)		25,649 (84.7%)	
Chronic kidney disease without dialysis	911 (12.0%)	26,833 (11.5%)		3,549 (11.7%)	
Chronic kidney disease with dialysis	274 (3.6%)	7,221 (3.1%)		1,078 (3.6%)	
Immune disease	147 (1.9%)	3,935 (1.7%)	0.0991	562 (1.9%)	0.6221
Abnormal liver function	904 (11.9%)	26,151 (11.3%)	0.061	3,603 (11.9%)	0.9178
Malignancy	683 (9.0%)	17,722 (7.6%)	<0.0001	2,811 (9.3%)	0.483
History of disease					
Prior stroke or systemic thromboembolism	977 (12.9%)	34,401 (14.8%)	<0.0001	3,928 (13.0%)	0.8784
Prior stroke	886 (11.7%)	31,779 (13.7%)	<0.0001	3,556 (11.7%)	0.9237
Old myocardial infarction	425 (5.6%)	10,307 (4.4%)	<0.0001	1,661 (5.5%)	0.6605
Medications					
ACEi/ARB	2,445 (32.3%)	91,378 (39.3%)	<0.0001	9,795 (32.4%)	0.9343
Calcium channel blockers	1,667 (22.0%)	58,046 (25.0%)	<0.0001	6,672 (22.0%)	0.9802
β -blockers	2,549 (33.7%)	75,683 (32.6%)	0.0418	9,531 (31.5%)	0.0002
Dipeptidyl peptidase 4 inhibitors	153 (2.0%)	4,518 (1.9%)	0.6308	568 (1.9%)	0.4081
Statins	832 (11.0%)	25,522 (11.0%)	0.975	3,095 (10.2%)	0.0496
Biguanides	697 (9.2%)	21,906 (9.4%)	0.5259	2,756 (9.1%)	0.7752
Sulfonylurea	758 (10.0%)	24,341 (10.5%)	0.2	3,004 (9.9%)	0.8099
Thiazolidinedione	106 (1.4%)	3,160 (1.4%)	0.7626	388 (1.3%)	0.415
Insulin	260 (3.4%)	7,366 (3.2%)	0.1944	946 (3.1%)	0.169
Antiplatelet	2,479 (32.8%)	104,861 (45.1%)	<0.0001	9,968 (32.9%)	0.776

ACEi, angiotensin converting enzyme inhibitors; AF, atrial fibrillation; AFL, atrial flutter; ARB, angiotensin II receptor blockers.

RESULTS

Study Population

We identified a total of 351,164 patients newly diagnosed with AF or AFL during 2001–2013 in the NHIRD. After exclusions, 232,425 patients with AF (aged 72.2 \pm 13.3 years) and 7,569 patients with AFL (aged 67.41 \pm 6.0 years) without receiving oral anticoagulants were eligible for analysis in the study cohort 1 (Table 1). The distribution of comorbidities was similar

between the patients with AF and AFL, except there was a higher prevalence of hypertension, ischemic heart disease, and history of thromboembolism/ischemic stroke in patients with AF compared with patients with AFL. After 4:1 ratio of PSM using all variables in Table 1 and there was a good balance between the patients with AF and AFL, except for β -blockers (right panel of Table 1), 30,276 patients with AF (aged 67.3 \pm 15.7 years) and 7,569 patients with AFL were analyzed for comparison of the risk of dementia.

TABLE 2 | Clinical outcomes between the patients with atrial fibrillation and atrial flutter not receiving warfarin therapy before and after PSM.

Variable	Before PSM			After PSM		
	AF (n = 232,425)	AFL (n = 7,569)	P-value	AF (n = 30,276)	AFL (n = 7,569)	P-value
Dementia						
Number of events, n (%)	22,833 (9.82)	521 (6.88)		2,435 (8.04)	521 (6.88)	
Incidence density§	3.04 (3.00–3.08)	2.05 (1.87–2.22)		2.33 (2.24–2.43)	2.05 (1.87–2.22)	
Hazard ratio (95% CI)	1.52 (1.39–1.66)	Reference	<0.0001	1.14 (1.04–1.25)	Reference	0.0064
Ischemic stroke						
Number of events, n (%)	18,475 (7.95)	297 (3.92)		2,095 (6.92)	297 (3.92)	
Incidence density§	2.54 (2.50–2.58)	1.18 (1.04–1.31)		2.07 (1.98–2.16)	1.18 (1.04–1.31)	
Hazard ratio (95% CI)	2.15 (1.92–2.42)	Reference	<0.0001	1.76 (1.56–1.98)	Reference	<0.0001

AF, atrial fibrillation; AFL, atrial flutter; PSM, propensity score matching.

§ Incidence density: number of events per 100 person-years.

Moreover, we further categorized the original 232,425 patients with AF and 7,569 patients with AFL in the cohort 1 into cohorts 2 and 3 according to history without (cohort 2) or with stroke (cohort 3) and performed the additional PSM in each cohort. After PSM, there were 26,732 patients with AF and 6,683 patients with AFL in the cohort 2, and 3,544 patients with AF and 886 patients with AFL in the cohort 3 (Figure 1).

Difference in the Risk of Dementia and Ischemic Stroke Between Patients With AF and AFL Before and After PSM (Cohort 1)

Before PSM, the incidence densities of dementia (hazard ratio [HR], 1.52; 95% CI, 1.39–1.66; $P < 0.0001$) and ischemic stroke (HR, 2.15; 95% CI, 1.92–2.42; $P < 0.0001$) were higher in patients with AF than in patients with AFL (Table 2). After PSM, the incidence densities of dementia (HR, 1.14; 95% CI, 1.04–1.25; $P = 0.0064$) and ischemic stroke (HR, 1.76; 95% CI, 1.56–1.98; $P < 0.0001$) were still higher in patients with AF than in patients with AFL (Table 2). The cumulative incidences of dementia in patients with AF and AFL before and after PSM were shown in Figures 2A,B.

Difference in the Risk of Dementia and Ischemic Stroke Between Patients With AF and AFL Without Previous History of Stroke (Cohort 2)

There were 200,646 patients with AF without previous history of stroke and 6,683 patients with AFL without previous history of stroke (Table 3, Figure 1). After 4:1 PSM, 26,732 patients with AF and 6,683 patients with AFL were well balanced, except for β -blockers (Table 3). Before PSM, the incidence densities of dementia (HR, 1.54; 95% CI, 1.40–1.69; $P < 0.0001$) and ischemic stroke (HR, 2.32; 95% CI, 2.04–2.64; $P < 0.0001$) were higher in patients with AF than in patients with AFL (Table 4). After PSM, the incidence densities of dementia (HR, 1.15; 95% CI, 1.03–1.27; $P = 0.0098$) and ischemic stroke (HR, 1.82; 95% CI, 1.59–2.09; $P < 0.0001$) were higher in patients with AF than in patients with AFL (Table 4).

The cumulative incidences of dementia in patients with AF and AFL without previous history of stroke before and after PSM were shown in Figures 3A,B.

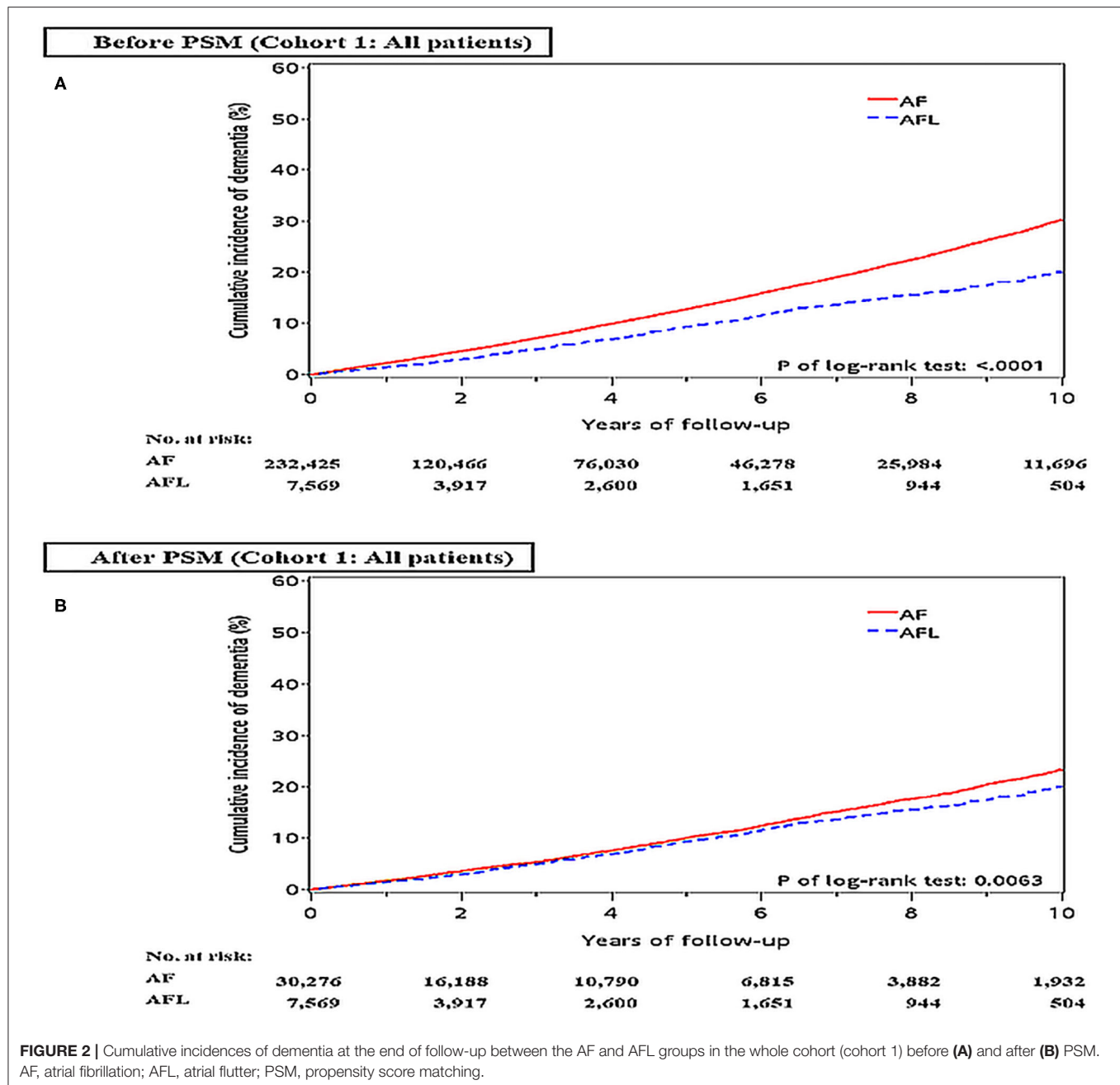
Difference in the Risk of Dementia and Recurrent Ischemic Stroke Between Patients With AF and AFL With Previous History of Stroke (Cohort 3)

There were 31,779 patients with AF with a history of stroke and 886 patients with AFL with a history of stroke (Table 5, Figure 1). After 4:1 PSM, 3,544 patients with AF and 886 patients with AFL were well balanced (Table 5). Before PSM, the incidence densities of dementia (HR, 1.25; 95% CI, 1.02–1.54; $P = 0.0351$) and recurrent ischemic stroke (HR, 1.39; 95% CI, 1.09–1.78; $P = 0.0083$) were higher in patients with AF than in patients with AFL (Table 6). After PSM, the incidence density of dementia did not differ between patients with AF and AFL (HR, 1.16; 95% CI, 0.92–1.45; $P = 0.2058$) (Table 6). However, the incidence density of recurrent ischemic stroke (HR, 1.40; 95% CI, 1.08–1.83; $P = 0.0118$) was higher in patients with AF than in patients with AFL (Table 6).

The cumulative incidences of dementia in patients with AF and AFL with a history of stroke before and after PSM were shown in Figures 3C,D.

The Risk of Dementia Stratified According to CHA₂DS₂-VASc Score Between Patients With AF and AFL (Cohort 1)

The risk of dementia in the cohort 1 was stratified according to CHA₂DS₂-VASc score. Before PSM, the incidence density of dementia was higher in patients with AF than in patients with AFL across CHA₂DS₂-VASc scores of 1–4 and 6–9 (Figure 4). After PSM, the cumulative incidences of dementia in patients with AF and AFL stratified according to different CHA₂DS₂-VASc scores were shown in Figure 5, and the cumulative incidences of dementia were significantly higher in patients with AF than patients with AFL in patients with CHA₂DS₂-VASc score ≤ 2 ($P < 0.0001$) and patients with CHA₂DS₂-VASc score between 3 and 4 ($P = 0.0104$), but not in patients with CHA₂DS₂-VASc score ≥ 5 .



Difference in the Risk of Dementia and Ischemic Stroke Between Patients With AF and AFL Receiving Oral Anticoagulants Therapy Before and After PSM

Because the usage of oral anticoagulant might affect the risk of dementia and ischemic stroke in patients with AF and AFL, we identified and analyzed the risk of dementia and ischemic stroke between patients newly diagnosed with AF ($n = 29,187$) and AFL ($n = 451$) who received oral anticoagulants (mainly vitamin K oral anticoagulant)

in another dataset during the same study period in the NHIRD.

Before PSM, the incidence densities of dementia (HR, 2.05; 95% CI, 1.35–3.11; $P = 0.0008$) and ischemic stroke (HR, 2.95; 95% CI, 1.86–4.62; $P < 0.0001$) were higher in patients with AF than in patients with AFL (Table 7). After PSM, the incidence density of dementia (HR, 1.57; 95% CI, 1.00–2.45; $P = 0.0501$) was higher with borderline significance in patients with AF than in patients with AFL, and the incidence density of ischemic stroke (HR, 2.54; 95% CI, 1.56–4.12; $P = 0.0002$) was significantly higher in patients with AF than in patients with AFL

TABLE 3 | Baseline characteristics of the atrial fibrillation and atrial flutter groups before and after PSM (without history of stroke).

Variables	Without history of stroke patients			Propensity score matched	
	AFL (n = 6,683)	AF (n = 200,646)	P-value	AF (n = 26,732)	P-value
Age (years; mean \pm SD)	66.41 \pm 6.3	71.51 \pm 3.6	<0.0001	66.51 \pm 6.0	0.8808
Age group			<0.0001		0.7103
< 65 years	2,694 (40.3%)	55,494 (27.7%)		10,751 (40.2%)	
65~74 years	1,640 (24.5%)	51,904 (25.9%)		6,457 (24.2%)	
\geq 75 years	2,349 (35.1%)	93,248 (46.5%)		9,524 (35.6%)	
Gender			<0.0001		0.6932
Male	4,130 (61.8%)	114,261 (56.9%)		16,590 (62.1%)	
Female	2,553 (38.2%)	86,385 (43.1%)		10,142 (37.9%)	
Comorbidities					
Hypertension	3,576 (53.5%)	116,639 (58.1%)	<0.0001	14,458 (54.1%)	0.398
Diabetes Mellitus	1,179 (17.6%)	35,701 (17.8%)	0.7504	4,790 (17.9%)	0.5972
Ischemic heart disease	2,168 (32.4%)	72,738 (36.3%)	<0.0001	8,717 (32.6%)	0.7928
Heart failure	705 (10.5%)	22,725 (11.3%)	0.0485	2,883 (10.8%)	0.5778
Dyslipidemia	918 (13.7%)	25,282 (12.6%)	0.006	3,670 (13.7%)	0.9873
Gout	662 (9.9%)	20,528 (10.2%)	0.3879	2,645 (9.9%)	0.9781
Chronic obstructive pulmonary disease	1,134 (17.0%)	37,762 (18.8%)	0.0001	4,489 (16.8%)	0.7311
Peripheral arterial disease	245 (3.7%)	8,668 (4.3%)	0.0095	960 (3.6%)	0.7692
Renal status			0.0191		0.809
Non-chronic kidney disease	5,702 (85.3%)	173,283 (86.4%)		22,725 (85.0%)	
Chronic kidney disease without dialysis	758 (11.3%)	21,634 (10.8%)		3,088 (11.6%)	
Chronic kidney disease with dialysis	223 (3.3%)	5,729 (2.9%)		919 (3.4%)	
Immune disease	132 (2.0%)	3,421 (1.7%)	0.0941	520 (1.9%)	0.8743
Abnormal liver function	812 (12.2%)	22,840 (11.4%)	0.0523	3,299 (12.3%)	0.6711
Malignancy	606 (9.1%)	15,458 (7.7%)	<0.0001	2,422 (9.1%)	0.9848
History of disease					
Prior systemic thromboembolism	91 (1.4%)	2,622 (1.3%)	0.6977	390 (1.5%)	0.5505
Old myocardial infarction	323 (4.8%)	7,614 (3.8%)	<0.0001	1,348 (5.0%)	0.4822
Medications					
ACEi/ARB	2,104 (31.5%)	78,575 (39.2%)	<0.0001	8,499 (31.8%)	0.6257
Calcium channel blockers	1,419 (21.2%)	48,585 (24.2%)	<0.0001	5,858 (21.9%)	0.2278
β -blockers	2,300 (34.4%)	67,200 (33.5%)	0.1155	8,644 (32.3%)	0.0012
Dipeptidyl peptidase 4 inhibitors	129 (1.9%)	3,748 (1.9%)	0.7115	458 (1.7%)	0.2272
Statins	736 (11.0%)	21,750 (10.8%)	0.6545	2,732 (10.2%)	0.0573
Biguanides	594 (8.9%)	18,483 (9.2%)	0.368	2,332 (8.7%)	0.6703
Sulfonylurea	645 (9.7%)	20,391 (10.2%)	0.1732	2,567 (9.6%)	0.904
Thiazolidinedione	95 (1.4%)	2,665 (1.3%)	0.5126	348 (1.3%)	0.4441
Insulin	188 (2.8%)	5,525 (2.8%)	0.77	798 (3.0%)	0.4572
Antiplatelet	2,118 (31.7%)	89,775 (44.7%)	<0.0001	8,524 (31.9%)	0.7601

ACEi, angiotensin converting enzyme inhibitors; AF, atrial fibrillation; AFL, atrial flutter; ARB, angiotensin II receptor blockers.

(Supplemental Table 5). The cumulative incidences of dementia in patients with AF and AFL before and after PSM were shown in **Figures 6A,B** and the cumulative incidences of dementia were significantly higher in patients with AF than in patients with AFL before and after PSM.

DISCUSSIONS

The main findings of this study were (1) among patients without oral anticoagulants, the incidence densities of dementia and

ischemic stroke were significantly higher in patients with AF than in patients with AFL before and after PSM; (2) among patients without oral anticoagulants, the incidence densities of dementia and ischemic stroke were significantly higher in patients with AF than in patients with AFL among patients without previous history of stroke before and after PSM; (3) among patients without oral anticoagulants, the incidence density of dementia did not differ between patients with AF and AFL among patients with previous history of stroke after PSM; (4) among patients without oral anticoagulants, the cumulative incidence

TABLE 4 | Clinical outcomes between the patients with atrial fibrillation and atrial flutter before and after PSM (without history of stroke).

Variable	Before PSM			After PSM		
	AF (n = 200,646)	AFL (n = 6,683)	P-value	AF (n = 26,732)	AFL (n = 6,683)	P-value
Dementia						
Number of events, n (%)	18,693 (9.32)	431 (6.45)		1,983 (7.42)	431 (6.45)	
Incidence density§	2.77 (2.73–2.81)	1.85 (1.67–2.02)		2.11 (2.02–2.20)	1.85 (1.67–2.02)	
Hazard ratio (95% CI)	1.54 (1.40–1.69)	Reference	<0.0001	1.15 (1.03–1.27)	Reference	0.0098
Ischemic stroke						
Number of events, n (%)	15,119 (7.54)	232 (3.47)		1,661 (6.21)	232 (3.47)	
Incidence density§	2.31 (2.28–2.35)	1.00 (0.87–1.13)		1.82 (1.73–1.91)	1.00 (0.87–1.13)	
Hazard ratio (95% CI)	2.32 (2.04–2.64)	Reference	<0.0001	1.82 (1.59–2.09)	Reference	<0.0001

AF, atrial fibrillation; AFL, atrial flutter; PSM, propensity score matching.

§ Incidence density: number of events per 100 person-years.

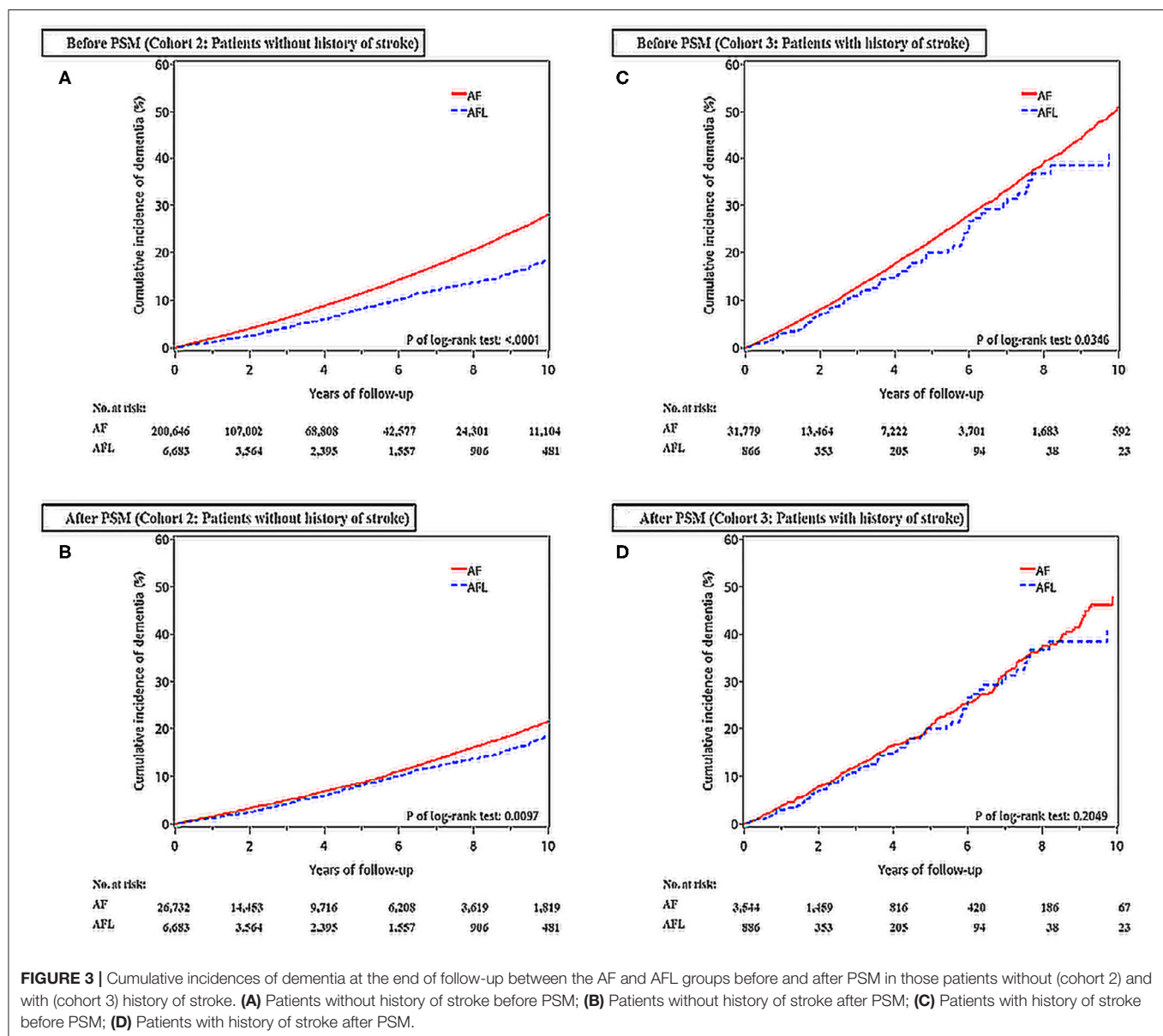
**FIGURE 3 |** Cumulative incidences of dementia at the end of follow-up between the AF and AFL groups before and after PSM in those patients without (cohort 2) and with (cohort 3) history of stroke. (A) Patients without history of stroke before PSM; (B) Patients without history of stroke after PSM; (C) Patients with history of stroke before PSM; (D) Patients with history of stroke after PSM.

TABLE 5 | Baseline characteristics of the atrial fibrillation and atrial flutter groups before and after PSM (with history of stroke).

Variables	With history of stroke patients			Propensity score matched	
	AFL (n = 886)	AF (n = 31,779)	P-value	AF (n = 3,544)	P-value
Age (years; mean ± SD)	75.01 ± 1.1	76.61 ± 0.0	<0.0001	75.01 ± 0.7	0.9391
Age group			0.0001		0.9949
< 65 years	147 (16.6%)	4,062 (12.8%)		589 (16.6%)	
65~74 years	243 (27.4%)	7,879 (24.8%)		966 (27.3%)	
≥ 75 years	496 (56.0%)	19,838 (62.4%)		1,989 (56.1%)	
Gender			<0.0001		0.9504
Male	559 (63.1%)	17,571 (55.3%)		2,232 (63.0%)	
Female	327 (36.9%)	14,208 (44.7%)		1,312 (37.0%)	
Comorbidities					
Hypertension	623 (70.3%)	22,798 (71.7%)	0.3536	2,478 (69.9%)	0.8185
Diabetes Mellitus	284 (32.1%)	8,971 (28.2%)	0.0127	1,167 (32.9%)	0.6197
Ischemic heart disease	378 (42.7%)	13,624 (42.9%)	0.9021	1,523 (43.0%)	0.8674
Heart failure	196 (22.1%)	7,116 (22.4%)	0.849	769 (21.7%)	0.7849
Dyslipidemia	168 (19.0%)	5,368 (16.9%)	0.1053	697 (19.7%)	0.6357
Gout	91 (10.3%)	3,567 (11.2%)	0.3747	360 (10.2%)	0.9208
Chronic obstructive pulmonary disease	226 (25.5%)	8,192 (25.8%)	0.8561	899 (25.4%)	0.9312
Peripheral arterial disease	76 (8.6%)	2,339 (7.4%)	0.1719	302 (8.5%)	0.9571
Renal status			0.233		0.7953
Non-chronic kidney disease	682 (77.0%)	25,088 (78.9%)		2,691 (75.9%)	
Chronic kidney disease without dialysis	153 (17.3%)	5,199 (16.4%)		645 (18.2%)	
Chronic kidney disease with dialysis	51 (5.8%)	1,492 (4.7%)		208 (5.9%)	
Immune disease	15 (1.7%)	514 (1.6%)	0.8604	65 (1.8%)	0.7779
Abnormal liver function	92 (10.4%)	3,311 (10.4%)	0.9731	345 (9.7%)	0.5623
Malignancy	77 (8.7%)	2,264 (7.1%)	0.0746	301 (8.5%)	0.8507
History of disease					
Old myocardial infarction	102 (11.5%)	2,693 (8.5%)	0.0014	423 (11.9%)	0.7274
Medications					
ACEi/ARB	341 (38.5%)	12,803 (40.3%)	0.2812	1,319 (37.2%)	0.4849
Calcium channel blockers	248 (28%)	9,461 (29.8%)	0.2528	1,039 (29.3%)	0.4367
β-blockers	249 (28.1%)	8,483 (26.7%)	0.3496	932 (26.3%)	0.2769
Dipeptidyl peptidase 4 inhibitors	24 (2.7%)	770 (2.4%)	0.5858	106 (3.0%)	0.6562
Statins	96 (10.8%)	3,772 (11.9%)	0.3473	460 (13.0%)	0.0848
Biguanides	103 (11.6%)	3,423 (10.8%)	0.4191	422 (11.9%)	0.8162
Sulfonylurea	113 (12.8%)	3,950 (12.4%)	0.7729	532 (15.0%)	0.0884
Thiazolidinedione	11 (1.2%)	495 (1.6%)	0.4524	71 (2.0%)	0.1324
Insulin	72 (8.1%)	1,841 (5.8%)	0.0035	234 (6.6%)	0.1097
Antiplatelet	361 (40.7%)	15,086 (47.5%)	<0.0001	1,461 (41.2%)	0.7952

ACEi, angiotensin converting enzyme inhibitors; AF, atrial fibrillation; AFL, atrial flutter; ARB, angiotensin II receptor blockers.

of dementia was significantly higher in patients with AF than patients with AFL only in patients with CHA₂DS₂-VASc score ≤4; (5) among patients who received oral anticoagulants, the cumulative incidences of dementia were significantly higher in patients with AF than in patients with AFL before and after PSM.

Atrial fibrillation provides five-fold increase in the risk of stroke and AF is associated with more severe ischemic strokes than emboli from carotid disease (18, 22). It has been reported that AF is associated with cognitive dysfunction and dementia, and may increase the risk of developing dementia (4, 23–26). Several pathophysiologic mechanisms have been proposed to

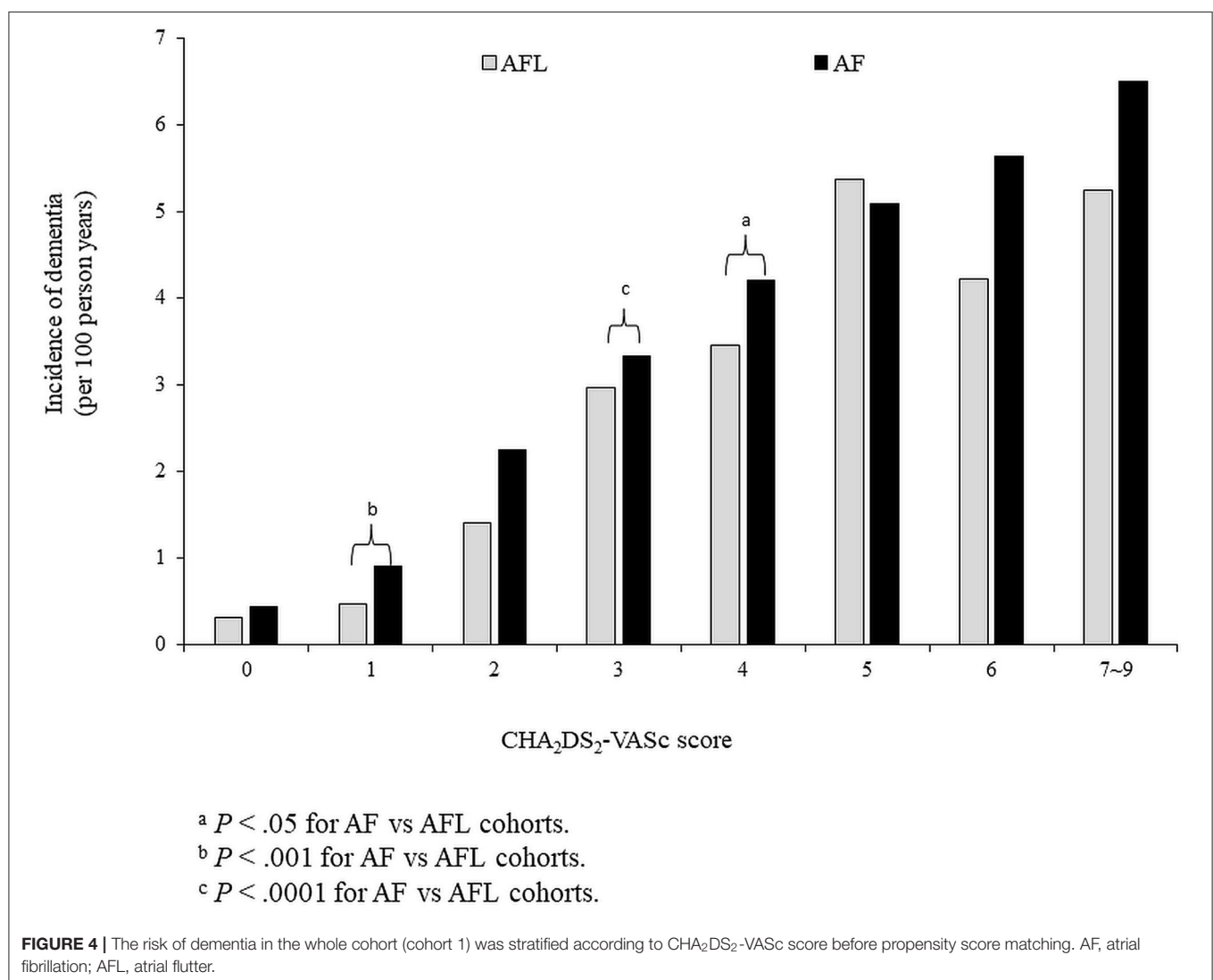
explain the complex association between AF and dementia, which include cerebral hypoperfusion, vascular inflammation, cerebral small vessel disease, microbleeds, brain atrophy, and AF-related ischemic stroke (3, 5, 23, 27, 28). AF and AFL share some common risk factors for cognitive dysfunction or dementia. When more cardiovascular disease risks coexist, the risk of dementia in patients with AF will increase significantly. CHA₂DS₂-VASc score has been reported to be useful for risk stratification regarding ischemic stroke and dementia among patients with AF and AFL (18–21, 29). Study conducted by Chou et al. reported that CHADS₂ score had a good prediction

TABLE 6 | Clinical outcomes between the patients with atrial fibrillation and atrial flutter before and after PSM (with history of stroke).

Variable	Before PSM			After PSM		
	AF (n = 31,779)	AFL (n = 886)	P-value	AF (n = 3,544)	AFL (n = 886)	P-value
Dementia						
Number of events, n (%)	4,140 (13.03)	90 (10.16)		427 (12.05)	90 (10.16)	
Incidence density§	5.33 (5.17–5.49)	4.31 (3.42–5.20)		4.97 (4.50–5.44)	4.31 (3.42–5.20)	
Hazard ratio (95% CI)	1.25 (1.02–1.54)	Reference	0.0351	1.16 (0.92–1.45)	Reference	0.2058
Ischemic stroke						
Number of events, n (%)	3,356 (10.56)	65 (7.34)		368 (10.38)	65 (7.34)	
Incidence density§	4.59 (4.43–4.74)	3.27 (2.48–4.07)		4.60 (4.13–5.07)	3.27 (2.48–4.07)	
Hazard ratio (95% CI)	1.39 (1.09–1.78)	Reference	0.0083	1.40 (1.08–1.83)	Reference	0.0118

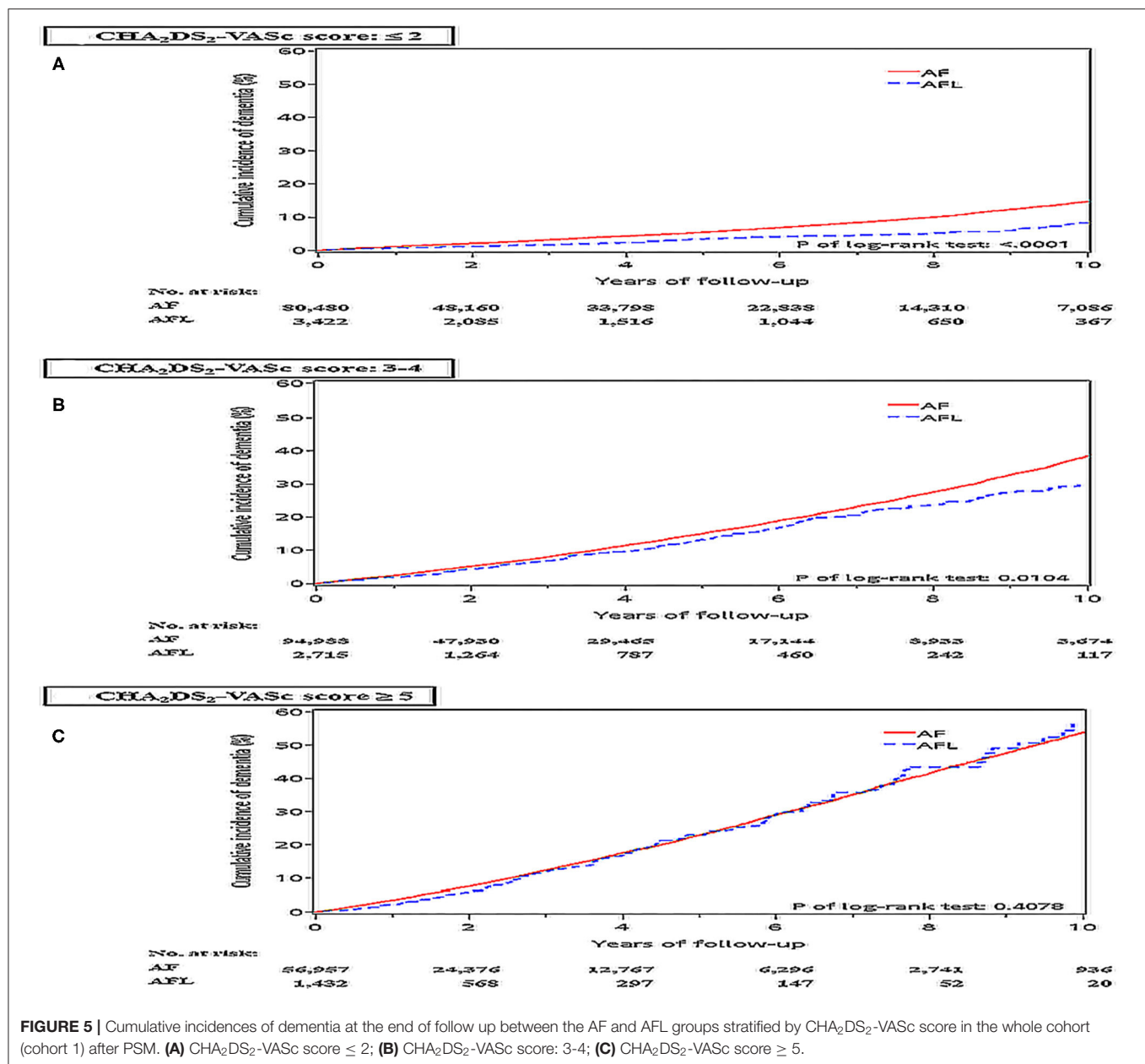
AF, atrial fibrillation; AFL, atrial flutter; PSM, propensity score matching.

§ Incidence density: number of events per 100 person-years.



of vascular dementia in patients with AF and AFL (3). The higher the score, the higher the number of patients suffering from dementia. CHA₂DS₂-VASc score is composed of chronic

diseases and also a history of stroke. Therefore, not only AF or AFL alone might contribute to the development of dementia, but all the comorbidities do. In this study, we also showed that



the risk of dementia is higher in both AF and AFL cohort with higher CHA₂DS₂-VASc scores. Based on our and other studies, the CHA₂DS₂-VASc score might be a useful risk scoring system for predicting future risk of development of vascular dementia and for risk stratification in patients with AF and AFL at risk of developing dementia (18). In this study, patients with AF were older than patients with AFL and had more comorbidities, including prior stroke, hypertension, ischemic heart disease, heart failure, and a higher average CHA₂DS₂-VASc score. All of these differences may contribute to a higher incidence of dementia in patients with AF than in patients with AFL before adjusting the comorbidities in this study. However, the incidence of dementia is still higher in patients with AF than in patients

with AFL even after adjusting the comorbidities and medications by PSM, especially in those patients without previous history of stroke. Therefore, differential risk of dementia existed between AF and AFL patients without previous history of stroke.

Although AF and AFL share some common risk factors and can both be risk stratified by CHA₂DS₂-VASc score, the pathophysiological mechanisms of AF and AFL are different. The initiation and maintenance of AF usually involved the pulmonary veins and depended on multiple chaotic rotors in the arrhythmogenic substrate in the left or right atrium for maintenance. In contrast, the maintenance of AFL depended on a single reentry circuit, such as single reentry circuit in the right atrium of a typical AFL. Our previous study showed that the

TABLE 7 | Clinical outcomes between the patients with atrial fibrillation and atrial flutter who received oral anticoagulants before and after PSM.

Variable	Before PSM			After PSM		
	AF (n = 29,187)	AFL (n = 451)	P-value	AF (n = 1,840)	AFL (n = 451)	P-value
Dementia						
Number of events, n (%)	2,830 (9.70)	22 (4.88)		144 (7.98)	22 (4.88)	
Incidence density§	2.81 (2.71–2.91)	1.39 (0.81–1.97)		2.20 (1.84–2.56)	1.39 (0.81–1.97)	
Hazard ratio (95% CI)	2.05 (1.35–3.11)	Reference	0.0008	1.57 (1.00–2.45)	Reference	0.0501
Ischemic stroke						
Number of events, n (%)	3,255 (11.15)	18 (3.99)		181 (10.03)	18 (3.99)	
Incidence density§	3.43 (3.31–3.54)	1.16 (0.62–1.70)		2.95 (2.52–3.38)	1.16 (0.62–1.70)	
Hazard ratio (95% CI)	2.95 (1.86–4.62)	Reference	<0.0001	2.54 (1.56–4.12)	Reference	0.0002

AF, atrial fibrillation; AFL, atrial flutter; PSM, propensity score matching.

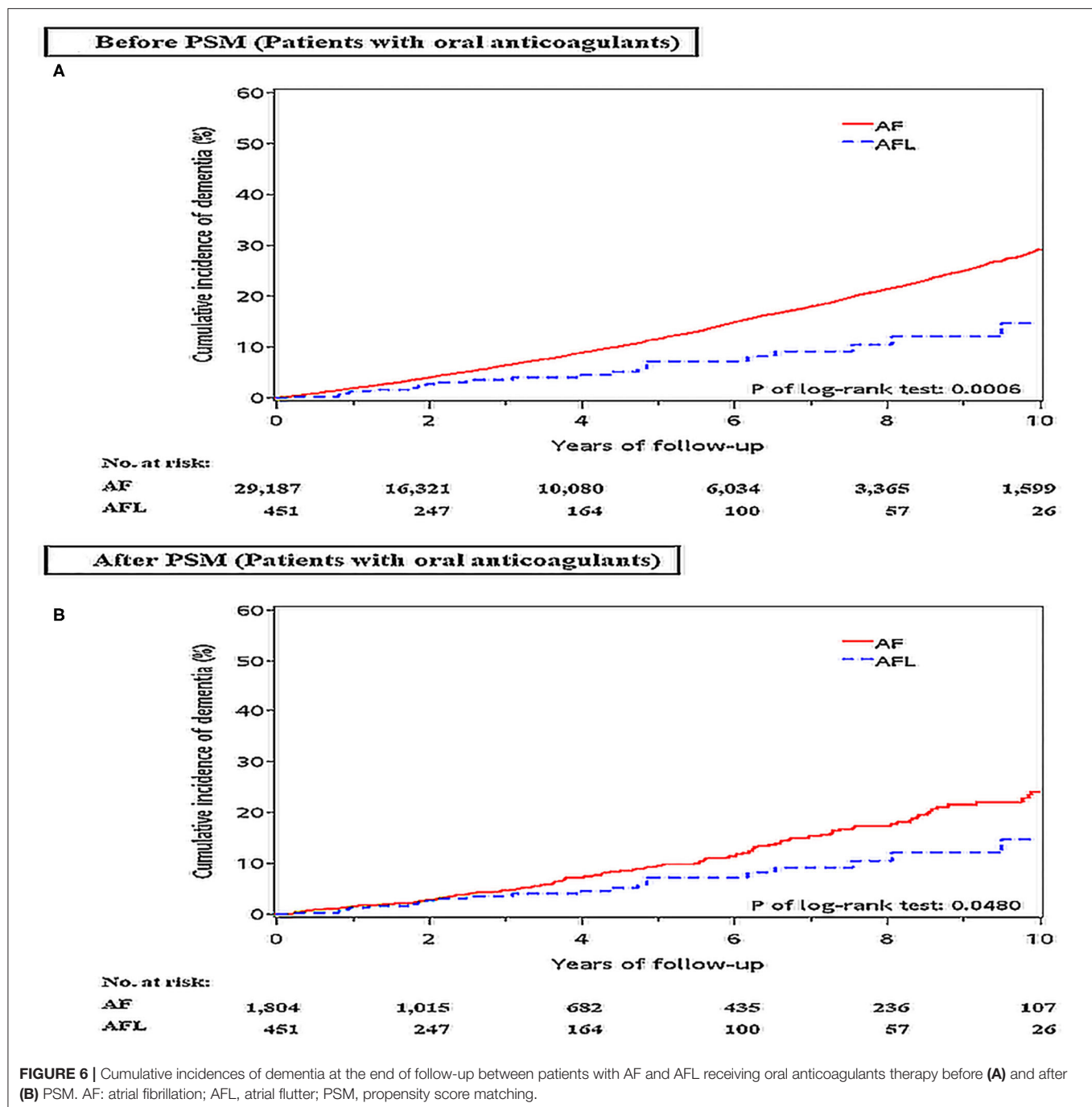
§ Incidence density: number of events per 100 person-years.

incidences of ischemic stroke, hospitalization for heart failure, and all-cause mortality are different between AF and AFL, which might be related to the different pathophysiological mechanisms between AF and AFL (8, 30). Although the risk of dementia is higher in patients with AF than in patients with AFL in this present study, further studies should be conducted to explore the pathophysiologic mechanisms of differential risk of dementia between patients with AF and AFL.

The previous studies showed that there were conflicting data regarding the therapeutic effect of oral anticoagulants on the risk of dementia in patients with AF (31). Although oral anticoagulants might decrease the risk of dementia by reducing the risk of silent infarct and microembolism, microhemorrhages, especially due to supratherapeutic range of anticoagulation, may have implications for the mechanism of dementia (32, 33). In contrast, non-vitamin K oral anticoagulants may significantly reduce the occurrence of dementia (33–35). This may explain the discrepancy of oral anticoagulants on the risk of dementia in patients with AF. Our studies showed that among patients who received oral anticoagulants (mainly vitamin K oral anticoagulant), the cumulative incidence of dementia was significantly higher in patients with AF than in patients with AFL. Accordingly, the usage of oral anticoagulants may not affect the differential risk of dementia between patients with AF and AFL. Further prospective randomized studies are warranted to validate our findings and to investigate the effect of different oral anticoagulants on the risk of dementia in patients with AF and AFL.

Several limitations are associated with epidemiologic data from the NHIRD. First, using ICD-9-CM codes for patient selection may result in some missing cases due to incorrect coding. Some patients who had dementia with indistinct symptoms may be also missed due to the unavailability of clinical characteristics or image studies. Second, certain misclassification of disease leading to the miscalculation of the CHA₂DS₂-VASc score may have occurred due to the retrospective nature of the study. However, the highly diagnostic accuracy of comorbidities in CHA₂DS₂-VASc score has been validated in previous studies. Third, we could not categorize the mechanisms of AFL into

typical or atypical AFL and right or left AFL by ICD-9-CM codes. The clinical outcome may be different between patients with typical and atypical AFL and also between patients with left and right AFL. Further investigation should be conducted to clarify this issue. Fourth, AF was developed in around 30% AFL during follow-up according to previous studies (36). Although we have excluded those patients who have concomitant AF and AFL, there is still the possibility that some patients with AFL who developed AF during follow-up might not be diagnosed in the clinical follow-up. Despite this concern, we showed that the incidence density of dementia was significantly higher in patients with AF than in patients with AFL without previous history of stroke. Fifth, this study showed that among patients who received oral anticoagulants (mainly vitamin K oral anticoagulants), the cumulative incidence of dementia was significantly higher in patients with AF than in patients with AFL. However, this study did not analyze the effect of non-vitamin K oral anticoagulants on the risk of dementia in patients with AF and AFL. A previous study showed that non-vitamin K oral anticoagulants may lower the risk of dementia in patients with AF (33–35). Further prospective randomized studies are warranted to investigate the effect of different non-vitamin K oral anticoagulants on the risk of dementia in patients with AF and AFL. Sixth, although HAS-BLED score (hypertension, abnormal renal/liver function, stroke, bleeding history or predisposition, labile international normalized ratio, elderly [age ≥65 years], drugs/alcohol concomitantly) and CHA₂DS₂-VASc score share many common risk factors, in a real-world population with AF, the CHA₂DS₂-VASc and HAS-BLED risk classifications are correlated but not exchangeable (37). Future research should be conducted to investigate the risk of dementia stratified according to HAS-BLED score alone or combined CHA₂DS₂-VASc score and HAS-BLED score between patients with AF and AFL. Seventh, although this study used PSM with all known variables to achieve a good balance between patients with AF and AFL, unavailable confounding risk factors might be still present. Finally, this study did not subclassify all subtypes of dementia. There might be a difference in the risk of developing different subtypes of dementia between



AF and AFL. Further studies could be conducted to clarify this issue.

CONCLUSIONS

This large nationwide cohort study showed that among patients without oral anticoagulants, the risk of dementia was higher in patients with AF than in patients with AFL. Among patients without oral anticoagulants, the risk of dementia was higher in patients with AF than in patients with AFL

with CHA₂DS₂-VASc score ≤ 4 . Among patients without oral anticoagulants, the risk of dementia was higher in patients with AF than in patients with AFL among patients without previous history of stroke before and after PSM but the risk did not differ between patients with AF and AFL among patients with a previous history of stroke after PSM. Among patients who received oral anticoagulants, the cumulative incidences of dementia were significantly higher in patients with AF than in patients with AFL before and after PSM. Our study implicated that CHA₂DS₂-VASc score might be useful

for risk stratification of dementia between patients with AF and AFL.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board of Chang Gung Memorial Hospital. Written informed consent for participation

was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

C-MC and M-CC led in the conception and design of the study, revised the draft of the manuscript, and supervised and validated the clinical work and results. H-TW, Y-LC, and Y-SL collected research data, prepared the draft of the manuscript, performed the statistical analysis, and drafted the manuscript. H-CC, S-ZC, and SH organized the collected data. All authors have read and agreed to the published version of the manuscript.

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Diagnostic Accuracy of the HAS-BLED Bleeding Score in VKA- or DOAC-Treated Patients With Atrial Fibrillation: A Systematic Review and Meta-Analysis

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Background: Several bleeding risk assessment models have been developed in atrial fibrillation (AF) patients with oral anticoagulants, but the most appropriate tool for predicting bleeding remains uncertain. Therefore, we aimed to assess the diagnostic accuracy of the Hypertension, Abnormal liver/renal function, Stroke, Bleeding history or predisposition, Labile international normalized ratio, Elderly, Drugs/alcohol concomitantly (HAS-BLED) score compared with other risk scores in anticoagulated patients with AF.

Methods: We comprehensively searched the PubMed and Embase databases until July 2021 to identify relevant pieces of literature. The predictive abilities of risk scores were fully assessed by the C-statistic, net reclassification improvement (NRI) and integrated discrimination improvement (IDI) values, calibration data, and decision curve analyses.

Results: A total of 39 studies met the inclusion criteria. The C-statistic of the HAS-BLED score for predicting major bleeding was 0.63 (0.61–0.65) in anticoagulated patients regardless of vitamin K antagonists [0.63 (0.61–0.65)] and direct oral anticoagulants [0.63 (0.59–0.67)]. The HAS-BLED had the similar C-statistic to the Hepatic or renal disease, Ethanol abuse, Malignancy, Older, Reduced platelet count or function, Re-bleeding risk, Hypertension (uncontrolled), Anemia, Genetic factors, Excessive fall risk, Stroke (HEMORR₂HAGES), the Anticoagulation and Risk Factors in Atrial Fibrillation (ATRIA), the Outcomes Registry for Better Informed Treatment of Atrial Fibrillation (ORBIT), the Global Anticoagulant Registry in the FIELD-Atrial Fibrillation (GARFIELD-AF), or the Age, Biomarkers, Clinical History (ABC) scores, but significantly higher C-statistic than the Congestive heart failure, Hypertension, Age ≥ 75 years, Diabetes mellitus, Stroke/transient ischemic attack history (CHADS₂) or the Congestive heart failure/left ventricular ejection fraction $\leq 40\%$, Hypertension, Age ≥ 75 years, Diabetes mellitus, Stroke/transient ischemic attack/thromboembolism history, Vascular disease, Age 65–74 years, Sex (female) (CHA₂DS₂-VASc) scores. NRI and IDI values suggested that the HAS-BLED score performed better than the CHADS₂ or the CHA₂DS₂-VASc scores and

had similar or superior predictive ability compared with the HEMORR₂HAGES, the ATRIA, the ORBIT, or the GARFIELD-AF scores. Calibration and decision curve analyses of the HAS-BLED score compared with other scores required further assessment due to the limited evidence.

Conclusion: The HAS-BLED score has moderate predictive abilities for bleeding risks in patients with AF regardless of type of oral anticoagulants. Current evidence support that the HAS-BLED score is at least non-inferior to the HEMORR₂HAGES, the ATRIA, the ORBIT, the GARFIELD-AF, the CHADS₂, the CHA₂DS₂-VASc, or the ABC scores.

Keywords: HAS-BLED, major bleeding, risk prediction, atrial fibrillation, meta-analysis

INTRODUCTION

Atrial fibrillation (AF), the most common cardiac arrhythmia in clinical practice, is associated with a 5-fold risk of ischemic stroke. Oral anticoagulation (OAC) is recommended to reduce the risk of thromboembolism among AF patients with the Congestive heart failure/left ventricular ejection fraction $\leq 40\%$, Hypertension, Age ≥ 75 years, Diabetes mellitus, Stroke/transient ischemic attack/thromboembolism history, Vascular disease, Age 65–74 years, Sex (female) (CHA₂DS₂-VASc) score of ≥ 2 in males or ≥ 3 in females (1). However, the use of OAC could increase the bleeding risks, especially major bleeding and intracranial bleeding, which are associated with increased rates of cardiovascular adverse events and death (2). Therefore, the risk assessment of bleeding after the initiation of OAC should be taken into consideration. The Hypertension, Abnormal liver/renal function, Stroke, Bleeding history or predisposition, Labile international normalized ratio, Elderly, Drugs/alcohol concomitantly (HAS-BLED) score is originated from the European Heart Survey database in 2010 (3), mainly focusing on the modifiable bleeding risk factors. The HAS-BLED score has been routinely recommended for predicting the bleeding risks in patients with AF who are taking anticoagulation (1).

In addition to the HAS-BLED score, several other bleeding risk assessment models have been developed in patients with AF (4–6). However, the differences in the predictive ability of the HAS-BLED score compared with other risk scores remain uncertain. Chang et al. (5) performed a network meta-analysis of 18 studies involving 321,888 patients and found that the HAS-BLED score was the most balanced bleeding risk prediction tool regarding sensitivity and specificity. Nevertheless, the sensitivity and specificity have limited guidance to clinicians when considering the probability of bleeding events in patients with AF (7). Zhu et al. (4) performed a meta-analysis including more critical measures (i.e., the C-statistic, reclassification, and calibration data), suggesting that the HAS-BLED score performed better for predicting major bleeding than other risk scores including the Hepatic or renal disease, Ethanol abuse, Malignancy, Older, Reduced platelet count or function, Re-bleeding risk, Hypertension (uncontrolled), Anemia, Genetic factors, Excessive fall risk, Stroke (HEMORR₂HAGES), the Anticoagulation and Risk Factors in Atrial Fibrillation (ATRIA), the Congestive heart failure, Hypertension, Age ≥ 75 years,

Diabetes mellitus, Stroke/transient ischemic attack history (CHADS₂), and the CHA₂DS₂-VASc scores. However, in this study, they assessed the HAS-BLED score only in patients with AF with the use of vitamin K antagonists (VKAs). Since direct oral anticoagulants (DOACs) including dabigatran, rivaroxaban, apixaban, and edoxaban are recommended as the preferred drugs for stroke prevention among patients with non-valvular AF (1, 8), whether the application of the HAS-BLED score could be extended to DOAC-treated patients is still unclear. Therefore, this systematic review and meta-analysis aimed to: (1) assess the diagnostic accuracy of the HAS-BLED score in anticoagulated (VKAs or DOACs) patients with AF and (2) compared the performances of the HAS-BLED score with other risk scores to determine the most appropriate tool for predicting bleeding risks.

METHODS

This meta-analysis and systematic review were carried out based on the Cochrane Handbook for systemic reviews. The results were presented according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement. Ethical approval was not necessary because the published studies of electronic databases were included.

Literature Search

We comprehensively conducted a search of the PubMed and Embase electronic databases until July 2021 to identify relevant pieces of literature reporting the HAS-BLED score in anticoagulated patients with AF. The following keywords in the search strategies were used: (1) AF, (2) VKAs or warfarin or coumadin or phenprocoumon or acenocoumarol or indandione or fluindione or phenindione or anisindione or non-VKAs or DOACs or dabigatran or rivaroxaban or apixaban or edoxaban, and (3) the HAS-BLED score. To obtain the qualified studies comprehensively, we also performed the cross-reference retrieval by screening the reference lists of included studies and prior meta-analyses. English language restrictions in the literature research were applied in this study.

Eligibility Criteria

Studies were included if they met the following inclusion criteria: (a) patients with non-valvular AF (aged ≥ 18 years) with anticoagulants (VKAs or DOACs); (b) *post-hoc* analyses

of randomized controlled trials (RCTs) or observational (prospective or retrospective cohorts) studies reported the diagnostic performance of the HAS-BLED score or focused on the predictive ability of the HAS-BLED score compared with any of other risk scores including the HEMORR₂HAGES, the ATRIA, the ORBIT, the CHADS₂, the CHA₂DS₂-VASc, the Global Anticoagulant Registry in the FIELD-Atrial Fibrillation (GARFIELD-AF), or Age, Biomarkers, Clinical History (ABC) (**Supplementary Table 1**); (c) the primary outcome was major bleeding and other bleeding events included any clinically relevant bleeding, any bleeding, intracranial bleeding, and gastrointestinal bleeding; and (d) at least one of the following data should be available: discrimination analysis (sensitivity/specificity or the C-statistic), net reclassification improvement (NRI) and integrated discrimination improvement (IDI) analyses, calibration data, and decision curve analyses. The sensitivity and specificity of the risk models have been analyzed by Chang et al. (5) in 2020 and, thus, were not included in this study.

We excluded studies that focused on the non-AF population or patients with AF with specific diseases (e.g., myocardial infarction, dialysis, ischemic stroke). In addition, we also excluded studies limiting to patients with AF with certain interventions (e.g., percutaneous coronary intervention, ablation). The bleeding risk prediction tools [e.g., the Rutherford score (9), mOBRI@@ (10), Adam score (11)] compared with the HAS-BLED score were not included, if they were analyzed for one bleeding endpoint in less than two independent studies. We also excluded studies reporting the modified HAS-BLED score version by adding additional factors (e.g., biomarkers, gene polymorphisms) into the original HAS-BLED score. Certain publication types with insufficient data such as reviews, case reports, editorials, or meeting abstracts were excluded.

Study Selection and Data Extraction

Potentially relevant studies were selected by two reviewers independently based on the predetermined criteria. Qualified articles were included after the title/abstract screenings and the full-text screenings. At this step, if two or more studies had the same data source, we selected the study that was more designed to meet our inclusion criteria. If both the studies meet the inclusion criteria, we selected the newly published study or the study with the longest follow-up or highest sample size. Disagreements were resolved through discussion between the two researchers or consultation with a third reviewer.

Data from included studies were extracted by two researchers independently. We abstracted the following baseline information including the authors, year of publication, study type, data source, demographic data, baseline characteristics of the patient [age, sex ratio, sample size, type of anticoagulants, concomitant antiplatelet drugs, or non-steroidal anti-inflammatory drugs (NSAIDs)], study endpoints and their definitions, and the follow-up time.

Quality Assessment

We applied the Prediction Model Risk of Bias Assessment Tool (PROBAST) (www.probast.org) to assess the risk prediction

models (12). The PROBAST consists of four domains, namely participants, predictors, outcomes, and analysis. Risk assessment was rated as low risk, high risk, or unclear.

Consistency Test and Publication Bias

The consistency of the included studies was assessed through the Cochrane Q test and I^2 index. Significant heterogeneity was considered if the p -value of the Cochrane Q test <0.1 or if the I^2 value of $>50\%$. We used the funnel plots to examine the publication bias and a visual inspection of asymmetry indicated a bias.

Statistical Analysis

All the statistical analyses were carried out by using the Review Manager 5.3 software (The Nordic Cochrane Center, The Cochrane Collaboration, 2014, Copenhagen, Denmark, UK) (<https://community.cochrane.org/>). $p < 0.05$ was considered as statistically significant.

The C-statistic and their 95% CIs were abstracted from the included studies for the discrimination analysis. The C-statistic of ≤ 0.5 indicated that discrimination was no better than chance. The pooled analyses were performed if at least two studies reported the C-statistic for the HAS-BLED score. A random-effects model with an inverse variance method was chosen in the pooled analysis due to the observed significant heterogeneity. For the primary major bleeding events, the subgroup analyses were conducted on the basis of study design, the OAC type, and the follow-up time. We also assessed the predictive ability of the HAS-BLED based on available vs. unavailable labile international normalized ratio (INR) in the score.

The Z-statistic was calculated to compare the two C-statistic of the HAS-BLED score vs. other risk prediction models (the HEMORR₂HAGES, the ATRIA, the ORBIT, the CHADS₂, the CHA₂DS₂-VASc, the GARFIELD-AF, or the ABC scores) (4). In addition, we assessed the improvement in predictive accuracy by the reclassification analyses including the NRI and IDI parameters. The probability of correctly predicting bleeding events by using the HAS-BLED score was reflected in the percentage of events correctly reclassified. Calibration data represented the extent to which predicted risks correspond to observed risks. The net benefits of the HAS-BLED vs. other risk scores were assessed by using the decision curve analyses. Narrative analyses were performed with respect to reclassification, calibration, and decision curve analyses due to the lack of numerical data.

RESULTS

Study Selection

The flowchart of document retrieval and screening process in this meta-analysis is shown in **Supplementary Figure 1**. We initially retrieved 1,601 studies through the electronic search of the PubMed and the Embase databases. After the screenings of the titles and abstracts, 97 studies were assessed for more detail. Furthermore, 58 of these studies were excluded because: (1) patients with OACs were not analyzed separately ($n = 5$); (2) duplicate data ($n = 9$); (3) the anticoagulated drugs were not

VKAs or DOACs or unknown OAC data ($n = 8$); (4) studies did not report the C-statistic and/or their CIs ($n = 8$); (5) non-AF population or AF patients with coexisting specific diseases ($n = 25$); and (6) the outcome of bleeding was not analyzed separately ($n = 3$). Finally, a total of 39 studies published from 2010 to 2021 met the inclusion criteria and were included in this study (**Supplementary Table 2**).

The baseline characteristics of patient of the included studies are summarized in **Table 1**. The variables of the HAS-BLED score in the included studies are presented in **Supplementary Table 3**. The component of “labile INR” in the HAS-BLED score was available in 10 included studies. “Labile INR” was not applicable in three studies (13–15) because they only included DOAC-treated patients for analysis. As shown in **Supplementary Table 4**, all the included studies had high ($n = 20$, 51%) or unclear ($n = 19$, 49%) risk of bias according to the PROBAST tool.

Diagnostic Accuracy of the HAS-BLED Score

In anticoagulated patients with AF, the C-statistic for the HAS-BLED score ranged from 0.56 to 0.80 for major bleeding (median 0.62), 0.53 to 0.62 for any clinically relevant bleeding (median 0.58), 0.51 to 0.64 for any bleeding (median 0.57), 0.53 to 0.64 for intracranial bleeding (median 0.57), and 0.61 to 0.74 for gastrointestinal bleeding (median 0.68). In the pooled analysis, the C-statistic for major bleeding, any clinically relevant bleeding, any bleeding, intracranial bleeding, and gastrointestinal bleeding were 0.63 (0.61–0.65), 0.58 (0.56–0.61), 0.57 (0.53–0.61), 0.58 (0.53–0.62), and 0.67 (0.55–0.82), respectively (**Figure 1**; **Supplementary Figure 2**). For this part, there were no potential publication biases when inspecting the funnel plots (**Supplementary Figure 3**).

For the risk of major bleeding, in the subgroup analysis based on the OAC type shown in **Figure 2**, the C-statistic for major bleeding in four subgroups of mixed DOACs or VKAs, DOACs, VKAs, and warfarin were 0.62 (0.61–0.63), 0.63 (0.61–0.65), 0.63 (0.59–0.67), and 0.61 (0.58–0.64), respectively ($P_{\text{interaction}} = 0.58$). The subgroup analysis based on study design (*post-hoc* analysis of RCT, prospective cohort, and retrospective cohort) indicated no interaction between them ($P_{\text{interaction}} = 0.14$; **Supplementary Figure 4**). In addition, the predictive ability for major bleeding was also similar between available vs. unavailable labile INR in the HAS-BLED score ($P_{\text{interaction}} = 0.19$; **Supplementary Figure 5**). However, there was a difference in the subgroup analysis based on the follow-up time, suggesting that the HAS-BLED score performed better in the group of ≤ 12 months compared with that of > 12 months ($P_{\text{interaction}} = 0.01$; **Supplementary Figure 6**).

Performances of the HAS-BLED Score With Other Risk Scores

Discrimination Analysis

As shown in **Table 2**, for comparisons of the C-statistic between two different risk scores, there were no statistically significant differences between the HAS-BLED vs. the HEMORR₂HAGES

scores (major bleeding: Z-statistic = 0.396; any clinically relevant bleeding: 0.321; intracranial bleeding: -0.408); vs. the ORBIT (major bleeding: -0.911 ; any clinically relevant bleeding: 0; intracranial bleeding: -0.158); vs. the ATRIA (major bleeding -0.502 ; any clinically relevant bleeding: 0.257; intracranial bleeding: 0); vs. the GARFIELD-AF (major bleeding: -0.448); or vs. the ABC scores (major bleeding: -1.09) ($p > 0.05$), suggesting similar discrimination performances. However, the HAS-BLED score had significantly higher C-statistic for predicting major bleeding than the CHADS₂ (Z-statistic = 2.19, $p < 0.05$) or the CHA₂DS₂-VASc scores (Z-statistic = 1.99, $p < 0.05$), suggesting that the HAS-BLED score performed better than the CHADS₂ or CHA₂DS₂-VASc scores.

Reclassification Analysis

As presented in **Table 3**, the HAS-BLED score for predicting major bleeding had both the significantly positive NRI and IDI values compared with the CHADS₂ (16–18) or the CHA₂DS₂-VASc scores (13, 16, 17), suggesting that the predictive ability of the HAS-BLED score was more dominant than the CHADS₂ or the CHA₂DS₂-VASc scores. The HAS-BLED score compared with the HEMORR₂HAGES (19–22), the ATRIA (16, 19–23), or the ORBIT score (20–22) had positive NRI and IDI values, although non-significant in some studies (19, 21). Only one study of Jaspers Focks et al. (24) reported non-significant negative NRI values between the HAS-BLED vs. the HEMORR₂HAGES or the ATRIA scores. In the study of Proietti et al. (25), the GARFIELD-AF compared with the HAS-BLED scores had both non-significant negative NRI and IDI values. Overall, the HAS-BLED score had at least non-inferior predictive ability for predicting major bleeding compared with the HEMORR₂HAGES, the ATRIA, the ORBIT, or the GARFIELD-AF scores.

The NRI values of the ABC score compared with the HAS-BLED score had the reverse effects in two included studies [$+13.8\%$ in Berg et al. (26) and -13.74% in Esteve-Pastor et al. (27)]. In the study of Esteve-Pastor et al. (27), the ABC score showed significant negative IDI values compared with the HAS-BLED score (-13.14% , $p = 0.002$). Further study should confirm the improvement in the predictive accuracy of ABC vs. HAS-BLED scores in anticoagulated patients with AF.

The values from the NRI and IDI analyses assessing the improvement in predictive accuracy for any clinically relevant bleeding, any bleeding, intracranial bleeding, and gastrointestinal bleeding are presented in **Table 3**. The results of these sections should be interpreted cautiously due to the limiting number of included studies.

Calibration and Decision Curve Analysis

A total of seven included studies provided the calibration analysis of the HAS-BLED score, but the findings were inconsistent (**Supplementary Table 5**). Jaspers Focks et al. (24) and Beshir et al. (10) found that the HAS-BLED score had an adequate calibration. Two studies demonstrated that compared with the rates in the derivation cohort, the HAS-BLED score overestimated (28) or underestimated (29) the risk of bleeding. The HAS-BLED score had a better calibration than the ATRIA

TABLE 1 | Baseline characteristics of patient of the included studies.

Study (author-year)	Data source	Study design	Sample size*	Type of anticoagulants analyzed	Concomitant antiplatelet or NSAIDS	Study endpoints	Bleeding scales	Comparisons of HAS-BLED vs. others	Follow-up time (y)
Pisters-2010	Euro Heart Survey on AF; 2003–2004	Retrospective cohort	1,722	VKAs	NA	Major bleeding	ISTH	HEMORR2HAGES	1.0
Olesen-2011	Danish National Patient Registry; 1997–2006	Retrospective cohort	44,771	VKAs (99.8%), heparins	33%	Major bleeding	ICD codes	HEMORR2HAGES	1.0
Friberg-2012	Swedish National Hospital Discharge Registry; 2005–2008	Retrospective cohort	48,599	Warfarin	NA	Major bleeding; Intracranial bleeding	ICD codes	HEMORR2HAGES	1.50
Apostolakis-2012	The AMADEUS trial	<i>Post-hoc</i> analysis of RCT	2,293	Warfarin	18%	Major bleeding; Any clinically relevant bleeding	ISTH	HEMORR2HAGES; ATRIA	1.18
Apostolakis-2013	The AMADEUS trial	<i>Post-hoc</i> analysis of RCT	2,293	Warfarin	18%	Any clinically relevant bleeding	ISTH	CHADS2; CHA2DS2-VASc	1.18
Senoo-2016	The AMADEUS trial	<i>Post-hoc</i> analysis of RCT	2,293	Warfarin	16.5%	Major bleeding; Any clinically relevant bleeding	ISTH	ORBIT	1.18
Proietti-2016	The SPORTIF III and V clinical trials	<i>Post-hoc</i> analysis of RCT	3,551	Warfarin	19.9%	Major bleeding	ISTH	ORBIT; ATRIA; HEMORRAGES	1.6
Proietti-2018a	The SPORTIF III clinical trial	<i>Post-hoc</i> analysis of RCT	3,550	Warfarin	19.9%	Major bleeding; Any clinically relevant bleeding; Any bleeding	ISTH	GARFIELD-AF	1.56
Roldan-2013a	Outpatient anticoagulation clinic; City Hospital, Birmingham, UK; 2007.03–2008.11	Prospective cohort	937	Acenocoumarol	17%	Major bleeding	ISTH	ATRIA	2.61
Roldan-2013b	Outpatient anticoagulation clinic; City Hospital, Birmingham, UK; 2007–2008	Prospective cohort	1,370	Acenocoumarol	18%	Major bleeding	ISTH	CHADS2; CHA2DS2-VASc	2.73
Barnes-2014	Michigan Anticoagulation Quality Improvement Initiative (MAQI2); 2009–2012	Prospective cohort	2,600	Warfarin	NA	Major bleeding	ISTH	HEMORR2HAGES; ATRIA; CHADS2; CHA2DS2-VASc	1.0
Esteve-Pastor-2016	The FANTASIIA registry; Spanish; 2013–2014	Prospective cohort	1,276	DOACs; VKAs	10.9%	Major bleeding	ISTH	ORBIT	1.0
Hijazi-2016	The ARISTOTLE derivation cohort	<i>Post-hoc</i> analysis of RCT	14,537	Apixaban; warfarin	39%	Major bleeding	ISTH	ABC; ORBIT	1.9
	The RE-LY validation cohort	<i>Post-hoc</i> analysis of RCT	8,468	Dabigatran; warfarin	44%	Major bleeding	ISTH	ABC; ORBIT	1.9

(Continued)

TABLE 1 | Continued

Study (author-year)	Data source	Study design	Sample size*	Type of anticoagulants analyzed	Concomitant antiplatelet or NSAIDs	Study endpoints	Bleeding scales	Comparisons of HAS-BLED vs. others	Follow-up time (y)
Proietti-2018b	The RE-LY trial, whole cohort	Post-hoc analysis of RCT	18,113	Dabigatran; warfarin	40%	Major bleeding; Intracranial bleeding	ISTH	HEMORR2HAGES; ATRIA; ORBIT	2.0
Berg-2019	The ENGAGE AF-TIMI 48 trial	Post-hoc analysis of RCT	8,705	Edoxaban; Warfarin	NA	Major bleeding	ISTH	ABC	2.8
Jaspers Focks-2016	The anticoagulation clinic in the region Arnhem/Nijmegen, the Netherlands	Prospective cohort	1,157	VKAs (acenocoumarol 90%)	4.1%	Major bleeding; Any clinically relevant bleeding; Any bleeding	ISTH	HEMORR2HAGES; ATRIA	2.5
Steinberg-2016	The ORBIT-AF registry; US outpatients	Prospective cohort	7,420	Dabigatran; Warfarin	NA	Major bleeding	ISTH	ATRIA	NA
Poli-2017	The START register, multicenter in Italy	Prospective cohort	4,579	DOACs; VKAs	16.5%	Major bleeding	ISTH	CHADS2; CHA2DS2-VASc	1.4
Caro Martínez-2017	Three Spanish hospitals; 2013–2014	Retrospective cohort	973	DOACs	NA	Major bleeding; Gastrointestinal bleeding	ISTH	ATRIA; ORBIT	1.77
Elvira-Ruiz-2020	Two hospitals in Spain; 2013–2016	Retrospective cohort	2,880	DOACs; VKAs	17.7%	Major bleeding	ISTH	ATRIA; ORBIT	1.5
Esteve-Pastor-2017	Single anticoagulation centre in a tertiary hospital in Murcia, Spain; 2007	Prospective cohort	1,120	Acenocoumarol	NA	Major bleeding; Intracranial bleeding; Gastrointestinal bleeding	ISTH	ABC	6.5
Rivera-Caravaca-2017	Single anticoagulation centre in a tertiary hospital in Murcia, Spain; 2007	Retrospective cohort	1,361	Acenocoumarol	18%	Major bleeding	ISTH	HEMORR2HAGES; ATRIA; ORBIT	6.5
Fox-2021	The GARFIELD-AF registry from 35 countries; 2010–2016	Retrospective cohort	52,032	DOACs; VKAs	> 12%	Major bleeding	ISTH	GARFIELD-AF	2.0
Beshir-2018	University of Malaya Medical Centre and Institut Jantung Negara or the National Heart Institute of Malaysia	Retrospective cohort	1,017	Warfarin, rivaroxaban, dabigatran	35%	Major bleeding; Clinically relevant non-major bleeding	ISTH	HEMORR 2HAGES, ATRIA; ORBIT	1.0
Chao-2018	National Health Insurance Research Database, Taiwan; 1998–2011	Retrospective cohort	40,450	Warfarin	22.7%	Major bleeding; Intracranial bleeding	NA	HEMORR2HAGES; ATRIA; ORBIT	4.6
Dalgaard-2019	Danish nationwide databases	Retrospective cohort	51,180	DOACs; VKAs	NA	Major bleeding	ICD codes	GARFIELD-AF	1.0
Lip-2018	Danish nationwide databases	Retrospective cohort	57,930	DOACs	39.1%	Any bleeding	ICD codes	ATRIA; ORBIT	2.5

(Continued)

TABLE 1 | Continued

Study (author-year)	Data source	Study design	Sample size*	Type of anticoagulants analyzed	Concomitant antiplatelet or NSAIDS	Study endpoints	Bleeding scales	Comparisons of HAS-BLED vs. others	Follow-up time (y)
Mori-2019	The DIRECT registry; single-center in Japan	Prospective cohort	2,216	DOACs	21.5%	Major bleeding	ISTH	ORBIT	0.86
O'Brien-2015	The ORBIT-AF registry; 176 sites in the USA	Prospective cohort	7,411	Dabigatran; Warfarin	37.9%	Major bleeding	ISTH	ATRIA; ORBIT	2.0
	The ROCKET-AF validation cohort	Post-hoc analysis of RCT	14,264	Warfarin, rivaroxaban	NA	Major bleeding	ISTH	ATRIA; ORBIT	1.9
Quinn-2016	The ATRIA Study; California; 1996–1997	Retrospective cohort	13,559	Warfarin	NA	Major bleeding	ISTH	CHADS ₂ ; CHA ₂ DS ₂ -VASc; ATRIA	NA
Yao-2017	OptumLabs Data Warehouse; US; 2010–2015	Retrospective cohort	39,539	DOACs	7%	Major bleeding; Intracranial bleeding	NA	CHADS ₂ ; CHA ₂ DS ₂ -VASc	0.6
Claxton-2018	The derivation (MarketScan, 2007–2014) and validation (Optum Clinformatics, 2009–2015) cohorts	Prospective cohort	81,285	DOACs; Warfarin	NA	Major bleeding	ISTH	HEMORR ₂ HAGES; ATRIA; ORBIT	1.0
Rutherford-2018	Norwegian Patient Registry and Norwegian Prescription Database; 2013–2015	Retrospective cohort	21,248	DOACs	52.8%	Any clinically relevant bleeding	ICD codes	ATRIA; ORBIT	0.5
Adam-2021	Multicenter cohort study in Switzerland (SWISS-AF)	Prospective cohort	2,147	DOACs; VKAs	18%	Any clinically relevant bleeding	ISTH	ATRIA; ORBIT	4.4
Siu-2014	Queen Mary Hospital, Hong Kong; 1997–2011	Retrospective cohort	1,912	Warfarin	NA	Intracranial bleeding	NA	NA	3.19
Suzuki-2014	Kameda Medical Center; Japan; 2005	Prospective cohort	231	Warfarin	36.9–50%	Major bleeding	ISTH	NA	7.1
Prochaska-2018	The thrombEVAL cohort. Denmark	Prospective cohort	1,089	Phenprocoumon	37.9%	Any clinically relevant bleeding	NA	NA	3.0
Schwartz-2019	Northwestern Healthcare system's Enterprise Database Warehouse; US; 2011–2017	Retrospective cohort	9,819	DOACs; VKAs	NA	Major bleeding	ISTH	NA	1.84
Ravvaz-2021	Longitudinal electronic health records in eastern Wisconsin and northern Illinois	Retrospective cohort	7,274	Warfarin	NA	Any bleeding	ICD codes	NA	0.93

*Number of anticoagulated patients.

HAS-BLED, Hypertension, Abnormal liver/renal function, Stroke, Bleeding history or predisposition, Labile international normalized ratio, Elderly, Drugs/alcohol concomitantly; HEMORR₂HAGES, Hepatic or renal disease, Ethanol abuse, Malignancy, Older, Reduced platelet count or function, Re-bleeding risk, Hypertension (uncontrolled), Anemia, Genetic factors, Excessive fall risk, Stroke; ATRIA, Anticoagulation and Risk Factors in Atrial Fibrillation; ORBIT, Outcomes Registry for Better Informed Treatment of Atrial Fibrillation; GARFIELD-AF, Global Anticoagulant Registry in the FIELD-Atrial Fibrillation; ABC, Age, Biomarkers, Clinical History; CHADS₂, Congestive heart failure, Hypertension, Age ≥ 75 years, Diabetes mellitus, Stroke/transient ischemic attack history; CHA₂DS₂-VASc, Congestive heart failure/left ventricular ejection fraction $\leq 40\%$, Hypertension, Age ≥ 75 years, Diabetes mellitus, Stroke/transient ischemic attack/thromboembolism history, Vascular disease, Age 65–74 years, Sex (female); ICD, International Classification of Diseases; ISTH, International Society of Thrombosis and Haemostasis; NSAIDS, non-steroidal anti-inflammatory drugs; NA, not available.

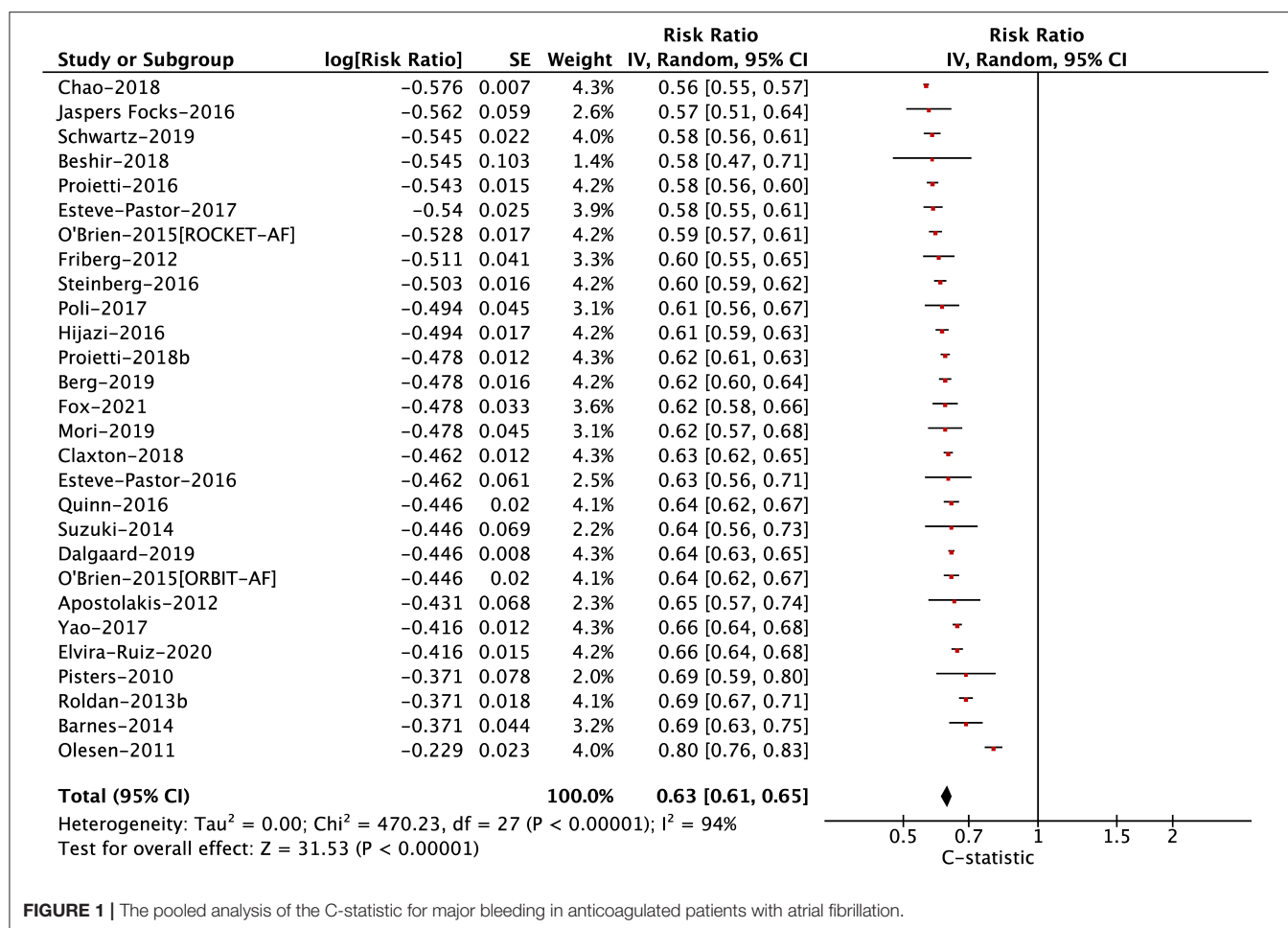


FIGURE 1 | The pooled analysis of the C-statistic for major bleeding in anticoagulated patients with atrial fibrillation.

score (13, 30), but showed a similar or lower calibration compared with the ORBIT score (14, 30).

The net benefits of the HAS-BLED score vs. other risk scores were assessed by using the decision curve analysis (**Supplementary Table 6**). The HAS-BLED score might have larger net benefits than the HEMORR₂HAGES, the CHADS₂, the CHA₂DS₂-VAsC, or the GARFIELD-AF scores (19, 25, 31). Two studies reported the net benefits between the HAS-BLED and the ABC scores, but reached the opposite conclusion (27, 32). The net benefits between the HAS-BLED and the ATRIA or the ORBIT scores might be related to the intervention thresholds (29).

DISCUSSION

In this study, our results suggested that the HAS-BLED score had moderate predictive abilities for bleeding risks in anticoagulated patients with AF regardless of the OAC type. We also observed the suitable application of the HAS-BLED score in patients with AF when the labile INR was unavailable. The discrimination performance of the HAS-BLED score assessed by the C-statistic was comparable to the HEMORR₂HAGES, the ATRIA, the ORBIT, the GARFIELD-AF, or the ABC scores, but performed better than the CHADS₂ or the CHA₂DS₂-VAsC scores. The NRI

and IDI data suggested that the HAS-BLED score performed better than the CHADS₂ or the CHA₂DS₂-VAsC scores and had similar or superior predictive ability compared with the HEMORR₂HAGES, the ATRIA, the ORBIT, or the GARFIELD-AF scores. Calibration and decision curve analyses of the HAS-BLED score compared with other risk models required further evidence-based assessment due to the different findings among the included studies.

The use of OAC effectively reduces the embolic risks but at cost of an increased risk of bleeding. Over the past few decades, VKAs such as warfarin have been confirmed to be effective for preventing stroke in patients with AF. Since the effectiveness and safety of DOACs are superior or non-inferior to warfarin in patients with AF, DOACs are increasingly used with time. However, the bleeding events and their related cardiovascular outcomes are not negligible. The optimal use of VKAs or DOACs in the management of AF should be based on a balanced risk-to-benefit assessment during anticoagulation. For this situation, it is vital that the potentially preventable risk factors of bleeding events should be monitored sufficiently and addressed appropriately. The HAS-BLED score has been currently recommended by current AF guidelines, where a score of ≥ 3 points indicates high-risk bleeding. Note that the

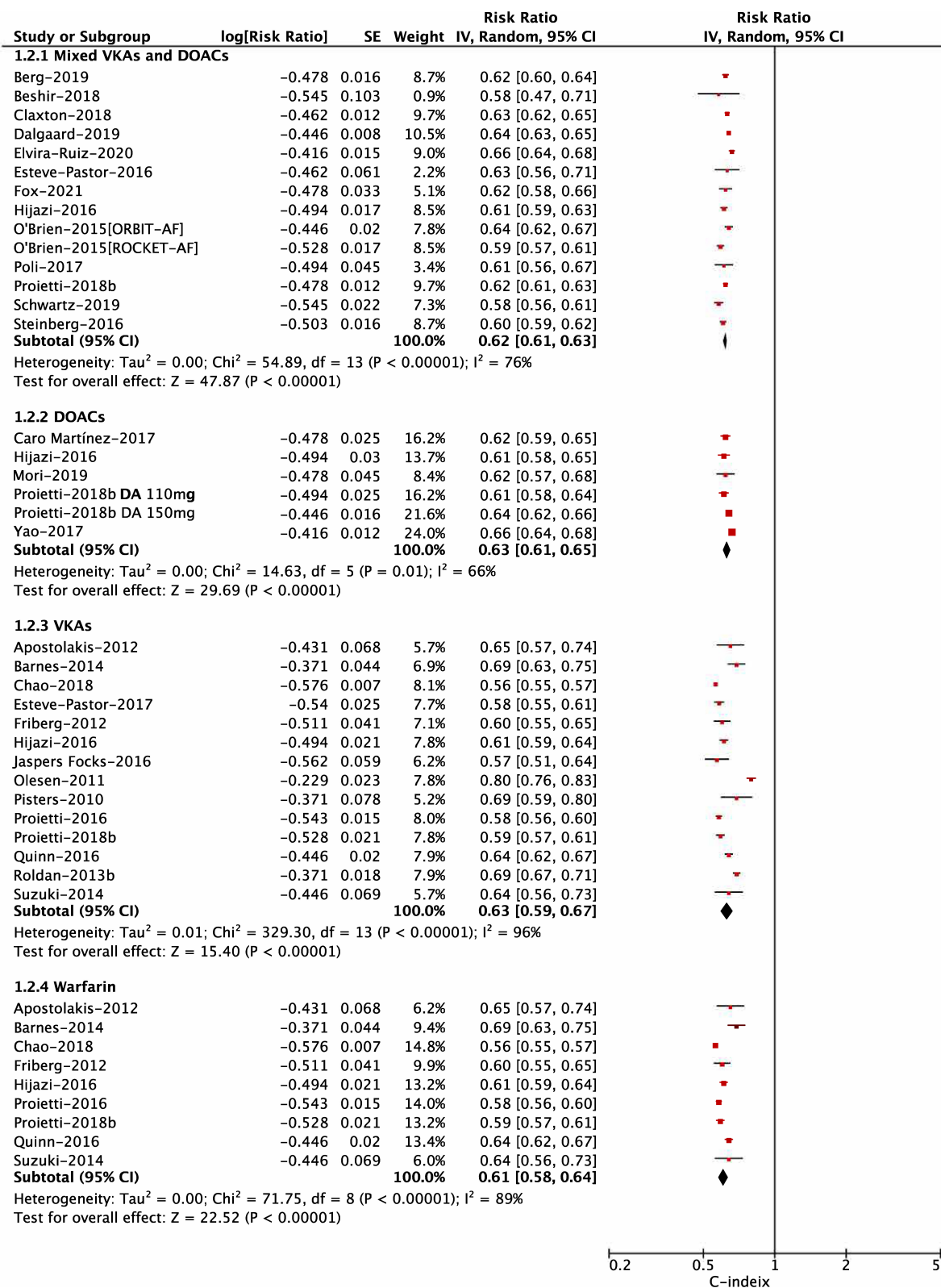


FIGURE 2 | Subgroup analysis for pooling the C-statistic of the Hypertension, Abnormal liver/renal function, Stroke, Bleeding history or predisposition, Labile international normalized ratio, Elderly, Drugs/alcohol concomitantly (HAS-BLED) score based on the types of oral anticoagulation.

TABLE 2 | Summary of the C-statistic and 95% CIs of the included studies.

	Major bleeding	Any clinically relevant bleeding	Intracranial bleeding
Overall			
No. of studies	28	7	6
C-statistic: HAS-BLED	0.63 [0.61, 0.65]	0.58 [0.56, 0.61]	0.58 [0.53, 0.62]
HAS-BLED vs. HEMORR₂HAGES			
No. of studies	12	3	3
C-statistic: HAS-BLED	0.63 [0.60, 0.67]	0.57 [0.54, 0.61]	0.56 [0.51, 0.63]
C-statistic: HEMORR ₂ HAGES	0.62 [0.58, 0.65]	0.56 [0.51, 0.61]	0.58 [0.51, 0.66]
Z-statistic	0.396 [#]	0.321 [#]	−0.408 [#]
HAS-BLED vs. ORBIT			
No. of studies	12	4	2
C-statistic: HAS-BLED	0.61 [0.59, 0.64]	0.59 [0.56, 0.63]	0.54 [0.51, 0.57]
C-statistic: ORBIT	0.63 [0.60, 0.67]	0.59 [0.53, 0.66]	0.55 [0.45, 0.69]
Z-statistic	−0.911 [#]	0 [#]	−0.158 [#]
HAS-BLED vs. ATRIA			
No. of studies	15	5	2
C-statistic: HAS-BLED	0.62 [0.60, 0.65]	0.59 [0.56, 0.62]	0.54 [0.51, 0.57]
C-statistic: ATRIA	0.63 [0.60, 0.66]	0.58 [0.51, 0.65]	0.54 [0.47, 0.62]
Z-statistic	−0.502 [#]	0.257 [#]	0 [#]
HAS-BLED vs. CHADS₂			
No. of studies	5	-	-
C-statistic: HAS-BLED	0.66 [0.64, 0.68]	-	-
C-statistic: CHADS ₂	0.61 [0.57, 0.65]	-	-
Z-statistic	2.19 [*]	-	-
HAS-BLED vs. CHA₂DS₂-VASc			
No. of studies	5	-	-
C-statistic: HAS-BLED	0.66 [0.64, 0.68]	-	-
C-statistic: CHA ₂ DS ₂ -VASc	0.61 [0.57, 0.66]	-	-
Z-statistic	1.99 [*]	-	-
HAS-BLED vs. GARFIELD-AF			
No. of studies	3	-	-
C-statistic: HAS-BLED	0.61 [0.57, 0.66]	-	-
C-statistic: GARFIELD-AF	0.63 [0.56, 0.71]	-	-
Z-statistic	−0.448 [#]	-	-
HAS-BLED vs. ABC			
No. of studies	4	-	-
C-statistic: HAS-BLED	0.61 [0.60, 0.63]	-	-
C-statistic: ABC	0.65 [0.58, 0.72]	-	-
Z-statistic	−1.09 [#]	-	-

The absolute value of Z-statistic more than 1.96 indicated a p-value of <0.05, suggesting a significant difference in the discrimination between the two risk scores. * $p < 0.05$; [#] $p > 0.05$. HAS-BLED, Hypertension, Abnormal liver/renal function, Stroke, Bleeding history or predisposition, Labile international normalized ratio, Elderly, Drugs/alcohol concomitantly; HEMORR₂HAGES, Hepatic or renal disease, Ethanol abuse, Malignancy, Older, Reduced platelet count or function, Re-bleeding risk, Hypertension (uncontrolled), Anemia, Genetic factors, Excessive fall risk, Stroke; ATRIA, Anticoagulation and Risk Factors in Atrial Fibrillation; ORBIT, Outcomes Registry for Better Informed Treatment of Atrial Fibrillation; GARFIELD-AF, Global Anticoagulant Registry in the FIELD-Atrial Fibrillation; ABC, Age, Biomarkers, Clinical History; CHADS₂, Congestive heart failure, Hypertension, Age ≥ 75 years, Diabetes mellitus, Stroke/transient ischemic attack history; CHA₂DS₂-VASc, Congestive heart failure/left ventricular ejection fraction $\leq 40\%$, Hypertension, Age ≥ 75 years, Diabetes mellitus, Stroke/transient ischemic attack/thromboembolism history, Vascular disease, Age 65–74 years, Sex (female).

HAS-BLED score is previously derived and validated mainly in VKA-treated patients with AF, whether it could be used in DOAC-treated patients remains unclear. In this study, we found that the HAS-BLED score had moderate predictive values for bleeding risks in anticoagulated patients with AF and the findings were consistent in the subgroups of mixed anticoagulated drugs, DOACs, VKAs, and warfarin. The variable of labile INR in the

HAS-BLED score was not available in all the included studies. Nevertheless, the predictive ability for major bleeding was not significantly changed, if we only included the studies with labile INR in the pooled analysis.

Although several other bleeding risk prediction models have been proposed in published studies, whether the predictive ability of these models is parallel to the guideline recommended

TABLE 3 | NRI and IDI analysis for predicting the bleeding risks in anticoagulated patients with AF.

Study (author-year)	NRI analysis	IDI analysis
Major bleeding		
Apostolakis-2012	HAS-BLED vs. HEMORR ₂ HAGES (+6.8%, $P = 0.42$); HAS-BLED vs. ATRIA(+9.0%, $P = 0.33$)	Not available
Roldan-2013a	Continuous: HAS-BLED vs. ATRIA(+13.6%, $P = 0.43$) Categorical: HAS-BLED vs. ATRIA(+19.6%, $P = 0.019$)	Continuous: HAS-BLED vs. ATRIA(+6.9%, $P = 0.033$) Categorical: HAS-BLED vs. ATRIA(+7.0%, $P = 0.001$)
Roldan-2013b	HAS-BLED vs. CHADS ₂ (+38.62%, $P < 0.001$); HAS-BLED vs. CHA ₂ DS ₂ -VASC (+37.6%, $P < 0.001$)	HAS-BLED vs. CHADS ₂ (+10.0%, $P < 0.001$); HAS-BLED vs. CHA ₂ DS ₂ -VASC (+12.0%, $P < 0.001$)
Barnes-2014	HAS-BLED vs. HEMORR ₂ HAGES (+26.0%, $P = 0.006$); HAS-BLED vs. ATRIA (+31.0%, $P = 0.001$); HAS-BLED vs. CHADS ₂ (+58.0%, $P < 0.001$); HAS-BLED vs. CHA ₂ DS ₂ -VASC (+36.0%, $P < 0.001$)	Not available
Berg-2019	ABC vs. HAS-BLED +13.8% (8.0–22.8%)	Not available
Chao-2018	HAS-BLED vs. HEMORR ₂ HAGES (+4.3%, $P < 0.001$); HAS-BLED vs. ATRIA (+4.9%, $P < 0.001$); HAS-BLED vs. ORBIT (+5.5%, $P < 0.001$)	Not available
Esteve-Pastor-2017	ABC vs. HAS-BLED (–13.74%, $P = 0.005$)	ABC vs. HAS-BLED (–13.14%, $P = 0.002$)
Jaspers Focks-2016	HAS-BLED vs. HEMORR ₂ HAGES (–3.60%, $P = 0.460$); HAS-BLED vs. ATRIA (–6.32%, $P = 0.894$)	Not available
Proietti-2016	ORBIT vs. HAS-BLED (–0.77%, $P = 0.392$); ATRIA vs. HAS-BLED (–8.83%, $P = 0.323$); HEMORR ₂ HAGES vs. HAS-BLED (–13.66%, $P = 0.119$)	ORBIT vs. HAS-BLED (0%, $P = 0.646$); ATRIA vs. HAS-BLED (0%, $P = 0.611$); HEMORR ₂ HAGES vs. HAS-BLED (–0.18%, $P = 0.039$)
Proietti-2018a	GARFIELD vs. HAS-BLED (–4.2%, $P = 0.448$)	GARFIELD vs. HAS-BLED (–0.2%, $P = 0.318$)
Rivera-Caravaca-2017	HAS-BLED vs. HEMORR ₂ HAGES (+15.74%, $P < 0.001$); HAS-BLED vs. ATRIA (+15.98%, $P < 0.001$); HAS-BLED vs. ORBIT (+12.12%, $P = 0.007$)	HAS-BLED vs. HEMORR ₂ HAGES (+3.11%, $P = 0.347$); HAS-BLED vs. ATRIA (+3.09%, $P = 0.142$); HAS-BLED vs. ORBIT (+2.4%, $P = 0.067$)
Quinn-2016	HAS-BLED vs. CHADS ₂ (+0.4%)	Not available
Yao-2017	HAS-BLED vs. CHA ₂ DS ₂ -VASC (+2.0%, $P < 0.001$)	Not available
Any clinically relevant bleeding		
Apostolakis-2012	HAS-BLED vs. HEMORR ₂ HAGES (+10.3%, $P < 0.001$); HAS-BLED vs. ATRIA(+13.0%, $P < 0.001$)	
Apostolakis-2013	Continuous: HAS-BLED vs. CHADS ₂ (+16.0%, $P = 0.017$); HAS-BLED vs. CHA ₂ DS ₂ -VASC (+29.0%, $P < 0.001$) Categorical: HAS-BLED vs. CHADS ₂ (+13.0%, $P = 0.001$); HAS-BLED vs. CHA ₂ DS ₂ -VASC (+10.0%, $P = 0.04$)	Not available
Jaspers Focks-2016	HAS-BLED vs. HEMORR ₂ HAGES (–5.61%, $P = 0.194$); HAS-BLED vs. ATRIA (–3.6%, $P = 0.119$)	Not available
Proietti-2018a	GARFIELD vs. HAS-BLED (+3.3%, $P = 0.756$);	GARFIELD vs. HAS-BLED (–0.1%, $P = 0.746$);
Intracranial bleeding		
Chao-2018	HAS-BLED vs. HEMORR ₂ HAGES (+3.0%, $P = 0.056$); HAS-BLED vs. ATRIA (+6.0%, $P < 0.001$); HAS-BLED vs. ORBIT (+4.8%, $P < 0.001$)	Not available
Esteve-Pastor-2017	ABC vs. HAS-BLED (–13.96%, $P = 0.075$)	ABC vs. HAS-BLED (–0.11%, $P = 0.536$)
Yao-2017	HAS-BLED vs. CHA ₂ DS ₂ -VASC (+7.0%, $P < 0.001$)	Not available
Gastrointestinal bleeding		
Esteve-Pastor-2017	ABC vs. HAS-BLED (–8.17%, $P = 0.362$)	ABC vs. HAS-BLED (–5.55%, $P = 0.164$)
Any bleeding		
Jaspers Focks-2016	HAS-BLED vs. HEMORR ₂ HAGES (–3.72%, $P = 0.334$); HAS-BLED vs. ATRIA (–8.51%, $P = 0.009$)	Not available
Proietti-2018a	GARFIELD vs. HAS-BLED (–8.7%, $P < 0.001$)	GARFIELD vs. HAS-BLED (–1.1%, $P < 0.001$)

HAS-BLED, Hypertension, Abnormal liver/renal function, Stroke, Bleeding history or predisposition, Labile international normalized ratio, Elderly, Drugs/alcohol concomitantly; HEMORR₂HAGES, Hepatic or renal disease, Ethanol abuse, Malignancy, Older, Reduced platelet count or function, Re-bleeding risk, Hypertension (uncontrolled), Anemia, Genetic factors, Excessive fall risk, Stroke; ATRIA, Anticoagulation and Risk Factors in Atrial Fibrillation; ORBIT, Outcomes Registry for Better Informed Treatment of Atrial Fibrillation; GARFIELD-AF, Global Anticoagulant Registry in the FIELD-Atrial Fibrillation; ABC, Age, Biomarkers, Clinical History; CHADS₂, Congestive heart failure, Hypertension, Age ≥ 75 years, Diabetes mellitus, Stroke/transient ischemic attack history; CHA₂DS₂-VASC, Congestive heart failure/left ventricular ejection fraction $\leq 40\%$, Hypertension, Age ≥ 75 years, Diabetes mellitus, Stroke/transient ischemic attack/thromboembolism history, Vascular disease, Age 65–74 years, Sex (female); NRI, net reclassification improvement; IDI, integrated discrimination improvement; AF, atrial fibrillation.

HAS-BLED score remains unclear. The HAS-BLED score has been previously assessed as the most balanced bleeding risk prediction tool in terms of sensitivity and specificity by using a meta-analytic approach (5, 33). However, discrimination outcome data (sensitivity/specificity or the C-statistic) are less critical than other predictive accuracy outcome measures. In combination with NRI and IDI values, calibration, and decision curve analyses, our current meta-analysis comprehensively assessed the predictive abilities of the HAS-BLED vs. the HEMORR₂HAGES, the ATRIA, the ORBIT, the GARFIELD-AF, or the ABC bleeding scores. Overall, as reflected by these multiple methods, the HAS-BLED score showed at least non-inferior abilities for bleeding risk prediction than the HEMORR₂HAGES, the ATRIA, the ORBIT, or the GARFIELD-AF scores in VKA- or DOAC-treated patients with AF. Although there was no significant difference in the C-statistic between the HAS-BLED and the ABC scores, data of reclassification, calibration, and decision curve analyses between them were still controversial and needed further clarifications. In addition, there is an overlap of risk factors such as age, hypertension, previous stroke, and diabetes between stroke and bleeding risk scores. As such, the CHADS₂ and the CHA₂DS₂-VASc stroke scores are also closely associated with the increased bleeding risks. Nevertheless, we found that the HAS-BLED score performed better than the CHADS₂ or the CHA₂DS₂-VASc scores in anticoagulated patients with AF.

Several published studies have modified the HAS-BLED score by revising the original variables or including additional factors. As shown in **Supplementary Table 7**, integration of additional factors (e.g., biomarkers, gene polymorphisms, aortic stenosis, area deprivation index) on the basis of the original HAS-BLED score has been taken into account. The modified HAS-BLED score might improve the predictive ability, but certainly at the expense of additional complexity, increased cost, and reduced practicality. The number and definition of variables may vary from study to study, potentially affecting the diagnostic performance of the HAS-BLED score.

The dynamic changes of bleeding risks should be assessed in the management of AF (34). Chao et al. (35) found that the prediction values of the follow-up or the delta HAS-BLED score were better compared with the baseline HAS-BLED score. The HAS-BLED score has been tested prospectively in the mobile atrial fibrillation application-II (mAFA-II) randomized trial, suggesting that dynamic monitoring management could reduce major bleeding and increase OAC uptake at 1 year (36). Therefore, the dynamic changes in modifiable risk factors for bleeding outcomes during the follow-up should be monitored and corrected timely to improve AF patient care (36). Current evidence supports the HAS-BLED score regularly used in patients with AF to identify patients at high risk of bleeding as early as possible. However, the HAS-BLED score is sometimes inappropriately used in clinical practice as an excuse to preclude the use of oral anticoagulants. For the majority of patients with AF, the benefits of OAC in reducing the stroke risk outweigh the bleeding risk. Clinicians should flag up high bleeding-risk patients (e.g., the HAS-BLED score of ≥ 3) for the early review and follow-up. Appropriate monitoring services and more efforts are necessary to be taken to correct modifiable bleeding risk

factors such as uncontrolled hypertension, poor control of INR (VKA users), concomitant use of medications such as aspirin or NSAIDs, and alcohol abuse.

The 2021 UK National Institute for Health and Care Excellence (NICE) guidelines tend to recommend the use of the ORBIT score in the bleeding risk prediction for patients with AF (especially for DOAC users) (37). This recommendation is mainly supported by the better calibration evidence than the HAS-BLED score although the low quality data. As the committee pointed out by using new risk models of the ORBIT score in current clinical practice, it remains a challenge potentially due to the unknown cost-effectiveness and extra resources. Clinicians are not familiar with the ORBIT score and learning and training may take time and cost. More importantly, the ORBIT bleeding score mainly consists of non-modifiable risk factors. In this context, a newly published study by Proietti et al. compared the abilities of the HAS-BLED vs. the ORBIT scores in contemporary patients with AF with DOACs based on the data from the European Society of Cardiology-European Heart Rhythm Association (ESC-EHRA) and the EURObservational Research Programme AF (EORP-AF) General Long-Term Registry (38). The authors found that the ORBIT score identified less patients at high bleeding risk, showed no improvements in predictive accuracy for major bleeding assessed by the NRI and IDI values, and had a poorer calibration compared with the HAS-BLED score (38). These findings seemingly do not support the use of the ORBIT over the HAS-BLED scores for bleeding risk prediction in patients with AF. The simple and practical use of the HAS-BLED score is still appropriate for assessing VKA- or DOAC- related bleeding risks and helps clinicians to make informed decisions in clinical practice.

Limitations

There were still several limitations in this study. First, due to the high heterogeneity observed across the included studies, the discrimination performances of bleeding prediction models evaluated by the C-statistic should be interpreted cautiously. More studies focusing on the improvement in predictive accuracy by the NRI and IDI analyses, calibration, or net benefits by decision curve analysis could be taken to fully assess the performances of risk scores. Second, compared to the primary major bleeding, our results suggested a relatively lower predictive value of the HAS-BLED score for any clinically relevant bleeding, any bleeding, or intracranial bleeding. Only two studies reported the C-statistic for gastrointestinal bleeding. Therefore, more studies should further assess the value of the HAS-BLED score for these other bleeding outcomes. Third, the bleeding risk prediction tools of interest were derived and validated in studies with different study types ranging from observational cohorts to clinical trials, potentially complicating the synthesis of the C-statistic. Nevertheless, we observed no significant interaction in the subgroup analysis based on the study design. Fourth, we provided the data of concomitant antiplatelet drugs, but the effect of these drugs on the predictive value of the HAS-BLED score could not be analyzed due to the unclear cutoff points. Finally, this study was performed based on most included studies with low-quality data, but we comprehensively assessed the role of the

HAS-BLED score vs. other risk models in predicting bleeding events in patients with AF, which had implications for clinical application and future research development.

CONCLUSION

The HAS-BLED score had moderate predictive abilities for bleeding risks in VKA- or DOAC-treated patients with AF. The HAS-BLED score was at least non-inferior to the HEMORR₂HAGES, the ATRIA, the ORBIT, the GARFIELD-AF, or the ABC scores, but performed better than the CHADS₂ or the CHA₂DS₂-VAsC scores.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

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All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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SUPPLEMENTARY MATERIAL

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Post-operative Atrial Fibrillation Impacts on Outcomes in Transcatheter and Surgical Aortic Valve Replacement

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Background: Atrial fibrillation (AF) in severe aortic stenosis (AS) has poor outcomes after transcatheter and surgical aortic valve replacement (TAVR and SAVR, respectively). We compared the incidence of AF after aortic valve replacement (AVR) according to the treatment method and the impact of AF on outcomes.

Methods: We investigated the incidence of AF and clinical outcomes of AVR according to whether AF occurred after TAVR and SAVR after propensity score (PS)-matching for 1 year follow-up. Clinical outcomes were defined as death, stroke, and admission due to heart failure. The composite outcome comprised death, stroke, and admission due to heart failure.

Results: A total of 221 patients with severe AS were enrolled consecutively, 100 of whom underwent TAVR and 121 underwent SAVR. The incidence of newly detected AF was significantly higher in the SAVR group before PS-matching (6.0 vs. 40.5%, $P < 0.001$) and after PS-matching (7.5 vs. 35.6%, $P = 0.001$). TAVR and SAVR showed no significant differences in outcomes except in terms of stroke. In the TAVR group, AF history did not affect the outcomes; however, in the SAVR group, AF history affected death (log rank $P = 0.038$). Post-AVR AF had a worse impact on admission due to heart failure (log rank $P = 0.049$) and composite outcomes in the SAVR group. Post-AVR AF had a worse impact on admission due to heart failure (log rank $P = 0.008$) and composite outcome in the TAVR group.

Conclusion: Post-AVR AF could be considered as a predictor of the outcomes of AVR. TAVR might be a favorable treatment option for patients with severe symptomatic AS who are at high-risk for AF development or who have a history of AF because the occurrence of AF was more frequent in the SAVR group.

Keywords: atrial fibrillation, incidence, aortic stenosis, transcatheter aortic valve replacement, surgical aortic valve replacement, outcome

INTRODUCTION

For decades, surgical aortic valve replacement (SAVR) has been the definitive treatment option for patients with severe symptomatic aortic valve stenosis (AS). However, patients with severe AS tend to be older, frail, and usually have multiple comorbidities. Therefore, a substantial proportion of AS patients are ineligible for SAVR. Transcatheter aortic valve replacement (TAVR) has emerged as a treatment option for severe symptomatic AS (1–4) and has taken the place of SAVR for some patients who are at high- or intermediate-risk for SAVR (1, 4). Recent data have also shown that TAVR is safe and effective for low-risk patients with SAVR (2, 3). Therefore, TAVR is now performed worldwide and is favored by some physicians because of the short duration of hospitalization and low periprocedural morbidity. With the progression of technology and learning curves, the number of TAVR procedures has also increased in Korea (5, 6).

Atrial fibrillation (AF) is the most common arrhythmia that requires appropriate management, such as rhythm control, rate control, and anticoagulation. AF is known to be associated with adverse clinical outcomes, such as heart failure, systemic embolism, stroke, or death. Because many cases of severe AS and AF are degenerative, their incidence is high in old patients, and they share similar risk factors contributing to worse clinical outcomes. Moreover, it is well-known that AF itself is an independent predictor of long-term mortality and heart failure in patients with AS (7). Previous studies have demonstrated that postoperative AF or pre-existing AF is associated with poor prognosis after TAVR (8, 9). Postoperative AF is also known to have a detrimental effect after cardiac surgery (10, 11). However, there are only a few studies comparing effects of AF on the outcomes of TAVR vs. SAVR.

Therefore, we aimed to compare the AF status in TAVR and SAVR, and its impact on the development of admission due to heart failure, death, stroke. Moreover, we compared the composite outcomes according to the management method for severe AS.

METHODS

Study Population

A total of 221 patients with severe symptomatic AS were consecutively enrolled from January 2016 to December 2019 at the Department of Cardiology and Thoracic and Cardiovascular Surgery at Chonnam National University Hospital in Gwangju, South Korea. Among them, 100 patients underwent TAVR and 121 underwent SAVR.

We retrospectively reviewed the medical records and analyzed the clinical data of all patients before and during the regular follow-up period. The data included previous medical history, echocardiographic parameters, laboratory results, and clinical outcomes. We analyzed the AF status and clinical outcomes according to the occurrence of AF in each aortic valve replacement (AVR) method. We performed propensity score (PS)-matching to homogenize the group characteristics. After PS-matching, the patient groups were matched 1:1 (TAVR, $n =$

53; SAVR, $n = 53$). The follow-up period was 1 year or until the first occurrence of any study outcome since enrollment.

The study was approved by the Ethics Committee of Chonnam National University Hospital, Gwangju, South Korea (IRB No., CNUH-2021-022). The requirement for informed consent was waived because the study was a retrospective analysis.

TAVR Procedure

Eligibility for TAVR was discussed by a multidisciplinary team, including cardiologists, cardiac surgeons, and anesthesiologists. The team decided to perform the procedure after reaching a consensus. All patients who underwent TAVR received either the balloon-expandable Edwards SAPIEN 3 transcatheter heart valves (Edwards LifeSciences, Irvine, CA, USA) or the self-expandable Medtronic Evolut R or Evolut PRO (Medtronic, Minneapolis, MN, USA). The approach route was mostly via the transfemoral ($n = 99$), except in one case, in which it was via the subclavian artery. The patient who was approached via the subclavian artery had very small, tortuous right and left superficial femoral arteries on computer tomographic angiography image, which would have made it difficult to insert the guiding catheters. All patients received aspirin (300 mg) and clopidogrel (300 mg) as loading doses before the TAVR procedure. Anticoagulation with unfractionated heparin was maintained during the procedure by monitoring the activation clotting time. Dual antiplatelet therapy (aspirin, 100 mg and clopidogrel, 75 mg) was maintained for at least 3 months after the procedure depending on the patient's clinical condition.

Definition and Outcomes

Severe AS was defined as: effective orifice area of the aortic valve $\leq 1.0 \text{ cm}^2$, effective orifice area index $\leq 0.8 \text{ cm}^2/\text{m}^2$, mean pressure gradient $\geq 40 \text{ mmHg}$, and/or jet velocity $\geq 4.0 \text{ m/s}$ by transthoracic echocardiography examination.

Post-AVR AF was defined as the occurrence of AF, irrespective of whether the patients had a history of AF post-operatively. Newly detected AF was defined as the documentation of AF during admission following the procedure without a history of AF.

The clinical outcome was assessed by death, stroke, admission due to heart failure, and implantation of a permanent pacemaker. The composite outcome was defined as the composition of death, stroke, and admission due to heart failure.

Statistical Analyses

Statistical analyses were performed using SPSS version 25.0 for Windows (SPSS, Inc., Chicago, Illinois, USA). Continuous variables were presented as the mean values \pm standard deviations. Student's t -test was used to evaluate the differences between continuous variables. Categorical variables were presented as percentages and frequencies, and were analyzed using the chi-square test or Fisher's exact test, as appropriate. PS-matching was performed to obtain similar baseline characteristics for each group. The PS was calculated using multivariable logistic regression incorporating frequently used variables and potential risk factors, including age, sex, hypertension, diabetes mellitus,

TABLE 1 | Baseline characteristics before and after propensity score matching.

	Before propensity score matching			After propensity score matching		
	TAVR (n = 100)	SAVR (n = 121)	P-value	TAVR (n = 53)	SAVR (n = 53)	P-value
Female sex, n(%)	57 (57.0)	54 (44.6)	0.067	25 (57.2)	24 (45.3)	0.846
Age, years	79.69 ± 6.1	68.25 ± 10.3	<0.001	77.50 ± 7.0	75.32 ± 5.8	0.083
>65 years	98 (98.0)	81 (66.9)	<0.001	51 (96.2)	50 (94.3)	0.647
Height (cm)	157.40 ± 9.3	159.29 ± 7.7	0.110	159.18 ± 9.4	158.51 ± 8.2	0.706
Weight (kg)	62.95 ± 14.8	60.65 ± 9.8	0.174	62.88 ± 14.6	58.34 ± 9.4	0.073
BMI (kg/m ²)	1.65 ± 0.2	1.63 ± 0.2	0.451	1.66 ± 0.2	1.60 ± 0.2	0.092
Medical history, n (%)						
Hypertension	77 (77.0)	66 (54.5)	0.001	39 (73.6)	31 (58.5)	0.151
Diabetes mellitus	30 (30.0)	33 (27.3)	0.655	20 (37.7)	15 (28.3)	0.409
Previous myocardial infarction	17 (17.0)	5 (4.1)	0.001	8 (15.1)	4 (7.5)	0.359
Previous heart failure	22 (22.0)	15 (12.4)	0.057	10 (18.9)	6 (11.3)	0.416
Previous CVA	14 (14.0)	14 (11.6)	0.589	11 (20.8)	8 (15.1)	0.613
CKD or ESRD	11 (11.0)	16 (13.2)	0.615	6 (11.3)	7 (13.2)	0.767
Smoking	20 (20.0)	33 (27.3)	0.208	13 (24.5)	15 (28.3)	0.826
STS score	8.10 ± 0.2	7.93 ± 0.5	0.201	7.84 ± 0.8	7.19 ± 0.9	0.105
Echocardiographic parameter						
Ejection fraction (%)	60.53 ± 12.6	61.43 ± 12.6	0.600	60.50 ± 12.7	60.86 ± 13.7	0.888
Mean pressure gradient (mmHg)	50.06 ± 64.6	54.49 ± 18.9	0.466	49.17 ± 14.0	52.76 ± 19.8	0.290
AoV area (mm ²)	0.77 ± 0.4	0.87 ± 0.5	0.120	0.80 ± 0.2	0.83 ± 0.2	0.418
AoV velocity (m/s)	4.68 ± 0.6	4.96 ± 0.6	0.438	4.55 ± 0.6	4.57 ± 0.8	0.856
LA size (mm)	47.32 ± 5.9	48.00 ± 7.3	0.516	47.22 ± 5.7	48.40 ± 7.0	0.416
Laboratory data						
Cr	1.10 ± 1.4	1.21 ± 1.0	0.495	1.31 ± 1.9	1.18 ± 0.8	0.654
ProBNP	3,391.79 ± 5227.3	3,283.34 ± 4745.6	0.909	4,165.02 ± 6895.8	4,736.16 ± 5853.5	0.742
CHA2DS2-VASc	4.20 ± 1.4	2.62 ± 1.6	<0.001	4.11 ± 1.7	3.43 ± 1.5	0.080

TAVR, Transcatheter aortic valve implantation; SAVR, Superficial aortic valve replacement; BMI, Body mass index; AoV, Aortic valve; CVA, Cerebrovascular accident; CKD, Chronic kidney disease; ESRD, End-stage renal disease; STS, Society of Thoracic Surgeons; LA, Left atrium.

chronic kidney disease, history of stroke or transient ischemic attack, previous myocardial infarction, or heart failure. Matching was performed using a greedy matching protocol (1:1 nearest neighbor matching without replacement). The 1 year survival rate was estimated using the Kaplan–Meier method, and the curves were compared using the log-rank test. Comparison of clinical outcomes was adjusted using Cox proportional hazards model. In all statistical tests, a two-sided $P < 0.05$ was considered statistically significant.

RESULTS

Baseline Characteristics

The baseline characteristics of the patients are shown in **Table 1**. Before PS-matching, 100 patients underwent TAVR and 121 patients underwent SAVR. There were no significant differences in sex, height, body weight, or body mass index between the groups. The mean age was higher in the TAVR group than in the SAVR group (79.69 ± 6.1 vs. 68.25 ± 10.3 ; $P < 0.001$), and the proportion of patients >65 years was higher in the TAVR group (98.0% vs. 66.9%; $P < 0.001$). The incidence of comorbidities, such as of diabetes mellitus, heart failure, stroke, and chronic kidney disease, was comparable between the two

groups. Hypertension and previous myocardial infarctions were more common in the TAVR group. The Society of Thoracic Surgeons (STS) score was similar between the two groups. There was no significant difference in echocardiographic parameters, such as ejection fraction and left atrium size, between the two groups. The CHA2DS2-VASc score was higher in the TAVR group than the SAVR group (4.20 ± 1.4 vs. 2.62 ± 1.6 ; $P < 0.001$). After PS-matching, the baseline characteristics were comparable between the two groups; there were no significant differences in sex, age, comorbidities, STS risk score, echocardiographic parameters, laboratory data, and CHA2DS2-VASc score.

AF Occurrence

Before PS-matching, the history of AF frequency was similar between the two groups. Post-AVR AF was more frequent in the SAVR group than in the TAVR group (55.4 vs. 23.0%; $P = 0.001$). Among the post-AVR AF, the number of newly detected cases of AF was 6 and 49, respectively (TAVR vs. SAVR, 6.0 vs. 40.5%; $P < 0.001$).

After PS-matching, the rates of previous AF were not significantly different between the two groups. Post-AVR AF was higher in the SAVR group than in TAVR group (60.4 vs. 22.6%; $P < 0.001$). The incidence of newly detected AF was also higher

TABLE 2 | Atrial fibrillation occurrence before and after procedure.

	Before propensity score matching			After propensity score matching		
	TAVR (n = 100)	SAVR (n = 121)	P-value	TAVR (n = 53)	SAVR (n = 53)	P-value
Past history of AF (%)	24 (24.0)	25 (20.7)	0.552	11 (20.7)	16 (30.2)	0.176
Paroxysmal	12 (12.0)	16 (13.2)	0.322	5 (9.4)	10 (18.9)	0.150
Persistent	12 (12.0)	9 (7.4)	0.322	6 (11.3)	6 (11.3)	1.000
Post-AVR AF (%)	23 (23.0)	67 (55.4)	0.001	12 (22.6)	32 (60.4)	<0.001
Newly detected	6 (6.0)	49 (40.5)	<0.001	4 (7.5)	19 (35.6)	0.001
Termination <1 month	3	45	0.022	3	17	0.001
Termination 1–3 months	2	0	0.010	1	0	1.000
Persistence >3 months	1	4	0.452	0	2	0.495
Known-paroxysmal	5 (5.0)	10 (8.3)	0.337	2 (3.8)	8 (15.1)	0.093
Known-persistent	12 (12.0)	7 (5.8)	0.101	6 (11.3)	5 (9.4)	0.750
Rhythm control						
Amiodarone	9 (9.0)	16 (14.9)	0.219	4 (7.5)	9 (17.0)	0.236
DC cardioversion	1 (1.0)	7 (5.8)	0.075	1 (1.9)	6 (11.3)	0.051
Anticoagulation	14 (14.0)	27 (22.3)	0.122	7 (13.2)	11 (20.8)	0.301
Warfarin	3 (3.0)	23 (19.0)	<0.001	1 (1.9)	7 (13.2)	0.060
NOACs	11 (11.0)	4 (3.3)	0.023	6 (11.3)	4 (7.5)	0.371

TAVR, Transcatheter aortic valve implantation; SAVR, Superficial aortic valve replacement; AF, Atrial fibrillation; AVR, Aortic valve replacement; NOACs, Novel oral anticoagulants.

TABLE 3 | Clinical event rates for 1 year follow-up.

	Before propensity score matching			After propensity score matching		
	TAVR (n = 21 [†] /79 [‡])	SAVR (n = 12 [§] /109)	P-value	TAVR (n = 21 [†] /32 [‡])	SAVR (n = 2 [§] /51)	P-value
Admission due to heart failure (%)	1 [†] /4 [‡] (5.0)	8 (6.6)	0.685	4 [‡] (7.5)	5 (9.4)	0.860
Stroke	1 [†] /4 [‡] (5.0)	3 (2.5)	0.436	1 [†] /2 [‡] (5.7)	0 (0)	0.350
Ischemic	1 [†] /3 [‡] (4.0)	1 (1.0)		1 [†] /2 [‡] (5.7)	0 (0)	
Hemorrhagic	0 (0)	2 (1.7)		0 (0)	0 (0)	
TIA	1 [†] (1.0)	0 (0)		0 (0)	0 (0)	
Death	1 [†] /8 [‡] (9.0)	6 (5.0)	0.212	1 [†] /5 [‡] (11.3)	4 (7.5)	0.429
PPM	1 [†] /12 [‡] (13.0)	10 (8.3)	0.459	1 [†] /6 [‡] (13.2)	4 (7.5)	0.270
Composite outcome*	3 [†] /12 [‡] (15.0)	17 (14.0)	0.524	2 [†] /8 [‡] (18.9)	10 (18.9)	0.564

TAVR, Transcatheter aortic valve implantation; SAVR, Superficial aortic valve replacement; TIA, Transient ischemic accident; PPM, Permanent pacemaker. *Composition of admission due to heart failure, stroke, or death; [†]balloon expandable aortic valve; [‡]self-expandable aortic valve; [§]mechanical aortic valve; ^{||}tissue aortic valve.

in the SAVR group than in the TAVR group (35.6 vs. 7.5%; $P = 0.001$). Additionally, newly detected AF was terminated relatively early; most of the AF was terminated within 1 month, especially in the SAVR group (Table 2).

Clinical Outcomes

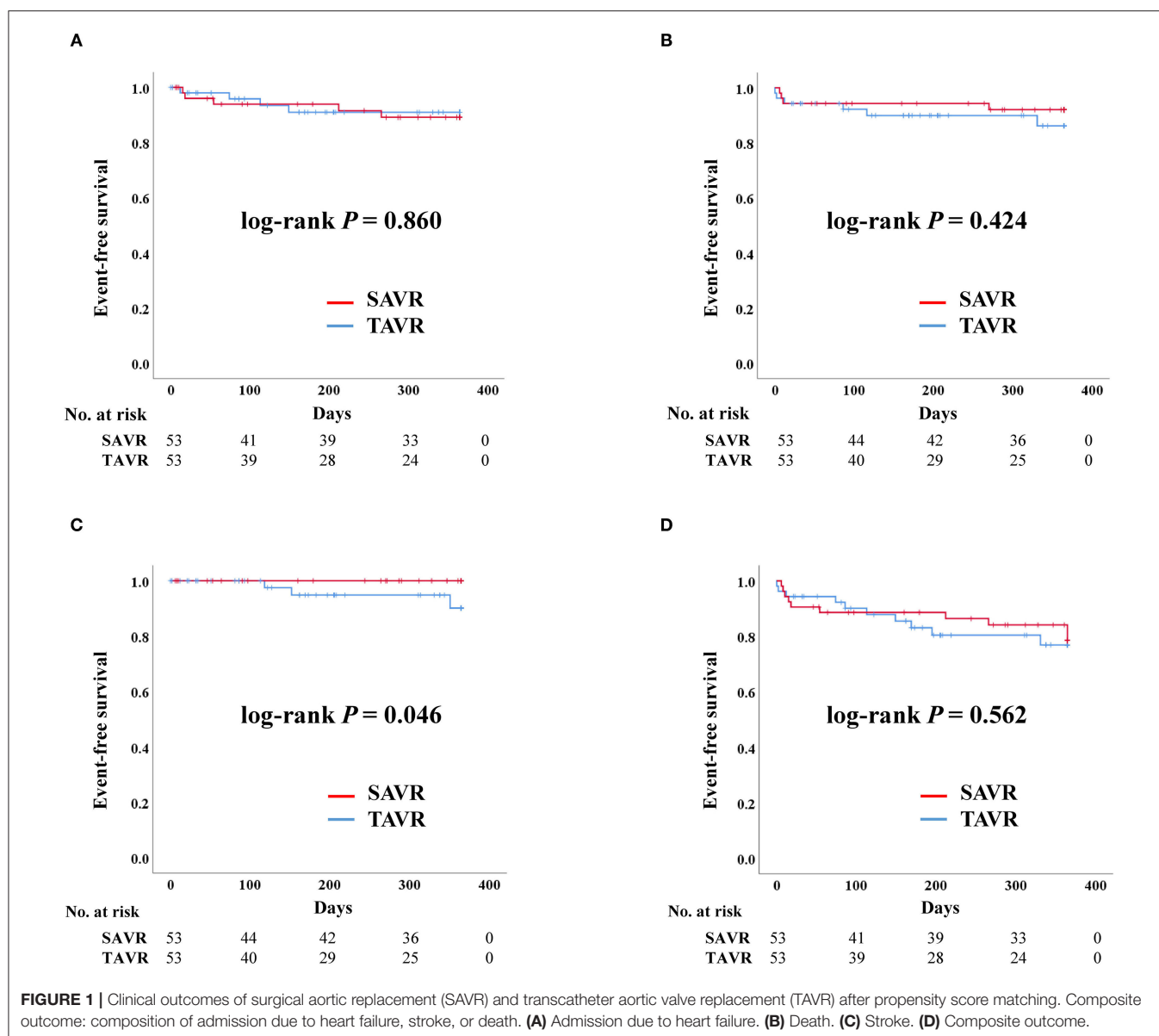
Adverse Event Rate: TAVR vs. SAVR

The rates of admission due to heart failure, stroke, death, and permanent pacemaker implantation were similar between the two groups before PS-matching. The composite outcomes were also comparable between the two groups (Table 3). These results were similar even after PS-matching. The Kaplan–Meier curve for event rates during the 1 year follow-up demonstrated that the rate of stroke was significantly higher in the TAVR than in the SAVR group. Otherwise, there were no significant

differences in terms of admission due to heart failure, death, and composite outcome (Figure 1). Twelve patients in the SAVR group underwent AVR with a mechanical aortic valve. After PS-matching, only two patients with mechanical AVR were included in the analysis. These patients received warfarin after SAVR. All adverse clinical outcomes occurred in patients who underwent tissue AVR.

Impact of AF History in TAVR

After PS-matching, the Kaplan–Meier curve demonstrated no significant difference in the rates of admission due to heart failure, death, stroke, and composite outcome, regardless of AF history. The occurrence of stroke tended to be higher in patients with a history of AF but there was no significant difference (Figure 2).



Impact of AF History in SAVR

For patients who underwent SAVR, there was no significant difference in admission due to heart failure according to AF history. Death was more frequent in patients with AF than in those without a history of AF (log-rank $P = 0.038$). However, there were no stroke events in the SAVR group after PS-matching. The composite outcome was worse in patients with a history of AF, although there was no statistically significant difference (Figure 3).

Impact of Post-AVR AF in TAVR

After PS-matching in the TAVR group, admission due to heart failure was significantly higher in patients with post-AVR AF than those without (log-rank $P = 0.008$) (Figure 4). Death and stroke development were not significantly different between the two groups. Moreover, the composite

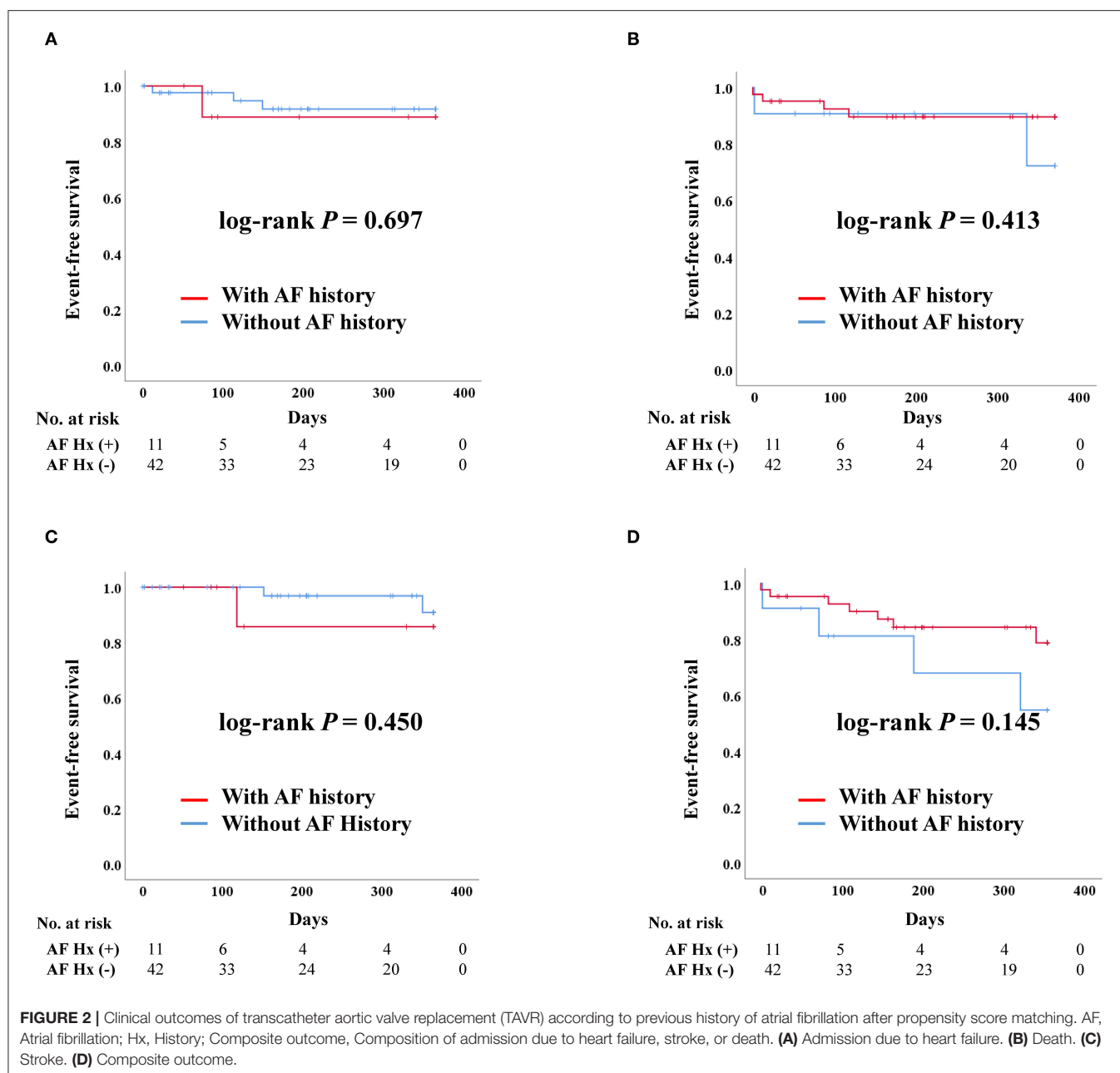
outcome was better in patients without post-AVR AF (log-rank $P = 0.031$).

Impact of Post-AVR AF in SAVR

After PS-matching in the SAVR group, admission due to heart failure and composite outcome was significantly worse in patients who developed post-AVR AF (Figure 5). There were no stroke events in the SAVR group after PS-matching. However, four deaths occurred, all of which were in the post-AVR AF group, although no statistical differences were observed in the log-rank analysis.

Risk Factors for Clinical Outcome After PS-Matching: TAVR vs. SAVR

In the univariate analysis using the Cox proportional model, post-AVR AF (hazard ratio [HR] = 3.58; confidence interval



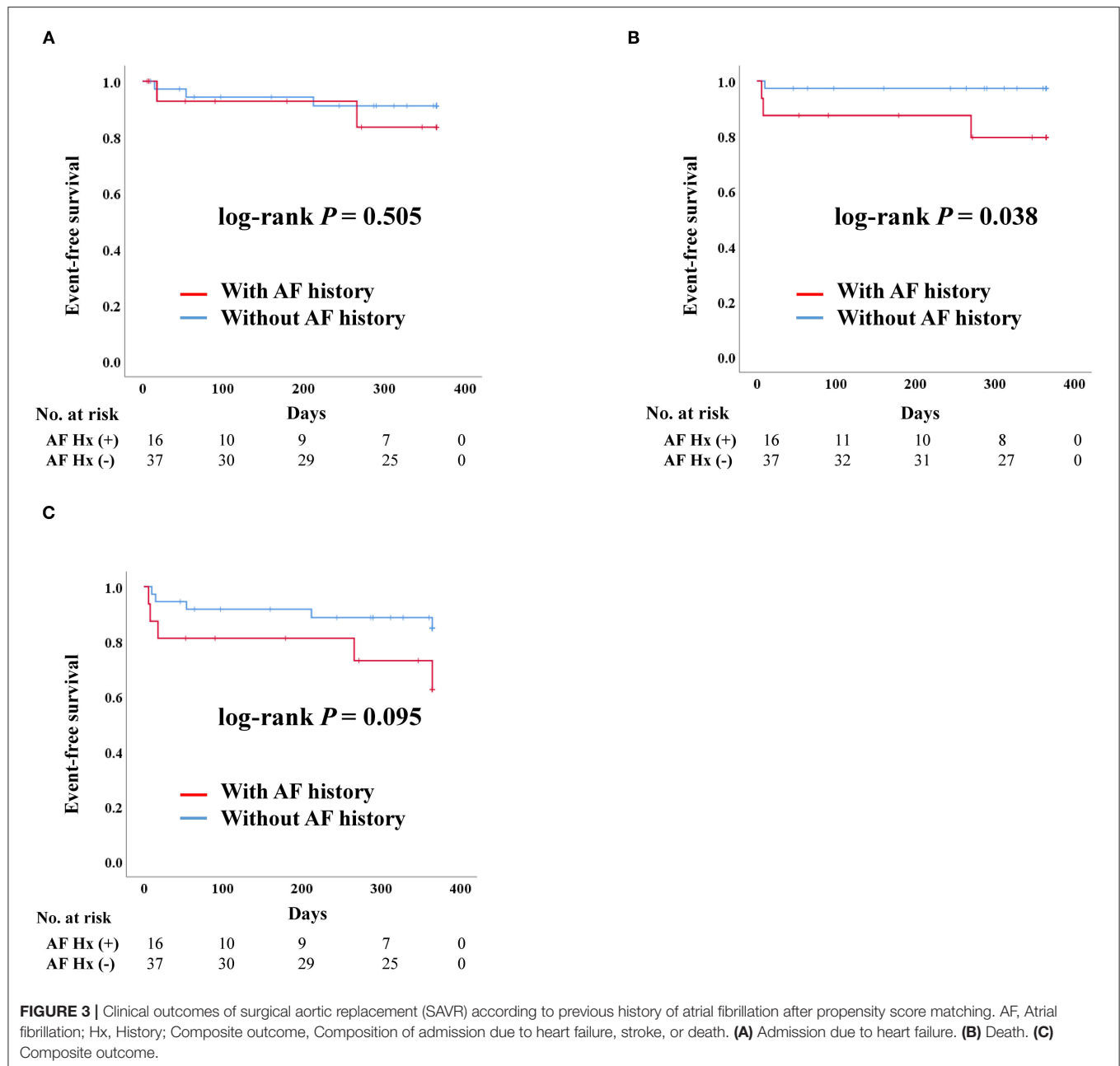
[CI] = 1.03–12.45; $P = 0.044$) and a previous history of myocardial infarction (HR = 4.98; CI = 1.39–17.78; $P = 0.013$) were associated with a worse composite outcome in the TAVR group. Conversely, no significant clinical factors were associated with worse outcomes in the SAVR group. In multivariate analysis, post-AVR AF (HR = 5.52; CI = 1.44–21.13; $P = 0.013$, HR = 20.13; CI = 1.78–228.43; $P = 0.015$) was associated with an adverse event for composite outcome. Additionally, a history of myocardial infarction (HR = 7.84; CI = 1.97–31.25; $P = 0.004$, HR = 14.82; CI = 2.18–100.75; $P = 0.006$) was also an independent risk factor for worse composite outcome in both TAVR and SAVR groups (Table 4).

Comparison of Clinical Outcomes in Patients With Post-AVR AF: TAVR vs. SAVR

There was no significant difference in admission due to heart failure, death, or composite outcome between TAVR and SAVR group in patients with post-AVR AF after PS-matching (Supplementary Figure 1).

DISCUSSION

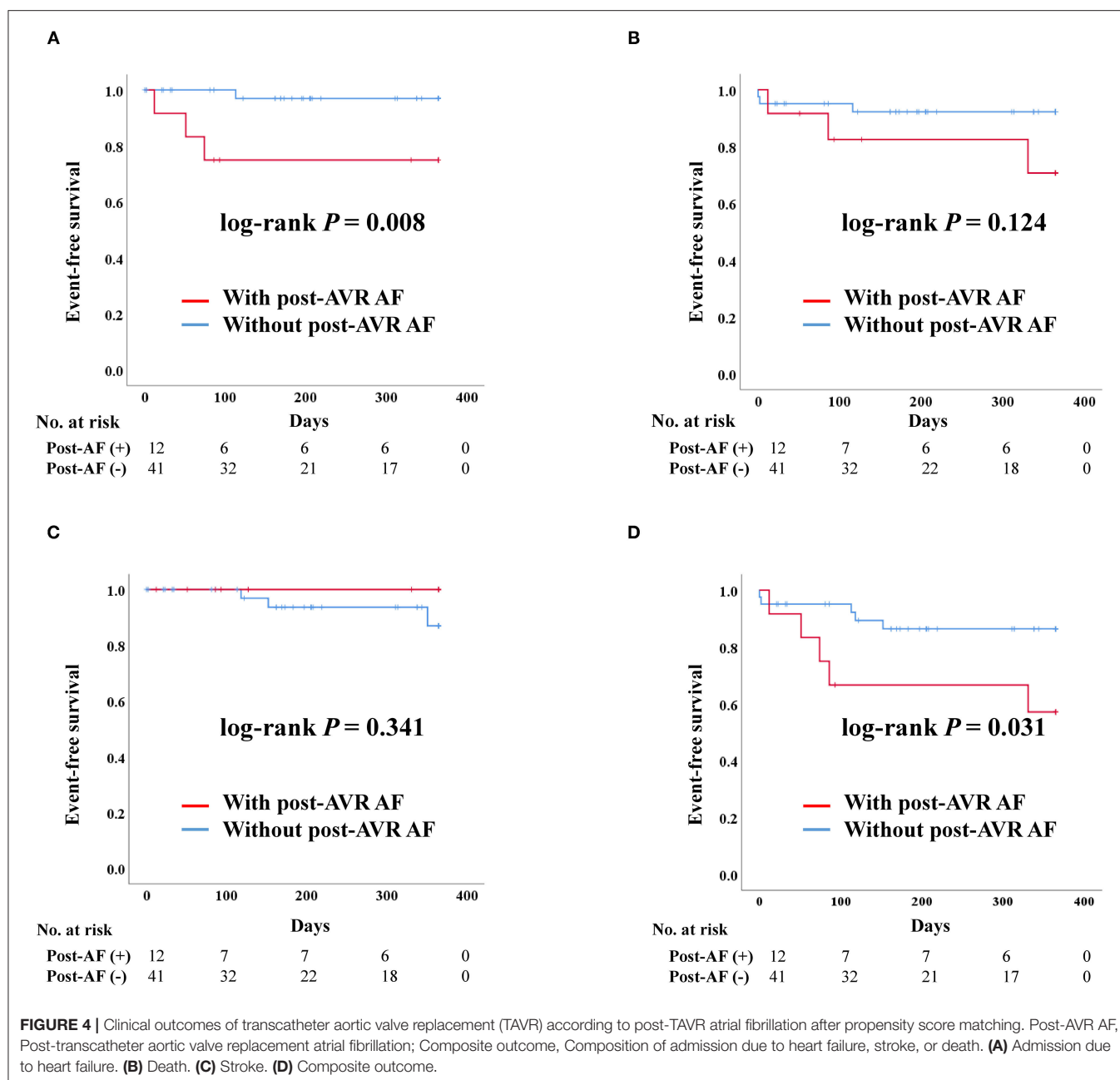
In the present study, we compared the incidence of AF between TAVR and SAVR after PS-matching, and analyzed the clinical outcomes according to the postoperative AF occurrence.



Our results demonstrated that the incidence of post-AVR AF, especially newly detected AF, was significantly higher in the SAVR group before and after PS-matching (Table 2). Moreover, our data showed that AF had a worse impact on both TAVR and SAVR outcomes after PS-matching.

The incidence of AF in our study was consistent with that reported in previous studies. The incidence of newly detected AF in the TAVR group before and after PS-matching was 6 and 7.5%, respectively, while in the SAVR group, it was 40.5 and 35.6%, respectively. Previous studies have reported that the incidence of newly detected AF in TAVR ranges from 7.2 to 11.7% (3, 4, 12) and that the incidence of postoperative AF after cardiac

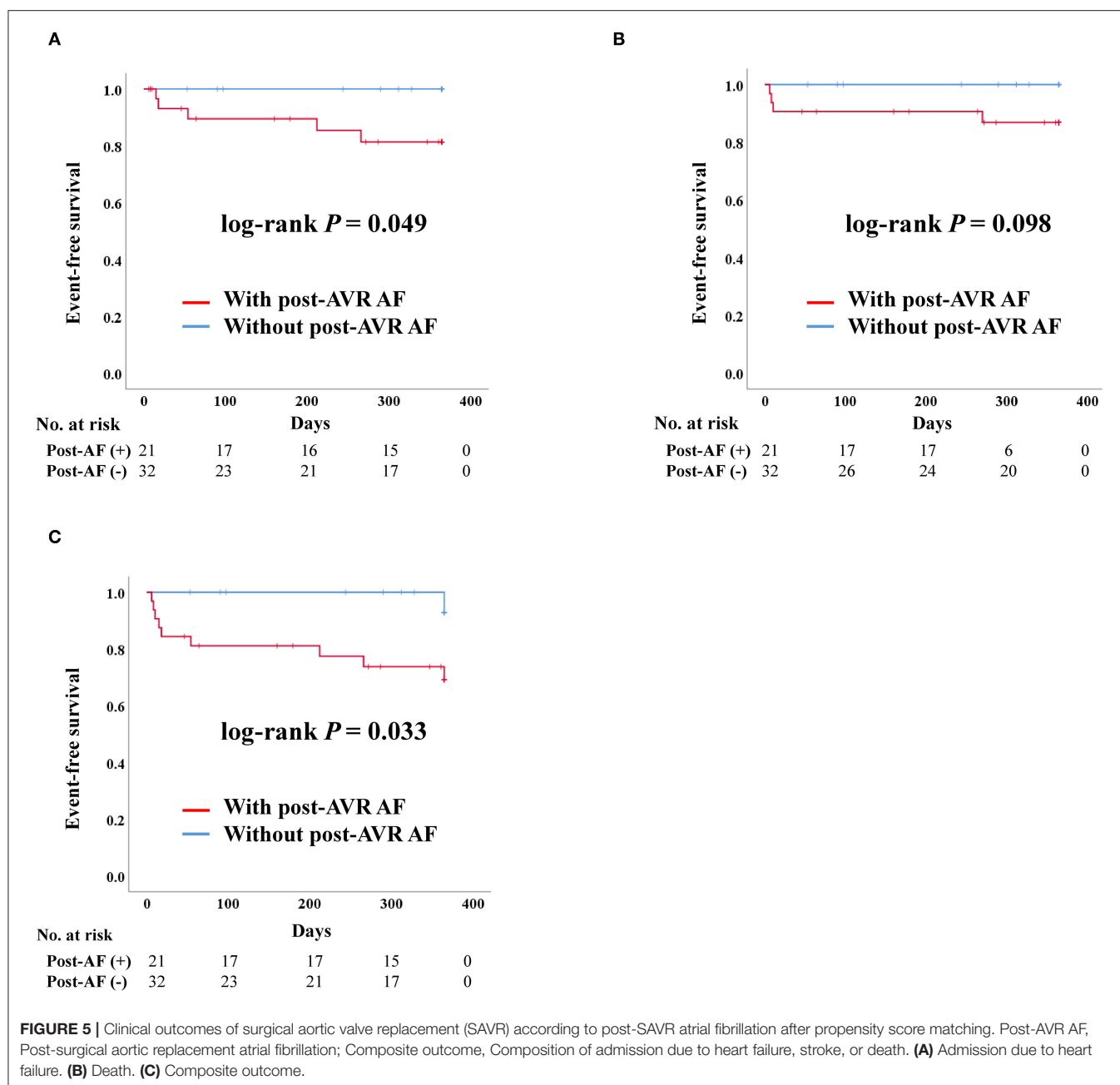
surgery ranges from 10 to 65% (10, 11). In the recent New York state inpatient database validation cohort, the incidence of AF was 14.1% in patients undergoing TAVR and 30.6% in patients undergoing SAVR. Compared to our data, the incidence of AF in TAVR was higher, but, the incidence of AF in SAVR was less (13). The exact mechanism of AF development after TAVR has not yet been elucidated clearly. Although several mechanisms have been suggested for its pathophysiology, much of the knowledge is dependent on the mechanism of postoperative AF occurrence (14). Maesen et al. suggested that the factors facilitating AF can be divided into acute and chronic factors. Acute factors are directly related to procedures such as adrenergic stimulation, and



chronic factors are associated with atrial remodeling, such as left atrium enlargement (15, 16). These factors may increase the risk of re-entry and ectopic activity, which may ultimately induce AF after cardiac surgery or TAVR (14). Urena et al. suggested that patients with severe AS develop AF because AF and AS share many common risk factors, such as age and hypertension. Moreover, they reported that one-third of the patients with AS were already affected by AF, which was more frequent than that of the TAVR group and less than that of the SAVR group in our study population (17).

The majority of postoperative AF is known to be terminated spontaneously within 4–6 weeks (10). Our results also

demonstrated that most postoperative AF was terminated within 1 month (89.4%, 17 of 19). However, there are little data on how long newly detected AF persists after TAVR. Patients with AF who underwent TAVR at our center usually had AF for <3 months, except for one patient, in whom AF persisted for more than 3 months despite having a newly detected AF. Among the patients with newly detected AF ($n = 6$) in the TAVR group, AF was terminated within 1 month in three patients, within 3 months in two patients. Even though post-AVR AF was terminated relatively early in both the TAVR and SAVR groups, the data showed that AF still had a worse impact on clinical outcomes. Moreover, we believe that the AF was simply



triggered by the procedure or surgery, and that the AF substrate had already existed. Therefore, the deleterious effects of AF would continue.

In one subgroup analysis of the PARTNER 3 trial, TAVR had a significantly lower frequency of post-AVR AF than did SAVR (18). This was similar to the present study's findings. Particularly, the authors divided AF into early and late postoperative AF. Only late postoperative AF, defined as AF occurring after hospital discharge up to 1 year, was associated with worse clinical outcomes regardless of treatment modalities. Although we did not categorize AF in detail, our results showed that post-AVR AF had a worse impact on clinical outcomes, which was consistent

with that study. Therefore, the present study may provide evidence of real-world data pertaining to patients with various risk factors, which may be comparable to the subgroup analysis of well-designed randomized trials results.

Another subgroup analysis of the PARTNER 3 trial showed that low-risk patients with preexisting AF had worse clinical outcomes (19). The authors defined the outcomes as death, stroke, rehospitalization, and a composite of all these outcomes. Only the composite outcome was significantly higher in patients with pre-existing AF regardless of the treatment modalities, which was mainly driven by rehospitalization. However, when the patients were classified into SAVR and TAVR groups,

TABLE 4 | Risk factors of worse composite outcome in patients with TAVR and SAVR after PS-matching.

	TAVR						SAVR					
	Unadjusted			Adjusted			Unadjusted			Adjusted		
	HR	95% CI	P-value	HR	95% CI	P-value	HR	95% CI	P-value	HR	95% CI	P-value
Age	1.09	0.99–1.19	0.070				1.05	0.93–1.19	0.402			
Sex	0.53	0.14–2.03	0.351				0.94	0.26–3.34	0.924			
Hypertension	3.51	0.45–22.71	0.234				0.75	0.22–2.61	0.656			
DM	0.66	0.17–2.55	0.547				1.79	0.50–6.35	0.369			
Heart failure	1.33	0.28–6.28	0.720				2.05	0.43–9.66	0.366			
Previous MI	4.98	1.39–17.78	0.013	7.84	1.97–31.25	0.004	2.92	0.62–13.79	0.176	14.82	2.18–100.75	0.006
Old CVA	1.42	0.37–5.49	0.614				1.80	0.38–8.56	0.460			
CKD/ESRD	1.77	0.38–8.36	0.469				0.81	0.10–6.38	0.839			
AF history	3.00	0.84–10.68	0.090				2.75	0.80–9.52	0.110			
Post-AVR AF	3.58	1.03–12.45	0.044	5.52	1.44–21.13	0.013	6.88	0.87–54.37	0.067	20.13	1.78–228.43	0.015

TAVR, Transcatheter aortic valve implantation; SAVR, Superficial aortic valve replacement; PS, propensity score; MI, Myocardial infarction; DM, Diabetes mellitus; CVA, Cerebrovascular accident; CKD, Chronic kidney disease; ESRD, End-stage renal disease; Post-AVR, Post-aortic valve replacement; HR, Hazard ratio; CI, Confidence interval.

AF did not significantly change the outcomes, although the absolute number of adverse outcomes was greater in the preexisting AF group. The present study also did not reveal significant differences in clinical outcomes according to AF history. However, considering the small sample size, the tendency of composite clinical outcome was similar to that reported in the recent study. Meanwhile, Zhang et al. suggested that preexisting AF is not an independent predictor of outcomes in severe AS (20). The authors assumed that the concomitant cardiac abnormalities may play a role in worse clinical outcomes. Therefore, whether preexisting AF has a substantially bad impact on the clinical outcome would require clarification through additional studies.

Usually, permanent pacemaker implantation rates are more frequent in TAVR than in SAVR. Interestingly, the rate of permanent pacemaker implantation was only 5.6% in the Korean data and 17% on average in other meta-analyses (21, 22). Yu et al. suggested that this was due to adequate device selection, meticulous procedures, high threshold for pacemaker implantation, relatively longer hospital stays, and low rate of pre-existing conduction disturbance (6). At our center, 13% of TAVR patients received a permanent pacemaker within 1 month, which was more frequent than that in the SAVR group, but the difference was not statistically significant.

The TAVR group had a higher incidence of stroke. In the present study, after PS-matching, there were no stroke events in the SAVR group, but there were three events in the TAVR group. Despite the significant difference, the data should be interpreted with caution because of the small sample size and lower event rates. We assumed that the occurrence of stroke in the TAVR group might have been attributable to guidewire and catheter manipulation through the aortic arch or calcified aortic valves. As TAVR is used in degenerative disease, many atherosclerotic changes in the aorta are likely to exist. Therefore, guidewire and catheter manipulation may induce devastating events, such as stroke. This finding is consistent with those of previous trials (23). However, a recent study showed that the stroke rate discrepancies between TAVR and SAVR dissipated 5 years after the procedure

(24). However, the mechanism was unclear, and further research is required to assess it precisely.

Some data have previously demonstrated a comparison between TAVR and SAVR. Moreover, some data suggests that AF has a worse impact on clinical outcomes regardless of the AVR method. In this context, the novelty of our study is the investigation of the AF incidence after PS-matching and the comparison of the difference in the impact of AF on the clinical outcomes in each group. Our data showed that AF had worse impact on clinical outcomes in both groups, similar to the results of previous studies, and revealed a far higher incidence of AF in the SAVR group. This finding suggests that if the probability of AF occurrence is high in some patients with severe AS due to old age, hypertension, or chronic kidney disease, TAVR may be a favorable choice because of lower incidence of post-AVR AF.

AF-sustaining duration is relatively longer in cases of non-paroxysmal AF. Thus, non-paroxysmal AF may be considered to affect clinical outcomes. A research revealed that different AF types may impact clinical outcomes after TAVR. Shaul et al. suggested that histories of non-paroxysmal AF were associated with the risk of stroke or death. Conversely, paroxysmal AF did not significantly differ from sinus rhythm (25). They assumed that short AF episodes may be relative to the other major comorbidities; therefore, it would only have a minor effect on the overall clinical outcomes. In our study, a total of 27 patients had a history of AF after PS-matching. Paroxysmal AF (TAVR, $n = 5$, SAVR, $n = 10$) was identified in 15 patients, which was not sufficient for statistical analyses.

Limitations

The present study had several limitations. First, the study population was enrolled from a single center in Korea. Therefore, it is not representative of all patients with severe AS. However, it is likely to reflect the effectiveness of TAVR or SAVR in Asian patients. Second, the sample size was relatively small, and discrepancies in the clinical outcomes between groups were smaller than those reported in previous studies, which revealed no statistical differences in some clinical outcomes. For example,

there was no significant difference in the rate of permanent pacemaker implantations between the SAVR and TAVR groups. Third, this was a retrospective observational study, and the baseline characteristics were heterogeneous between the TAVR and SAVR groups. We conducted PS-matching to homogenize the baseline characteristics. However, as the differences in characteristics, such as age and previous myocardial infarction, were substantial, a large portion of the patients were excluded as a result.

Future Directions

Large-scale, multicenter, and prospective studies would be beneficial in overcoming the abovementioned limitations.

CONCLUSION

TAVR and SAVR had similar clinical outcomes in patients with severe symptomatic AS, and post-AVR AF had a worse impact on both the TAVR and SAVR groups compared with that on patients without AF. Therefore, post-AVR AF could be considered a predictor of the outcomes of AVR. TAVR may be a more favorable treatment option than SAVR for patients with severe symptomatic AS who are at high-risk for AF-development or who have a history of AF because the occurrence of AF was less frequent in the TAVR group.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Chonnam National University

Hospital, Gwangju, South Korea. The ethics committee waived the requirement of written informed consent for participation.

AUTHOR CONTRIBUTIONS

HKJ conducted the conception, investigation, and formal analysis and wrote the first draft of the manuscript. NY contributed to the design, literature search, experimental studies, data acquisition, data analysis, manuscript preparation, manuscript editing, manuscript review, approval of the final version of the manuscript, and agreement of all aspects of the work. All authors participated in the conception, investigation, manuscript review, approval of the final version of the manuscript, agreement of all aspects of the work, provided constructive criticism and comments, and read and approved the final manuscript.

FUNDING

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcvm.2021.789548/full#supplementary-material>

Supplementary Figure 1 | Transcatheter aortic valve replacement (TAVR) vs. surgical aortic valve replacement (SAVR) in patients with post aortic valve replacement (AVR) atrial fibrillation (AF) after propensity score (PS)-matching. There was no significant difference in the occurrence of heart failure, death or composite outcome in patients with post-AVR AF between TAVR and SAVR groups after PS-matching. It was impossible to compare stroke rates because there was no stroke event in SAVR group.

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Absence of Obesity Paradox in All-Cause Mortality Among Chinese Patients With an Implantable Cardioverter Defibrillator: A Multicenter Cohort Study

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Background: The results of studies on the obesity paradox in all-cause mortality are inconsistent in patients equipped with an implantable cardioverter-defibrillator (ICD). There is a lack of relevant studies on Chinese populations with large sample size. This study aimed to investigate whether the obesity paradox in all-cause mortality is present among the Chinese population with an ICD.

Methods: We conducted a retrospective analysis of multicenter data from the Study of Home Monitoring System Safety and Efficacy in Cardiac Implantable Electronic Device-implanted Patients (SUMMIT) registry in China. The outcome was all-cause mortality. The Kaplan–Meier curves, Cox proportional hazards models, and smooth curve fitting were used to investigate the association between body mass index (BMI) and all-cause mortality.

Results: After inclusion and exclusion criteria, 970 patients with an ICD were enrolled. After a median follow-up of 5 years (interquartile, 4.1–6.0 years), in 213 (22.0%) patients occurred all-cause mortality. According to the Kaplan–Meier curves and multivariate Cox proportional hazards models, BMI had no significant impact on all-cause mortality, whether as a continuous variable or a categorical variable classified by various BMI categorization criteria. The fully adjusted smoothed curve fit showed a linear relationship between BMI and all-cause mortality (p -value of 0.14 for the non-linearity test), with the curve showing no statistically significant association between BMI and all-cause mortality [per 1 kg/m² increase in BMI, hazard ratio (HR) 0.97, 95% CI 0.93–1.02, $p = 0.2644$].

Conclusions: The obesity paradox in all-cause mortality was absent in the Chinese patients with an ICD. Prospective studies are needed to further explore this phenomenon.

Keywords: obesity paradox, body mass index, all-cause mortality, implantable cardioverter-defibrillator, Chinese

INTRODUCTION

Being overweight or obese is a global health problem, with almost two-thirds of American adults experiencing overweight or obesity (1) and the latest epidemiological survey data from China show that the proportion of adults in China who are overweight and obese is 28.1 and 5.2%, respectively (2). Being overweight or obese can promote inflammatory responses, cardiac hypertrophy, and fibrosis, which can lead to an increased incidence of cardiovascular disease (CVD) and is associated with numerous adverse CVD prognostic events (3–5). Although obesity is a risk factor for CVD, a phenomenon known as the “obesity paradox” has been identified. “Obesity paradox” means that patients who have already suffered from many types of CVD may have a better prognosis if they are classified as overweight or obese (6). The obesity paradox in all-cause mortality has been identified when better survival is seen in people with higher body mass index (BMI) among the patients with hypertension, coronary heart disease, atrial fibrillation, and heart failure (3–5, 7–13).

For patients equipped with an implantable cardioverter defibrillator (ICD), the results of studies on the obesity paradox in all-cause mortality are inconsistent (14–17). Moreover, the previous studies have focused on European and American populations, and there is a lack of studies on the Chinese population with large sample size. This study retrospectively analyzed multicenter data from China to investigate whether the obesity paradox all-cause mortality is present in the Chinese population with an ICD.

METHODS

Study Population

Based on data from the Study of Home Monitoring System Safety and Efficacy in Cardiac Implantable Electronic Device-implanted Patients (SUMMIT) registry, we conducted a retrospective cohort analysis enrolling the patients between May 2010 and May 2015 in China. Inclusion criteria were as follows: (i) patients aged more than 18 years; (ii) patients met indications of primary or secondary prevention of sudden cardiac death (SCD) according to clinical practice standards (18–20); and (iii) patients were implanted with an ICD or cardiac resynchronization therapy defibrillator (CRT-D) (collectively referred to as ICD) (Biotronik, Germany) device with home monitoring (HM). Exclusion criteria were as follows: (i) patients under the age of 18 years and (ii) patients with missing clinical data. **Figure 1** depicts the flowchart of the research population. The study protocols were approved by the Ethics Committee of Fuwai Hospital, the Chinese Academy of Medical Sciences (the lead institute), and all other collaborating organizations (Zhongshan Hospital, Fudan University, and so on). The protocols followed the Declaration of Helsinki.

Before the registry, all patients signed informed permission forms. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) principles were followed for all reporting (21).

Data Collection

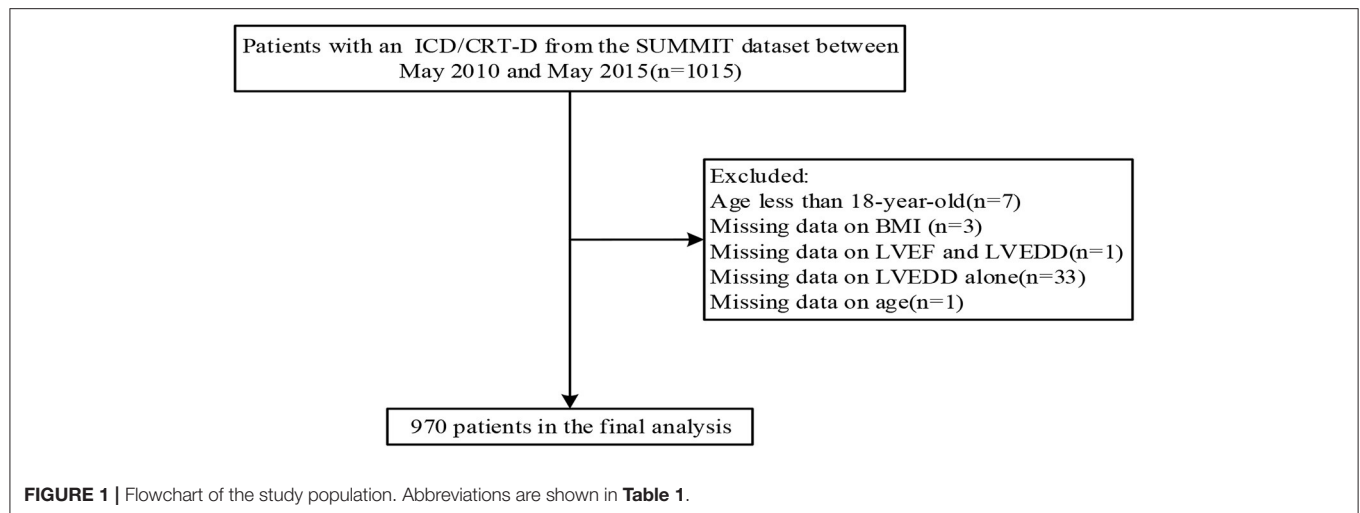
The BMI was determined by dividing the patient's weight (kg) by the square of his or her height (m^2), and the result was expressed as kg/m^2 . BMI grouped according to tertiles, WHO criteria (22), Asian criteria (23), or Chinese criteria (24). Before ICD implantation, baseline clinical information was obtained from the patients' medical records.

Device Programming Settings and Outcome

The device programming settings technique was the same as in our prior study (25), which was described in detail in **Supplementary Material**. The outcome was all-cause mortality. Routine follow-ups were undertaken by phone calls and outpatient clinic visits. In the case that the transmission of their data was disrupted, and patient status was confirmed *via* phone calls. The last follow-up visit was in June 2018.

Statistical Analysis

The means \pm SD or proportions are used to present the data. To compare the all-cause mortality of different BMI groups, we used Kaplan–Meier curves (log-rank test). A Cox proportional hazards model was used to furtherly assess the association between BMI and all-cause mortality. A univariate Cox proportional hazards model was first used to explore the factors influencing all-cause mortality. The multivariate Cox proportional hazards models were conducted by adjusting for factors that had a statistically significant effect on death at the 0.05 level in the univariate Cox model or all baseline factors. To further explore the dose-response effect of BMI on all-cause mortality, a cubic spline function model and smooth curve fitting (penalized spline method) were conducted. To ensure the robustness of the result, we did the following sensitivity analysis. First, we converted the BMI into a categorical variable by tertiles and calculated the *P* for trend. Second, we performed the same analysis based on WHO criteria, Asian criteria, or Chinese criteria for BMI grouping. Third, we did subgroup analyses in various groups. To maximize the exploration of other possible risk factors for all-cause mortality, the univariate and multivariate Cox models were used to do the analysis. R version 4.0.0 (R Foundation for Statistical Computing, Vienna, Austria) and Empower (R) were used for all the analyses (X&Y Solutions, Inc., Boston, MA, USA). All *P*-values were considered statistically significant if they were <0.05 (two-sided).

**TABLE 1 |** Baseline characteristics of study population.

Variables	Total (n = 970)	BMI < 24 kg/m ² (n = 567)	BMI: 24–28 kg/m ² (n = 352)	BMI ≥ 28 kg/m ² (n = 51)	p
Male, n (%)	707 (72.9)	392 (69.1)	275 (78.1)	40 (78.4)	0.008
Age at implantation, years	60.3 ± 13.5	60.3 ± 13.7	60.7 ± 13.0	58.3 ± 15.0	0.498
NYHA, Class III/IV, n (%)	484 (49.9)	288 (50.8)	170 (48.3)	26 (51)	0.753
SBP, mmHg	124.5 ± 17.4	123.5 ± 17.1	125.0 ± 17.3	131.9 ± 19.9	0.004
DBP, mmHg	76.9 ± 10.9	76.1 ± 10.6	77.5 ± 10.7	80.7 ± 13.8	0.005
Primary prevention, n (%)	576 (59.4)	343 (60.5)	199 (56.5)	34 (66.7)	0.273
CRT-D, n (%)	266 (27.4)	163 (28.7)	90 (25.6)	13 (25.5)	0.548
Ischemic cardiomyopathy, n (%)	324 (33.4)	174 (30.7)	127 (36.1)	23 (45.1)	0.046
Dilated cardiomyopathy, n (%)	238 (24.5)	142 (25)	84 (23.9)	12 (23.5)	0.908
Hypertrophic cardiomyopathy, n (%)	37 (3.8)	19 (3.4)	13 (3.7)	5 (9.8)	0.091
Long QT syndrome, n (%)	12 (1.2)	7 (1.2)	5 (1.4)	0 (0)	0.888
Hypertension, n (%)	305 (31.4)	163 (28.7)	114 (32.4)	28 (54.9)	< 0.001
Diabetes mellitus, n (%)	101 (10.4)	51 (9)	42 (11.9)	8 (15.7)	0.164
Stroke, n (%)	18 (1.9)	5 (0.9)	10 (2.8)	3 (5.9)	0.01
Atrial fibrillation, n (%)	104 (10.7)	60 (10.6)	38 (10.8)	6 (11.8)	0.965
Pre-implant syncope, n (%)	194 (20.0)	114 (20.1)	78 (22.2)	2 (3.9)	0.01
LVEF, %	42.5 ± 14.9	42.1 ± 15.0	42.8 ± 14.7	44.4 ± 15.5	0.503
LVEDD, mm	58.8 ± 13.1	58.4 ± 13.2	59.7 ± 13.0	57.3 ± 13.3	0.252
β-Blocker, n (%)	566 (58.4)	326 (57.5)	209 (59.4)	31 (60.8)	0.8
Amiodarone, n (%)	290 (29.9)	171 (30.2)	109 (31)	10 (19.6)	0.248
ACE or ARB, n (%)	360 (37.1)	207 (36.5)	132 (37.5)	21 (41.2)	0.79
Diuretic, n (%)	382 (39.4)	212 (37.4)	149 (42.3)	21 (41.2)	0.318
Loop diuretic, n (%)	280 (28.9)	159 (28)	106 (30.1)	15 (29.4)	0.794
Aldosterone antagonist, n (%)	363 (37.4)	210 (37)	138 (39.2)	15 (29.4)	0.385

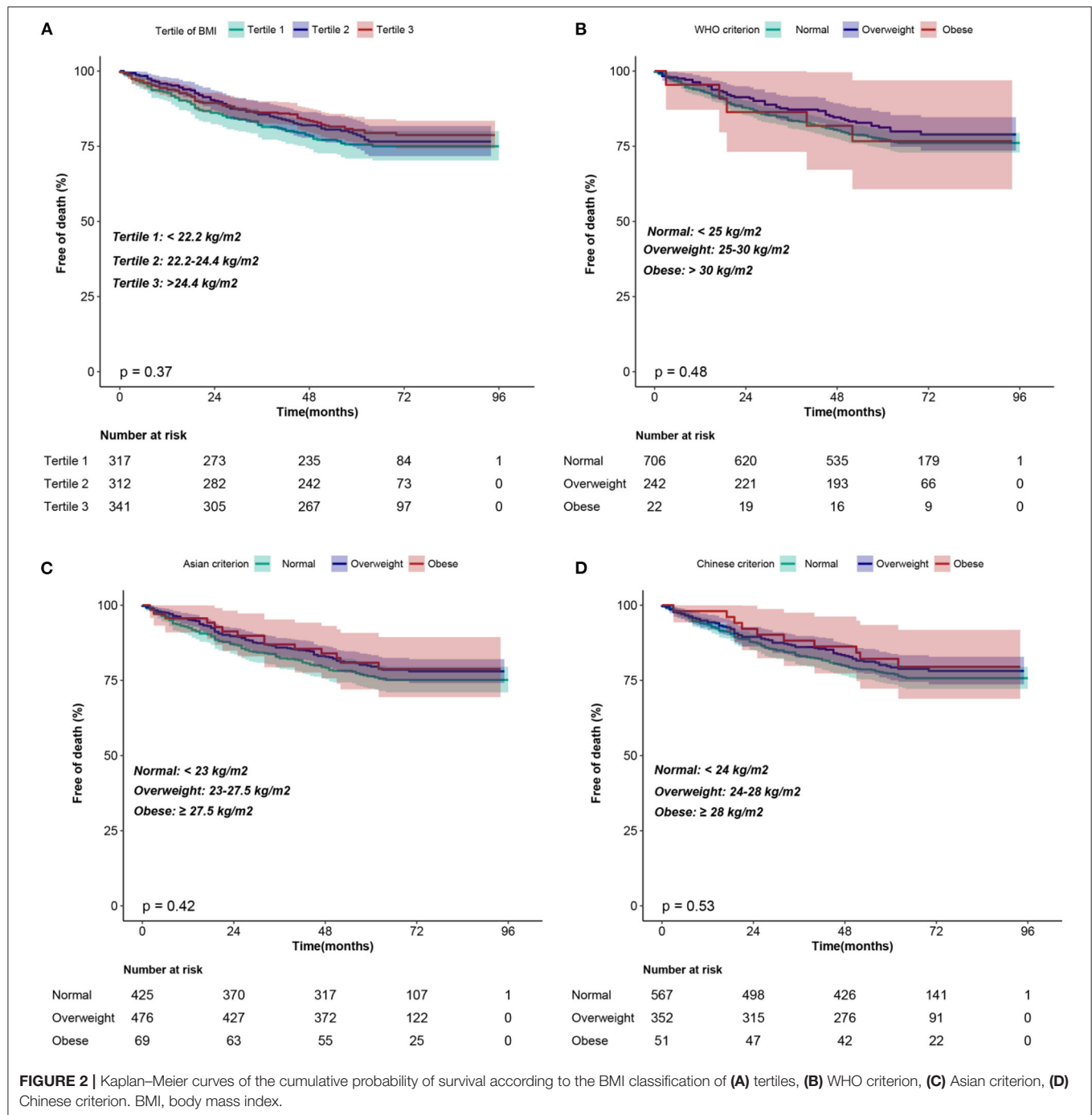
ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CRT-D, cardiac resynchronization therapy defibrillator; DBP, diastolic blood pressure; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-systolic dimension; NYHA, New York Heart Association; SBP, systolic blood pressure.

RESULTS

Baseline Characteristics of the Study Population

A total of 1,015 patients from the SUMMIT dataset between May 2010 and May 2015 were initially included. After inclusion

and exclusion criteria, 970 patients were enrolled. **Table 1** shows the overall baseline characteristics of the study population. The average age of the study population was 60.3 years, with 72.9% male. The percentage of New York Heart Association [NYHA] class III/IV was 49.9 and 27.4% of patients were implanted with CRT-D. In total, 394 patients met the secondary



prevention of SCD criteria. Among these patients, 236 (60%) had documented sustained ventricular tachycardia (VT), 98 (25%) had documented ventricular fibrillation (VF) and resuscitated SCD, and 60 (15%) experienced unexplained syncope and may be induced to VT or VF during the electrophysiological study. We performed a comparison of baseline characteristics based on the Chinese grouping criteria for BMI. Most of the variables were not significantly different, except that the obese population had

a higher proportion of men, higher systolic and diastolic blood pressure, a greater proportion of ischemic cardiomyopathy, a higher proportion of hypertension, and a greater history of stroke (all $P < 0.05$).

Influence of BMI on All-Cause Mortality

The median follow-up was 5.0 years (interquartile, 4.1–6.0 years). During follow-up, 213 (22.0%) patients experienced all-cause

TABLE 2 | Association of BMI with all-cause mortality in different Cox proportional hazards models.

BMI (kg/m ²)	No. of death	Model 1		Model 2		Model 3		Model 4	
		HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Continuous	213	0.98 (0.94, 1.03)	0.4405	0.98 (0.93, 1.02)	0.3507	0.97 (0.92, 1.02)	0.1937	0.97 (0.93, 1.02)	0.2644
Tertiles									
<22.1	96	Reference		Reference		Reference		Reference	
22.1–24.4	126	0.87 (0.63, 1.20)	0.4002	0.86 (0.62, 1.19)	0.3560	0.89 (0.64, 1.25)	0.5163	0.89 (0.63, 1.25)	0.4949
> 24.4	130	0.79 (0.57, 1.10)	0.4405	0.77 (0.55, 1.07)	0.1175	0.72 (0.51, 1.01)	0.0571	0.73 (0.52, 1.04)	0.0793
<i>P_{trend}-value</i>			0.1645		0.1178		0.0560		0.0791
WHO criterion									
<25	245	Reference		Reference		Reference		Reference	
25–30	100	0.82 (0.59, 1.13)	0.2313	0.80 (0.57, 1.10)	0.1692	0.72 (0.52, 1.01)	0.0570	0.76 (0.54, 1.06)	0.1048
≥30	7	0.99 (0.40, 2.40)	0.9757	1.02 (0.42, 2.49)	0.9585	0.89 (0.36, 2.20)	0.8004	0.99 (0.40, 2.40)	0.9757
<i>P_{trend}-value</i>			0.3221		0.2701		0.0941		0.1296
Asian criterion									
< 23	138	Reference		Reference		Reference		Reference	
23–27.5	183	0.84 (0.64, 1.11)	0.2186	0.82 (0.62, 1.08)	0.1583	0.79 (0.59, 1.06)	0.1129	0.84 (0.64, 1.11)	0.2186
≥ 27.5	31	0.81 (0.46, 1.42)	0.4679	0.77 (0.44, 1.34)	0.3515	0.71 (0.40, 1.26)	0.2433	0.72 (0.40, 1.29)	0.2726
<i>P_{trend}-value</i>			0.2181		0.1420		0.0870		0.0968
Chinese criterion									
< 24	197	Reference		Reference		Reference		Reference	
24–28	131	0.87 (0.65, 1.16)	0.3286	0.84 (0.63, 1.12)	0.2364	0.79 (0.59, 1.06)	0.1105	0.80 (0.60, 1.08)	0.1520
≥ 28	24	0.80 (0.42, 1.52)	0.4888	0.79 (0.42, 1.50)	0.4739	0.72 (0.38, 1.39)	0.3335	0.73 (0.38, 1.42)	0.3574
<i>P_{trend}-value</i>			0.2694		0.2074		0.0901		0.1219

Model 1: adjusted for none. Model 2: adjusted for age, gender. Model 3: adjusted for variables in Model 2 plus NYHA, Class III/IV, primary prevention, Ischemic cardiomyopathy, hypertension, diabetes mellitus, atrial fibrillation, LVEF, LVEDD, β -Blocker, ACEI or ARB, a loop diuretic, aldosterone antagonists, dilated cardiomyopathy. Model 4 adjusted for all covariates presented in **Table 1**. HR, hazard ratio; other abbreviations are shown in **Table 1**.

mortality. Kaplan–Meier curves were plotted to compare the cumulative probability of survival for different BMI groups (**Figure 2**). The cumulative probability of survival did not differ between the groups regardless of the criteria of BMI grouping (log-rank, all $p > 0.05$).

Table 2 shows the effect of BMI on all-cause mortality using four Cox proportional risk models. In the unadjusted model (model 1), each 1 kg/m² increase in BMI was associated with a 2% increase in the risk of death, but this was not statistically significant. Further, in model 2, the association between BMI and death was again not statistically significant after adjusting for age and sex. After adjustment for additional covariates in model 3 (for age, New York Classification of Cardiac Function (NYHA) III/IV, primary prevention, ischaemic cardiomyopathy, hypertension, diabetes, atrial fibrillation, left ventricular ejection fraction (LVEF), left ventricular end-systolic dimension (LVEDD), β -blocker, angiotensin-converting enzyme inhibitor (ACEI)/angiotensin receptor blocker (ARB), a loop diuretic, aldosterone antagonist, and dilated cardiomyopathy) and all covariates in **Table 1** in model 4, the results underwent negligible changes. In addition, we converted BMI from a continuous variable to a categorical variable. There was no significant increase in the risk of death in patients with tertile 2 or tertile 3 in all models (models 1–4) compared with tertile 1 as a reference. The test for trend was not significant in any of the models. In addition, the sensitivity analyses were performed

using different clinical classification criteria for BMI and the results remained consistent. Additionally, we analyzed different subgroups and the results were still robust (**Figure 3**).

A cubic spline function model and smoothed curve fitting (penalized spline approach) were performed to assess the dose-response association between BMI and all-cause mortality. The fully adjusted smoothed curve fit showed a linear relationship between BMI and mortality (p -value of 0.14 for the non-linearity test) (**Figure 4**), with the curve showing no statistically significant association between BMI and all-cause mortality (per 1 kg/m² increase in BMI, hazard ratio (HR) 0.97, 95% CI 0.93–1.02, $p = 0.2644$).

Univariate and Multivariate Risk Factors of All-Cause Mortality

Table 3 shows the univariate Cox proportional hazards models of all-cause mortality. Older age, NYHA class III/IV, primary prevention, ischaemic cardiomyopathy, hypertension, diabetes, atrial fibrillation, lower LVEF, wider LVEDD, β -blocker, ACEI/ARB, a loop diuretic, aldosterone antagonist, and dilated cardiomyopathy were the univariate predictors of all-cause mortality in the overall group. Older age (HR 1.02; 95% CI 1.01–1.04; $P < 0.001$), NYHA Class III/V (HR 1.55; 95% CI 1.1–2.19; $P < 0.012$), ischemic cardiomyopathy (HR 1.54; 95% CI 1.14–2.07; $P < 0.005$), wider LVEDD (HR 1.02; 95% CI

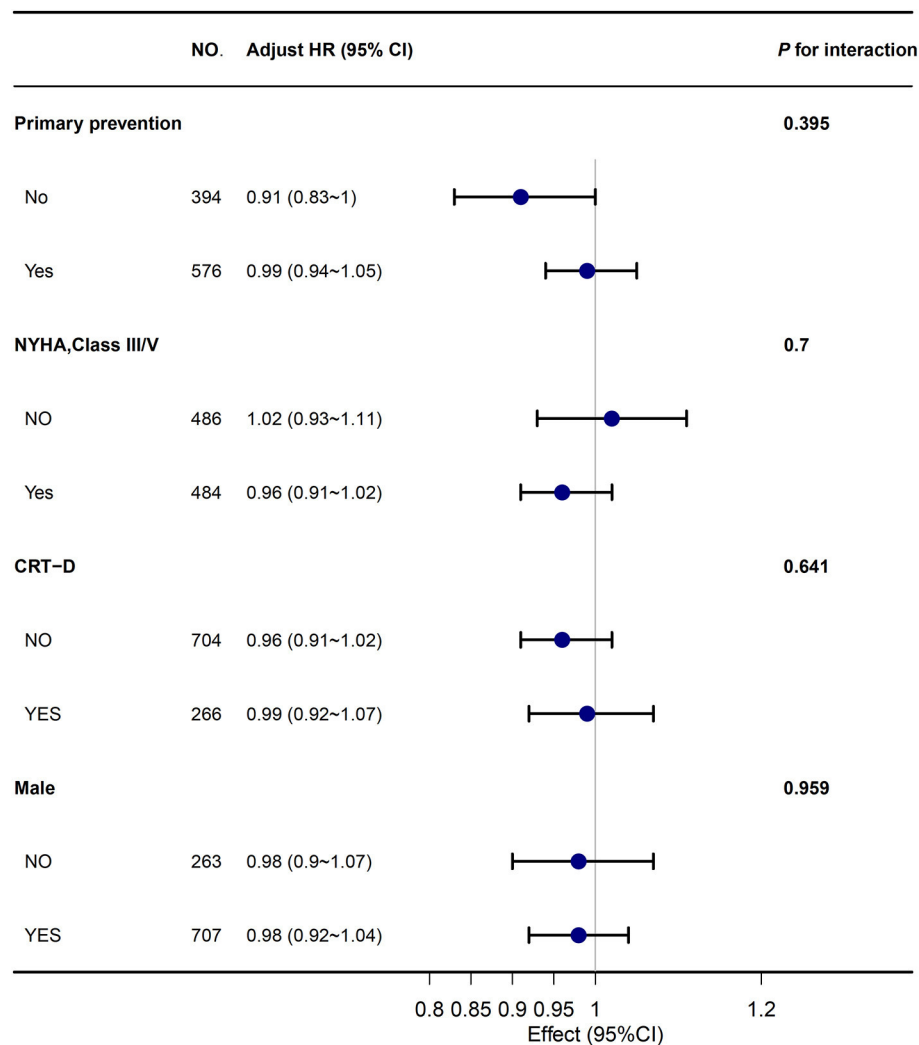


FIGURE 3 | The association between BMI and all-cause mortality in subgroups. Abbreviations are shown in **Table 1**.

1.01–1.04; $p = 0.01$) were independent predictors of increased all-cause mortality.

DISCUSSION

Major Findings

The following are the main findings of study: (1) according to Kaplan–Meier curves and Cox proportional hazards models, BMI had no significant impact on all-cause mortality in the patients with ICD, whether as a continuous variable or a categorical variable classified by various BMI categorization criteria. (2) A linear relationship between BMI and all-cause mortality was identified with the curve showing no statistically significant association between BMI and all-cause mortality.

Compared With Previous Studies

This study applied various statistical methods for data analysis, all of which indicated that there was no obesity paradox

in the Chinese ICD population. This is consistent with the results of a Spanish study (14). However, another research from the United States reported that low BMI was independently associated with death at 1 year (15). In the Multicenter Automatic Defibrillator Implantation Trial-II (MADIT II) study, a study of a retrospective analysis of patients with left ventricular dysfunction after myocardial infarction, obese patients ($\text{BMI} \geq 30 \text{ kg/m}^2$) had a higher survival rate than non-obese patients (16). Another study in the United States found a greater benefit of higher BMI on survival in patients with ICDs, particularly in the older patients (17). The inconsistent results may be due mainly to differences in demographic characteristics, ethnic groups, sample size, or adjusted covariates. In addition, from the MADIT era (26), the pharmacological treatment and prevention strategies for heart failure optimize over the years (27), which may contribute to a lower rate of all-cause mortality. This may make it harder to observe associations of BMI and all-cause mortality. However, the obesity paradox does not appear to be present in

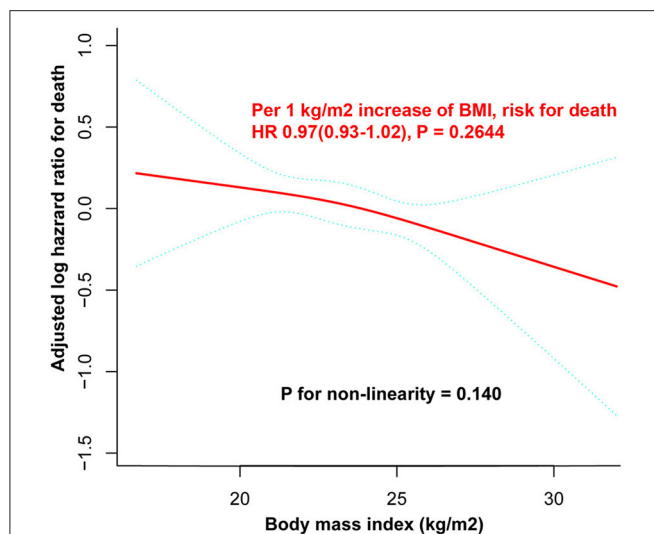


FIGURE 4 | Dose-response curve between BMI and all-cause mortality. There was a linear relationship between BMI and all-cause mortality (P for non-linearity = 0.140). The adjusted log HR and its 95% CI are represented by the solid blue and dashed blue lines, respectively. All the covariates listed in **Table 1** were used as adjustment factors. No statistically significant association between BMI and all-cause mortality was observed (for every increase of 1 kg/m^2 BMI, HR 0.97, 95% CI 0.93–1.02, P = 0.2644). BMI, body mass index; CI , confidence interval; HR , hazard ratio.

the Chinese ICD population as far as the results of this study are concerned.

Risk Factors Related to All-Cause Mortality Among Patients With an ICD

Our study found that older age, NYHA Class III/V, ischemic cardiomyopathy, and wider LVEDD were independent predictors of increased all-cause mortality. Compared with the patient in NYHA Class I/II, patients with NYHA Class III/V had a 55% increased risk of all-cause mortality. Higher NYHA Class was reported to be associated with a higher rate of 1-year all-cause mortality (15). Thus, our study extended the above findings to a 5-year follow-up, suggesting that the effect of cardiac function class, a very clinically assessable index, on all-cause mortality can last that long. This suggests that we need to pay more attention to the assessment and management of cardiac function class in patients with ICD. Similarly, we should improve the evaluation and management of patients with advanced age, ischemic cardiomyopathy, and left ventricular enlargement.

Clinical Implications

This study had some clinical implications. First, it clarified that the obesity paradox was not found in the Chinese with an ICD for the time being, adding evidence from the Chinese population to this controversial topic. Second, it illustrated that

TABLE 3 | The univariate and multivariate risk factors of all-cause mortality.

Variable	Crude model		Adjust model*	
	HR (95% CI)	P-value	HR (95% CI)	P-value
BMI	0.98 (0.94–1.03)	0.44	0.97 (0.93–1.02)	0.262
Male	1.2 (0.88–1.65)	0.246	1.05 (0.75–1.48)	0.778
Age	1.03 (1.02–1.04)	<0.001	1.02 (1.01–1.04)	<0.001
NYHA, Class III/V	2.49 (1.86–3.32)	<0.001	1.55 (1.1–2.19)	0.012
SBP	0.99 (0.98–1)	0.072	0.99 (0.98–1)	0.054
DBP	0.99 (0.98–1.01)	0.398	1 (0.99–1.02)	0.586
Primary prevention	1.41 (1.06–1.87)	0.018	0.88 (0.61–1.27)	0.492
CRT-D	1.59 (1.2–2.1)	0.001	0.9 (0.62–1.31)	0.58
Ischemic cardiomyopathy	1.91 (1.46–2.5)	<0.001	1.54 (1.14–2.07)	0.005
Dilated cardiomyopathy	1.47 (1.1–1.96)	0.01	1.02 (0.73–1.44)	0.897
Hypertrophic cardiomyopathy,	0.46 (0.17–1.23)	0.121	0.87 (0.31–2.42)	0.793
Long QT syndrome	0.33 (0.05–2.36)	0.271	0.61 (0.08–4.5)	0.627
Hypertension	1.7 (1.29–2.23)	<0.001	1.36 (0.99–1.86)	0.054
Diabetes mellitus	1.84 (1.28–2.66)	0.001	1.19 (0.8–1.77)	0.379
Stroke	1.58 (0.7–3.56)	0.27	1.09 (0.46–2.56)	0.852
Atrial fibrillation	1.61 (1.11–2.33)	0.012	1.19 (0.81–1.76)	0.382
Pre-implant syncope	0.84 (0.59–1.19)	0.326	0.95 (0.65–1.39)	0.788
LVEF	0.97 (0.96–0.98)	<0.001	1 (0.98–1.01)	0.609
LVEDD	1.03 (1.02–1.04)	<0.001	1.02 (1.01–1.04)	0.001
β -Blocker	1.46 (1.1–1.94)	0.009	1.28 (0.94–1.73)	0.113
Amiodarone	0.84 (0.62–1.13)	0.253	0.81 (0.58–1.13)	0.212
ACE or ARB	1.43 (1.09–1.87)	0.009	0.92 (0.68–1.25)	0.599
Diuretic	2.01 (1.54–2.64)	<0.001	0.78 (0.42–1.44)	0.43
Loop diuretic	1.83 (1.39–2.41)	<0.001	1.26 (0.8–1.97)	0.324
Aldosterone antagonists	1.95 (1.49–2.55)	<0.001	1.33 (0.84–2.1)	0.229

*Adjusted for all covariates presented in **Table 1** except the independent variable itself. Abbreviations are shown in **Tables 1, 2**.

risk stratification for all-cause mortality based on baseline BMI was not desirable in the Chinese population with an ICD and that there was no need to focus specifically on the value of baseline BMI for the time being. Third, this study suggested that we should pay more attention to the patients with advanced age, ischemic cardiomyopathy, NYHA Class III/IV, and left ventricular enlargement.

Strengths and Limitations

The study had the following strengths. First, the study was a multicenter study with a relatively large sample size and good generalizability. Second, the study used multiple statistical methods to maximize the exploration of the relationship between BMI and all-cause mortality. Third, the study used various BMI grouping criteria to enhance the robustness of the results. However, the study had some limitations. First, the study was a retrospective observational study and there was some selection bias. Second, the study did not collect data on the blood tests, ECG, etc. that may have influenced the effect of BMI on mortality. We were unable to adjust for these substantial confounders; therefore, prospective studies that collect more variables are needed for further in-depth study. Third, in our study, we used conventional programming setting otherwise the proposed high-rate therapy and delayed ICD therapy were proposed in the Multicenter Automatic Defibrillator Implantation Trial-Reduce Inappropriate Therapy (MADIT-RIT) study (28). The effect of different programming settings on all-cause mortality should be considered. However, all the patients in our study received the same programming setting. So, the prognostic impact of the programming setting on each individual was close. Fourth, the relatively small number of obese patients in the study limited the generalizability of the findings. Last, in our investigation, we did not gather data on adiposity distribution (waist-to-hip ratio or waist circumference) or body fat percentage which were also indicators for obesity. However, it is undeniable that it is used in a wide range of studies (8, 10, 13–17) as a commonly used and easily accessible indicator. In the future, prospective studies that include larger sample sizes to ensure a balanced sample across groups and collect more indicators that respond to obesity are awaited to better illustrate the obesity paradox of all-cause mortality in the Chinese population with an ICD.

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CONCLUSIONS

Using various statistical methods of analysis and different BMI grouping criteria, the obesity paradox in all-cause mortality did not emerge in the Chinese population with an ICD. Prospective studies are still needed to further explore this topic.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Fuwai Hospital, the Chinese Academy of Medical Sciences (the lead institute), and all other collaborating organizations (Zhongshan Hospital, Fudan University, and so on). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

The study was conceived and designed by SZhang, MT, and BZ. The ICD was implanted by MT, KC, WH, YS, JY, ZL, and WX. The data were collected by SZhao. The data were analyzed and the manuscript was written by BZ, XS, and NY. The manuscript was revised by SZhang and MT. The final manuscript was read and approved by all authors.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcvm.2021.730368/full#supplementary-material>

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Effects of Hot Balloon vs. Cryoballoon Ablation for Atrial Fibrillation: A Systematic Review, Meta-Analysis, and Meta-Regression

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Background: Balloon-based catheter ablations, including hot balloon ablation (HBA) and cryoballoon ablation (CBA), have rapidly emerged as alternative modalities to conventional catheter atrial fibrillation (AF) ablation owing to their impressive procedural advantages and better clinical outcomes and safety. However, the differences in characteristics, effectiveness, safety, and efficacy between HBA and CBA remain undetermined. This study compares the characteristic and prognosis differences between HBA and CBA.

Methods: Electronic search was conducted in six databases (PubMed, Embase, Cochrane Library, Web of Science, ClinicalTrial.gov, and medRxiv) with specific search strategies. Eligible studies were selected based on specific criteria; all records were identified up to June 1, 2021. The mean difference, odds ratios (ORs), and 95% confidence intervals (CIs) were calculated to evaluate the clinical outcomes. Heterogeneity and risk of bias were assessed using predefined criteria.

Results: Seven studies were included in the final meta-analysis. Compared with CBA, more patients in the HBA group had residual conduction and required a higher incidence of touch-up ablation (TUA) [OR (95% CI) = 2.76 (2.02–3.77), $P = 0.000$]. The most frequent sites of TUA were the left superior pulmonary veins (PVs) in the HBA group vs. the right inferior PVs in the CBA group. During HBA surgery, the left and right superior PVs were more likely to have a higher fluid injection volume. Furthermore, the procedure time was longer in the HBA group than in the CBA group [weighted mean difference (95% CI) = 14.24 (4.39–24.09), $P = 0.005$]. Patients in the CBA group could have an increased risk of AF occurrence, and accepted more antiarrhythmic drug therapy; however, the result was insignificant.

Conclusions: HBA and CBA are practical ablation approaches for AF treatment. Patients who received HBA had a higher incidence of TUA and longer procedure time. Clinical outcomes during the mid-term follow-up between HBA and CBA were comparable.

Systematic Review Registration: https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=259487, identifier: CRD42021259487.

Keywords: catheter ablation, cryoballoon ablation, hot balloon ablation, atrial fibrillation, prognosis, meta-analysis

INTRODUCTION

Catheter ablation has been the most effective therapeutic approach and has been awarded the highest-level guideline recommendation for the treatment of atrial fibrillation (AF) since the decades (1). Pulmonary vein (PV) isolation is the most commonly used, and fundamental strategy in contemporary clinical practice. Several balloon-based catheter ablations, including hot balloon ablation (HBA) and cryoballoon ablation (CBA), have rapidly emerged as alternatives to conventional radiofrequency (RF) catheter-based AF ablation. As previous studies have reported, balloon-based ablations not only have equal clinical utility (2, 3) but also provide several advantages over traditional surgery, such as a more extensive wide-area ablation (4, 5), shorter procedure duration (6), and less frequent dormant PV conduction (7). Moreover, compared with antiarrhythmic drug therapy, balloon-based ablations possess the advantage of reducing AF recurrence and improving the patients' quality of life without increasing the incidence of adverse events (8, 9). However, the differences in characteristics, effectiveness, safety, and efficacy between HBA and CBA remain undetermined. This meta-analysis aimed to compare these two approaches in AF management and guide the optimum selection of balloon-based catheter ablation as the initial rhythm control strategy in patients with AF during routine clinical practice.

METHODS

Search Strategy

This work was registered in the International Prospective Register of Systematic Reviews, and was identified as CRD42021259487. Our electronic search was conducted in six databases, including PubMed, Embase, Cochrane Library, Web of Science, ClinicalTrials.gov, and medRxiv, with specific search strategies using keywords "[Cryosurgery OR Cryoablation OR cryoballoon] AND [HotBalloon OR thermal balloon OR radiofrequency thermal balloon catheter OR hot balloon] AND [Atrial Fibrillation]." Results from the time of database establishment to June 1, 2021 were included. We also analyzed reference lists of relevant studies to identify potentially eligible articles. No restrictions on language were applied. The search strategies are listed in **Supplementary Table 1**.

Study Selection

Eligible studies were selected according to the following inclusion criteria: (1) prospective or retrospective cohort studies, (2) studies of adult patients diagnosed with AF treated with HBA or CBA; and (3) studies reporting surgical complications and clinical outcomes. Exclusion criteria were (1) studies

with inaccessible or incomplete full texts, and (2) those with incomplete data reports. Our analysis did not include duplicate articles, conference abstracts, case reports, review articles, comments, letters, animal studies, or *in vitro* studies. Using the above criteria, two authors (Peng and Chen) independently reviewed all the articles by browsing abstracts and titles for selecting relevant studies, which were then subjected to further screening. In case of any difference in opinion or disagreement between the two authors, the corresponding author (Li) was consulted.

Literature Quality Evaluation

The quality and bias of cohort studies were assessed using the original Newcastle-Ottawa Scale (NOS). The total score was 9; studies with 4 points or less were considered low-quality literature, while those with 5 points or more were deemed high-quality.

Data Extraction and Statistical Analyses

Data from all eligible studies were extracted by one author (Peng) and given to another author (Chen) for cross-checking. Extracted data included the following: first author's name, date of publication, type of study, study location, study characteristics (sample size, sex, age, follow-up days, comorbidities, baseline characteristics), clinical manifestations, surgical characteristics, complications, and clinical outcomes. Data analysis was conducted using Stata software version 15.0, and pooled using the fixed-effects models; if the heterogeneity was significant, random-effect models were applied. Results of data analysis were odds ratios (ORs) and 95% confidence intervals (CIs) for dichotomous variables, weighted mean difference (WMD), and 95% CI for continuous outcomes. Finally, tests of heterogeneity were performed using the I^2 statistic (I^2 25% = low, 50% = medium, 75% = high; $p < 0.10$ indicating statistically significant heterogeneity). When heterogeneity was statistically significant, the source of heterogeneity was comprehensively identified through subgroup or sensitivity analyses. Publication bias was analyzed using a funnel plot and Egger's test when more than five eligible studies were included. Statistical significance was set at $P < 0.05$.

Meta-Regression

To evaluate the potential source of heterogeneity in this meta-analysis, a meta-regression analysis was performed using a random-effects model. The selected variables were as follows: year of publication, number of patients, age, proportion of males, body mass index (BMI), proportion of comorbidities, baseline level of left atrial diameter (LAD), left ventricular ejection fraction (LVEF), and CHA2DS2-VASc score.

RESULTS

Study Characteristics

Following the literature search strategy, we retrieved 214 potentially relevant records. Sixty duplicate articles were eliminated, and 154 records were independently screened by title and abstract. A total of 139 articles were excluded, including

Abbreviations: AF, atrial fibrillation; BMI, body mass index; CB, cryoballoon; CBA, cryoballoon ablation; CI, confidence interval; DM, diabetes mellitus; HB, hot balloon; HBA, hot balloon ablation; LA, left atrium; LAD, left atrial diameter; LIPV, left inferior pulmonary vein; LSPV, left superior pulmonary vein; LVEF, left ventricular ejection fraction; NOS, Newcastle-Ottawa Scale; OR, Odds ratio; PV, pulmonary vein; PVI, pulmonary vein isolation; RF, radiofrequency; RIPV, right inferior pulmonary vein; RSPV, right superior pulmonary vein; SD, standard deviation; TIA, transient ischemic; TUA, touch-up ablation; WMD, weighted mean difference.

obviously irrelevant articles ($n = 80$), conference abstracts ($n = 43$), review articles ($n = 12$), and case reports or animal experiments ($n = 4$). Furthermore, after carefully reviewing the remaining 15 results with full text, eight studies were excluded owing to incomplete data or irrelevant clinical outcomes. Therefore, seven studies that satisfied our inclusion and exclusion criteria were finally included in this meta-analysis (10–16). The stepwise selection process is illustrated in **Figure 1**.

Table 1 shows the main characteristics of the seven eligible studies, while **Table 2** summarizes the baseline characteristics of the patients involved in the seven studies. We included both prospective and retrospective cohort studies involving 679 patients with AF who underwent HBA or CBA procedures.

Most studies reported surgical parameters and clinical outcomes with short- and mid-term follow-up, including procedure time, fluoroscopy time, incidence of touch-up ablation (TUA), AF recurrence, and major complications. Several studies have also recorded total mapping points, residual PV potential, dormant conduction, and different antiarrhythmic therapies. All of the above results are included in this meta-analysis.

All seven studies were judged to be of high quality; the literature quality evaluation form is shown in **Supplementary Table 2**.

Overall Meta-Analysis

Variables Related to TUA

All seven included studies recorded the number of patients or PVs that required touch-up RF ablation, and the HBA group had a higher incidence of TUA [OR (95% CI) = 2.76 (2.02–3.77), $P = 0.000$] with low heterogeneity ($I^2 = 0.0\%$, $p = 0.525$). No significant change was observed in the subgroup analysis of paroxysmal AF and non-paroxysmal AF (**Figure 2A**).

Two studies evaluated the number of residual PV potentials and dormant conduction. The pooled analysis showed that, compared with the CBA group, more patients in the HBA group had residual PV potential [OR (95% CI) = 3.01 (1.53–5.88), $P = 0.001$, $I^2 = 0.0\%$, $p = 0.839$] and dormant conduction [OR (95% CI) = 1.91 (0.75–4.83), $P = 0.174$, $I^2 = 0.0\%$, $p = 0.903$] (**Figure 2B**).

Meta-regression regarding TUA of all involved studies revealed that the ablation strategy was not influenced by the publication year ($P = 0.506$), sample size ($P = 0.129$), age ($P = 0.947$), proportion of males (%) ($P = 0.699$), BMI ($P = 0.242$), proportion of comorbidity (%) (including paroxysmal AF, $P = 0.154$; hypertension, $P = 0.981$; DM, $P = 0.319$; stroke or TIA, $P = 0.760$; heart failure, $P = 0.359$; vascular disease, $P = 0.433$), baseline level of LAD (mm) ($P = 0.252$), LVEF (%) ($P = 0.460$), and CHA2DS2-VASc score ($P = 0.317$) (**Supplementary Figure 1**).

The incidence of TUA following CBA and HBA procedures in PVs is shown in **Figure 3**. A noticeable increase in the incidence of ablation was observed in the HBA group ($P < 0.001$). More TUA was applied in the anterior carina and anterior ridge of the left superior pulmonary vein (LSPV) in patients with HBA. In contrast, more ablations were performed in the inferior aspect of the left inferior pulmonary vein (LIPV) and right inferior pulmonary vein (RIPV) in patients with CBA, especially for

RIPV. A significant difference between the two procedures was observed in the LSPV ($P = 0.005$) and RIPV ($P = 0.028$).

The distribution of TUA sites following HBA and CBA was reported in five studies and is summarized in **Figure 4**. In the HBA group, the left PVs had the highest incidence of TUA (LSPV, 50.0%; LIPV, 21.5%). The specific procedure was often required at the anterior aspect of the LSPV (carina, 48.6% of LSPV; ridge, 32.7% of LSPV) and the bottom of the LIPV (45.7% of LIPV). In the CBA group, ablation was often applied to the inferior PVs (RIPV, 60.8%; LIPV, 28.9%). Among the inferior PVs, the ablation incidences and sites were concentrated at the base (57.6% of the RIPV, 42.9% of the LIPV).

Procedure-Related Data

The procedure data for CBA and HBA were summarized in detail, including fluoroscopy time, total mapping points, and procedure time. A random-effects model was applied owing to significant heterogeneity.

The procedure time was reported in five studies, with the results showing that patients in the HBA group had a longer procedure duration than those in the CBA group [WMD (95% CI) = 9.69 (−2.78 to −22.16), $P = 0.128$]. No statistical difference was found between the two groups, and the heterogeneity was moderate ($I^2 = 62.6\%$, $p = 0.030$).

Four studies reported the results considering the fluoroscopic time. Compared with the HBA group, the pooled fluoroscopic duration was longer in the CBA group [WMD (95% CI) = −1.03 (−9.50 to −7.44)]; however, the results were statistically insignificant ($P = 0.812$) and the heterogeneity was considerable ($I^2 = 87.5\%$, $p = 0.000$).

The overall value of the total mapping points summarized from the three included studies was 525.79 [WMD (95% CI) = (−56.56 to −1108.13), $P = 0.077$, $I^2 = 90.5\%$, $p = 0.000$]. The above results indicated that the total number of mapping points was higher in the HBA group, with considerable heterogeneity.

Lesion Size

Two studies reported the ablation lesion size; the pooled results showed that the lesion was larger in the CBA group [WMD (95% CI) = −2.22 (−16.52, 12.08), $P = 0.761$], although no statistical difference was observed. However, the heterogeneity was significant ($I^2 = 95.5\%$, $p = 0.000$). The above two studies also explored differences in lesion area (%). The pooled analysis revealed a neutral result [WMD (95% CI) = −0.16 (−19.96, 19.63), $P = 0.987$, $I^2 = 98.1\%$, $p = 0.000$]. The results are shown in **Supplementary Figure 2**.

Sensitivity Analysis and Meta-Regression

To further assess the sources of intertrial heterogeneity, sensitivity analysis was performed by sequentially omitting each study.

Considering the procedure time, based on the one-study removed model, we found that when we omitted the Wakamatsu's study, the analysis carried out on the four remaining studies revealed a noticeable decrease in heterogeneity from 62.6 to 26.0% [WMD (95% CI) =

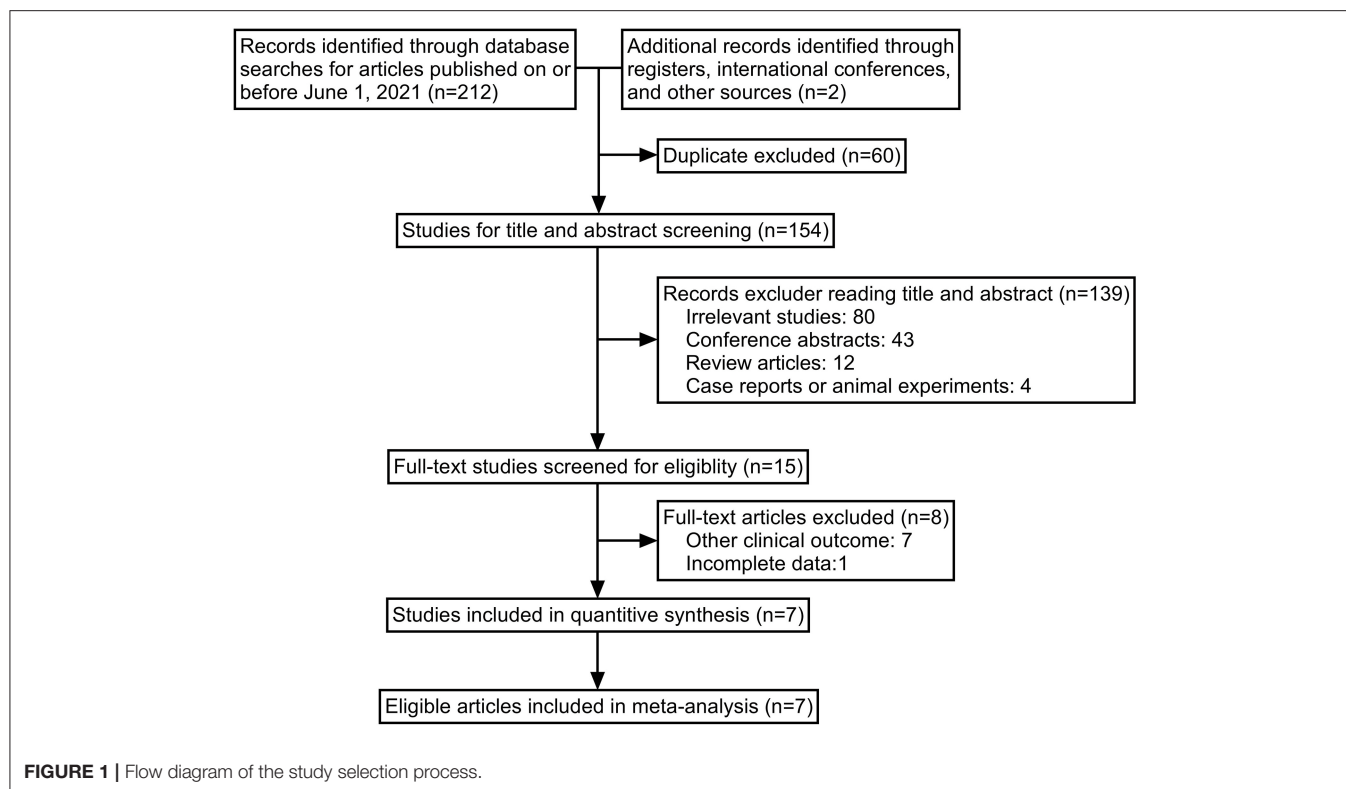


TABLE 1 | Main characteristics of 7 eligible studies included in the meta-analysis.

No.	References	Type of study	Region	Number of Patients (HBA/CBA, n)	Follow up period	Evaluated parameters
No. 1	Nagashima et al. (10)	Retrospective	Japan	37/37	11.8 m	Total mapping points, Touch-up ablation, Residual PV potential, Dormant conduction, AF Recurrence, Major complication, Antiarrhythmic therapy
No. 2	Nakamura et al. (11)	Prospective	Japan	58/65	46 ± 31 d	Procedure time, Touch-up ablation, Major complication
No. 3	Wakamatsu et al. (12)	Retrospective	Japan	46/46	12 m	Total mapping points, Touch-up ablation, Residual PV potential, Dormant conduction, AF Recurrence, Antiarrhythmic therapy
No. 4	Wakamatsu et al. (13)	Retrospective	Japan	79/79	18 m	Procedure time, Fluoroscopy time, Total mapping points, Touch-up ablation, AF Recurrence, Major complication, Antiarrhythmic therapy
No. 5	Hojo et al. (14)	Prospective	Japan	46/46	73 d	Procedure time, Fluoroscopy time, Touch-up ablation, AF Recurrence, Major complication
No. 6	Suruga et al. (15)	Retrospective	Japan	30/30	365 ± 102 d	Procedure time, Fluoroscopy time, Touch-up ablation, AF Recurrence
No. 7	Akita et al. (16)	Retrospective	Japan	40/40	12 m	Procedure time, Fluoroscopy time, Touch-up ablation, AF Recurrence

AF, Atrial fibrillation; CBA, cryoballoon ablation; d, days; HBA, hot balloon ablation; m, month; PV, pulmonary vein.

14.24 (4.39–24.09), $P = 0.005$, $I^2 = 26.0\%$, $p = 0.256$] (Figure 5A). The results of the sensitivity analysis are shown in Supplementary Figure 3.

There was no significant change in heterogeneity following sensitivity analysis of fluoroscopic time and total mapping points, and the point estimate and 95% CI of the results were not appreciably altered. The forest plot of the fluoroscopic time and total mapping points is shown in Figures 5B,C.

Meta-regression was conducted to determine the potential source of heterogeneity. However, no potential source of heterogeneity was related to fluoroscopy time, total mapping points, and procedure time.

Fluid Injected Into the HBA

Four studies reported the fluid injection volume of HBA for PV occlusion. The amount of injected fluid is summarized in

TABLE 2 | Evaluated parameters, baseline characteristics of 7 eligible studies included in the meta-analysis.

No.	References	Age (years)	Gender (male %)	BMI (kg/m ²)	Paroxysmal AF (%)	HT (%)	DM (%)	Stroke or TIA (%)	HF (%)	Vascular disease (%)	LAD (mm)	LVEF (%)	CHA2DS2-VASc
No. 1	Nagashima et al. (10)	62 ± 10.03	74.32	25 ± 4	62	59.46	24.32	13.51	9.46	4.05	40 ± 5.58	66 ± 11	2 ± 1.47
No. 2	Nakamura et al. (11)	65 ± 10	68.3	NG	95.1	46.3	17.1	6.5	3.3	4.9	38 ± 5	63 ± 8	1.9 ± 1.4
No. 3	Wakamatsu et al. (12)	62.63 ± 9.75	76.09	24.72 ± 4.26	63.04	48.91	19.57	9.78	6.52	5.43	39.22 ± 6.32	66.04 ± 8.76	1.78 ± 1.69
No. 4	Wakamatsu et al. (13)	64 ± 9.48	77.22	25 ± 4	0	53.16	16.46	12.03	13.92	5.7	41 ± 5.51	62 ± 12.00	2 ± 1.48
No. 5	Hojo et al. (14)	65.15 ± 9.31	81.52	23.95 ± 3.19	100	NG	NG	NG	NG	NG	NG	64.5 ± 6.36	1.75 ± 1.54
No. 6	Suruga et al. (15)	63.5 ± 10.43	85	24.55 ± 3.10	100	48.33	21.67	5	8.33	3.33	39.5 ± 5.16	65 ± 7.65	1.75 ± 1.54
No. 7	Akita et al. (16)	64.55 ± 9.47	78.75	24 ± 2.93	93.75	NG	NG	NG	NG	NG	38.5 ± 6.39	62 ± 5.93	NG

BMI, Body mass index; DM, diabetes mellitus; HF, heart failure; HT, hypertension; LAD, left atrial diameter; LVEF, left ventricular ejection fraction; NG, not given; AF, paroxysmal atrial fibrillation; PV, pulmonary vein; TIA, transient ischemic attacks; VD, vascular disease.

Figure 6; among the 4 PVs, the left superior and right superior PVs are more likely to be agitated with a higher volume of fluid.

Clinical Outcomes

Six studies analyzed the AF recurrence and found that more patients in the CBA group were likely to have AF occurrence [OR (95% CI) = 0.75 (0.44–1.27), $P = 0.281$, $I^2 = 0.0\%$, $p = 0.651$] and accepted more antiarrhythmic drug therapy [OR (95% CI) = 0.70 (0.45, 1.09), $P = 0.114$, $I^2 = 0.0\%$, $p = 0.446$], although the result was statistically insignificant (**Figures 7A,B**).

There was no significant change in the AF recurrence during the subgroup analysis of paroxysmal or non-paroxysmal AF and follow-up periods (**Supplementary Figures 4A,B**).

Among all the antiarrhythmic drug therapies, both class I drugs and bepridil were more likely to be used in the CBA group [OR (95% CI) = 0.56 (0.25–1.27), $P = 0.165$, $I^2 = 45.6\%$, $p = 0.159$; OR (95% CI) = 0.59 (0.31–1.12), $P = 0.107$, $I^2 = 0.0\%$, $p = 0.857$, respectively]. Amiodarone was more frequently used in the HBA group [OR (95% CI) = 5.25 (0.60–45.96), $P = 0.134$, $I^2 = 0.0\%$, $p = 0.996$] (**Figure 7C**).

Complications were compared in four studies. The pooled analyses showed a higher prevalence of complications in the HBA group [OR (95% CI) = 2.36 (0.71–7.79), $P = 0.160$, $I^2 = 0.0\%$, $p = 0.647$]; however, the result was insignificant (**Figure 7D**).

Publication Bias

We performed a funnel plot and Egger's test to examine the publication bias. Egger's test resulted in a P -value of 0.773, suggesting no significant publication bias in this meta-analysis (**Supplementary Figure 5**).

DISCUSSION

Main Findings

This meta-analysis included seven studies with a total of 679 patients. To the best of our knowledge, no previous meta-analysis has compared the characteristics of the clinical outcomes between these balloon modalities.

The main findings were as follows:

- 1) Compared with CBA, patients in the HBA group had more residual conduction, higher incidence of TUA and longer procedural time,
- 2) The most frequent sites of TUA were the left superior PVs in the HBA group vs. the right inferior PVs in the CBA group, and
- 3) The clinical outcomes during the mid-term follow-up between the HBA and CBA groups were comparable. Although patients in the CBA group had a higher risk of AF occurrence and accepted more antiarrhythmic therapies, the HBA group had more surgical complications; the between-group difference was insignificant.

Residual Conduction and TUA

As revealed in this study, more cases with residual PV potentials and dormant conduction were observed in the HBA group, which

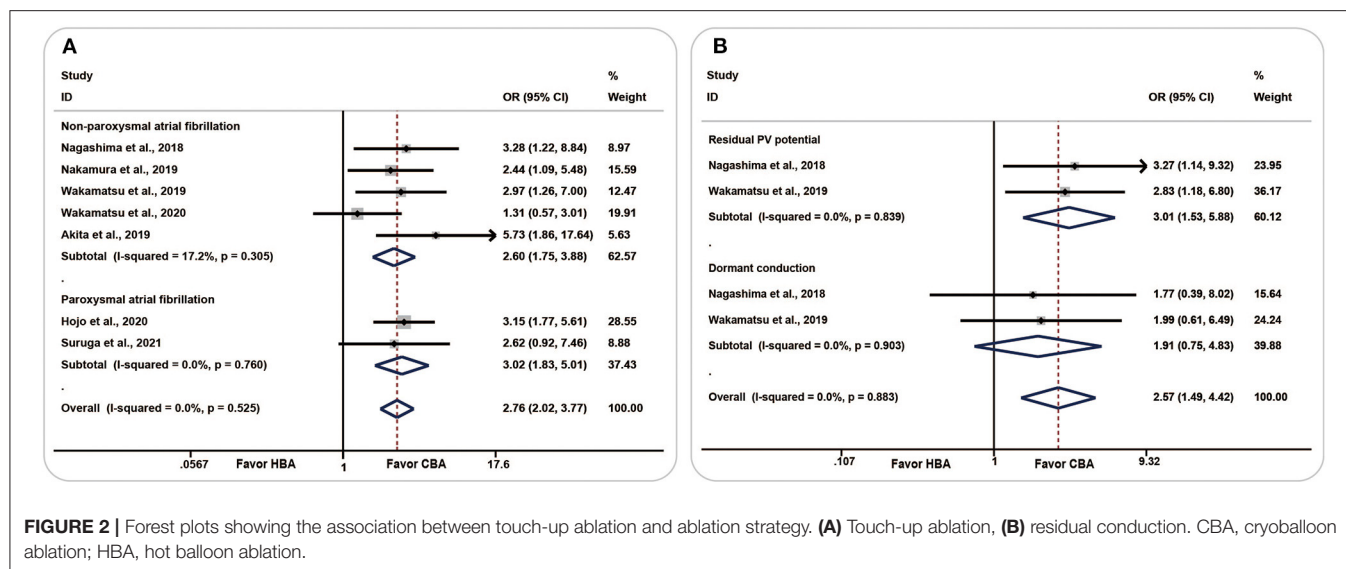


FIGURE 2 | Forest plots showing the association between touch-up ablation and ablation strategy. **(A)** Touch-up ablation, **(B)** residual conduction. CBA, cryoballoon ablation; HBA, hot balloon ablation.

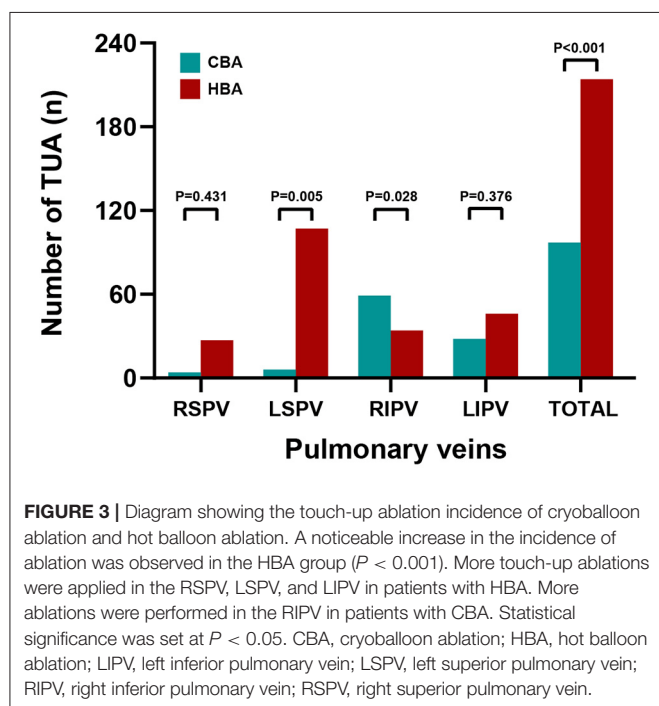


FIGURE 3 | Diagram showing the touch-up ablation incidence of cryoballoon ablation and hot balloon ablation. A noticeable increase in the incidence of ablation was observed in the HBA group ($P < 0.001$). More touch-up ablations were applied in the RSPV, LSPV, and LIPV in patients with HBA. More ablations were performed in the RIPV in patients with CBA. Statistical significance was set at $P < 0.05$. CBA, cryoballoon ablation; HBA, hot balloon ablation; LIPV, left inferior pulmonary vein; LSPV, left superior pulmonary vein; RIPV, right inferior pulmonary vein; RSPV, right superior pulmonary vein.

required a significantly higher incidence of additional touch-up RF ablation. Based on the findings of the present studies (10, 17, 18), the unequal comparison of the TUA between the HBA and the CBA could be attributed to characteristic balloon compliance and ablation size.

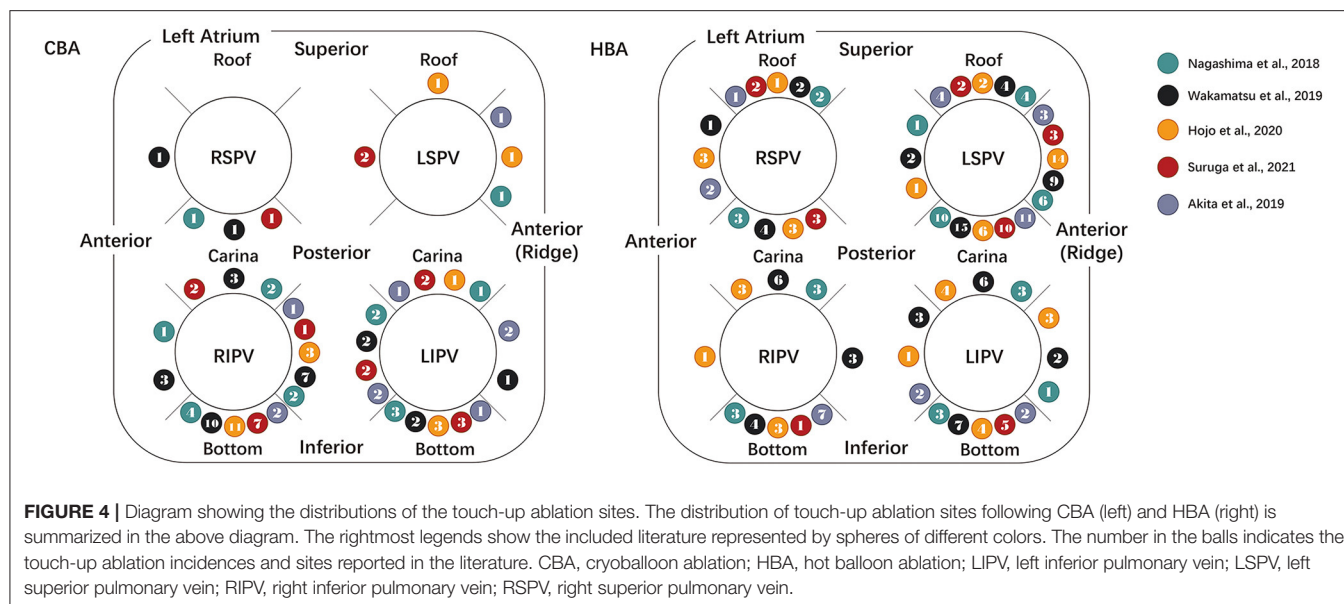
Considering the compliance of the balloon modalities, Yamasaki et al. reported that the hot balloon (HB) had better compliance and adjustability during the procedure, in which the size and shape of the balloon were easily altered to accommodate variations in the PV geometry and achieve optimal occlusion and isolation of the PVs (18). By fluently inflating inside the

PV ostium, the HB could facilitate the occlusion of the more distal and deeper portions of the PVs (10). However, this elastic feature could lead to a suboptimum contact area of the PV ridge, which corresponds to the balloon ablation lesion, and results in inadequate occlusion of the PV antrum. These could be indicative of the need for additional RF ablation of the ridge following the HB system.

Compared to the HB, the cryoballoon (CB) was non-conforming and did not vary in size and shape (17). This feature stiffened the CB during energy deliveries and allowed it to expand outside the PV before advancing to the PV port, resulting in distension of the PV ridge and a larger ablation area of the antrum.

Additionally, the left atrium (LA) ablation lesion size could also be related to the need for TUA. It has been proven that balloon-based pulmonary vein isolation (PVI) could produce a wider ablation lesion than standard RF-based ablation (19). However, studies have shown that CBA might produce an even larger LA lesion size, lesion width, and lesion gap than the HBA (10, 16), which is consistent with the results of our analysis. This could be explained by the relatively large size of the second-generation CB, which had a large contact area and was capable of creating a larger freezing surface. In contrast to the cryothermal energy, the further the HB expanded compared to the standard inflated size; the less thermal energy it could deliver to the distal tissue, which could explain the narrow lesion created by the HBA. Although the lesion of the HB system was narrower, its durability rate was as constant as that of CBA. However, owing to limited research focusing on lesion differences between the two procedures, more studies are warranted for an incontestable conclusion.

Wakamatsu et al. (12) stated that HBA often could not provide ideal occlusion because the HB surface did not adhere well to all the tissues of the PV walls during PVI. This problem could not be solved by further advancing the enlarged HB to distend the



orifice or extend the contact area since the overinflation could lead to dislodgment of the catheter from the PV ostium.

In contrast to HB, the stiffer CB remained fixed around the antrum, allowing for better and larger balloon surface–tissue contact by covering both small and large PVs, allowing for extensive PV distention, and creating wide-area antral lesion sets efficiently. This conclusion was consistent with a previous study showing that the area coverage in the left atrial was more stable, and the lesion creation of the CB was relatively larger (4). Therefore, the smaller isolation areas created by the HB systems could lead to further TUA.

The learning curve of HBA could also influence the incidence of TUA. The HB system has recently been introduced into the AF treatment and has not been widely applied in the arrhythmia centers, which limits the further development of the procedure. As Nagashima et al. (4) suggested, an improved ablation outcome and less demand for TUA were observed following an accumulation of HB experience. However, the results have been reported for experienced CBA operators. This phenomenon suggests the influence of a learning curve on the requirement of TUA in HBA surgery.

Distribution of the TUA Sites

Apparently, in the above diagram, in the CBA group, the superior PVs and the anterior aspects of the inferior PVs were less needed for additional touch-up RF ablation. The procedure was prominently distributed in the inferior aspect of the RIPV and LIPV, especially for the RIPV. The potential reason for the above consequences could be the imperfect alignment of the inferior PVs (10, 12). The angle of the inferior PVs, the short distance between the puncture site and the vein, or the tight space between the dorsal vertebrae and the vein could lead to unstable and inadequate contact between the CB and the LA myocardium. During the inferior PVs' balloon inflation, the CB stretched in the superior direction and led to suboptimal contact and less PV

distention in the inferior aspect, resulting in limited frozen tissue and smaller PV ostial lesions. Lesion creation in the superior and carina regions is particularly effective. Compared with superior PVs, the inferior PVs were relatively slimsy and small, resulting in a greater contact area between the balloon surface and blood flow. Owing to the CB, the freezing temperature was markedly influenced by the surrounding blood flow, and the larger surrounding area counteracted the optimal tissue freezing. These balloon features could cause specific characteristics and distributions of CB lesions.

Interestingly, among the HBA procedures, the right PVs were less likely to receive TUA, and the applications were frequently required in the anterior carina and anterior ridge of the LSPV, which was obviously different from the CBA procedure. This could be related to two reasons. A possible explanation for the group difference in TUA at the anterior ridge could be related to the compliance mentioned above. A dominant TUA site was observed near the anterior ridge in the HBA group; compared with HB, the characteristically non-compliant CB could distend the ridge better and produce a more extensive isolation area. In contrast, the adjustable HB inflated into the ostium easily so that the distension of the PV antrum was inadequate, resulting in more TUA near the anterior ridge. Another possible explanation for the distribution differences was the anatomic features. In the HBA procedure, the frequency of TUA was especially higher in the anterior ridge and carina areas of the LSPV. This result could perhaps be explained by the anatomic features, such as the left atrial wall thickness. The mechanism of lesion creation underlying the HBA was capacitive energy transfer, which was notably affected by the thickness of the LA myocardial tissue. Under temperature control, the heating fluid was agitated to ablate the LA tissue by conductive heating energy from the balloon surface and finally create a wide planar antral isolation area. The temperature decreases gradually as the endocardial tissue moves further away from the balloon surface. Among

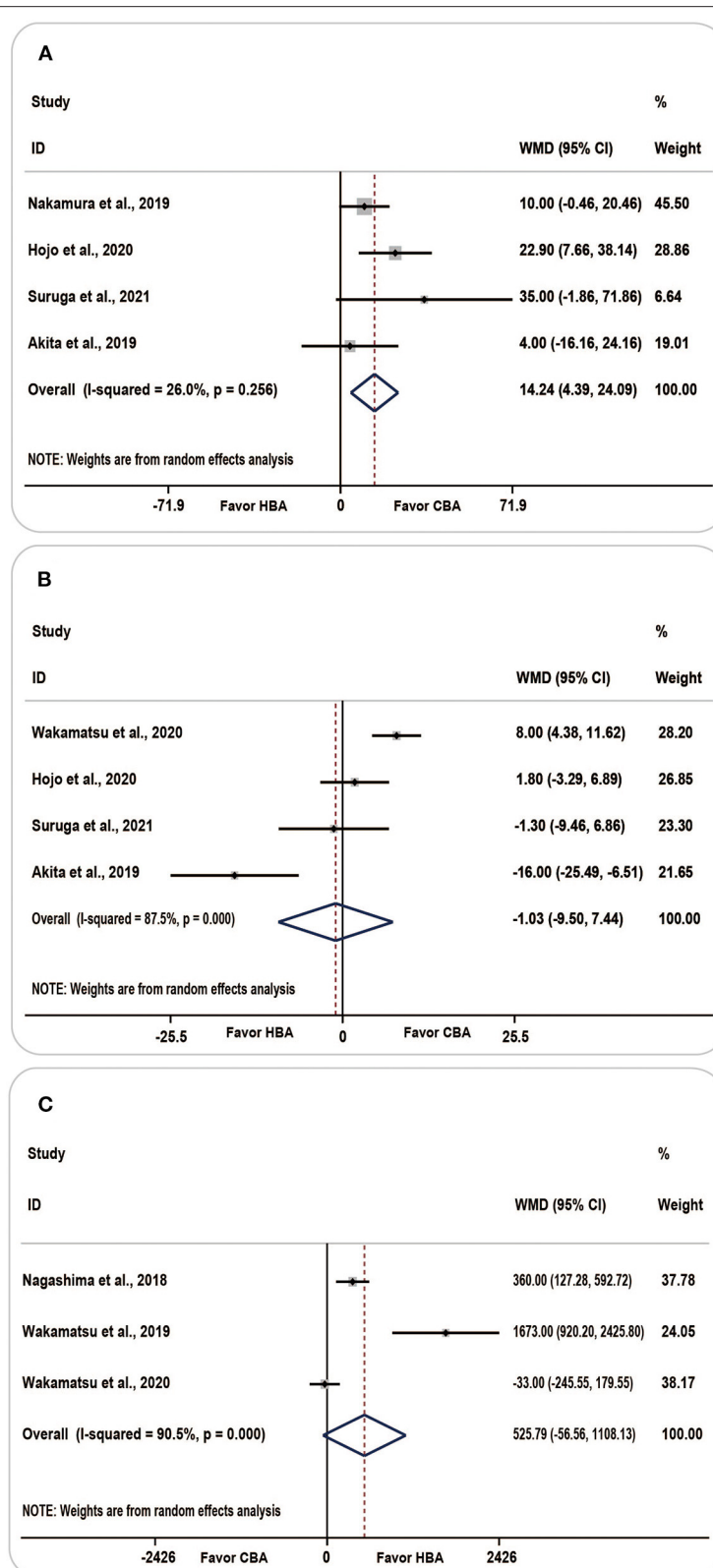


FIGURE 5 | Forest plots showing the association between procedure-related parameters and ablation strategy. **(A)** Procedure time, **(B)** fluoroscopy time, **(C)** total mapping points. CBA, cryoballoon ablation; HBA, hot balloon ablation.

Fluid injected to the hotballoon (Mean±SD)

First Author, year	Sample size	LSPV	LIPV	RSPV	RIPV
Nagashima et al., 2018	37	12.3±1.7	11.2±1.3	12.4±1.3	11.9±1.4
Wakamatsu et al., 2020	79	11.7±1.6	11.6±1.5	11.7±1.3	11.1±1.5
Hojo et al., 2020	46	11.3±1.7	10.2±0.8	10.8±1.5	10.3±0.7
Suruga et al., 2021	30	11.5±2.1	10.0±0.3	11.8±1.9	10.4±1.4
Total	192	11.69±1.75	10.94±1.37	11.63±1.54	10.95±1.43

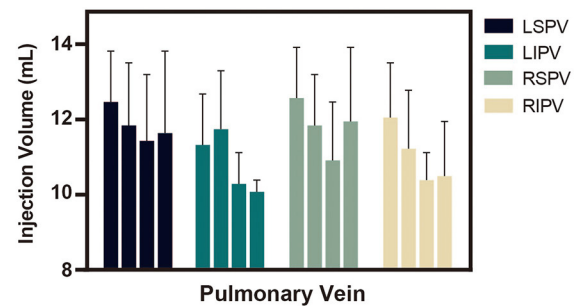


FIGURE 6 | Fluid injected into the hot balloon (Mean ± standard deviation). The above table and diagram summarize the amount of fluid injected into the left atrial pulmonary veins during the hot balloon procedure. As shown in the chart, the LSPV and RSPV received more fluid injections during the procedure. LIPV, left inferior pulmonary vein; RIPV, right inferior pulmonary vein; RSPV, right superior pulmonary vein; RIPV, right inferior pulmonary vein; SD, standard deviation.

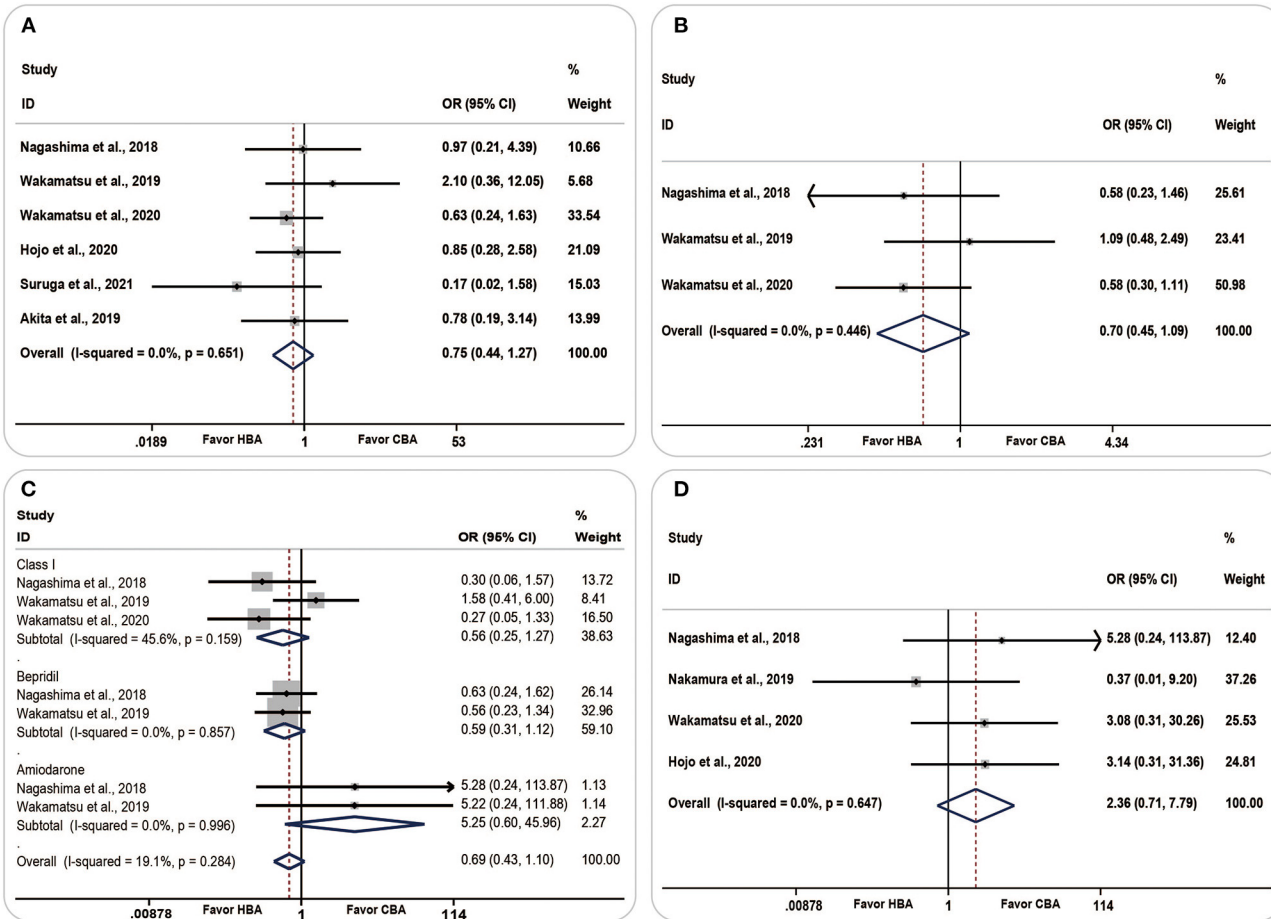


FIGURE 7 | Forest plots showing the association between clinical outcomes and ablation strategy. (A) Atrial fibrillation recurrence, (B) antiarrhythmic therapy, (C) antiarrhythmic drug therapies in detail, (D) complications. CBA, cryoballoon ablation; HBA, hot balloon ablation.

the PVI procedures, the anterior ridge and carina area of the LSPV is the thickest area, which profoundly cuts down the energy penetration depth, hinders the creation of a transmural lesion, and concentrates more PV reconnections (20). Hojo et al. reported that a balloon temperature of 70–73°C could reduce TUAs, thereby improving the outcomes (14).

Considering the above discussion focusing on the characteristics of TUA, several aspects are worth learning to guide future clinical applications. In terms of HBA, as an anatomy-dependent procedure, broader coverage between the balloon and PV antral tissue was crucial for optimal ablation. Further investigation and development concerning the

techniques to enhance heat transfer and improve balloon-tissue contact are warranted. Second, more attention should be paid to the anterior ridge and the LSPV area, which warrants better balloon contact and deeper capacitive-type heating to achieve wider and more durable lesions. As for the CBA, in order to perform preferable ablation, precise atrial septal puncture site, the inflation angle, and the dorsal vertebrae space are warranted. Further, longer freezing times could be required in the inferior aspect of the PVs to realize optimal ablation lesions, decrease the TUA rate, shorten the procedure duration, and increase the PVI success rate.

Ablation Outcomes

Our study compared the clinical outcomes reported in the published literature. First, the between-group difference in complications did not significantly differ between the modalities. Although several published articles reported procedural complications, such as fatal atriobronchial fistula formation and ice formation, which were closely related to the balloon location (21, 22), were higher in the CBA and the PV stenosis was higher in the HBA, our pooled analysis revealed that the difference was comparable. To avoid HB complications, a more appropriate balloon position and added balloon injection volume should be considered (21). As the clinical implication of CBA, a more proximal location between CB and LSPV and relatively counterclockwise rotation are strongly recommended to optimize the cryothermal injury.

Furthermore, middle-term outcomes, including AF occurrence and antiarrhythmic drug therapy, also revealed a similar incidence. Further, subgroup analysis revealed that AF occurrence was not significantly associated with persistent AF percentage or follow-up length.

Interestingly, even though HBA had more residual conduction and a higher TUA rate, the AF occurrence was comparable to that of the CBA procedure. A possible explanation could be that the soft HB can be modulated to fit the antral region, ablated inside the PV orifice, and created more lesions in the PVs, which covered the shortage of inadequate antrum ablation. Although the HBA lesions were small, they were as durable as those achieved by CBA (23). Another underlying reason could be related to the study design. Since the balloon-based technique is relatively novel, there is a lack of larger prospective multicenter randomized studies with long-term follow-up. Future comparative studies are warranted to reveal the differences, elucidate the clinical efficacy, and validate these two approaches.

LIMITATION

Certain limitations of this study need to be acknowledged. First, potential confounding factors were not easy to control

in a non-randomized comparative study. Although most studies have matched the propensity score between the two groups, unknown confounding factors could still exist. In some cases, meaningful information, such as the CHA2DS2-VASc score and echocardiographic parameters, could be missing. Second, this meta-analysis included only Japanese studies. The results could differ according to the race and country; the relatively small sample size could limit the representativeness of the samples, which could bias our conclusions. Finally, the follow-up period of some included studies was relatively short, and some patients might not have reached clinical endpoints, which could have influenced the clinical outcomes. However, as the first meta-analysis to investigate the difference in procedure-related data and clinical outcomes between the two balloon systems, we believe that the results of our study could provide guidance for clinical applications and promote further technological developments.

CONCLUSION

To summarize, this meta-analysis shows that both HBA and CBA are practical ablation approaches for the treatment of AF. Patients who received HBA had a higher incidence of TUA and longer procedure time. The clinical outcomes during the mid-term follow-up were equivalent between HBA and CBA.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

XP contributed to the literature search, data extraction, data analysis, and manuscript drafting. XLiu and HT contributed to the data extraction and manuscript drafting. YC contributed to the data extraction and data analysis. XLi contributed to the study conception, provided statistical expertise, revised the paper, and approved the final manuscript. All authors participated in the interpretation of the results, critical revision of the manuscript, contributed to the article, and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcvm.2021.787270/full#supplementary-material>

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Clinical Features of Patients Undergoing the Head-Up Tilt Test and Its Safety and Efficacy in Diagnosing Vasovagal Syncope in 4,873 Patients

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Background: The head-up tilt test (HUTT) is a useful diagnostic tool in patients with suspected vasovagal syncope (VVS).

Objectives: We aimed to investigate the direct drug-potentiated HUTT in patients with recurrent syncope or precursor syncope and to assess the diagnostic value of the direct drug-potentiated HUTT.

Methods: The medical history and direct drug-potentiated HUTT records of patients who complained of syncope or precursor syncope and who visited The Xianyang Central Hospital from January 2016 to December 2020 were retrospectively reviewed.

Results: A total of 4,873 patients (age = 43.8 ± 17.6 years; male = 2,064 [42.4%]) were enrolled in our study. Overall, 2,343 (48.1%) showed positive responses as follows: 1,260 (25.9%) with the mixed type, 34 (0.7%) with the cardioinhibitory type, 580 (11.9%) with the vasodepressor type, 179 (3.7%) with postural tachycardia syndrome (POTS), and 290 (6.0%) with orthostatic hypotension (OH). The study showed that prior to syncope or near-syncope symptoms, patients first presented an increase in heart rate (HR), followed by decreases in blood pressure (BP) and HR successively. Among the patients in the syncope group, the sensitivity of the HUTT was 65.9%, which was significantly higher than a sensitivity of 44.8% for patients in the non-syncope group ($P < 0.01$). The sensitivity of the HUTT was higher for females than males in both the syncope group (52.6% in males and 77.9% in females, $P < 0.01$) and the non-syncope group (36.5% in males and 50.6% in females, $P < 0.01$). Within the four age groups (<20, 21–40, 41–60, and >60 years old), the sensitivities were 74.7%, 67.7%, 45.6%, and 31.2%, respectively. And all gender, age and symptom (whether suffered from a syncope or not) significantly affected the positive responses of HUTT. There were two adverse events and no deaths during the HUTT in this study.

Conclusion: The direct drug-potentiated HUTT is a safe and highly sensitive tool with which to diagnose VVS. Patients with precursor syncope symptoms without syncope should undergo a HUTT, especially young females presenting with weakness and sweating, which can decrease the probability of a misdiagnosis or a missed diagnosis.

Keywords: neurocardiovascular, diagnosis, drug-potentiated head-up tilt test, syncope, vasovagal syncope, heart rate, blood pressure

INTRODUCTION

Vasovagal syncope (VVS) is one of the most common causes of convulsive syncope, which accounts for 50% of all syncope cases, and is characterized by a transient loss of consciousness and a cascade of associated symptoms (1, 2). The pathophysiology of VVS is not completely understood, but it seems to be derived from a neurocardiogenic reflex-mediated inhibition of sympathetic activity, the overexcitation of parasympathetic activity, heart rate (HR) deceleration, and hypotension resulting in sudden peripheral and cerebral hypoperfusion due to a variety of precipitating factors, such as orthostatic (upright) stress, pain, or emotional triggers (3, 4).

The head-up tilt test (HUTT), as a class IIa recommendation, is widely used and has remained a practical tool in the diagnosis and management of VVS (5, 6). A previous study showed that patients with VVS had many autonomic symptoms, such as nausea, vomiting, abdominal discomfort, pallor, sweating and palpitations, yawning, stridor, salivation, pupillary dilatation, and urinary incontinence, in addition to syncope. Additionally, other symptoms related to cerebral and retinal hypoperfusion were commonly observed in VVS patients, such as dizziness, light-headedness, and blurred vision (7). Therefore, patients presenting with the symptoms mentioned above should be evaluated for VVS. To enrich the relevant data, we performed the HUTT on 4,873 patients from January 2016 to December 2020 at The Xianyang Central Hospital, and we aimed to explore the application of the direct drug-potentiated HUTT in patients with a complaint of recurrent or precursor syncope.

STUDY POPULATION AND METHODS

Study Population and Data Collection

The study population consisted of all patients undergoing a HUTT due to recurrent unexplained syncope or precursor syncope from January 2016 to December 2020 at The Xianyang Central Hospital. The symptoms of precursor syncope were identified as dizziness, light-headedness, blurred vision, nausea, vomiting, pallor, diaphoresis, chest tightness, and palpitations. We mainly excluded patients who had a history of cardiomyopathies, intracranial disease, seizure, psychogenic pseudosyncope, carotid sinus syndrome, and any HUTT-associated contraindication as determined by a thorough evaluation, including a careful medical history, physical examination, electrocardiogram (ECG)/Holter ECG, echocardiography, chest X-ray, electroencephalogram, and brain computed tomography (CT)/magnetic resonance imaging

(MRI). Patients who took any medications that impacted the autonomic nervous system or circulatory system were also excluded from the analysis. The study complied with the Helsinki Declaration and was approved by the Institutional Ethics Committee of The Xianyang Central Hospital (No. 282000010). All patients provided their or their parents' written informed consent.

HUTT Protocol

The HUTT was performed using an electrically controlled tilt table (SHUT-100), and the blood pressures (BP) and HR of the patients were continuously monitored. The direct drug-potentiated HUTT was performed without a basic HUTT stage. We used a protocol that included 5–10 min of rest in the supine position, after which 500 μ g (4–6 μ g/kg for children) of nitroglycerin was administered sublingually, followed by a phase of 20 min with the patient's head positioned with a tilt of 60–70 degrees (patients ≤ 18 or ≥ 75 years old underwent a 60-degree HUTT, and the other patients underwent a 70-degree HUTT). The HUTT was completed if a positive response occurred or after 20 min. After 30 min of rest, patients with a negative response who were highly suspected of having VVS underwent an isoprenaline-stimulated HUTT, and the patients spent another 20 min with their head tilted up to the same degree. They were initially treated with isoprenaline 1 μ g/min as an intravenous drip, which was increased by 1 μ g/min at 5-min intervals, up to a maximum of 3 μ g/min. The HUTT with isoprenaline infusion was halted when a positive response occurred, the average HR increased by 20–25% from the baseline value, or the fastest HR was over 150 beats per min (bpm) at 20 min (8).

Positive responses of the patients were due to VVS, postural tachycardia syndrome (POTS), and orthostatic hypotension (OH). Vasovagal syncope was defined as syncope or near-syncope, simultaneously accompanied by the following hemodynamic changes: a BP decrease [systolic BP (SBP) ≤ 80 mmHg and/or a diastolic BP (DBP) ≤ 50 mmHg or a mean arterial pressure decrease $\geq 25\%$] and/or bradycardia (HR < 50 bpm) or cardiac arrest > 3 s (9–11). Vasovagal syncope was further classified into three responses based on the classification of the Vasovagal Syncope International Study (VASIS) (12): mixed type (VASIS I), cardioinhibitory type (VASIS II), and vasodepressor type (VASIS III). Postural tachycardia syndrome (except OH) was identified as an HR increase of > 30 bpm or an HR ≥ 120 bpm if the BP did not decrease significantly (the decrease was $< 20/10$ mmHg) (10). Orthostatic hypotension was defined by an SBP decrease of ≥ 20 mmHg and/or a DBP decrease of ≥ 10 mmHg (within the first 3 min of the HUTT) if

the HR did not significantly decrease (11). The criteria of positive HUTT in the study were coincident for both non-syncope and syncope patients.

Finally, the patients were mainly classified based on the different clinical symptoms that were presented at the time of referral, and the patients were classified into two groups (the syncope group and non-syncope group). In the non-syncope group, the patients were mainly classified into five subgroups, namely, the dizziness group, chest tightness/palpitation group, intermittent weakness group, sweating group, and intermittent unexplained arrhythmia group. In addition, all patients were divided into four groups (<20, 21–40, 41–60, and > 60 years old) based on age. The positive rate of the HUTT and the percentage of each positive class were compared within groups or subgroups.

Statistical Analysis

Statistical analysis was performed using SPSS 22.0. Continuous variables are presented as the mean \pm standard deviation (SD) for normally distributed data and were compared using Student's *t*-test. Medians (P25, P75) were used to describe the non-normally distributed continuous variables and were compared using the Mann-Whitney U-test. Categorical data are presented as frequencies (percentages) and were compared using the χ^2 -test. Binary logistic regression was used to analyze the related factors for positive HUTT results. A *p*-value of < 0.05 was considered statistically significant.

RESULTS

Baseline Characteristics

A total of 4,873 patients who underwent the HUTT due to syncope or near-syncope were included in the study. The mean age at referral was 48.3 ± 17.6 years, and 2,064 (42.4%) patients were males. There were 768 patients (365 [47.5%] males) aged 41.6 ± 19.2 years in the syncope group and 4,105 patients (1,699

[41.4%] males) aged 49.6 ± 17.0 years in the non-syncope group. Patients in the syncope group were younger than the patients in the non-syncope group ($P < 0.001$). The proportion of males in the syncope group was higher than that in the non-syncope group ($P = 0.002$). The common symptoms at referral were syncope (15.8%), dizziness (60.7%), chest tightness/palpitation (21.0%), weakness (3.1%), sweating (1.9%), and intermittent unexplained arrhythmia (3.3%). The first department in which the patients chose to be examined was the Department of Neurology (50.4%), followed by the Department of Cardiology (35.9%), and other departments (13.7%), including the Departments of Ophthalmology and Otorhinolaryngology, Orthopedics and Psychiatry in the syncope group, and the Department of Neurology (49.4%), the Department of Cardiology (43.2%), and other departments (8.3%) in the non-syncope group. Compared with the non-syncope group, the proportion of patients who initially visited other departments in the syncope group was higher ($P < 0.001$) (Table 1).

HUTT Results

Among all 4,873 patients, 2,343 (48.1%) exhibited a positive response (1,260 [25.9%] with the mixed type, 34 [0.7%] with the cardioinhibitory type, 580 [11.9%] with the vasodepressor type, 179 [3.7%] with POTS, 290 [6.0%] with OH), and 2,530 (51.9%) patients exhibited a negative reaction. Of the 768 patients with syncope, a positive response was observed in 506 (65.9%) patients, including mixed type in 331 (43.1%) cases, cardioinhibitory type in 9 (1.2%) cases, vasodepressor type in 107 (13.9%) cases, POTS in 26 (3.4%) cases, and OH in 33 (5.1%) cases. Among the 4,105 patients without syncope, 1,837 (44.8%) exhibited a positive response, including mixed type in 929 (22.6%) cases, cardioinhibitory type in 25 (0.6%) cases, vasodepressor type in 473 (11.5%) cases, POTS in 153 (3.7%) cases, and OH in 257 (6.3%) cases. Additionally, the positive rate

TABLE 1 | Basic clinical features and HUTT results of the patients.

Parameter	Syncope group (<i>n</i> = 768)	Nonsyncope group (<i>n</i> = 4,105)	<i>P</i> -value
Age (years)	41.6 ± 19.2	49.6 ± 17.0	<0.001
Male	365 (47.5)	1,699 (41.4)	0.002
First consultation department			
Department of Cardiology	276 (35.9)	1,738 (42.3)	0.001
Department of Neurology	387 (50.4)	2,026 (49.4)	0.598
Other departments	105 (13.7)	341 (8.3)	<0.001
Positive HUTT	506 (65.9)	1,837 (44.8)	<0.001
Positive types of HUTT			
Mixed type	331 (43.1)	929 (22.6)	<0.001
Cardioinhibitory type	9 (1.2)	25 (0.6)	0.085
Vasodepressor type	107 (13.9)	473 (11.5)	0.058
POTS	26 (3.4)	153 (3.7)	0.644
OH	33 (4.3)	257 (6.3)	0.035
Decrease in SBP (mmHg)	43.8 ± 18.7	42.8 ± 18.5	0.707
Decrease in HR (bpm)	44.7 ± 18.0	40.7 ± 18.3	0.782

HUTT, head-up tilt test; POST, postural tachycardia syndrome; OH, orthostatic hypotension; SBP, systolic blood pressure; HR, heart rate; bpm, beats per min.

of the HUTT in the syncope group was higher than that in non-syncope group ($P < 0.001$). Compared with the non-syncope group, a positive HUTT in the syncope group was significantly more frequent in the mixed type group ($P < 0.01$). There was no significant difference in the frequency of positive response to the HUTT between the syncope and non-syncope groups with the cardioinhibitory type ($P = 0.085$) and vasodepressor type ($P = 0.058$) (Table 1).

Both the decreases in HR and SBP during the HUTT were similar between the syncope and non-syncope groups (Table 1). The study showed that there were changes in HR and BP prior to syncope or near-syncope symptoms, which presented as an increase in HR first, followed by successive decreases in BP and HR. The changes in HR and BP in the mixed-type and vasodepressor-type patients were as follows: the HR initially increased rapidly to the maximal HR when the patients had their head in the upward position, and the BP subsequently rapidly decreased to the lowest BP, followed by a decrease in HR. The changes in HR and BP in patients who had positive responses to the HUTT were described in Figure 1. A majority of positive responses (96.8%) were observed in the first 15 min of the HUTT, and the positive responses of POTS and OH mainly occurred in the first 5 min (Figure 2). These parameters usually returned to completely normal after 5 min of rest while the patient was in the supine position.

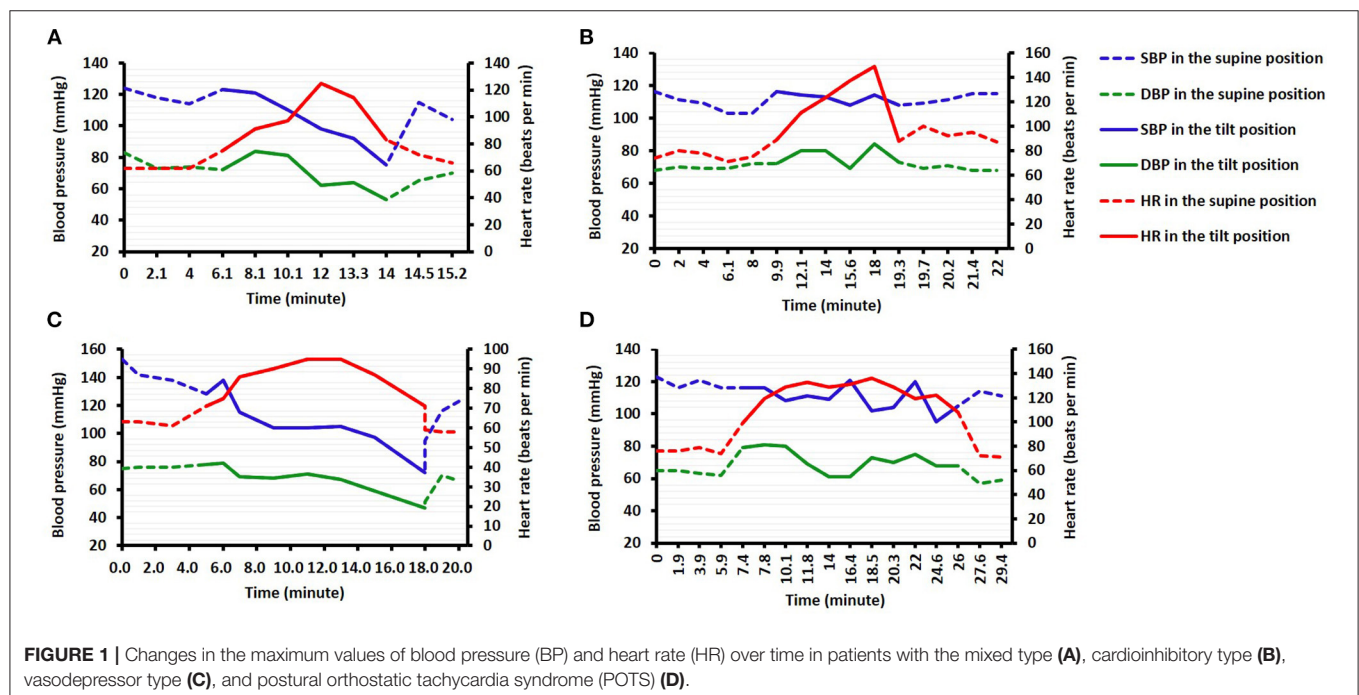
Diagnostic Sensitivity of the HUTT

A total of 2,343 (48.1%) patients displayed a positive response. In other words, the overall sensitivity of the HUTT with pharmacological intervention was 48.1%. Among the patients in the syncope group, the sensitivity was 65.9% (52.6% in males and 77.9% in females), which was significantly higher than that in

patients in the non-syncope group, with a sensitivity of 44.8% (36.5% in males and 50.6% in females) ($P < 0.01$). The sensitivity of the HUTT was higher in females than in males in both the syncope group ($P < 0.01$) and the non-syncope group ($P < 0.01$). Within the 4 age groups (<20, 21–40, 41–60, and >60 years old), the sensitivities were 74.7, 67.7, 45.6, and 31.2%, respectively. And all gender, age and symptom (whether suffer from a syncope or not) significantly affected the positive responses of HUTT (Table 2). Furthermore, in the non-syncope group, the sensitivities of the five subgroups, including the dizziness group, chest tightness/palpitation group, intermittent weakness group, sweating group, and intermittent unexplained arrhythmia group, were 38.6, 34.2, 44.6, 44.6, and 41.0%, respectively.

Safety and Adverse Events

The HRs that were detected during the HUTT ranged from 29 to 178 bpm, and patients whose HRs were <40 bpm accounted for 1.5% of the positive patients, 94.3% of whom had an isoprenaline-stimulated HUTT. The lowest SBP in this study was 54 mmHg, which was detected from the brachial artery. Patients who developed significant hypotension (could not be detected) accounted for 1.0% of the positive patients, and 95.7% of these patients had an isoprenaline-stimulated HUTT. There were two adverse events during the HUTT: one patient (60 years old, male) did not recover from the positive response after 2 min of rest in the supine position and had a junctional escape rhythm with significant hypotension (60–70/30–40 mmHg). Then, he recovered after 30 min following administration of 1 mg of intravenous atropine and an infusion of dopamine at a constant rate of 5–10 $\mu\text{g/kg/min}$. Another patient (65 years old, male) had asystole for 9 s, accompanied by loss of consciousness and hyperspasmia, but returned to the baseline value quickly after



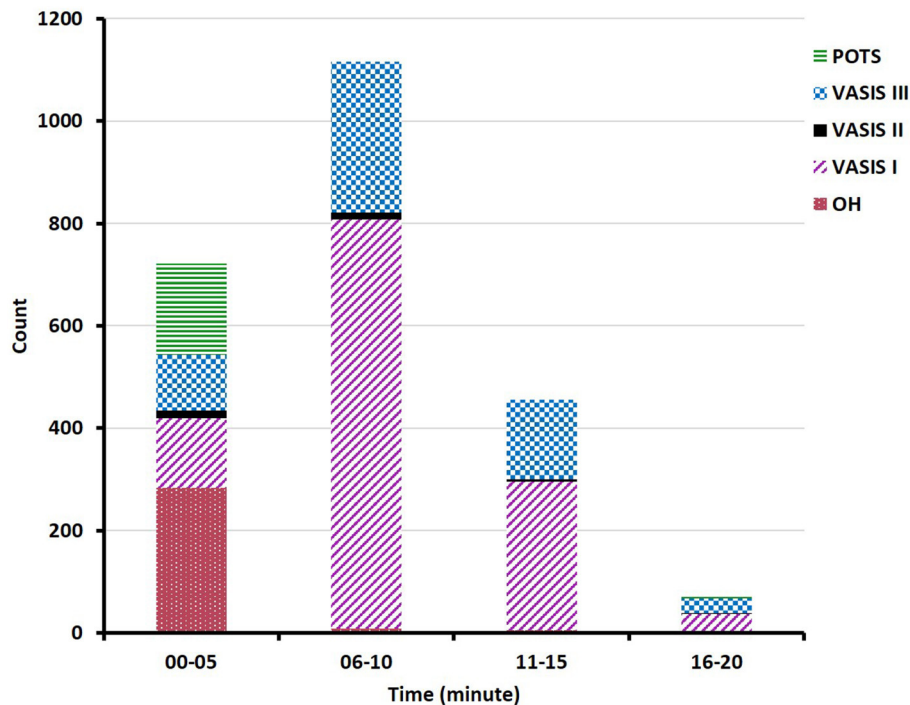


FIGURE 2 | Bar graph showing the distribution of all of the positive types. POST, postural tachycardia syndrome; VASIS III, vasodepressor type; VASIS II, cardioinhibitory type; VASIS I, mixed type; OH, orthostatic hypotension.

TABLE 2 | Binary logistic regression analysis of related factors for positive HUTT results.

Related factors	Wald value	P-value	OR	95%CI
Age	40.048	<0.001	0.989	0.986–0.992
Gender				
Male	336.483	<0.001	0.247	0.212–0.286
Female	–	–	–	–
Symptom				
Syncope	329.681	<0.001	6.223	5.116–7.595
Nonsyncope	–	–	–	–

HUTT, head-up tilt test.

being placed in the supine position. However, none of these patients died during the HUTT process.

DISCUSSION

In this study, 4,873 patients with unexplained syncope or precursor syncope were enrolled. We found that the age of the syncope patients was younger than that of the non-syncope patients, and the proportion of males in the syncope group was higher. A positive HUTT was recorded in 48.1% of the study population, and the mixed type was the most frequently observed, followed by the vasodepressor type. Our study showed that the changes in HR and BP prior to syncope or near-syncope symptoms were preceded by an increase in HR first, followed by a successive decrease in BP and HR. A majority of the

positive responses were observed in the first 15 min of the HUTT. Our HUTT protocol had a higher sensitivity in young females with syncope. Among the non-syncope population, patients who presented with weakness and sweating more frequently displayed a positive response (higher sensitivity). There were no serious adverse events or deaths during the HUTT process.

Compared with precursor syncope patients, the patients with syncope were younger, which showed that there was more exaggerated vagal activity in the younger patients. This result was supported by a report from Noormand et al. that showed that the overexcitation of vagal activity became less frequent with age (13). We also found that the proportion of females included in the non-syncope group was higher, the reasons for which were as follows. Precursor syncope symptoms, such as dizziness, chest tightness, palpitation, and weakness, were subjective and non-specific and may be associated with anxiety and depression. Previous studies have shown that females were more likely than males to suffer from anxiety and depression (14, 15), which probably led to inappropriate HUTT performance, which decreased the sensitivity of the HUTT in our study. Moreover, the proportion of patients with syncope who visited other departments initially was higher than that of the non-syncope patients, which may be attributed to the presence of wounds that occurred secondary to syncope, and this helps to remind surgeons that they should be cautious of VVS when they see patients with wounds secondary to syncope.

Of the positive patients, the mixed type was the most frequently observed in our study, which was similar to the result of a previous study (16). And the previous study also suggested

that the cardioinhibitory type was the second most frequently observed, which was different from our result (vasodepressor type). A possible reason was that the patients in our study were older (48.3 ± 17.6 vs. 34.0 ± 11.2 years), as the cardioinhibitory response decreases with age (13, 16). Our study revealed that the HR initially increased in patients identified as having VVS during the HUTT, followed by successive decreases in BP and HR. This regular pattern seemed to be due to a series of autonomic changes as reported in a previous paper (17), namely, increased excitability of the sympathetic nerves, decreased excitability of the sympathetic nerves, and increased excitability of the parasympathetic nerves. Based on the pathophysiological mechanism, many drugs, such as β -blockers, scopolamine, and etilefrine, have been tested for the treatment of VVS (18). In addition, we also found that a majority of positive responses were observed in the first 15 min of the HUTT, demonstrating that the time of observation during the HUTT was reasonable. This result serves as a reminder that more attention should be paid within the first 15 min during the HUTT.

The sensitivity of the HUTT has been reported to range from 46 to 65% for glyceryl trinitrate and 21 to 65% for isoprenaline (16, 19–23). Among the patients in the syncope group, the sensitivity was 65.9% in our study, which is higher than that reported previously. This result could be explained by the followings. First, any nitroglycerin-potentiated HUTT or isoprenaline-potentiated HUTT with a positive response was identified as a positive response in our study, which could lead to an increase in the sensitivity. Second, the patients were evaluated by a thorough evaluation, including an electrocardiogram, ECG, echocardiography, electroencephalogram, and cranial CT/MRI, which helped exclude the presence of cardiogenic syncope and neurological disease. In other words, the patients we included were suspected to have significant VVS. In addition, there was a higher sensitivity in young females, which was similar to the results reported by previous studies (13, 24). In this study, we also included precursor syncope patients, approximately half of whom displayed a positive response, suggesting that it is necessary to perform a HUTT for patients with precursor syncope who do not have evidence of cardiogenic disease or neurological disease, especially young females that have weakness and sweating. This probably resulted in a decrease in the numbers of misdiagnoses and missed diagnoses.

The HUTT protocol that we adopted was safe, as few serious adverse events were encountered with either protocol, which was similar to the results from previous studies revealing that many patients tolerated glyceryl trinitrate well. However, some patients tolerated isoprenaline poorly (16, 21, 22). The underlying mechanism that makes patients tolerate isoprenaline poorly is as follows. Isoprenaline exaggerates sympathetic nerve activity, which causes a significant decrease in the left ventricular volume, inducing myocardial ischemia and hypotension (22, 25). Graham et al. showed that there was an association between older age and a high adverse event rate. Therefore, physicians must be cautious of older patients with cardiovascular comorbidities when an isoprenaline-potentiated HUTT is performed. In this

study, two patients over 60 years of age suffered from an adverse event, which may be attributed to cardiovascular comorbidities.

LIMITATIONS

There were several limitations in our study. First, any nitroglycerin-potentiated HUTT or isoprenaline-potentiated HUTT presenting as a positive response was identified as a positive response in our study, which may have caused an increase in the sensitivity and a decrease in the specificity. A previous paper revealed that the specificities varied between 71 and 94.7% for glyceryl trinitrate and between 64 and 89.4% for isoprenaline (16, 20, 22). However, we could not determine the specificity of the HUTT protocol, as no healthy subjects were included in our study. Second, there is no gold standard test for the diagnosis of VVS, and the positive response rate of the HUTT, which was used to calculate the sensitivity, cannot reflect the real sensitivity of the HUTT. Thus, the false-positive and false-negative rates of the HUTT could not be acquired. Third, many patients with precursor syncope were included in this study and underwent a HUTT, which may lead to an excessive use of the HUTT. More studies are needed to identify the clinical characteristics of precursor syncope patients who need to undergo a HUTT.

CONCLUSION

The direct drug-potentiated HUTT is a feasible substitution for the conventional HUTT and has a high sensitivity and low risk. Young females presenting with near-syncope symptoms, especially weakness and sweating, who are highly suspicious of having VVS (although not suffering from syncope), should undergo a HUTT to decrease the chance for a misdiagnosis or a missed diagnosis.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Ethics Committee of The Xianyang Central Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

LX, FG, and CS: proposal. LX and XC: drafting. RW, YD, YY, JH, JW, BC, XX, and BZ: data gathering. HM, LX, FG, and CS: supervision and revision. LX: statistical analysis. All authors approved the final draft.

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Exploring the Correlation Between Fibrosis Biomarkers and Clinical Disease Severity in PLN p.Arg14del Patients

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Background: Pathogenic variants in *phospholamban* (PLN, like p. Arg14del), are found in patients diagnosed with arrhythmogenic (ACM) and dilated cardiomyopathy (DCM). Fibrosis formation in the heart is one of the hallmarks in PLN p.Arg14del carriers. During collagen synthesis and breakdown, propeptides are released into the circulation, such as procollagen type I carboxy-terminal propeptide (PICP) and C-terminal telopeptide collagen type I (ICTP).

Aim: To investigate if PICP/ICTP levels in blood are correlative biomarkers for clinical disease severity and outcome in PLN p.Arg14del variant carriers.

Methods: Serum and EDTA blood samples were collected from 72 PLN p.Arg14del carriers (age 50.5 years, 63% female) diagnosed with ACM ($n = 12$), DCM ($n = 14$), and preclinical variant carriers ($n = 46$). PICP levels were measured with an enzyme-linked immune sorbent assay and ICTP with a radio immuno-assay. Increased PICP/ICTP ratios suggest a higher collagen deposition. Clinical data including electrocardiographic, and imaging results were adjudicated from medical records.

Results: No correlation between PICP/ICTP ratios and late gadolinium enhancement (LGE) was found. Moderate correlations were found between the PICP/ICTP ratio and end-diastolic/systolic volume (both $r_s = 0.40$, $n = 23$, $p = 0.06$). PICP/ICTP ratio was significantly higher in patients with T wave inversion (TWI), especially in leads V4–V6, II, III, and aVF ($p < 0.022$) and in patients with premature ventricular contractions (PVCs) during an exercise tolerance test ($p = 0.007$).

Conclusion: High PICP/ICTP ratios correlated with clinical parameters, such as TWI and PVCs. Given the limited size and heterogeneity of the patient group, additional studies are required to substantiate the incremental prognostic value of these fibrosis biomarkers in PLN p.Arg14del patients.

Keywords: biomarkers, fibrosis, phospholamban (PLN), cardiomyopathy, collagen

INTRODUCTION

Pathogenic variants in multiple genes encoding proteins that are crucially important for a proper functioning of cardiomyocytes predispose to, or are directly causative for several forms of cardiomyopathy. Phospholamban (PLN) is such a key protein and is involved in calcium (Ca^{2+}) handling in cardiomyocytes. It is located at the sarcoplasmic reticulum (SR), where it regulates the function of sarco/endoplasmic reticulum Ca^{2+} -ATPase2a (SERCA2a). Dephosphorylated PLN tightly binds to SERCA2a and thereby inhibits SR Ca^{2+} influx. On the other hand, phosphorylation of PLN relieves the inhibitory binding of PLN to SERCA2a and facilitates Ca^{2+} influx into the SR (1–3). Therefore, PLN plays a crucial role in contractility of the heart. There are several pathogenic variants known in the *PLN* gene that might cause cardiomyopathy which can ultimately lead to heart failure (HF) and (potentially) lethal ventricular arrhythmias (VA). One of such variants is a Dutch founder variant, a heterozygous deletion of arginine at position 14 (p.Arg14del). This variant is found both in patients diagnosed with arrhythmogenic (ACM) as well as dilated cardiomyopathy (DCM) (1). Phenotypically, these patients typically show low QRS voltages and T wave inversions (TWI) on electrocardiogram (ECG) recordings in the precordial leads, HF, VA, and myocardial fibrosis (1–4).

Fibrosis formation is one of the early hallmarks of disease in patients with a p.Arg14del variant (3). Progressive fibrosis formation appears especially in the epicardial left ventricular (LV) free wall (5). Pathological fibrosis formation is a reaction to replace apoptotic cardiomyocytes or excessive production of extracellular matrix (ECM) components, like collagens. The ECM, or interstitium, consists of interstitial fluid and several proteins (e.g., lamins, dystrophin) most of them being produced by fibroblasts, such as proteoglycans and collagen, the latter being the main part of the cardiac ECM (6). The most abundant types of collagen in the heart are collagen type I (85%) and collagen type III (11%) (7, 8). There is continuous turnover of collagen synthesis and breakdown as the half-life of collagen in the human heart is 90–120 days (8). In a healthy heart, collagen forms a network that provides structure to the cardiac muscle and strength during contraction (9). Under pathological conditions such as in *PLN* cardiomyopathy, collagen production is increased, which results in excessive fibrosis formation in the heart. In this setting, fibrosis may act as a substrate for VA (7), but it also disturbs contractile performance by affecting both contraction and relaxation. Collagen is synthesized by cardiac fibroblasts as pre-procollagen. In the ECM, procollagen is cleaved to collagen, and thereby the amino (PINP and PIINP)- and carboxy (CICP, CIIICP) pro-peptides are released into the circulation. Degradation of collagen occurs with the help of metalloproteinases (MMPs), where MMPs cleave collagen fibers into fragments for further degradation. In the myocardium, different MMPs are expressed. MMP-1, MMP-8, and MMP-13, also known as collagenases, are highly specific for collagen types I and III and cleave the collagen into 2 fragments. These fragments will denature at physiological conditions. The denatured collagen fragments can be further digested by MMPs known as gelatinases, particularly MMP-2 and MMP-9 (10, 11). One such a breakdown

fragment, C-terminal telopeptide collagen type I (ICTP), is also released into the circulation, similarly to propeptides during synthesis of collagen (8).

In the clinical setting, cardiac fibrosis is detectable *via* late gadolinium enhancement on cardiac magnetic resonance imaging (LGE-CMR), CMR-based T1 mapping or in tissue biopsies. A drawback of LGE-CMR is the inability to detect small patches and diffuse fibrosis, including the more subtle types of fibrosis where collagen heterogeneously intermingles with cardiomyocytes and is most often responsible for arrhythmogenesis (7, 9). T1 mapping is a relatively new approach that enables detection of subtle and diffuse fibrosis in high resolution, although this technique currently is not yet standardized and not routinely available (3, 9). Moreover, tissue biopsies are not frequently used due to their highly invasive character. Given these shortcomings, the field would benefit from new additional approaches to detect fibrosis that are fast, non-invasive, and cheap. For that purpose, assessment of biomarkers that reflect on total collagen turnover in relation to clinical outcome are of interest (12–16). When successful, such an approach facilitates frequent analysis which will support early detection of disease onset and disease progression in carriers (including family members), and potentially also identification of patients at risk for severe clinical outcomes [such as ventricular tachycardia (VT) and sudden cardiac death (SCD)]. In this study, we measured procollagen type I carboxy-terminal pro-peptide (PICP, as a marker of collagen synthesis) and ICTP (as a marker of collagen breakdown) in serum obtained from *PLN* p.Arg14del patients. The ratio between PICP/ICTP levels is proposed to reflect the balance between collagen synthesis and breakdown (17, 18). As such, we hypothesized that higher PICP/ICTP levels correlate to increased myocardial fibrosis formation in hearts of these patients, and in turn are related to clinical outcomes. Therefore, the aim of this study was to investigate the correlation of PICP/ICTP levels with clinical and outcome parameters in *PLN* variant carriers.

MATERIALS AND METHODS

Study Population and Diagnosis

Serum and EDTA blood samples were obtained from 72 *PLN* p.Arg14del carriers, and were all collected on the same day during an event day for *PLN* p.Arg14del carriers. These were either carriers with an ACM or DCM diagnosis, or preclinical pathogenic variant carriers not fulfilling diagnostic criteria for a cardiomyopathy. The latter group consisted of patients that were either preclinical/asymptomatic and some with an intermediate phenotype. Diagnosis of ACM followed the 2010 Task Force Criteria score (TFC), where a TFC score ≥ 4 led to a definite ACM diagnosis (19). Diagnosis of DCM followed criteria, which included left ventricular dilatation (end-diastolic diameter (LVEDD) $>112\%$ of predicted for age and body surface area (BSA), or LV end-diastolic volume $>2\text{SD}$ from reference value) and a reduced LV function (ejection fraction (LVEF) $<45\%$ or fractional shortening (FS) $<25\%$) (20). Patients with a history of heart transplantation were excluded from this study. All subjects

provided written informed consent. The study was conducted according to the Declaration of Helsinki. Blood samples were stored (AUMC-Durrer center Biobank number DC17-006) at -80°C until use.

Clinical Data Collection

Clinical data were extracted from the ACM and PLN Registry as hosted in REDCap. These registries collect clinical data including demographics, symptoms, medication use, family history and genetic analysis (21).

Clinical parameters were categorized into functional/structural and electrical parameters. Functional/structural parameters included LGE-CMR, EF, end-diastolic and end-systolic volume (EDV, ESV, respectively). Dysfunction and dilatation parameters were qualitative assessments of ventricular function and diameter. For example, dilatation of the right ventricle (RV) was based on the RV diameter compared to the LV.

For electrical parameters QRS duration, TWI and low QRS voltages were assessed *via* ECG. A T wave was considered inverted if the voltage shifted ≥ 0.1 mV below baseline (21). A low QRS voltage was defined as a QRS peak-to-peak amplitude in leads I, II and III < 0.5 mV or amplitude in all precordial leads < 1.0 mV (22). Terminal activation duration (TAD) was defined as the longest value in V1–V3 from the nadir of the S wave to the end of all depolarization deflections in absence of a complete right bundle branch block, and was considered prolonged if ≥ 55 ms. During an exercise tolerance test the presence of premature ventricular contractions (PVCs) was assessed. Non-sustained VT was defined as three or more consecutive PVCs with a rate $> 100/\text{min}$, lasting < 30 s recorded on a Holter. For PVC amount in 24 h, all PVCs were counted except for VTs recorded on a Holter (22). Lastly, HF was defined as NYHA class II or higher, or ACC/AHA Stage C or higher (21).

Timepoint of serum collection was considered as “baseline” ($T = 0$) in this study. Clinical information around this date (with a time window of maximally 2 years prior to or following serum collection) were extracted from REDCap. To validate this chosen time window of 2 years, we did a sensitivity analysis including only patients who underwent clinical testing within a maximum of 5.5 weeks around blood sampling to get a closer picture of the reflection of PICP/ICTP levels and their relationship to clinical outcome ($n = 12$). Only a limited number of patients could be included into this sub-analysis, however, their PICP/ICTP levels were in line with the obtained overall picture (time window ± 2 years), as depicted in red dots in **Supplementary Figures 1A–D**.

Measurements of PICP and ICTP

PICP levels were measured using a commercially available enzyme-linked immune sorbent assay (ELISA) (MicroVue Bone, CICP, Cat #8005) according to the manufacturer’s protocol. In short, diluted EDTA serum was administrated to murine monoclonal anti-CICP antibody coated strips. After incubation with rabbit anti-CICP antibodies, enzyme conjugate and substrate solution were added to the wells in different steps. After administrated stop solution to the wells, optical density was

measured at 405 nm using the Bio-plex[®] 200 system (Bio-Rad, Hercules, CA).

ICTP levels were measured using a commercially available radioimmunoassay (RIA) (UniQ[®], Orion Diagnostica, Cat #68601). Serum was incubated with Tracer, Antiserum and Procollagen Separation Reagent to test tubes. After centrifugation and decantation of the supernatant, tubes were counted using a gamma counter (Wizard^{2®}, PerkinElmer). PICP and ICTP levels are shown as ng/mL.

Statistics

Biomarker data were not normally distributed. Therefore, when continuous variables between two groups were compared, Mann-Whitney *U*-test was performed. For testing of multiple groups, Kruskal Wallis test with adjusted *p*-values by Bonferroni correction were used. Correlation between variables were assessed using Spearman’s correlation coefficient. Data were considered significant if $p < 0.05$, correlations were considered weak between 0.10 and 0.40, moderate between 0.40 and 0.60, strong between 0.60 and 0.80 and very strong between 0.80 and 1.00. Data are shown as median [interquartile range]. Statistical analysis was performed using PRISM 9.0 (GraphPad Software, La Jolla, CA, USA, RRID:SCR_002798).

RESULTS

Patient Characteristics

Patient characteristics are summarized in **Table 1**. The median age of all PLN variant carriers was 50.5 years [37–59 years], and 45/72 (63%) were female. In total, 12/72 (17%) patients were diagnosed with ACM according the 2010 TFC score [median TFC score 5 (4–7)], while 14/72 (19%) patients were diagnosed with DCM and 46/72 (64%) were preclinical variant carriers without phenotypic expression of cardiomyopathy (yet). No significant differences in age and sex were found between the three groups. The proband status (first affected family member) was significantly different between these subgroups ($p = 0.01$). Also, medication use such as betablockers and ACE-inhibitors significantly differed between the subgroups ($p < 0.03$). Similarly, ICD implantation varied between patients with ACM, patients with DCM, and preclinical variant carriers ($p < 0.003$).

No significant difference in EF, EDV, or ESV of the ventricles was found between the three subgroups. However, the median of LVEF in DCM group was 40%, for ACM diagnosed 54% and preclinical pathogenic variant carriers 55%. The median LV EDV for DCM patients was 308 mL, for ACM 170 mL and for preclinical variant carriers 158 mL. For LV ESV, the median for the DCM diagnosed patients was 186 mL, ACM diagnosed 75 mL and for preclinical pathogenic variant carriers 73 mL. Furthermore, nine out of 23 patients showed LGE in the LV, while one out of 22 patients showed LGE in the RV. As expected, in ACM classified PLN patients the RV was significantly more dysfunctional, while in DCM diagnosed PLN patients primarily the LV was affected, as ventricular dysfunction was assessed *via* echocardiogram analysis.

TABLE 1 | Patient characteristics of included *PLN* variant carriers.

	All <i>PLN</i> (<i>n</i> = 72)	ACM diagnosed (<i>n</i> = 12)	DCM diagnosed (<i>n</i> = 14)	Preclinical variant carrier (<i>n</i> = 46)	Adjusted <i>p</i> -value
Demographics					
Age (years)	50.50 [37–59]	53 [48.75–56]	56 [43.50–61.25]	46 [37–58.75]	>0.999
Female	45/72 (63)	7/12 (58)	7/14 (50)	31/46 (67)	>0.999
Proband status	21/72 (29)	6/12 (50)	9/14 (64)	5/46 (11)	0.01**
TFC score	2 [0–3]	5 [4–7]	2 [1.25–3]	1 [0–2]	<0.003**
Treatment					
Betablockers	29/71 (41)	5/12 (42)	13/14 (93)	11/45 (24)	<0.003**
Antiarrhythmics	6/71 (8)	2/12 (17)	3/14 (21)	1/45 (2)	>0.999
Diuretics	16/71 (23)	4/12 (33)	6/14 (43)	6/45 (13)	>0.999
ACE-inhibitors	26/71 (37)	5/12 (42)	11/14 (79)	10/45 (22)	0.024*
ICD implantation	27/72 (38)	7/12 (58)	11/14 (79)	9/46 (20)	<0.003**
Imaging/MRI					
	1.03 years				
LGE LV	9/23 (39)	2/4 (50)	2/2 (100)	5/17 (29)	>0.999
LVEF (%)	54 [51.55–57.75]	54 [53–55]	39.50 [38.75–40.25]	55 [52–58]	>0.999
LV EDV (mL)	161.29 [152–189.40]	170 [157–175]	307.50 [272.75–342.25]	158 [152–178.80]	>0.999
LV ESV (mL)	74.14 [66–90.50]	75 [69.75–80]	185.50 [162.25–208.75]	73 [66–76]	>0.999
LGE RV	1/22 (5)	0/4 (0)	0/1 (0)	1/17 (6)	>0.999
RVEF (%)	53 [47–56]	55 [49.50–56.50]	-	54 [50.50–57.50]	>0.999
RV EDV (mL)	167 [123–193.70]	181 [151–191.50]	-	154.50 [121.50–181]	>0.999
RV ESV (mL)	81 [56.25–109]	81 [66–97]	-	76 [55.82–100.50]	>0.999
Echocardiogram					
	0.64 years				
LV dysfunction	24/61 (39)	5/10 (50)	11/13 (85)	8/38 (21)	0.007**
LV dilatation	11/59 (19)	4/10 (40)	7/13 (54)	0/36 (0)	<0.003**
RV dysfunction	11/61 (18)	7/10 (70)	3/13 (23)	1/38 (3)	<0.003**
RV dilatation	7/57 (12)	4/8 (50)	0/11 (0)	3/38 (8)	0.065
ECG-monitoring					
	0.49 years				
QRS duration (ms)	92 [80.50–112.25]	97.50 [93–102.50]	98 [84.50–118.25]	89 [78–108.50]	>0.999
T wave inversion	36/56 (64)	7/9 (78)	10/14 (71)	19/33 (58)	>0.999
Number of leads with TWI out of 9	2 [0–5]	3 [1–5]	3 [0.5–5]	1 [0–2]	>0.999
Low QRS voltage	22/63 (35)	5/11 (45)	10/14 (71)	7/38 (18)	0.058
TAD duration ≥55 ms	6/50 (12)	3/8 (38)	1/13 (8)	2/29 (7)	>0.999
Exercise tolerance test					
	0.60 years				
PVCs	30/43 (70)	5/7 (71)	7/7 (100)	18/29 (62)	>0.999
Holter					
Non-sustained VT	5/33 (15)	2/7 (29)	2/2 (100)	1/24 (4)	0.03*
PVC amount in 24 h	387 [7.5–682.5]	534.5 [412.25–3,190.25]	5,850.5 [5,172.75–6,528.25]	163 [–491]	>0.999
Outcomes					
Heart failure	11/70 (14)	2/12 (17)	8/14 (57)	0/44 (0)	<0.003**
VT/VF event or appropriate ICD shock	13/62 (21)	3/9 (33)	6/11 (55)	4/42 (10)	0.112

Data is depicted as median [interquartile range], *n* (%), or *n*/*N* (%). *PLN*, phospholamban; *ACM*, arrhythmogenic cardiomyopathy; *DCM*, dilated cardiomyopathy; *TFC*, 2010 Task Force Criteria; *ACE-inhibitors*, angiotensin-converted enzyme inhibitors; *ICD*, implantable cardioverter-defibrillator; *LGE*, late gadolinium enhancement; *LVEF*, left ventricular ejection fraction; *EDV*, end-diastolic volume; *ESV*, end-systolic volume; *RVEF*, right ventricular ejection fraction; *TAD*, terminal activation duration; *PVCs*, premature ventricular contractions; *VT/VF*, ventricular tachycardia/ventricular fibrillation. Kruskal wallis test is performed, Bonferroni correction is applied to correct *p*-value, ***p* < 0.01, **p* < 0.05.

No difference in QRS duration, TWI, number of leads with TWI and the presence of a TAD of ≥55 ms or low QRS voltage were found with ECG-recordings between the subgroups. The presence of PVCs at baseline and during an exercise tolerance test did not differ between the subgroups. Detection of non-sustained VTs with a Holter-recording showed significant differences between ACM or DCM diagnosed and

preclinical variant carriers (*p* = 0.03). The median amount of PVCs recorded in 24 h on a holter for DCM patients was 5,850.5 [5,172.75–6,528.25], for ACM patients 534.5 [412.25–3,190.25] and for preclinical variant carriers 163 [–491] Lastly, a significant difference between the incidence of HF (*p* < 0.003) was found between ACM, DCM patients, and preclinical variant carriers.

TABLE 2 | Fibrosis biomarkers concentrations in all *PLN* variant carriers ($n = 72$) or divided into ACM diagnosed ($n = 12$), DCM diagnosed ($n = 14$) or preclinical pathogenic variant carriers ($n = 46$).

	All <i>PLN</i> ($n = 72$)	ACM diagnosed ($n = 12$)	DCM diagnosed ($n = 14$)	Preclinical variant carrier ($n = 46$)	Adjusted <i>p</i> -value
PICP (ng/mL)	120.18 [98.32–146.98]	112.55 [95.19–135.32]	133.44 [104.91–156.28]	120.18 [98.62–144.72]	>0.999
ICTP (ng/mL)	3.14 [2.42–3.81]	3.34 [2.68–4.76]	3.36 [2.38–3.87]	3.07 [2.43–3.54]	>0.999
PICP/ICTP	42.40 [29.70–51.63]	37.74 [23.54–45.37]	43.70 [27.90–51.88]	42.57 [31.53–54.12]	>0.999

Data is shown as median [interquartile range]. *PLN*, phospholamban; *ACM*, arrhythmic cardiomyopathy; *DCM*, dilated cardiomyopathy; *PICP*, procollagen type I carboxy-terminal pro-peptide; *ICTP*, C-terminal telopeptide collagen type I. Kruskal wallis test is performed. Bonferroni correction is applied to correct *p*-value.

Fibrosis Biomarkers

The median level of PICP for all 72 *PLN* p.Arg14del patients was 120.18 [98.32–146.98] ng/mL, for ICTP 3.14 [2.42–3.81] ng/mL and a PICP/ICTP ratio of 42.40 [29.70–51.63]. There was no influence of sex or medication use on the PICP/ICTP levels (Supplementary Figure 2).

Furthermore, no differences were observed in PICP, ICTP, and PICP/ICTP levels within ACM (PICP; 112.55 [95.19–135.32] ng/mL, ICTP; 3.34 [2.68–4.76] ng/mL, and PICP/ICTP; 37.74 [23.54–45.37], $p > 0.999$), DCM (PICP; 133.44 [104.91–156.28] ng/mL, ICTP; 3.36 [2.38–3.87] ng/mL, and PICP/ICTP; 43.70 [27.90–51.88], $p > 0.999$) patients and preclinical pathogenic variant carriers [PICP; 120.18 (98.62–144.72) ng/mL, ICTP; 3.07 (2.43–3.54) ng/mL, and PICP/ICTP; 42.57 (31.53–54.12) $p > 0.999$], as summarized in Table 2.

Correlation of Fibrosis Biomarkers With Structural/Functional Parameters

LGE-CMR was performed in a subset of 23 patients included in our cohort. No differences between PICP/ICTP ratios were found between patients that showed LV fibrosis and the patients who did not; 43.30 [30.56–54.43] compared to 34.36 [29.01–45.27], $p = 0.60$ (Figure 1A). For the RV, only one preclinical variant carrier revealed significantly enhanced LGE (Figure 1B).

As for functional parameters, LVEF showed a weak, but not significant, inverted correlation ($r_s = -0.28$, $p = 0.188$) with PICP/ICTP ratio (Figure 1C). For RVEF, no correlation was found ($r_s = -0.10$, $p = 0.67$) with PICP/ICTP, Figure 1D.

For the morphologic parameters (Figures 1E,F), moderate positive correlations were found with LV EDV and LV ESV volume (both $r_s = 0.40$, $p = 0.06$). Weak, albeit not significant, positive correlations were found for RV EDV ($r_s = 0.30$, $p = 0.297$) and RV ESV ($r_s = 0.24$, $p = 0.304$) as shown in Figures 1G,H. To summarize, weak to moderate, but not significant, correlations were found with structural/functional parameters and fibrosis biomarkers.

Correlation of Fibrosis Biomarkers With Electrical Parameters

Previous research revealed an association between the proportion of myocardial fibrosis and QRS duration in a population of SCD patients (23). In our study, only a weak and not significant correlation between QRS duration and PICP/ICTP ratio was found ($r_s = 0.13$, $p = 0.331$), Figure 2A. On the other hand, the group of patients who presented with TWI had a significantly

higher total collagen turnover [PICP/ICTP ratio 43.53 (33.77–52.02)] compared to the group of patients that did not have TWI [PICP/ICTP 27.11 (21.14–50.85), $p = 0.044$], Figure 2B. Moreover, when the different leads of the ECG (in particular leads V4–V6) were assessed, the presence of TWI was associated with a significantly higher collagen turnover [lead V4; 44.92 (41.52–56.67) compared to 34.07 (23.43–50.06), $p = 0.022$, lead V5; 45.12 (41.91–56.81) compared to 32.96 (23.09–45.46), $p = 0.002$, lead V6; 46.76 (43.33–57.43) compared to 32.36 (23.16–44.99), $p = 0.001$], as exemplified for lead V6 in Figure 2C. Also, significantly higher PICP/ICTP ratios were found when TWIs were present in lead II [52.54 (44.92–59.97) compared to 32.96 (23.34–44.69), $p < 0.001$], lead III [46.58 (36.16–55.29) compared to 34.84 (23.43–45.36), $p = 0.022$] and lead aVF [49.92 (44.73–55.57) compared to 34.10 (23.51–45.27), $p = 0.004$] were observed in *PLN* variant carriers, Figure 2D and summarized in Table 3.

Another clinical manifestation in *PLN* p.Arg14del carriers is the presence of low QRS voltages (3). Our data did not identify a correlation between the presence of these low voltages and high collagen turnover, Figure 2E. However, PICP/ICTP ratios were significantly higher in patients who presented with PVCs during an exercise tolerance test [45.29 (38.62–54.09)] as compared to patients in which no PVCs occurred [32.96 (21.64–43.05), $p = 0.007$], as depicted in Figure 2F.

Subpopulation Analysis of Electrical Parameters

Since we included a heterogeneous study population, with some patients diagnosed with ACM or DCM, and preclinical variant carriers without a clinical diagnosis of a cardiomyopathy, a subpopulation analysis was performed. For preclinical variant carriers, a weak but not significant correlation between prolonged QRS duration and increased PICP/ICTP ratios ($r_s = 0.31$, $p = 0.066$) was found, Figure 3A. Patients diagnosed with an ACM phenotype showed a similar correlation as the preclinical variant carriers ($r_s = 0.45$, $p = 0.192$), whereas on the other hand DCM diagnosed *PLN* patients showed an inverted relationship with profibrotic biomarkers ($r_s = -0.56$, $p = 0.076$), Figure 3B. When the presence of low QRS voltages was compared among groups, we found a significant higher collagen turnover in preclinical variant carriers that showed low QRS voltages, but this discrimination was absent in the ACM and DCM diagnosed patients, Figures 3C,D. Similarly to the whole group analysis, preclinical *PLN* p.Arg14del variant carriers had a significantly

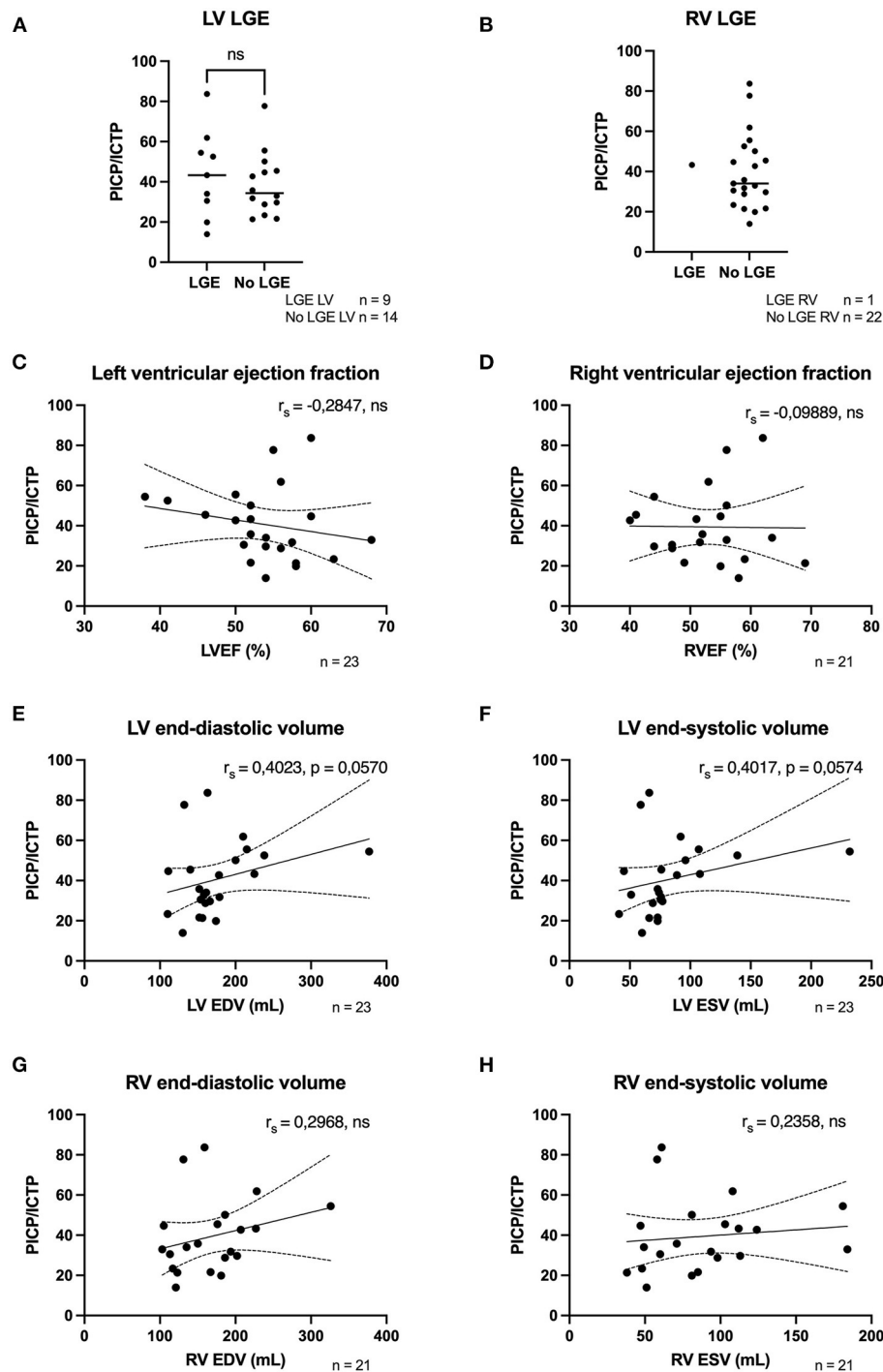


FIGURE 1 | Fibrosis biomarkers correlated weakly to moderately to structural/functional parameters. **(A)** PICP/ICTP ratio was not different in patients with LV LGE ($n = 9$) compared to patients who did not have LV LGE ($n = 14$). **(B)** Only one patient had LGE in the RV, therefore no comparison could be made. **(C)** A weak negative correlation was found with LVEF ($n = 23$). **(D)** No correlation was found with RVEF and fibrosis biomarkers ($n = 21$). **(E,F)** Moderate positive correlations were found with LV EDV and ESV ($n = 23$). **(G,H)** Weak positive correlations were found with RV EDV and ESV ($n = 21$). LGE, late gadolinium enhancement; LVEF, left ventricular ejection fraction; RVEF, right ventricular ejection fraction; EDV, end-diastolic volume; ESV, end-systolic volume; PICP, procollagen type I carboxy-terminal pro-peptide; ICTP, C-terminal telopeptide collagen type I; r_s , Spearman's rho; ns, not significant.

higher total collagen turnover when they experienced PVCs during an exercise tolerance test, **Figure 3E**. In contrast, no significant difference was observed in the ACM or DCM

diagnosed group. Of note, the small group of only 7 DCM patients all showed PVCs with high PICP/ICTP biomarker levels, as shown in **Figure 3F**.

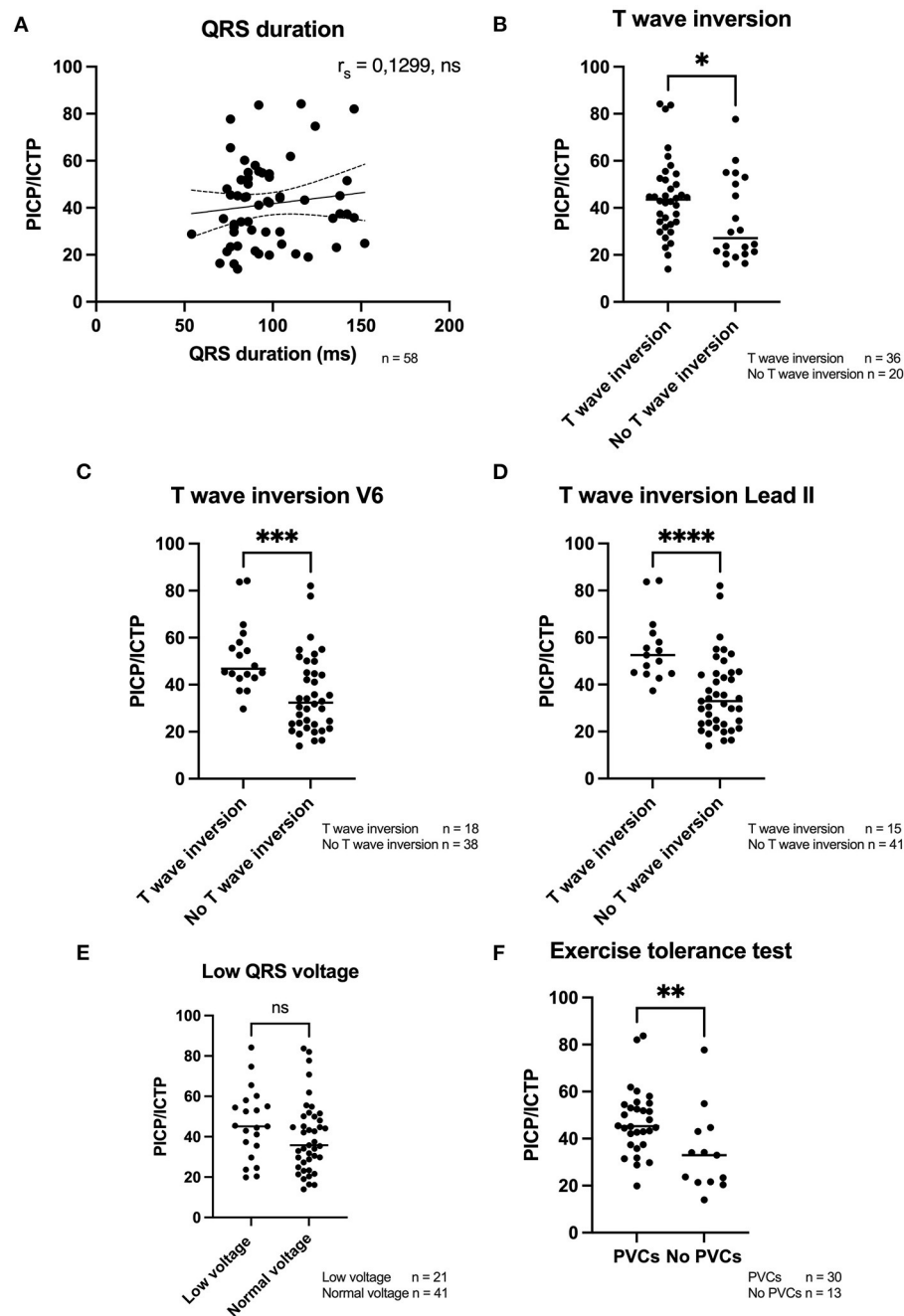


FIGURE 2 | Fibrosis biomarkers in relation to electrical parameters. **(A)** A weak positive correlation was found with QRS duration ($n = 58$). **(B–D)** A significant higher PICP/ICTP ratio was found when a T wave inversion was present, overall, but especially when detected in lead V4–V6 and lead II, III, and aVF. **(E)** No difference was found in the ratio of fibrosis biomarkers and the presence or absence of low QRS voltages. **(F)** PICP/ICTP ratio was significantly higher when PVCs were detected during an exercise tolerance test. PVCs, premature ventricular contractions; PICP, procollagen type I carboxy-terminal pro-peptide; ICTP, C-terminal telopeptide collagen type I; rs, Spearman's rho. **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

DISCUSSION

To date, visualization of especially patchy and diffuse fibrosis is difficult with current clinical techniques, so an experimental approach using biomarkers might help to provide better

insights into profibrotic remodeling of the heart under pathophysiological conditions. In this study, we found weak to moderate correlations of profibrotic biomarkers with QRS duration, EF, EDV, and ESV in both LV and RV in *PLN* p.Arg14del carriers. Of note, our study population was quite

TABLE 3 | The PICP/ICTP ratios of patients who showed a T wave inversion compared to patients who did not show a T wave inversion in different leads of the ECG.

	T wave inversion	No T wave inversion	p-value
Lead V4	44.92 [41.52–56.67]	34.07 [23.43–50.06]	0.022*
Lead V5	45.12 [41.91–56.81]	32.96 [23.09–45.46]	0.002**
Lead V6	46.76 [43.33–57.43]	32.36 [23.16–44.99]	0.001***
Lead II	52.54 [44.92–59.97]	32.96 [23.34–44.69]	<0.001****
Lead III	46.58 [36.16–55.29]	34.84 [23.43–45.36]	0.022*
Lead aVF	49.92 [44.73–55.57]	34.10 [23.51–45.27]	0.004**

Data is depicted as median [interquartile range]. PICP, procollagen type I carboxy-terminal pro-peptide; ICTP, C-terminal telopeptide collagen type I; ECG, electrocardiogram. Mann-Whitney U-test is performed. **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

heterogeneous, as it included patients diagnosed with ACM or DCM, but the majority concerned pathogenic variant carriers that did not have a clinical diagnosis of cardiomyopathy (yet). Therefore, an additional subpopulation analysis was performed. This showed a weak but not significant correlation of prolonged QRS duration with profibrotic markers in the blood, but only for preclinical variant carriers. Additionally, this group of preclinical *PLN* p.Arg14del variant carriers that showed low QRS voltages and TWI had significant higher PICP/ICTP ratios compared to the group that did not have these ECG changes.

An excessive production of collagen hinders cardiac function by: (1) hampering electrical conduction and thereby increasing the risk for VA, (2) reducing contractile performance of the ventricles, and (3) increasing ventricular stiffness thereby impairing the filling and relaxation capacity of the ventricle (7, 24). Electrical parameters, such as prolonged QRS duration and the presence of low QRS voltages on the ECG are suggested to reflect myocardial fibrosis (4, 23, 25). Furthermore, TWI can indicate the presence of a repolarization defect whereas also abnormal depolarization can result in a TWI. In our study, both these low QRS voltages and TWI in lead V5, V6, II, and aVF were also found in *PLN* p.Arg14del variant carriers without an ACM or DCM diagnosis, and thereby might reflect an early electrical transformation in still relatively unaffected patients (3, 4). The observation that significantly increased PICP/ICTP ratios correlated to TWI especially in ECG leads V4–V6 might suggest that the LV is more affected in most *PLN* p.Arg14del patients. When we compared ventricular dysfunction in *PLN* p.Arg14del carriers diagnosed with ACM or DCM, as expected, the ACM patients showed predominantly RV dysfunction while DCM diagnosed patients showed more LV dysfunction (see **Table 1**). More proof of electrical instability in *PLN* p.Arg14del patients with a high total collagen turnover adhered to the presence of PVCs during an exercise tolerance test. PVCs are early depolarizations of the ventricle which are linked to increased risk of severe arrhythmias and SCD (26). Transgenic mice expressing the *PLN* p.Arg14del variant also showed accumulation of myocardial fibrosis and contractile dysfunction by reduced LVEF and increased EDV and ESV. The contractile impairment most likely results from inhibition of

SERCA by the pathogenic *PLN* variant, which causes a distortion in Ca^{2+} cycling. Moreover, these *PLN* mutated mice showed lower QRS voltages and increased incidence of VA *ex vivo* (1).

Previous research compared PICP levels in blood to the extent of myocardial fibrosis in endocardial biopsies in HF patients. PICP levels were elevated in blood obtained from both the coronary artery and peripheral blood, which suggest that PICP levels measured in peripheral blood reflects collagen turnover in the heart. Although values obtained from coronary blood overall were higher than measured in peripheral blood, a direct correlation was found between the degree of endocardial fibrosis and the PICP levels both in coronary blood and peripheral blood. More importantly, a direct correlation existed between PICP levels measured in coronary and peripheral blood and collagen type I deposition in the heart (16). This suggests that PICP levels measured in peripheral blood (like we analyzed in our study) indeed reflects collagen turnover in the heart. Substantiating the findings in our study, elevated PICP levels were shown to be highly sensitive and specific for identification of severe myocardial fibrosis in a study of patients suffering from arterial hypertension (16). Similarly a study of Aguiar et al. identified a correlation of PICP/ICTP with LGE in patients with Fabry disease cardiomyopathy (27), but this correlation with LGE was absent in a cohort of overt hypertrophic cardiomyopathy (HCM) patients and HCM pathogenic variant carriers without left ventricular hypertrophy (17).

In this cohort of HCM patients, it was however shown that the PICP/ICTP ratio was increased in overt HCM patients, but not in pathogenic variant carriers, suggesting that in overt disease state, collagen synthesis exceeded collagen breakdown, while in preclinical variant carriers this increased collagen synthesis is stabilized by increased collagen breakdown (17). Another explanation, as formulated by Lombardi et al., suggested that collagen turnover is only robustly upregulated during a temporal progressive/hot phase of the disease (14). In Fabry disease cardiomyopathy it was shown that PICP/ICTP ratio was increased in patients with severe disease expression. Weak but significant correlations were found with fibrosis biomarkers and clinical outcome such as LV mass (28). Other studies also revealed weak, but significant, correlations between LVEF or left atrial (LA) diameter and PICP or ICTP levels in a population of elderly HF patients (13). Similarly, in hypertensive patients with chronic HF, weak but significant correlations were found with pulmonary capillary wedge pressure, a way to estimate filling pressure in these hypertensive patients (15). On the contrary, the study of Querejeta et al. did not find any significant correlations of PICP levels with clinical outcome in patients suffering from arterial hypertension, however this study population only consisted of 26 patients (16). In DCM patients, it was found that increased ICTP levels were associated with higher relative risk of advanced clinical disease progression and heart transplantation (12). In addition, it was found that increased ICTP levels correlated to a lower degree of cardiac event-free events (29).

In our study, we found weak to moderate correlations of PICP/ICTP levels to different parameters of clinical outcome. To our knowledge, this is the first time that these levels were measured in *PLN* p.Arg14del patients, and the degree in

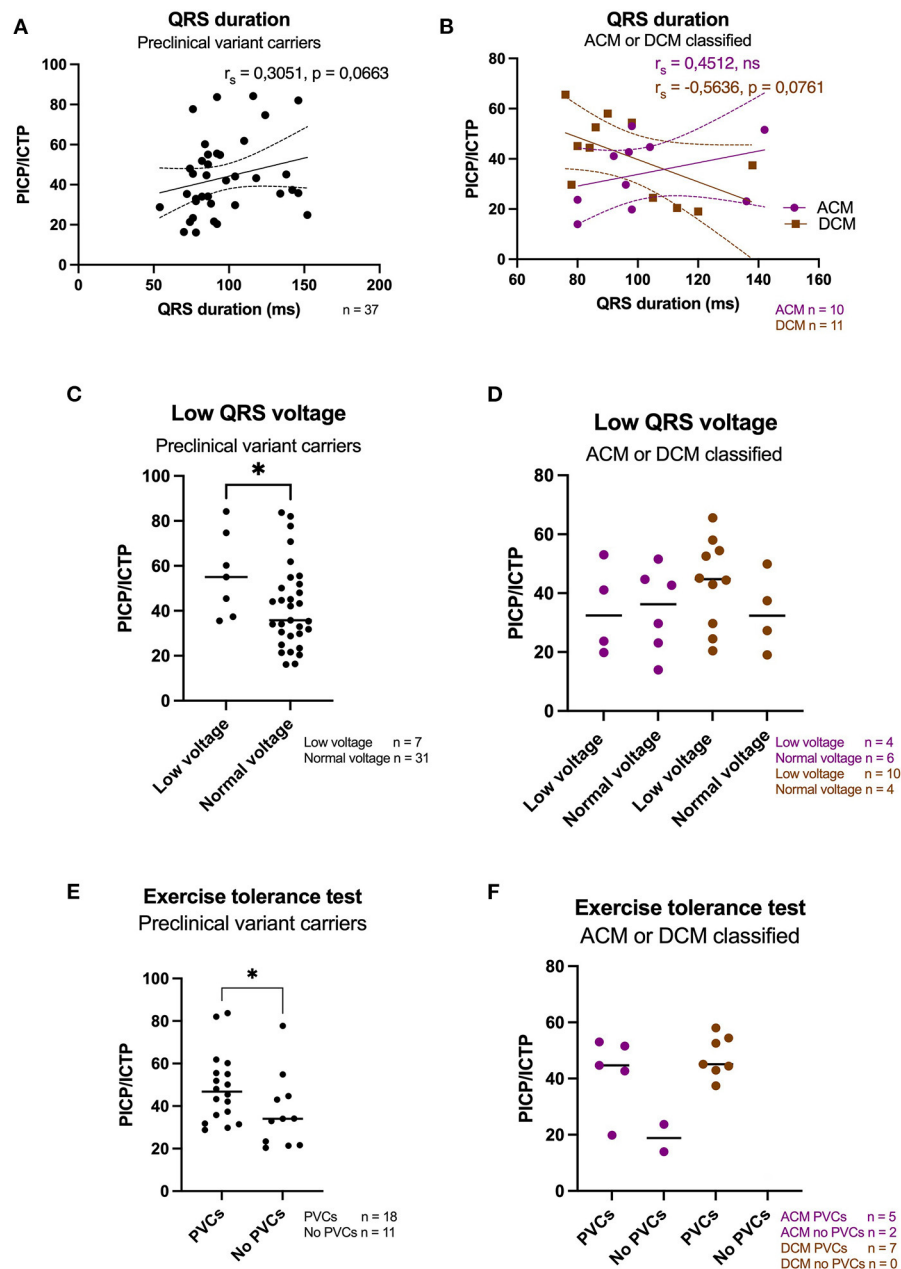


FIGURE 3 | Subpopulation analysis of electrical parameters. **(A)** A weak positive correlation was found with QRS duration in preclinical pathogenic variant carriers ($n = 37$). **(B)** A moderate positive correlation was found with QRS duration in patients diagnosed with ACM ($n = 10$), while a moderate negative correlation was found with QRS duration in DCM diagnosed patients ($n = 11$). **(C)** A significant higher PICP/ICTP ratio was found in preclinical variant carriers that presented with low QRS voltages. **(D)** No significant difference was found in ACM or DCM diagnosed *PLN* patients with total collagen turnover and the presence of low QRS voltages. **(E)** A significant higher PICP/ICTP ratio was found when PVCs were detected during an exercise tolerance test in preclinical variant carriers. **(F)** No significant difference was found in PICP/ICTP ratios and the detection of PVCs in ACM or DCM classified *PLN* patients. Of note, all DCM patients presented with PVCs during an exercise tolerance test ($n = 7$). ACM, arrhythmogenic cardiomyopathy; DCM, dilated cardiomyopathy; PVCs, premature ventricular contractions; PICP, procollagen type I carboxy-terminal pro-peptide; ICTP, C-terminal telopeptide collagen type I; r_s , Spearman's rho. * $p < 0.05$.

correlations fits with the correlation coefficients reported in the studies mentioned above. In a previous study performed in our group, the correlation of PICP/ICTP levels with myocardial fibrosis was investigated in which levels of fibrosis markers were

measured at baseline, at 6 weeks and around 5 months after myocardial infarction (MI) in patients. We found that total collagen turnover was significantly increased after 6 weeks and 5 months. In addition, moderate correlations were found with

increased PICP levels and scar size, as measured with LGE (manuscript under consideration). The stronger correlations in that particular study population most likely reflect the more severe degree of fibrosis formation upon MI.

LIMITATIONS

Limitations of this study include the difference in timepoints between blood sampling and clinical assessment. Therefore, direct effects of analyzed PICP and ICTP levels cannot signal a temporal hot phase of the disease, but we assumed that the PICP and ICTP levels reflect a continuous progression of collagen deposition. Another limitation of the study is that we could not correct for other abnormalities potentially leading to collagen deposition, since collagen type I is not only produced in the heart but found in many other organs in the body. Therefore, it is possible that in some cases the PICP and ICTP levels do not exclusively reflect myocardial fibrosis formation. Finally, to strengthen the predictive character of PICP/ICTP ratio as biomarker, inclusion of more patients would have been preferred but this was unfortunately hampered by the fact that blood samples of patients with this rare pathogenic variant is limited. Therefore, additional preferable correlations made to fibrosis parameters such as to LGE appeared not to be possible. Although not considered as a real limitation, and in order to be clear, following the aim of this study to correlate the collagen biomarkers to clinical outcome we did not implement healthy control subjects. This is logical due to the fact that from this tentative additional group clinical records are obviously lacking.

CONCLUSION

In conclusion, our data show that total collagen turnover correlates weakly to moderately with clinical parameters in *PLN* p.Arg14del patients. Contractile impairment of the LV also correlated weakly to moderately to total collagen turnover. In addition, we found a higher total collagen turnover in patients with PVCs and TWI, especially for leads V4–V6. However, to gain insights into the predictive value of PICP/ICTP ratio as a biomarker for clinical outcome, further investigation with a higher number of patients is required, especially in *PLN* variant carriers being diagnosed with either ACM or DCM.

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DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by AUMC-Durrer Center Biobank number DC17-006. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SV, CR, and TABV contributed to study design, data collection, data analysis, and writing the manuscript. MB, AR, KT, RB, TEV, and RB contributed to the data collection and interpretation. All authors have read the manuscript and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcvm.2021.802998/full#supplementary-material>

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Baseline Corrected QT Interval Dispersion Is Useful to Predict Effectiveness of Metoprolol on Pediatric Postural Tachycardia Syndrome

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Objectives: The study was designed to explore the role of baseline-corrected QT interval dispersion (QTcd) in predicting the effectiveness of metoprolol on pediatric postural tachycardia syndrome (POTS).

Methods: There were two groups in the study, the discovery group and the validation group. The children with POTS in the discovery group were treated with oral metoprolol, with the completed necessary medical records, head-up tilt test (HUTT), blood chemistry, and 12-lead ECG before treatment at the pediatrics of Peking University First Hospital, China. According to whether the symptom score (SS) was reduced by more than 2 points after administration with oral metoprolol as compared with that before treatment, the children with POTS were separated into responders and non-responders. The demographic characteristics, hemodynamic indicators, and the QTcd of the two groups were compared, and the estimate of the baseline QTcd in predicting the treatment response to metoprolol was tested through a receiver operating characteristic (ROC) analysis. Other 24 children suffering from POTS who were, administrated with metoprolol at the pediatrics of Peking University First Hospital were included in the validation group. The sensitivity, specificity, and accuracy of the baseline QTcd in the prediction of the effectiveness of metoprolol on POTS were validated in children.

Results: The pre-treatment baseline QTcd in responders treated with metoprolol was longer than that of the non-responders in the discovery group [(66.3 ± 20.3) ms vs. (45.7 ± 19.9) ms, $p = 0.001$]. The baseline QTcd was negatively correlated with SS after metoprolol treatment ($r = -0.406$, $p = 0.003$). The cut-off value of baseline QTcd for the prediction of the effectiveness of metoprolol on pediatric POTS was 47.9 ms, yielding a sensitivity of 78.9% and a specificity of 83.3%, respectively. The validation group showed

that the sensitivity, specificity, and accuracy of the baseline QTcd ≥ 47.9 ms before treatment for estimating the effectiveness of metoprolol on POTS in children were 73.7, 80.0, and 75.0%, respectively.

Conclusion: Baseline QTcd is effective for predicting the effectiveness of metoprolol on pediatric POTS.

Keywords: corrected QT interval dispersion, postural tachycardia syndrome, metoprolol, orthostatic intolerance, children

INTRODUCTION

Postural tachycardia syndrome (POTS), as one of the clinical subtypes of orthostatic intolerance (OI), mainly manifests an increase in heart rate (HR) in the standing position and OI symptoms. It greatly influences the quality of life of children both physically and mentally (1, 2). Its main pathogenesis includes high catecholamine levels in blood circulation, overexcitement of sympathetic nerve function, vasodilation, or low central blood volume (3–6). Beta-adrenoceptor blocker metoprolol was currently one of the main drugs for the treatment of children with POTS. Metoprolol exerts its therapeutic effect on POTS in children by inhibiting excessive sympathetic nerve function, decreasing HR, and the mechanical activation of ventricular wall baroreceptors, and inhibiting the effect of excessive plasma catecholamine content (7–10). However, the effective rate of metoprolol on POTS in children ranged from 57.1 to 57.89% (11). The main reason was that beta-adrenoceptor blockers could ameliorate the symptoms only for POTS children whose pathogenesis consisted of high catecholamine levels (11). It could be inferred that if POTS children whose pathogenesis involves high circulating catecholamine levels could be predicted before treatment, they could be given a targeted treatment of beta-adrenoceptor blockers, which would significantly improve the efficacy of POTS in children. Therefore, it is of great important significance to find out the useful, easy-to-perform, and inexpensive indicators or markers to predict the effectiveness of beta-adrenoceptor blocker in children suffering from POTS before treatment.

Previously, the investigators examined the baseline values of plasma norepinephrine (12), copeptin (13), C-type natriuretic peptide (CNP) levels (14), and 24-h heart rate variability (HRV) in predicting the efficacy of metoprolol on pediatric POTS (15), and then guided the clinical use of metoprolol in the treatment with the useful markers detected. However, the plasma norepinephrine is extremely variable, which limits the accuracy of the prediction. The detection of plasma copeptin requires enzyme-linked immunosorbent assay, the operation process of which is complicated, limiting the popularization of the technique for general practitioners. In addition, the determination of plasma norepinephrine, copeptin, and CNP

levels is invasive because the blood samples are obtained by venipuncture. The collection and recording of 24-h HRV are time-consuming and non-economic. As such, all the above-mentioned predictive techniques have obvious limitations in clinical application. Therefore, looking for stable, non-invasive, and easy-to-perform indicators before treatment to predict the efficacy of metoprolol on POTS in children is a critical issue in this field.

The previous reports showed that the corrected QT interval dispersion (QTcd) obtained by 12-lead ECG could quantitatively assess the activity of autonomic nerve function, which was non-invasive, easier to operate, and inexpensive (15, 16). Accordingly, the present study was designed to reveal if the baseline QTcd was useful in predicting the efficacy of metoprolol on POTS in children with POTS.

MATERIALS AND METHODS

Population

In this retrospective cohort study, there were 53 children suffering from POTS who were hospitalized in the Department of Pediatrics, Peking University First Hospital, Beijing, China from March 8, 2012 to August 3, 2018, with complete data of 12-lead ECG prior to the treatment and treated with metoprolol. Furthermore, 3 months after the first metoprolol therapy, telephonic, outpatient, or inpatient follow-up was conducted. The information of 3 children with POTS was missing during the follow-up or had incomplete follow-up data. The lost follow-up rate was 5.7%. Therefore, this study finally included 50 children with POTS treated with oral metoprolol in the discovery group [28 boys and 22 girls, mean age of 12.5 ± 2.1 years].

In addition, 26 children with POTS, who had completed 12-lead ECG data before treatment and treated with metoprolol in the Department of Pediatrics, Peking University First Hospital from August 31, 2018 to December 10, 2020, were recruited in the validation group. After 3 months of the treatment with metoprolol, the outpatient or inpatient follow-up was conducted, and 2 cases of children were lost to follow-up or with incomplete data in follow-up. The loss rate of follow-up was about 7.7%. Therefore, 24 children with POTS were finally included in the treatment with oral metoprolol in the validation group [9 boys and 15 girls, at a mean age of 11.9 ± 2.1 years]. **Figure 1** shows the flowchart for the subject inclusion in this investigation.

This research was granted by the ethics committee of the Peking University First Hospital [2018(202)]. The guardians or parents of the children were notified of the study aim and were requested to sign an informed consent form.

Abbreviations: QTd, QT interval dispersion; QTc, corrected QT interval; QTcd, corrected QT interval dispersion; POTS, postural tachycardia syndrome; ECG, electrocardiogram; SS, symptom score; OI, orthostatic intolerance; HR, heart rate; CNP, C-type natriuretic peptide; HRV, heart rate variability; BP, blood pressure; HUTT, head-up tilt test; AC, adenylate cyclase; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A.

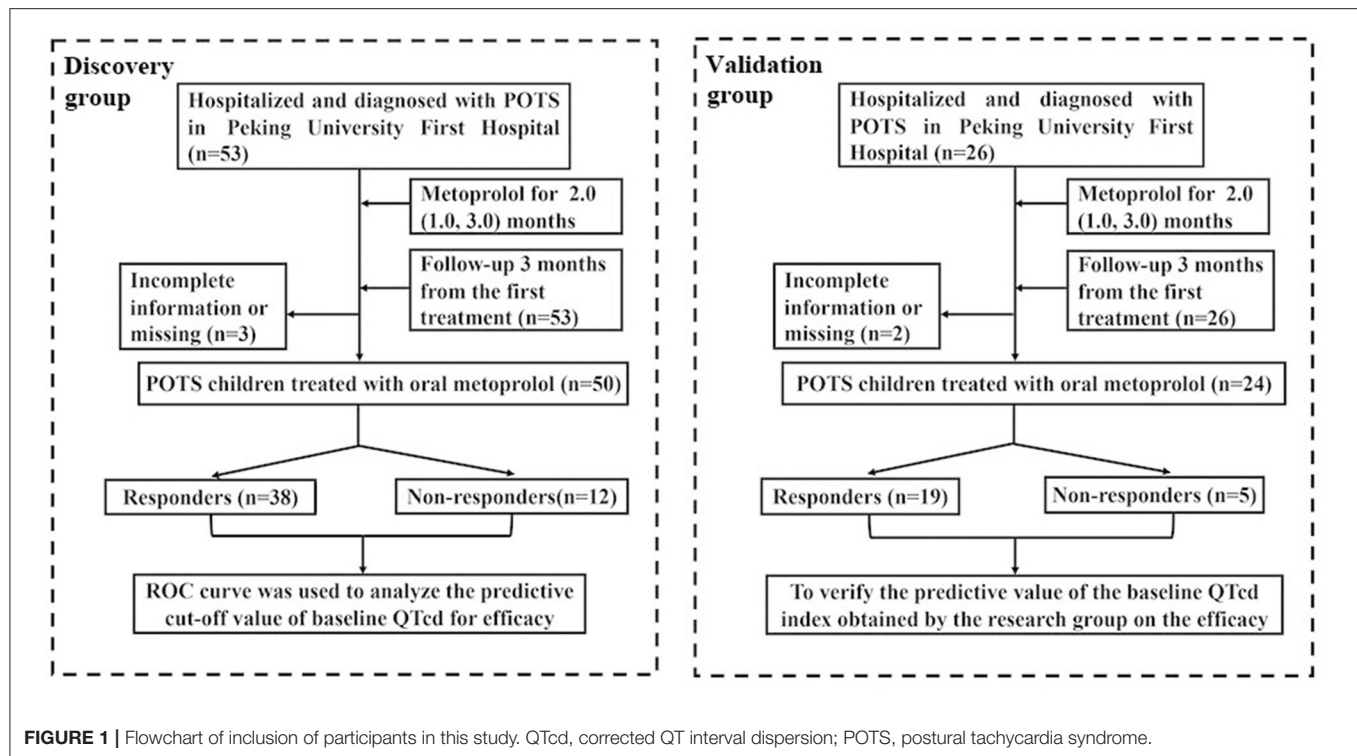


FIGURE 1 | Flowchart of inclusion of participants in this study. QTcd, corrected QT interval dispersion; POTS, postural tachycardia syndrome.

Inclusion Criteria of Children

The inclusion criteria are: children with POTS who were hospitalized in Peking University First Hospital; the clinical manifestations after admission, complete 12-lead ECG examination data, and necessary laboratory investigations before metoprolol treatment were all recorded; the age range was 6–18 years old; and the children with POTS were treated with oral metoprolol.

Diagnostic Criteria for POTS in Children

Children with POTS were often accompanied by quick changes in body position (from lying to standing), persistent standing, muggy environment, and other predisposing factors. They had OI symptoms, such as dizziness, headache, palpitations, and syncope. During the standing test or basic head-up tilt test (BHUTT), within 10 min from supine to the standing, the maximum heart rate (HR) when standing increases by 40 bpm compared with that of the supine position or the maximum of HR (HR max) within 10 min of upright position reaches the standard (≥ 130 bpm for 6–12 years old, or ≥ 125 bpm for 13–18 years old), and the blood pressure (BP) drops $< 20/10$ mmHg (17, 18).

Exclusion Criteria for POTS in Children

Patients suffering from cardiovascular disorders, such as arrhythmia, myocarditis, cardiomyopathy, pulmonary hypertension, and pseudo-syncope diseases, such as metabolic diseases, psychogenic pseudosyncope, or epilepsy, were excluded. In addition, children with incomplete data of 12-lead ECG before treatment and children who were lost to follow-up after metoprolol treatment were excluded.

Standing Test

The test requires a suitable temperature, dim light, and quiet environment. First, the patients were required to rest for 10 min in the supine position, and then to stand actively for 10 min. During the examination, ECG, HR, and BP were consecutively recorded with a multi-channel physiological monitor (Dash 2000, General Electric Company, NY, USA). During the standing process, if the child had syncope or pre-syncope, the test was terminated and then immediately the child was assisted to a supine position (19).

Basic Head-Up Tilt Test

All patients were asked to terminate the medicine that affects autonomic nerve function for longer than 5 half-life time prior to the test, and needed to fast for over 4 h. Under a noiseless, suitable temperature, and low-light condition, the child was required to lie supine on a tilted bed (HUT-821, Juchi Company, Beijing, China) for 10–30 min. And HR and ECG were constantly recorded using a multi-lead ECG monitor (General Electric, NY, USA). Continuous BP was recorded by a non-invasive arterial blood pressure monitoring system (Finapres Medical System, Finometer PRO, FMS company, the Netherlands). When HR, BP, and ECG were all stable, the inclined bed started to raise with an angle of 60 degrees, and HR, BP, and ECG were recorded till the whole process of the BHUTT was completed (20).

Twelve-Lead ECG Examination and QTcd Measurement

Before treatment, all children received a 12-lead ECG test with an electrocardiograph (FX-7402, Fukuda, Japan). The children

were required to lie and have a rest for about 10 min in the supine position, keep breathing steady, and take a regular 12-lead continuous tracing. The paper speed of ECG was set to 25 mm s⁻¹, and the amplitude to 10 mm (mV)⁻¹. After that, the stable and clear baseline ECG graph was recorded. And QT interval was measured manually by a full-time researcher.

The QT interval stands for the interval from the starting point of QRS wave to the end of T wave. The average of 3 consecutive QT intervals in each lead was obtained, and the maximum QT interval (QTmax) and the minimum QT interval (QTmin) were measured in the 12-lead ECG. In addition, the maximum RR interval (RRmax) and the minimum RR interval (RRmin) were calculated. Then, the QT dispersion (QTd) was calculated as $QTd = QTmax - QTmin$. Additionally, the corrected QTmax (QTcmax) and corrected QTmin were calculated using the Bazetts formula. The specific calculation was performed as $QTcmax = QTmax \div RRmax^{1/2}$ and $QTcmin = QTmin \div RRmin^{1/2}$. The calculation formula of corrected QTd (QTcd) was $QTcd = QTcmax - QTcmin$.

Symptom Score in Children With POTS

Symptom score (SS) is based on the frequency of the OI symptoms, which are as follows: dizziness, syncope, headache, chest tightness, palpitation, sweating, nausea, hand tremor, blurred vision, and inattention. The scoring criteria of any symptom frequency were as follows (21–25): 0 score stands for no symptom; 1 score, symptoms once a month; 2 scores, symptoms 2–4 times per month; 3 scores, symptoms 2–7 times per week; 4 scores, symptoms > once a day. The score of each symptom was recorded, and the total SS was the sum of the scores of each symptom for a child.

Treatment and Follow-Up

After all children in the study were first diagnosed with POTS, they were treated with metoprolol for 2 (1, 3) months. The initial dosage of metoprolol was used as 0.5 mg·kg⁻¹·day⁻¹, two times a day, and it was increased gradually, according to the tolerable dose (the maximum dose is 2 mg·kg⁻¹·day⁻¹).

All subjects suffering from POTS were followed-up for a period of 3 months after the treatment with metoprolol and it was recorded by the trained personnel. In addition, questionnaire surveys were conducted through outpatient, in-patient, and telephonic follow-up. The drug compliance, frequency of OI symptoms, and drug adverse effects should be carefully monitored during the follow-up.

Grouping Based on Therapeutic Effect of Metoprolol at Follow-Up

When POTS was first diagnosed in children, SS was calculated for the patients as the pre-treatment SS. Post-treatment SS was calculated after the follow-up.

If the post-treatment SS of the child was decreased by ≥ 2 scores as compared with the pre-treatment SS, the child was defined as a “responder.” On the contrary, when post-treatment SS was decreased by <2 scores, the child was defined as a “non-responder” (22–24).

The POTS children in the validation group were divided into “predicted responders” and “predicted non-responders,” according to the baseline QTcd prediction cut-off value obtained by the discovery group. According to the actual follow-up results, the children in the validation group were divided into “actual responders” and “actual non-responders.” Thereby, the sensitivity, specificity, and accuracy of baseline pre-treatment QTcd in estimating the effectiveness of metoprolol on POTS in children were validated.

Statistical Analysis

The statistical data analysis software used in this study is SPSS 23.0 (IBM, Armonk, NY, USA). The measurement data are represented by mean \pm SD, and the count data are represented by the number of cases (*n*). The Shapiro–Wilk test was used by the normality test of measurement data. When the data in the two groups were in a normal distribution, the independent *t*-test was utilized for the comparison between the groups, otherwise, the Mann–Whitney *U*-test was used. The χ^2 -test was applied for comparing count data between the groups. Pearson analysis was applied to test the correlation between baseline QTd or QTcd and post-treatment SS. A receiver operating characteristic (ROC) curve was applied to estimate the cut-off values of baseline QTd or QTcd in predicting the effectiveness of metoprolol on pediatric POTS in the discovery group. When the maximum value of the Youden index was taken, its predictive sensitivity and specificity reached the best. Therefore, according to the outcome of the 3-month-follow-up after metoprolol treatment, the QTcd cut-off value from the discovery group was calculated. The sensitivity, specificity, and accuracy of baseline QTcd to predict the therapeutic efficacy were verified in the validation group. The standard for statistically significant differences was $p < 0.05$.

RESULTS

Analysis of Demographic Characteristics, Baseline Hemodynamic Indexes, and Pre-treatment SS Between Responders and Non-responders in the Discovery Group

There were no marked differences in gender, age, weight, height, body mass index (BMI), HR, systolic blood pressure (SBP), and diastolic blood pressure (DBP) in the supine position, HR max, Δ HR, and pre-treatment SS between 38 responders [20 boys (52.6%), mean age (12.4 ± 2.3) years] and 12 non-responders [8 boys (66.7%), the mean age (12.8 ± 1.5) years] in discovery group (all $p > 0.05$; Table 1).

Analysis of Baseline ECG Indexes Between the Responders and the Non-responders in the Discovery Group

The comparison of baseline ECG indexes revealed that there were no obvious differences in pre-treatment baseline QTmax, RRmax, RRmin, QTcmax, and QTcmin between responders and

TABLE 1 | Comparison of demographic and hemodynamic parameters between responders and non-responders in the discovery group.

Groups	Cases [n (%)]	Sex (M/F)	Age (yrs)	Height (cm)	Weight (kg)	BMI (kg/m ²)	Supine HR (bpm)	Supine SBP (mmHg)	Supine DBP (mmHg)	Δ HR bpm	HR max (bpm)	Pre- Treatment SS (points)
Responders	38 (76.0)	20/18	12.4 \pm 2.3	159.3 \pm 15.5	53.4 \pm 20.5 ^a	20.3 \pm 4.9 ^a	72 \pm 14	110 \pm 17 ^a	70 \pm 16	47 \pm 7	123 \pm 10	7 \pm 4 ^a
Non-responders	12 (24.0)	8/4	12.8 \pm 1.5	162.3 \pm 7.5	55.9 \pm 17.9	20.9 \pm 5.2	76 \pm 15	110 \pm 12	65 \pm 11 ^a	47 \pm 8 ^a	123 \pm 12	9 \pm 5
$t/Z/\chi^2$	-	0.271	0.591	0.927	-0.466	-0.454	0.797	-0.057	-0.841	-0.421	-0.170	-1.759
P-Value	-	0.603	0.557	0.359	0.641	0.650	0.429	0.955	0.400	0.674	0.866	0.079

POTS, postural tachycardia syndrome; M/F, male/female; BMI, body mass index; kg/m², kilogram per square meter; HR, heart rate; bpm, beats per minutes; SBP, systolic blood pressure; DBP, diastolic blood pressure; Δ HR, the difference between the maximum heart rate within 10 min of standing and the supine position; HR max, maximum heart rate within 10 min of standing; SS, symptom score. Data are mean \pm SD. ^aNon-normal distribution.

non-responders (all $p > 0.05$; **Table 2**). While the pre-treatment QTmin in the responders was shorter than the non-responders [(330.5 \pm 26.8) ms vs. (357.5 \pm 26.9) ms, $t = 3.034$, $p = 0.004$]. Furthermore, the baseline QTd (**Figure 2A**) and QTcd (**Figure 2B**) in the responders were markedly longer than the non-responders [(50.4 \pm 18.3) ms vs. (38.3 \pm 21.2) ms, $Z = -2.455$, $p = 0.014$; (66.3 \pm 20.3) ms vs. (45.7 \pm 19.9) ms, $Z = -3.339$, $p = 0.001$].

Correlation Analysis Between Baseline QTd and QTcd and Post-treatment SS in the Discovery Group

Pre-treatment QTd ($r = -0.291$, $p = 0.041$; **Figure 3A**) or QTcd ($r = -0.406$, $p = 0.003$; **Figure 3B**) was negatively correlated with post-treatment SS.

ROC Analysis of Pre-treatment Baseline QTd and QTcd in the Prediction of Efficacy of Metoprolol

Since the baseline QTd and QTcd significantly differed between the responders and non-responders, the value of predicting the efficacy of metoprolol on POTS children was further analyzed with an ROC curve. The results demonstrated that the area under the curve (AUC) of baseline QTd (**Figure 4A**) and QTcd (**Figure 4B**) in predicting the efficacy of metoprolol on POTS in children was 0.736 [95% CI 0.552–0.920] and 0.822 (95% CI 0.653–0.992), respectively. When the Youden index was the largest, the predictive cut-off values were 37.0 and 47.9 ms, the sensitivity was 76.3 and 78.9%, and the specificity was 66.7 and 83.3%, respectively (**Tables 1, 2**). Considering the influence of HR on the QTd index and the better index of the predictive ability between QTd and QTcd, the baseline QTcd served as a useful index of the effectiveness of metoprolol on POTS in children.

Validation of Predictive Value of Baseline QTcd

In validation group, 24 pediatric POTS (9 boys and 15 girls, mean age of [11.9 \pm 2.1] years) who were treated with metoprolol were included. Based on the predictive cut-off value of baseline QTcd by the discovery study, children with POTS in the validation group were divided into QTcd \geq 47.9 ms group and QTcd $<$ 47.9 ms group. Additionally, through follow-up results of the validation group at 3 months after metoprolol treatment, it was found that the sensitivity, specificity, and accuracy of baseline QTcd \geq 47.9 ms in predicting the effectiveness of metoprolol on pediatric POTS were 73.7, 80.0, and 75.0%, respectively (**Table 3**).

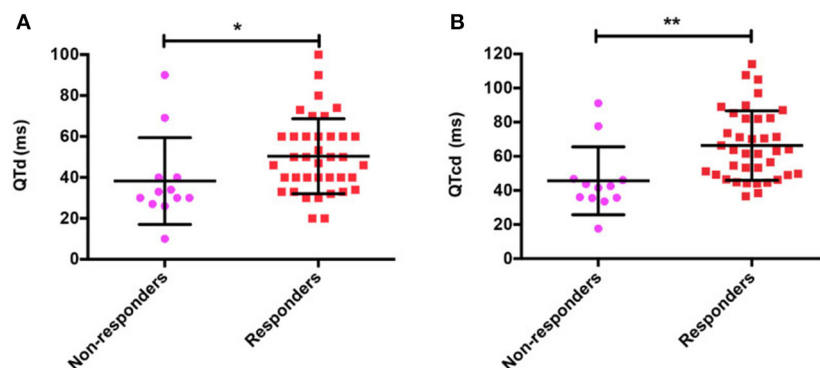
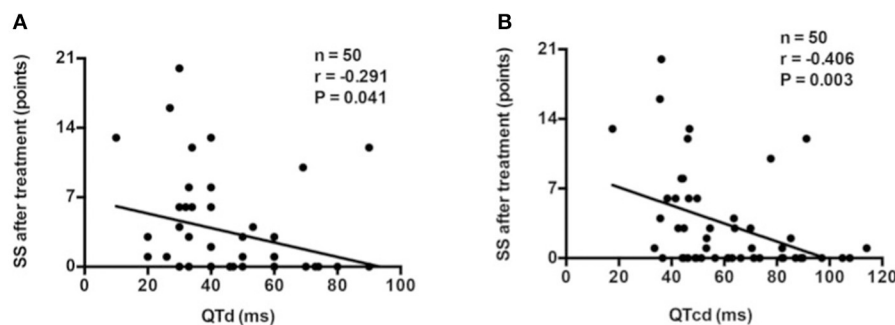
DISCUSSION

We indicated that the baseline QTd and QTcd of the responders were evidently longer than that of the non-responders. Pre-treatment baseline QTd and QTcd were both negatively associated with the post-treatment SS. The baseline QTcd of 47.9 ms had a sensitivity and specificity

TABLE 2 | Comparison of baseline ECG parameters between responders and non-responders in the discovery group.

Groups	QTmax (ms)	QTmin (ms)	RRmax (ms)	RRmin (ms)	QTcmax (ms)	QTcmin (ms)
Responders	380.4 ± 26.7	330.5 ± 26.8	771.6 ± 127.0	812.6 ± 130.1	435.1 ± 22.0	368.8 ± 28.1
Non-responders	395.8 ± 23.7	357.5 ± 26.9	841.1 ± 122.2	859.7 ± 128.0	433.7 ± 24.0	388.0 ± 33.4
<i>t</i>	1.789	3.034	1.734	1.096	−0.199	1.966
<i>P</i> -Value	0.080	0.004	0.089	0.279	0.843	0.055

POTS, postural tachycardia syndrome; ms, millisecond; QTmax, the maximum value of QT interval in 12 leads of ECG; QTmin, the minimum value of QT interval in 12 leads of ECG; RRmax, the maximum value of RR interval in 12 leads of ECG; RRmin, the minimum value of RR interval in 12 leads of ECG. Data are mean ± SD.

**FIGURE 2** | QT interval dispersion (QTd) (A) and corrected QT interval dispersion (QTcd) (B) in responders and non-responders with POTS treated with metoprolol. POTS, postural tachycardia syndrome; QTd, QT interval dispersion; QTcd, corrected QT interval dispersion; ms, millisecond, **p* < 0.05; ***p* < 0.01.**FIGURE 3** | Correlation analysis between baseline QTd (A) or QTcd (B) and SS after treatment in the discovery group. QTd, QT interval dispersion; QTcd, corrected QT interval dispersion; SS, symptom score.

of 78.9 and 83.3%, respectively, to predict the effectiveness of metoprolol in POTS children. Furthermore, the validation analysis showed that the sensitivity, specificity, and accuracy to predict the effectiveness of metoprolol in children suffering from POTS with a baseline QTcd ≥ 47.9 ms were 73.7%, 80.0%, and 75.0%, respectively. Therefore, baseline QTcd ≥ 47.9 ms could be used as a simple, easy-to-obtain, and inexpensive indicator for predicting the efficacy of metoprolol on pediatric POTS.

Postural tachycardia syndrome is common in children, with an incidence rate of about 6.8%, which seriously damages the physical and psychological health of children, and increases the burden on the family and society (26, 27). Therefore, it was

of great significance to give effective intervention on such a disease (28).

The β -adrenergic receptor blockers are important treatment drugs for children with POTS. They play a critical role in blocking the β -adrenergic receptors of the myocardium, but the overall therapeutic effect is not satisfactory (29). The reason includes the complexity and diversity of the pathogenesis of POTS in children, such as overexcitement of sympathetic nerve function, excessive vasodilation, low central blood volume, or endothelial dysfunction (3–6). Metoprolol, as a β -adrenergic receptor blocker, is only effective for POTS children with overexcitement of sympathetic nerve function or high catecholamine status by acting on β_1 receptor (30). The patients of POTS with

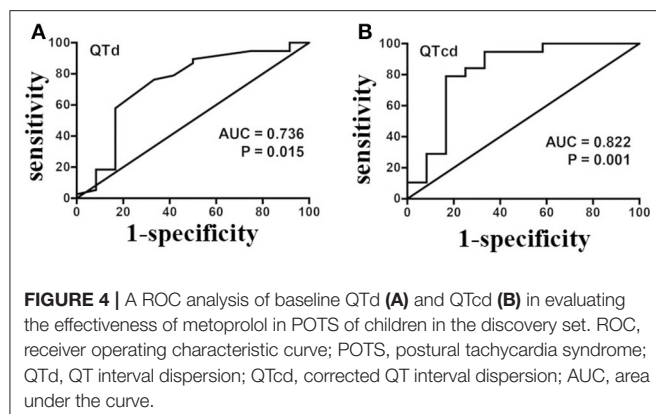


TABLE 3 | Baseline QTcd predicts the efficacy of metoprolol in children with POTS in the validation group ($n = 24$).

Differential ways to predict the efficacy of metoprolol in children with POTS		Clinical standard-based follow-up efficacy outcome	
		Responders	Non-responders
Baseline QTcd-based predictive efficacy outcome (QTcd \geq 47.9 ms)	Responders	14 (73.7%)	1 (20.0%)
	Non-responders	5 (26.3%)	4 (80.0%)

QTcd, corrected QT interval dispersion; POTS, postural tachycardia syndrome; ms, millisecond.

other mechanisms as mentioned above might be the non-responders to the treatment of metoprolol. To find out the useful predictors of the therapeutical response to β -adrenergic receptor blocker, we previously showed several markers to predict the usefulness of β -adrenergic receptor blocker in pediatric POTS (11–14). While, these markers had some limitations, such as the instability, the invasiveness to obtain the samples, the time-consuming or non-economic characteristics, which limits their extensively clinical application. Therefore, it is urgent to explore the non-invasive, easy-to-operate, and inexpensive indexes for the prediction of metoprolol effectiveness for POTS children.

A QT interval is regulated by the autonomic nervous system and the speed of HR. The autonomic nerve exerts an indirect effect on QT interval. When the sympathetic nerve is excited, HR increases and QT interval shortens. While, when the vagus nerve is activated, HR slows down and the QT interval prolongs (31). Cappato et al. reported that vagal tone increased intrinsic dependence of QT at increasing cycle length, whereas sympathetic tone did not seem to interfere significantly (32). Venugopala et al. reported that QTc in healthy children changed little within 24 h, and it was kept constant over a whole day. QTc prolongation indicated impaired cardiac autonomic nerve function and delayed ventricular

repolarization (33). QTd in children is increased by the activation of sympathetic nerve or the weakness of vagus nerve. The QTd in children with vasovagal syncope and POTS is significantly higher than that of the healthy children, which indicates that these patients had autonomic nerve dysfunction (31, 34, 35).

Autonomic nerve dysfunction plays an important role in the pathogenesis of pediatric POTS (36–38). It can influence the regulation of autonomic nerves in pediatric POTS. The change of ECG waveform reflects the interaction between the sympathetic nerve and the vagus nerve. QTd reflects the excitability of sympathetic nerves and the release of catecholamines. QTd in children is increased with the activation of sympathetic nerve and the release of excessive catecholamines. The excitement of sympathetic nerve can promote the norepinephrine release that acts on the adrenergic β receptor of the myocardial cell membrane to activate adenylate cyclase (AC)-cyclic adenosine monophosphate (cAMP)-protein kinase A (PKA) (AC-cAMP-PKA) signaling pathway. This phosphorylates the action potential-related ion channels, such as L-type Ca^{2+} channel and K^{+} channels, and then results in the increase of Ca^{2+} influx and acceleration of K^{+} outflow, so that the depolarization and repolarization processes are accelerated and the action potential duration phase is shortened. QTd is referred to the time difference between the earliest repolarization and the latest repolarization of the heart. The distribution of sympathetic nerve in the heart from the bottom to the apex is uneven. As such, when the sympathetic nerve is excited, the value of QT dispersion increases. Therefore, the excessive activation of sympathetic nerve accelerates the Ca^{2+} influx and K^{+} outflow, shortens the time of QT interval, and increases the QTd (36, 37, 39). Therefore, QTd reflects the excitability of sympathetic nerves and the release of catecholamines.

Metoprolol exerts its therapeutic effect on the patients with POTS with the excessive sympathetic nerve function as the main pathogenesis, other than those with different pathogenesis. While QTd obtained by 12-lead ECG is reflective of excessive sympathetic nerve function. Therefore, in the present study, we aimed to examine if the baseline QTd and QTcd could predict the therapeutic effectiveness of metoprolol in children with POTS whose main pathogenesis was the excessive sympathetic nerve function.

QT interval is considered as the sum time of depolarization and repolarization of central ventricular muscle in each cardiac cycle (38). QTd is the difference between QT intervals of each lead on ECG, which refers to the time difference between the earliest repolarization and the latest repolarization of the heart. QTcd is the corrected QTd, a useful index to reflect the cardiac autonomic nerve function, reflecting the asynchrony and electrical instability of ventricular repolarization (40–43). Previous studies on the relationship between QTd and HRV showed that the increased sympathetic or weakened vagal tone could lead to an increased QTd in healthy people (44). In addition, QTd could be increased by enhancing the sympathetic activity and/or reducing vagal activity in the healthy individuals in the process of BHUTT (16).

The QTd of children with OI was significantly longer than that of healthy controls (45–47). QTd and QTcd can be obtained by 12-lead ECG, and have the advantages of non-invasiveness, easy-to-operate, and inexpensiveness (48–51).

This study showed that the baseline QTd and QTcd in the responders to metoprolol were significantly longer than the non-responders, indicating that the sympathetic nerve activity and catecholamines release in the responders might be significantly higher than the non-responders. We showed that baseline QTd and QTcd before treatment were negatively correlated with post-treatment SS, indicating that when baseline QTd or QTcd before treatment increased, symptoms would be significantly relieved after metoprolol treatment. On the contrary, if the baseline QTd or QTcd did not increase in POTS of children before treatment, suggesting that the POTS cases did not have marked increase in the sympathetic nerve activity and catecholamines release, the therapeutic effects of metoprolol would not be satisfied. Further analysis showed that the baseline QTcd before treatment successfully predicted the effectiveness of metoprolol in children suffering from POTS. The predictive cut-off value of 47.9 ms yielded the sensitivity and specificity of 78.9 and 83.3%, respectively. Validation studies confirmed that the sensitivity, specificity, and accuracy of a baseline QTcd \geq 47.9 ms to predict the effectiveness of metoprolol on POTS in children were 73.7, 80.0, and 75.0%, respectively. In conclusion, the baseline QTcd \geq 47.9 ms before treatment can be regarded as a preliminary clinical indicator of predicting the efficacy of metoprolol in pediatric POTS.

However, this study has certain limitations. It is single-center-based research, and the sample size is not large enough. The specificity and sensitivity of the predictive value of QTd and QTcd are not high enough. Therefore, it would be meaningful to

conduct multi-centered and large sample-sized long-term follow-up studies and promote the clinical application of the study results in the future.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the Peking University First Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

JD, HJ, CT, YuaW, and YS contributed to conception and design of the study. YuaW, YS, QZ, CZ, and PL contributed to the conduction of the research. YuaW, YS, QZ, CZ, YulW, and PL contributed to the data acquisition and revision of the article for important intellectual content. YuaW contributed to analysis and interpretation of the data. YuaW, YS, HJ, and JD contributed to drafting the article. YuaW, YS, QZ, CZ, PL, YulW, CT, HJ, and JD contributed to final approval of the version to be submitted. All authors contributed to the article and approved the submitted version.

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Leukocyte Telomere Length Predicts Progression From Paroxysmal to Persistent Atrial Fibrillation in the Long Term After Catheter Ablation

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Background: Aging is significantly associated with the incidence and progression of atrial fibrillation (AF) incidence. This study aimed to evaluate the potential predictive value of leukocyte telomere length (LTL) for progression from paroxysmal AF (PAF) to persistent AF (PsAF) after catheter ablation.

Methods and Results: A total of 269 patients with AF (154 patients with PAF and 115 patients with PsAF, respectively) were prospectively enrolled, and all patients with PAF at baseline were regularly followed up to determine whether and when they should progress to PsAF after catheter ablation therapy. Baseline relative LTL was measured by quantitative real-time PCR (rt-PCT). There was a significant negative association between LTL and age ($r = -0.23, p < 0.001$). Patients with PsAF had significantly shorter LTL than those with PAF. After a mean follow-up of 854.9 ± 18.7 d, progression events occurred in 35 out of the 154 patients with PAF. Those progressed patients with PAF were older (70.9 ± 8.0 vs. $62.3 \pm 10.3, p < 0.001$) and had shorter LTL (1.2 ± 0.3 vs. $1.5 \pm 0.3, p < 0.001$) than those who did not. The receiver operating characteristic (ROC) curve analysis showed a significant value of LTL in distinguishing patients with PAF from patients with PsAF, with an area under the ROC curve (AUC) of 0.63 (95% CI 0.56–0.70, $p < 0.001$), and the optimal cut-off value of LTL was 1.175, with a sensitivity and specificity of 56.03 and 82.04%, respectively. All patients with PAF were divided into two subgroups according to the optimal cut-off point of LTL calculated by the ROC curve analysis: high LTL group (≥ 1.175) and low LTL group (< 1.175). Kaplan-Meier curve analysis showed that PAF patients with shorter LTL had a significantly higher rate of progression after catheter ablation (40.5% vs. 18.8%, log-rank test $p < 0.001$). Multivariate Cox proportional-hazards model indicated that LTL [hazard ratio (HR): 2.71, 95% CI 1.36–5.42, $p = 0.005$] was an independent predictor for progression from PAF to PsAF after catheter ablation therapy, but HATCH score was not (HR: 1.02, 95% CI: 0.68–1.52, $p = 0.923$).

Conclusion: Leukocyte telomere length was significantly associated with AF types. LTL was independently associated with progression from PAF to PsAF after catheter ablation therapy.

Chinese Clinical Trial Registry, Registration Number: ChiCTR1900021341.

Keywords: telomere length, atrial fibrillation, progression, biomarker, catheter ablation

INTRODUCTION

Atrial fibrillation (AF) is the most common sustained tachycardia in clinical practice (1). AF is significantly correlated with increased adverse cardiovascular events, including heart failure (HF), stroke, myocardial infarction (MI), all-cause death, and decreased quality of life (2, 3). Advanced age is currently recognized as the most important risk factor for the incidence of AF. The AF prevalence was merely <1% in subjects younger than 55-year old and significantly increased to ~10% in subjects over 80-year old (4, 5). With the development of aging worldwide, it is estimated that the total subjects with AF would be double in the year 2050 than the present (6, 7).

The natural disease history of AF often starts with a short and self-terminating paroxysmal AF (PAF) status and gradually transforms into a longer persistent AF (PsAF) type (8, 9). The factors contributing to AF occurrence have been well established, while the factors predicting AF progression from PAF to PsAF were not. However, it is thought to be more important because of the significantly worse prognosis for patients with PsAF than those patients with PAF (10, 11). Numerous risk factors, including modifiable and non-modifiable factors, have been identified to be significantly associated with AF progression, among which aging has been considered as the dominant risk factor associated with AF progression (12, 13).

Telomeres, which are located at both ends of chromosomes, are specific repeated DNA sequences [TTAGGG] $_n$ that function to prevent DNA degradation during cell replication (14). Telomere length (TL) is shortened with cell division because of the non-complete DNA replication. In addition to the influence of cell division, the shortening rate of TL, which is also termed telomere attrition, could be accelerated by several genetic and environmental factors, such as inflammation and oxidative stress (15, 16). When the TL reaches a certain point, the DNA damage response would be activated, which would lead to the upregulation of p-53 and thus cell apoptosis (17).

Catheter ablation is reported better than guideline-directed antiarrhythmic drug (AAD) therapy in delaying progression from PAF to PsAF (18, 19). Catheter ablation has been proposed as the first-line therapy for patients with PAF in restoring sinus rhythm (20). Despite the critical role of aging in perpetuating AF, the relation between TL and AF is still controversial. Our former study which enrolled 277 patients with AF indicated that shorter leukocyte telomere length (LTL) was significantly associated with AF recurrence after catheter ablation (21). In the present study, we aimed to investigate the association of LTL with AF types and determine the predictive value of LTL for the progression from PAF to PsAF after catheter ablation by a cross-sectional analysis method.

METHODS

Study Design

This is an observational prospective cross-sectional study. We obtained informed consent from all subjects recruited in this study and approval from the local research ethics committee of the Beijing Chaoyang Hospital, Capital Medical University.

The protocols applied in this study were in accordance with the ethical guidelines of the 1957 Declaration of Helsinki, as reflected in *a priori* approval by the Institution's Human Research Committee. One technician, who was blinded to both the baseline information and clinical endpoint, performed all analyses of the LTL measurement.

Study Subjects

A total of 269 subjects with symptomatic nonvalvular AF (154 PAF and 115 PsAF, respectively) were prospectively selected for this study from June 2016 to December 2017. Subjects with AF were diagnosed by the documented AF episodes detected by 12-lead ECG or 24-h Holter monitor. Those who met any of the following conditions were excluded from this study: acute or chronic inflammation conditions, MI or stroke history (6 months), thyroid dysfunction, congenital heart disease, heart surgery history (6 months), and valvular disease (moderate-to-severe valvular stenosis). In addition, patients with PAF who underwent catheter ablation before were also excluded. In the present study, all patients with PAF received catheter ablation to restore sinus rhythm, and rhythm control [converted by catheter ablation or anti-arrhythmia drugs (AADs)] or rate control therapy was according to the clinical conditions for patients with PsAF. The details of catheter ablation procedures of the present study were described in the former study.

All peripheral blood samples were drawn into ethylenediaminetetraacetic acid (EDTA) tubes from consenting subjects at administration after overnight fasting. All whole blood samples were processed with centrifugation within 4 h and stored as blood cells at -80°C until later LTL analysis.

DNA Extraction

We used QIAamp DNA Blood Midi Kits (Qiagen, Hilden, Germany) to manually extract the DNA from blood cells centrifugated from the peripheral whole blood samples. Erythrocyte was lysed and removed by a series of washing steps. Moreover, the remaining leukocytes were lysed, and other solubilized protein was removed by precipitation and centrifugation. DNA quality was evaluated by ultraviolet absorption at 260 nm/280 nm (Nanodrop 2000).

LTL Measurement

The relative LTL was measured by quantitative PCR, which was modified from the method reported by Cawthon et al. (22) before. The telomere repeats (primers: forward-acactaaggttgggttgggttgggttgggttgggttagtgt; reverse-tgttaggtatccctatccctatccctatccctatccctaaca) of a single gene (*HBG*, hemoglobin; primers: forward-cttcaccagcttcacctgt; reverse-gaggagaagctgcccgtt) in each sample were measured using quantitative real-time PCR (rt-PCT). The relative LTL was calculated as the ratio of telomere DNA repeats to single-copy gene (SCG) copies (t/s ratio), with *HBG* being designated as the SCG. All rt-PCR experiments were conducted in triplicate for each sample.

Follow-Up Approaches

All patients with PAF were regularly followed up for 36 months in the present study. During follow-ups, patients would receive 12-lead ECG or 24-h Holter at baseline and 1, 3, 6, 12 months after discharge and every 12 months thereafter in scheduled clinical visits. If patients exhibit AF episodes, they would be monitored by a 7-d Holter. There would also be symptoms that triggered those visits that triggered by symptoms would also be recorded accordingly. Patients or their care providers were taught to appreciate their pulse to identify AT (atrial tachycardia)/AF at home as a supplementary for any suspicious AF episodes, while any self-reported AF episodes would be further determined by ECG. Besides, some of the patients were equipped with a smartwatch capable of recording pulse rate and morphology. When recorded AT/AF, by the above measures, lasted for more than 7 d and would not be converted by chosen cardioversion measures, the patients would be defined as AF progression. Progression of AF in the present study was defined as follows: patients first diagnosed as paroxysmal, with documented ECG as the baseline, become persistent during 3-year follow-up, regardless of the managing strategies. PAF was defined as self-terminating AF episodes or terminated by any cardioversions means within 7 d, including AADs and direct current cardioversion (DCC). AF episodes lasting beyond 7 d were defined as PsAF.

We also calculated the HATCH score for each patient with PAF in this study according to described before (23): 1 point for either history of hypertension, chronic obstructive pulmonary disease (COPD), or age ≥ 75 years; 2 points for combining either history of transient ischemic attack or stroke or HF.

Statistical Analysis

Continuous variables were expressed as mean \pm SD or median (25, 75th interquartile range), according to the variable distribution. Shapiro-Wilk test was used to evaluate the distribution of the continuous variables. For continuous variables, statistical differences between groups were tested by Student's *t*-test or the Mann-Whitney *U* test appropriately. Categorical variables were shown as frequencies (percentage) and tested by the chi-square test. Association between continuous variables was evaluated by Spearman correlation analysis. The receiver operating characteristic (ROC) curve was applied to evaluate the value of LTL in distinguishing subtypes of AF and the area the under curve (AUC), optimal cut-off value, sensitivity, and specificity were calculated, respectively. According to the cut-off value of LTL, all subjects with PAF were divided into two subgroups: LTL shortened group (LTL ≤ 1.175) and non-shortened LTL group (LTL > 1.175). The rate of free from AF progression was estimated by the Kaplan-Meier curve method, and any difference in free from AF progression was tested by the log-rank test. Multivariable Cox proportional hazards model analysis was used to evaluating the prognostic value of LTL on progression from PAF to PsAF. In this study, all statistical analyses were performed by SPSS software, version 24.0 (SPSS, Chicago, IL, USA), and a two-tailed *p*-value of <0.05 was considered to be statistically significant. All graphs in this study were drawn by GraphPad prism software, version 8.0.

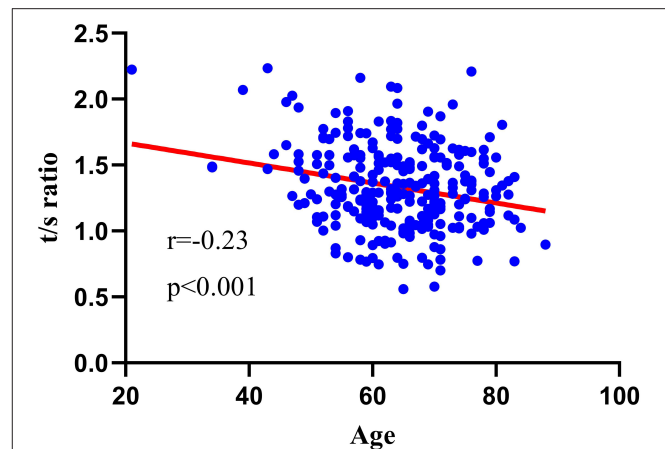


FIGURE 1 | Relationship between LTL and age. TLT was negatively associated with advanced age ($r = -0.23$, $p < 0.001$).

RESULTS

LTL Was Significantly Associated With AF Type

A total of 269 patients with AF were prospectively enrolled in this study (154 patients with PAF and 115 patients with PsAF, respectively). Baseline characteristic comparisons between different AF types are summarized in **Table 1**. Patients with PAF had significantly longer LTL than patients with PsAF (1.4 ± 0.3 vs. 1.2 ± 0.3 , $p = 0.003$), while the age (64.3 ± 10.4 vs. 64.3 ± 9.1 , $p = 0.998$) was comparable between the two groups. In addition, patients with PsAF had significantly increased levels of high-sensitivity C-reactive protein (hs-CRP) (2.0 vs. 1.1 , $p = 0.001$), N-terminal pro-natriuretic peptide (NT-proBNP) (953.7 vs. 221.2 , $p < 0.001$), left atrial diameter (LAD) (45.2 ± 5.9 vs. 39.1 ± 4.8 , $p < 0.001$), and decreased level of left ventricular ejection fraction (LVEF) (60.6 ± 11.3 vs. 65.4 ± 7.2 , $p < 0.001$). A higher proportion of HF (56.5% vs. 24.0%, $p < 0.001$) and stroke history (24.3% vs. 12.3%, $p = 0.010$) were also observed in a group with PsAF.

Spearman correlation analysis was used to evaluate the association between age and LTL, and the result is as shown in **Figure 1**. Advanced age was negatively associated with LTL ($r = -0.23$, $p < 0.001$).

Shorter LTL Was Significantly Associated With PAF Progression

Baseline characteristics comparison between patients with PAF with and without progression are as shown in **Table 2**. During a median follow-up of 854.9 ± 18.7 days, AF progression occurred in 35 (22.7%) out of 154 patients with PAF who were successfully followed up. Patients with AF progression were older (70.9 ± 8.0 vs. 62.3 ± 10.3 , $p < 0.001$), had significantly increased levels of NT-proBNP (484.5 vs. 183.6 , $p = 0.004$), HATCH score (2 vs. 1, $p = 0.002$), and LAD (40.7 ± 4.8 vs. 38.7 ± 4.7 , $p = 0.028$), and decreased level of e-GFR (80.5 ± 16.8 vs. 91.5 ± 15.1 , $p < 0.001$) and LTL (1.2 ± 0.3 vs. 1.5 ± 0.3 , $p < 0.001$), and higher proportion

TABLE 1 | Baseline characteristics by AF type.

Variables	PAF (n = 154)	PsAF (n = 115)	p-value
Age (year)	64.3. ar 0.4	64.3 ± 9.1	0.998
Male	90(58.4%)	67(58.3%)	0.976
BMI (kg/m ²)	25.8 ± 3.8	26.9 ± 4.3	0.023
Smoking	60(39.2%)	44(38.3%)	0.874
CAD	35(22.7%)	32(27.8)	0.339
HF	37(24.0%)	65(56.5%)	<0.001
DM	41(26.6%)	26(22.6%)	0.451
hypertension	94(61.0%)	74(64.3%)	0.579
stroke	19(12.3%)	28(24.3%)	0.010
COPD	5(3.2%)	3(2.6%)	0.761
ACEI/ARB	59(38.3%)	50(43.5%)	0.393
β-blocker	63(40.9%)	58(50.4%)	0.120
Statins	81(52.6%)	68(59.1%)	0.286
hs-CRP (mg/L)	1.1(0.6,2.5)	2.0(1.1,4.7)	<0.001
NT-proBNP (pg/ml)	221.2(82.3,814.4)	953.7(499.0, 1,964.0)	<0.001
eGFR (ml/min. 1.73 m ²)	89.0 ± 16.1	85.0 ± 17.7	0.054
t/s ratio	1.4 ± 0.3	1.2 ± 0.3	0.002
LAD (mm)	39.1 ± 4.8	45.2 ± 5.9	<0.001
LVEF (%)	65.4 ± 7.2	60.6 ± 11.3	<0.001

BMI, body mass index; CAD, coronary artery disease; HF, heart failure; DM, diabetes mellitus; COPD, chronic obstructive pulmonary disease; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blocker; hs-CRP, high-sensitivity C-reactive protein; NT-proBNP, N-terminal proatriuretic peptide; e-GFR, estimated glomerular filtration rate; t/s ratio, the ratio of telomere repeats to a single-copy gene (SCG) copies; LAD, left atrial diameter; LVEF, left ventricular ejection fraction.

TABLE 2 | Baseline characteristics by AF progression.

variables	Progression (n = 35)	No progressions (n = 119)	p-value
Age (year)	70.9. ru .0	62.3. ru 0.3	<0.001
Male	26(59.1%)	88(58.3%)	0.923
BMI (kg/m ²)	25.7 ± 3.9	25.7 ± 3.7	0.995
Smoking	13(38.2%)	47(39.5%)	0.894
CAD	7(20.0%)	28(23.5)	0.661
HF	13(37.1%)	24(20.2%)	0.039
DM	12(34.3%)	29(24.4%)	0.243
Hypertension	24(68.6%)	70(58.8%)	0.299
Stroke	5(14.3%)	14(11.8%)	0.690
COPD	3(8.6%)	2(1.7%)	0.043
ACEI/ARB	14(40.0%)	45(37.8%)	0.815
β-blocker	17(48.6%)	46(38.7%)	0.294
Statins	15(42.9%)	66(55.5%)	0.189
hs-CRP (mg/L)	1.3(0.7,3.5)	1.1(0.6,2.0)	0.197
NT-proBNP (pg/ml)	484.5(116.9,1624.0)	183.6(69.4,680.2)	0.004
eGFR(ml/min. 1.73 m ²)	80.5 ± 16.8	91.5 ± 15.1	<0.001
t/s ratio	1.2 ± 0.3	1.4 ± 0.3	<0.001
HATCH score	2(1,3)	1(0,2)	0.002
LAD (mm)	40.7 ± 4.8	38.7 ± 4.7	0.028
LVEF (%)	66.5 ± 8.0	65.1 ± 7.0	0.302

BMI, body mass index; CAD, coronary artery disease; HF, heart failure; DM, diabetes mellitus; COPD, chronic obstructive pulmonary disease; ACEI, Angiotensin-Converting Enzyme Inhibitors; ARB, angiotensin receptor blocker; hs-CRP, high-sensitivity C-reactive protein; NT-proBNP, N-terminal proatriuretic peptide; e-GFR, estimated glomerular filtration rate; t/s ratio, the ratio of telomere repeats to a single-copy gene (SCG) copies; LAD, left atrial diameter; LVEF, left ventricular ejection fraction.

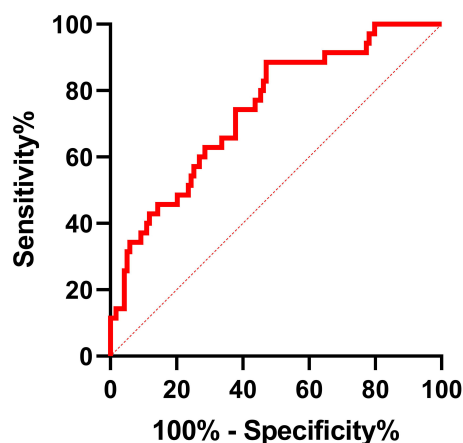


FIGURE 2 | ROC curve analysis for LTL in distinguishing PsAF patients from patients with PAF. AUC of shorter LTL was 0.63 (95% CI: 0.56–0.70, $p < 0.001$), with sensitivity and specificity of 56.03% and 82.04%, respectively. The optimum cut-off value for LTL was 1.175.

of HF (38.2% vs. 20.2% $p = 0.039$) and COPD history (8.6% vs. 1.7% $p = 0.043$) than those who did not progress.

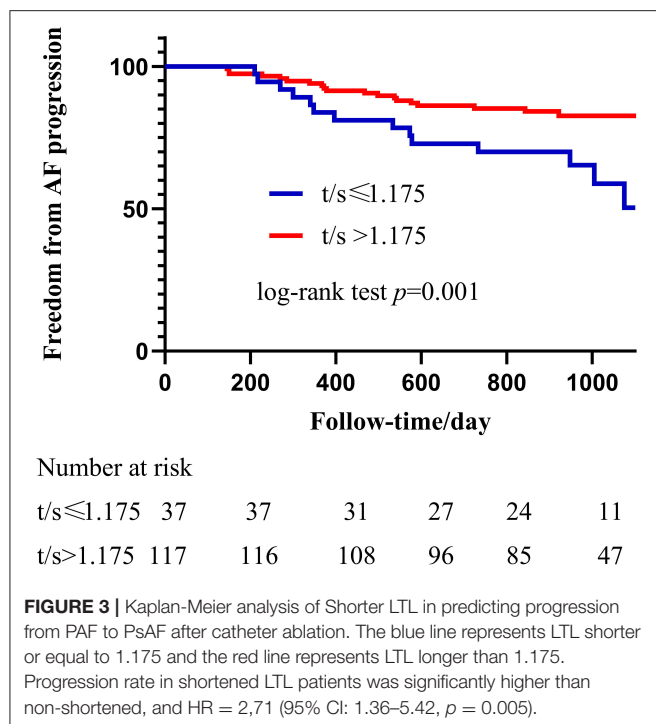
Shorter LTL Was a Potential Risk Factor for Progression From PAF to PsAF

A receiver operating characteristic curve was used in this study to evaluate the value of LTL in distinguishing patients with

PsAF from patients with PAF. As shown in **Figure 2**, LTL shows a significant diagnostic value for distinguishing patients with PsAF from patients with PAF, with AUC = 0.63 (95% CI: 0.56–0.70), and the optimal cut-off point was calculated as 1.175 with sensitivity and specificity of 56.03 and 82.04%, respectively. All patients with PAF were divided into two subgroups according to the optimal cut-off point calculated by the ROC curve: shorten LTL group (LTL ≤ 1.175) and non-shorten LTL group (LTL > 1.175). Kaplan-Meier curve analysis was used to estimate the difference of the cumulative proportional probabilities of the occurrence of progression between the two groups, as shown in **Figure 3**. Patients with shorter LTL had a significantly increased cumulative probability of AF progression than those with longer LTL (55.3% vs. 14.6%, log-rank test $p < 0.001$).

Shorter LTL Was an Independent Predictor for Progression From PAF to PsAF

A Cox proportion hazard model by the stepwise forward method was used to determine the risk factors for predicting progression from PAF to PsAF and results are shown in **Table 3**. Multivariate Cox regression analysis showed that shorter LTL (HR 2.71, 95% CI 1.36–5.42, $p = 0.005$), advanced age (HR 1.06, 95% CI 1.01–1.13, $p = 0.043$), COPD (HR 4.29, 95% CI 1.14–16.15, $p = 0.031$), and LAD (HR 1.09, 95% CI 1.00–1.19, $p = 0.047$) were

**TABLE 3 |** Cox regression analysis for progression of AF.

variables	Univariate		Multivariate	
	HR (95% CI)	p-value	HR (95%CI)	p-value
t/s ratio	2.87 (1.48–5.59)	0.002	2.71(1.36–5.42)	0.005
age	1.09(1.05–1.13)	<0.001	1.06(1.01–1.13)	0.043
HF	2.13(1.07–4.24)	0.032	1.16(0.37–3.67)	0.800
COPD	4.89(1.48–16.11)	0.009	4.29(1.14–16.15)	0.031
stroke	1.17(0.45,3.03)	0.744		
NT-proBNP	1.00(1.00–1.00)	<0.001		
LAD	1.10(1.02–1.18)	0.017	1.09(1.00–1.19)	0.047
e-GFR	0.97(0.95–0.98)	<0.001	0.99(0.96–1.02)	0.413
HATCH score	1.36(1.11, 1.66)	0.003	1.02(0.68–1.52)	0.923

HR, hazard ratio; CI, confidence interval; t/s ratio, the ratio of telomere repeats to a single-copy gene (SCG) copies; HF, heart failure; COPD, chronic obstructive pulmonary disease; NT-proBNP, N-terminal proatriuretic peptide; LAD, left atrial diameter; e-GFR, estimated glomerular filtration rate.

independent predictors for progression from PAF to PsAF, even after adjustment of other confounding risk factors.

DISCUSSION

In this study, we investigated the predictive value of LTL for progression from PAF to PsAF after catheter ablation therapy. The main findings in the present study were as follows: (1) LTLs were significantly associated with AF types; (2) LTLs were significantly shorter in patients with AF progression than those without; (3) LTL was an independent predictor for progression from PAF to PsAF, even after adjustments with other confounding risk factors.

In the present study, we found a significantly shorter LTL in the PsAF group than in the PAF group. Carlquist et al. (24) found that in a cross-sectional cohort, shorter LTL was significantly associated with AF, even after adjusting for age and other risk factors. However, in the further analysis of AF subtype, just PAF, not PsAF, was significantly associated with LTL. In addition, LTL was shorter in patients with PAF than sinus rhythm (SR) controls, patients with PsAF, and even permanent AF individuals. The possible explanation for the results in the Carlquist et al. was the study population selection. The subjects in that study came from a population who underwent angiography and the majority of the subjects had coronary heart disease. In fact, in Carlquist et al.'s study, the rate of CAD in subjects with PAF was significantly higher than that in patients with PsAF or permanent AF (82.7% vs. 78.5% vs. 65.1, $p = 0.01$). Previous studies have found a significant association between shorter TL and CAD, which indicates that CAD may influence the TL. Thus, the selection bias in Carlquist et al.'s study may affect the findings. Besides, by following up on the patients with PAF, we have also found that shorter LTL was independently associated with PAF progression from PAF to PsAF. This could in part underlie the association of worse AF phenotype (in this study, the propensity to PsAF, not to say the worse PsAF and Permanent AF) with shorter LTL. However, further studies are still in need to render more pieces of evidence for either of the conclusions.

The rate of AF progression in our present study was 8.4% (13/154) in the first year of follow-up and the overall rate was 22.7% (35/154), which was similar to a previous study constructed by Gareth et al. in the Canadian Registry of AF (13). The rate of AF progression in the present study was higher than the study constructed by Cees et al. (23), who also developed the HATCH score, which was used to predict progression from PAF to PsAF in the clinical context. The discrepancies of progression rates between their study and ours may be explained by the length of follow-up: the follow-up periods of the present study last for 3 years, which is longer than 1 year in the study of Cees et al. Besides, the progression rate in the present study in 1 year was lower than their study, perhaps due to the different strategy of restoring SR: patients with PAF were restored by catheter ablation in this study, while all patients were restored by AADs in that study.

In this study, we also investigated the value of the HATCH score for predicting progression from PAF to PsAF. We found that those patients with PAF who progressed to PsAF status indeed had higher HATCH scores than those who did not, however, the HATCH score was not an independent predictor for AF progression in this study (HR 0.89, 95% CI: 0.58–1.37, $p = 0.597$). Jongnarangsin et al. (25) have evaluated the predictive value of HATCH score in AF progression after catheter ablation and revealed that HATCH score was not an independent predictor for AF progression after ablation therapy. Tang et al. (26) also reported that the HATCH score was not independently associated with AF recurrence after catheter ablation.

Inflammation is one of the most important risk factors for accelerating telomere attrition and promoting the aging process. In total, 15–20 cell divisions with naive T cells may be involved in an immune response and lead to telomere loss. Jurk et al.

(27) demonstrated that an *nfkbl*^{-/-} mice model with chronic inflammation conditions significantly upregulated the reactive oxygen species (ROS) and accelerated telomere shortening. Besides, Amsellem et al. (28) observed a comparable pro-inflammatory state in late-generation *Terc*^{-/-} and *Tert*^{-/-} mice models that were used for telomere dysfunction-driven senescence analysis, which was often seen in late chronic inflammation conditions.

Recent studies have found that shortening TL was significantly associated with various tissue fibrosis, including pulmonary fibrosis (29), liver fibrosis (30), and renal fibrosis (31). Despite the fact that there was no current evidence indicating the association of shorter TL with cardiac fibrosis, a recent study that investigated the mechanisms between TL and pulmonary fibrosis may provide some insights for us. Ying-Ying Liu et al. (32) found that *Terc*^{-/-} mice with shorter telomeres developed significantly aggravated pulmonary fibrosis than wide-type mice with normal telomeres. In addition, TGF- β /Smad signaling, which plays a key role in promoting tissue fibrosis, was also markedly activated in the lungs of G3 *Terc*^{-/-} mice, indicating that shortening telomeres might enhance fibrosis via activation of TGF- β /Smads signaling, which is also the key pathway in cardiac fibrosis and AF persistency (33).

Catheter ablation therapy has been proved more effective in maintaining sinus rhythm and delaying AF progression than AADs (18, 19). A recent meta-analysis indicated that compared to the medication rate-control therapy, catheter ablation therapy significantly improves cardiac function evaluated by LVEF, exercise capacity, and quality of life (34). Thus, catheter ablation was proposed as the first-line strategy to restore sinus rhythm for patients with AF, especially for those patients with PAF in recent years. Kuo-Li Pan et al. found that shorter LTL was significantly associated with cardiac remodeling and AF recurrence after catheter ablation in younger patients (≤ 55 years) (35). A recent study found that shorter LTL was an independent predictor for progression from PAF into PsAF (36). In this study, we confirmed that shorter LTL was independently associated with progression from PAF to PsAF. The main discrepancies between their study and this study were the intervention means of restoring sinus rhythm. Only 35.3% (42/119) of patients with PAF had accepted catheter ablation in that study, while all patients with PAF had received catheter ablation therapy. In addition, the rate of progression in that study was relatively low than our study (16.8% vs. 22.7%), which may be due to the shorter length of follow-up (18 months vs. 36 months).

LIMITATIONS

Several limitations should be mentioned in this study. First, although we did find a significant association between LTL and AF progression, we could not obtain a direct causal-effect

relationship. Second, in our study, the relative LTL length was measured based on an rt-PCR analysis instead of Southern blotting, which has been the gold standard in LTL measuring. However, the relative length of LTL has long been a recognized mean in determining LTL/disease association studies. It may be necessary to utilize the absolute length of LTL as measured by Southern blotting in clinical settings, but this did not undermine the conclusions of the present study. Third, we did not obtain direct evidence of cardiac fibrosis, further studies are still needed. Finally, self-reported AF episodes may be inaccurate, which may affect the progression rate. Thus, all self-reported AF episodes were requested to confirm by ECG examination in this study.

CONCLUSION

Leukocyte telomere length was significantly associated with AF type, and patients with PsAF had significantly shorter LTL than patients with PAF. LTL was an independent predictor for progression from PAF to PsAF after catheter ablation.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Beijing Chaoyang Hospital, Capital Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MC and YG: designed the study. QW: collected the data. YD and QW: validated and inspected the data and performed the statistical analysis. QW, ZL, and YG: wrote the manuscript. MC, XY, and YG: revised the manuscript. All authors contributed to the article and approved the submitted version.

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Modified Taiwan Atrial Fibrillation Score for the Prediction of Incident Atrial Fibrillation

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Background: We have proposed the Taiwan AF score consisting of age, male sex, hypertension, heart failure, coronary artery disease, end-stage renal disease, and alcoholism to predict incident atrial fibrillation (AF) in Asian population. We hypothesized that the modified Taiwan AF score (mTaiwan AF score) excluding alcoholism remained useful for predicting new onset AF.

Methods: A total of 7,220,654 subjects aged ≥ 40 years without a past history of cardiac arrhythmia were identified from a national cohort, and 438,930 incident AF occurred during a 16-year follow-up with an incidence of 0.42 per 100 person-years. The mTaiwan AF score ranging between -2 and 14 and its predictive accuracy of incident AF was analyzed.

Results: The areas under the receiver operating characteristic curve (AUCs) of the mTaiwan AF scores in predicting AF are 0.861 for 1-year follow-up, 0.829 for 5-year follow-up, 0.795 for 10-year follow-up, and 0.751 for 16-year follow-up. The risk of incident AF increased from 0.05%/year for patients with a score of -2 to 6.98%/year for those having a score of 14 . Patients were classified into three groups based on the tertile values of the mTaiwan AF scores—group 1 (score -2 -3), group 2 (score 4-9) and group 3 (score 10-14). The annual risks of incident AF were 0.20, 1.33, and 3.36% for group 1, 2, and 3, respectively. Compared to patients in group 1, the hazard ratios of incident AF were 5.79 [95% confidence interval (CI) 3.75-7.75] for group 2 and 8.93 (95% CI 6.47-10.80) for group 3.

Conclusions: We demonstrated that the mTaiwan AF score based on age and clinical comorbidities could be used to predict incident AF in Asian population.

Keywords: incident atrial fibrillation, modified Taiwan AF score, prediction, Asian population, national cohort

BACKGROUND

AF is a worldwide epidemic (1) with significant effects on morbidity and mortality (2, 3). With the trend of worldwide aging, improved diagnostic tools and better public realization, the predicted prevalence of AF keeps rising substantially (4, 5), and there is no exception for Asians (2, 3, 6). Even through, the prevalence of AF might still be underestimated based on some real-world observations and device studies (5). Therefore, it is important to efficiently identify subjects with a potential risk of AF development and to employ more aggressive strategies for cardiac rhythm screening, so that a prompt diagnosis and associated interventions can be done in time. Several risk schemes are developed for the prediction of new onset AF, and most of them were developed from non-Asian population (7–9). Recently, we have proposed a clinical scheme, the Taiwan AF score, to predict the risk of incident AF based on the national cohort analysis including 7,220,654 subjects aged ≥ 40 years (10). The Taiwan AF score included age, male sex and important comorbidities [hypertension, heart failure (HF), coronary artery disease (CAD), end-stage renal disease (ESRD), and alcoholism], and is a straightforward scheme obviating the use of personal information, electrocardiogram and echocardiography data (10). However, the factor “alcoholism” is somewhat difficult to be accurately quantified, which might prevent this scheme from extensive clinical use. Therefore, the present study aims to investigate whether a modified Taiwan AF score (mTaiwan AF score) excluding “alcoholism” can be used for AF prediction in Asian population with long-term follow-up.

METHODS

Database

This study used the “National Health Insurance Research Database (NHIRD)” provided by Health and Welfare Data Science Center (HWDC), Ministry of Health and Welfare (MOHW), Taiwan. The National Health Insurance (NHI) system is a mandatory universal health insurance program providing comprehensive medical care coverage to all Taiwanese residents. NHIRD consists of detailed data of health care from January 1st, 1996, to December 31st, 2016, from >23 million enrollees, representing $>99\%$ of Taiwan’s population. In this cohort dataset, patients’ original identification numbers have been encrypted to protect their privacy, and the encrypting procedure was consistent, so that linkage of the claims belonging to the same patient was feasible. Therefore, patients can be followed continuously within the NHI database. The details about Taiwan NHIRD have been reported in our previous studies (3, 10–17). The present study was approved by the Institutional Review Board at Taipei Veterans General Hospital, Taipei, Taiwan.

Study Population

The study design was the same as our previous study (10). In general, a total of 7,220,654 patients aged ≥ 40 years without a history of cardiac arrhythmias from January 1st, 2000 to December 31st, 2000 were identified from Taiwan

NHIRD. Important comorbidities of each individual were confirmed based on the International Classification of Diseases (ICD), Ninth Revision, Clinical Modification (ICD-9-CM) codes from the NHIRD. The diagnostic accuracies of important comorbidities in NHIRD have been validated (18, 19). AF was confirmed using the ICD-9-CM code (427.31) registered by the physicians responsible for the care of patients. The diagnostic accuracy of AF based on ICD-9-CM code in Taiwan NHIRD has been validated previously (20). During a 16-year follow-up, 438,930 patients had incident AF with an incidence of 0.42 per 100 person-years.

The Modified Taiwan AF Score

The development of the original Taiwan AF score followed the TRIPOD (Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis) Statement (21) and the details have been described in our previous study (10). Potential variables were identified from the Cox proportional hazards modeling and forced into an initial saturated Cox proportional hazards model. An α level of 0.1 from the saturated model was used as a threshold to enter a variable predictor into a backward elimination model. β coefficients were derived from the final Cox regression model and used to calculate the score weights of each significant predictor in the multivariable Cox regression based on the method proposed by Sullivan et al. (22). The score weight for each predictor was rounded to its closest integer as the score point. Age, male sex, hypertension, HF, CAD, ESRD, and alcoholism were thus identified with different score weight and together constitute the Taiwan AF score ranging between -2 and 15 (Table 1). In the present study, we excluded alcoholism from the prediction scheme and proposed the mTaiwan AF (Table 1). The incidence of AF (%/year) after 1-year, 3-year, 5-year, 7-year, 10-year, 12-year, and 16-year follow-up for each mTaiwan AF score was calculated. Patients were classified into three groups based on the tertile values of the mTaiwan AF scores of patients who developed AF—group 1 (score -2 – 3), group 2 (score 4 – 9), and group 3 (score 10 – 14).

Statistical Analysis

The incidence of AF was calculated from dividing the number of events by person-time at risk. The Kaplan-Meier method were used to plot the cumulative incidence curves of AF for different risk groups, with statistical significance examined by the log-rank test. The diagnostic accuracy of the mTaiwan AF score in the prediction of incident AF was assessed by calculating c-indexes, based on the receiver operating characteristic (ROC) curve. All statistical significances were set at $p < 0.05$ and all statistical analyses were carried out by SPSS 17.0 (SPSS Inc. USA).

RESULTS

The distributions of each mTaiwan AF scores are shown in Figure 1. There were 5,407,576 (74.9%), 1,701,688 (23.6%) and 111,390 (1.5%) patients in group 1, 2, and 3, respectively (Figure 1).

Discrimination of mTaiwan AF Score in the Prediction of AF

The areas under the ROC curve (AUCs) of original Taiwan AF score and mTaiwan AF score in predicting incident AF after different follow-up durations are shown in **Table 2**. The AUCs of the mTaiwan AF scores are 0.861 [95% confidence interval (CI)

0.859-0.862] for 1-year follow-up, 0.829 (95% CI 0.827-0.832) for 5-year follow-up, 0.795 (95% CI 0.793-0.798) for 10-year follow-up and 0.751(95% CI 0.748-0.753) for 16-year follow-up (**Figure 2**).

Incidence and Risk of AF Stratified by mTaiwan AF Score

The incidences of AF (%/year) of each mTaiwan AF score during different follow-up periods are shown in **Table 3**. After a 16-year follow-up, the risk of incident AF increased from 0.05%/year

TABLE 1 | Calculations of Taiwan AF score and modified Taiwan AF score.

Variables	Taiwan AF score*	mTaiwan AF score
Age, years		
40-44	-2	-2
45-49	-1	-1
50-54	0	0
55-59	1	1
60-64	2	2
65-69	3	3
70-74	4	4
75-79	5	5
≥80	8	8
Male gender	1	1
Hypertension	1	1
Heart failure	2	2
Coronary artery disease	1	1
ESRD	1	1
Alcoholism	1	-
Total score	-2-15	-2-14

AF, atrial fibrillation; ESRD, end-stage renal disease.

*The calculation rule of Taiwan AF score was based on the paper by Chao et al. (10).

TABLE 2 | AUCs of Taiwan AF score and mTaiwan AF score in the prediction of AF after different follow-up durations.

Follow up duration	Taiwan AF score*		mTaiwan AF score	
	AUC (95%CI)	P-value	AUC (95%CI)	P-value
1 year	0.857 (0.855-0.860)	<0.001	0.861 (0.859-0.862)	<0.001
3 years	0.838 (0.837-0.840)	<0.001	0.830 (0.828-0.832)	<0.001
5 years	0.825 (0.824-0.826)	<0.001	0.829 (0.827-0.832)	<0.001
7 years	0.814 (0.813-0.815)	<0.001	0.814 (0.812-0.817)	<0.001
10 years	0.797 (0.796-0.798)	<0.001	0.795 (0.793-0.798)	<0.001
12 years	0.786 (0.785-0.787)	<0.001	0.785 (0.783-0.787)	<0.001
16 years	0.756 (0.755-0.757)	<0.001	0.751 (0.748-0.753)	<0.001

AF, atrial fibrillation; AUC, area under the receiver operating characteristic curve; CI, confidence interval.

*The AUCs of Taiwan AF score in the prediction of AF were adopted from the paper by Chao et al. (10).

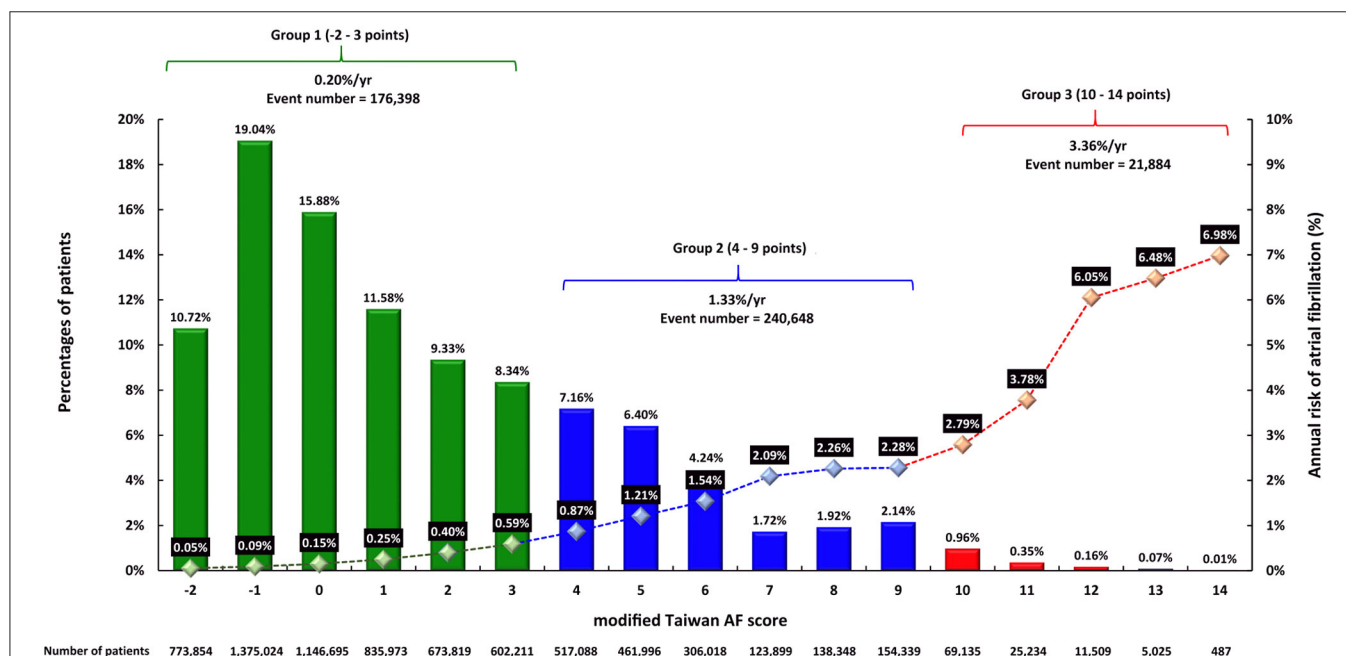


FIGURE 1 | The distributions of mTaiwan AF score and the risks of incident AF during 16-year follow-up. The mTaiwan AF score ranged between -2 and 14. After a 16-year follow-up, the annual risk of incident AF increased from 0.05% for patients with a score of -2 to 6.98% for patients with a score of 14. The annual risks of incident AF were 0.20, 1.33, and 3.36% for groups 1, 2, and 3, respectively. AF, atrial fibrillation.

for patients with a score of -2 to 6.98%/year for those having a score of 14 (**Figure 1**). The annual risks of incident AF are 0.20% for group 1, 1.33% for group 2, and 3.36% for group 3, respectively (**Figure 1**). The cumulative incidence curves of incident AF of groups 1, 2, 3 are shown in **Figure 3**. The 2-year risks of AF were 0.08, 2.04, and 7.82% for groups 1, 2, 3, respectively. The 4-year risks of AF were 0.31, 4.12, and 13.58% for groups 1, 2, 3, respectively. The 10-year risks of AF were 1.26, 11.13, and 27.89% for groups 1, 2, 3, respectively. Compared to group 1, the hazard ratios (HRs) of incident AF were 5.79

(95% CI 3.75-7.75) for group 2 and 8.93 (95% CI 6.47-10.80) for group 3.

DISCUSSION

In the present study, we proposed the mTaiwan AF score which excluded alcoholism from the original Taiwan AF score for incident AF prediction using a nationwide cohort including 7,220,654 subjects with 438,930 incident AF during a 16-year

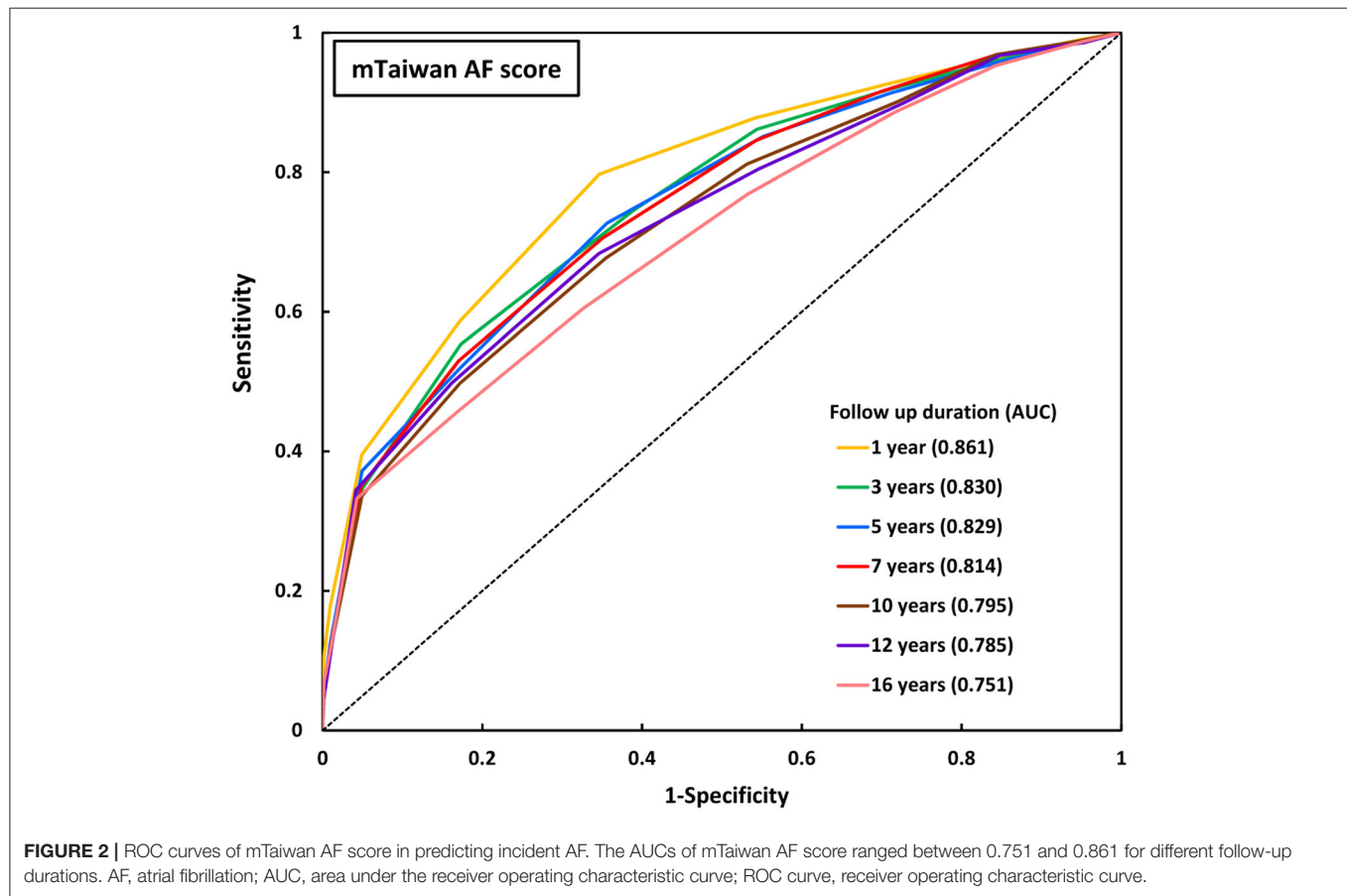
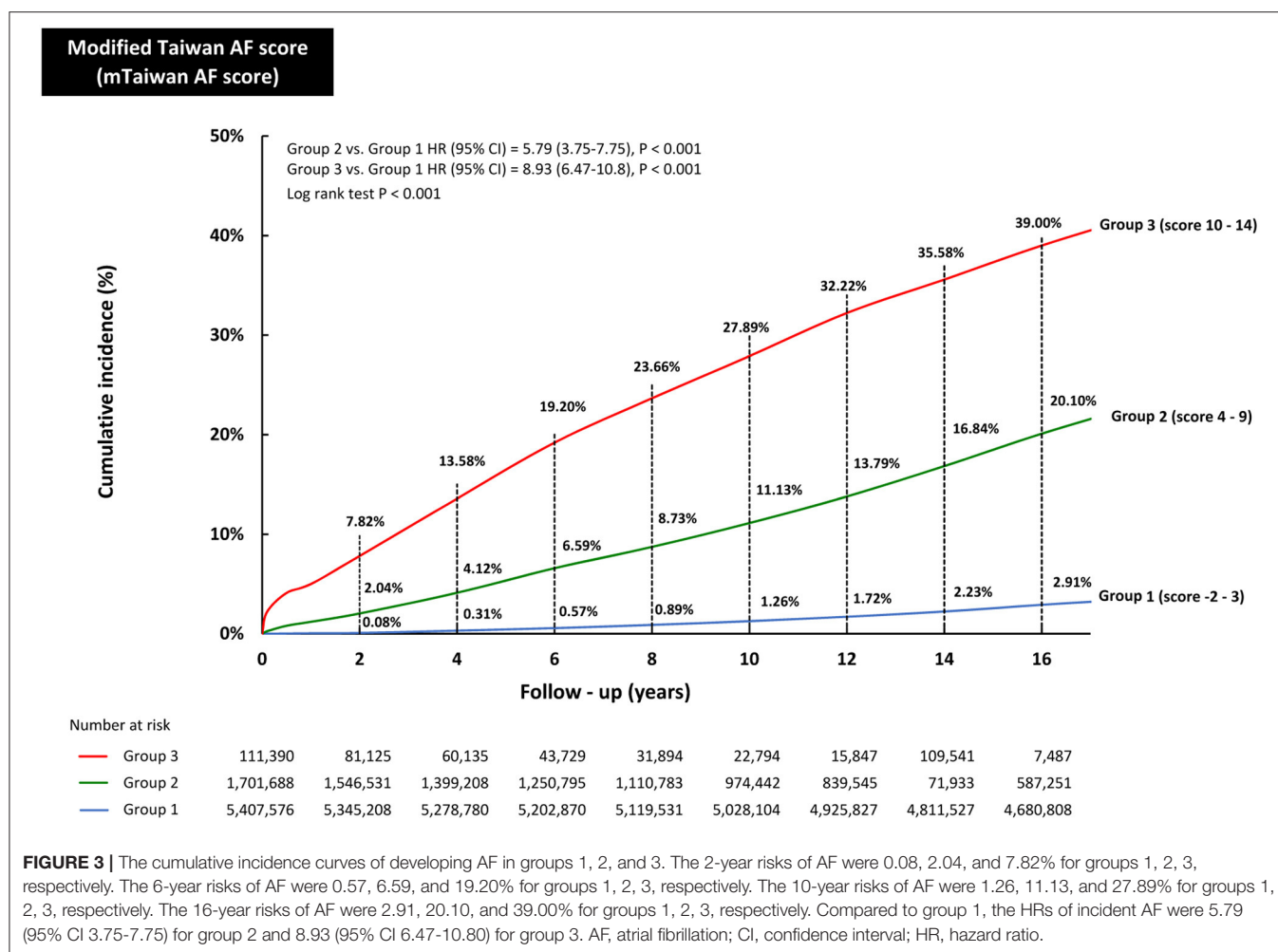


TABLE 3 | Incidence of AF stratified by mTaiwan AF score after different follow-up durations.

	Annual risk (%/year) of AF stratified by mTaiwan AF score																
	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1-year follow up	0.02	0.03	0.05	0.09	0.15	0.23	0.38	0.62	1.04	2.18	1.83	1.65	2.68	5.25	9.80	11.99	11.26
3-year follow up	0.02	0.04	0.06	0.12	0.20	0.33	0.55	0.81	1.20	2.05	2.08	2.12	2.85	4.55	8.05	8.97	9.36
5-year follow up	0.02	0.04	0.07	0.13	0.22	0.37	0.56	0.87	1.26	1.99	2.11	2.15	2.82	4.39	7.18	8.01	8.03
7-year follow up	0.03	0.05	0.08	0.15	0.25	0.38	0.60	0.90	1.30	1.97	2.14	2.17	2.81	4.12	6.73	7.38	7.77
10-year follow up	0.03	0.06	0.10	0.17	0.28	0.45	0.66	0.99	1.36	1.98	2.18	2.21	2.79	3.94	6.42	6.91	7.42
12-year follow up	0.04	0.06	0.11	0.19	0.31	0.50	0.72	1.06	1.42	2.04	2.21	2.24	2.79	3.89	6.22	6.72	7.38
16-year follow up	0.05	0.09	0.15	0.25	0.40	0.59	0.87	1.21	1.54	2.09	2.26	2.28	2.79	3.78	6.05	6.48	6.98

AF, atrial fibrillation.



follow-up. We confirmed the usefulness of this modified scheme to predict incident AF for Asian population.

Overview of Risk Factors of Incident AF

Risk factors for incident AF have been identified since long ago and results are somewhat variable across different studies. Potential reasons underlying the differences of reported risk factors remain unknown. Common risk factors include baseline demographics, underlying comorbidities, and cardiac structural abnormalities, such as age, male gender, obesity, hypertension, diabetes, and increased left ventricular wall thickness (23). Although the age distribution of AF differs between regions (5), the increase in AF prevalence with advancing age seems to be a worldwide phenomenon (5). Gender difference in AF incidence is also a consistent observation because a higher prevalence of AF in men than in women has been observed in most studies (5). Hypertension is the most common medical condition associated with AF worldwide, affecting 29-78% of patients with AF and there is no significant variation in the risk of AF associated with hypertension according to ethnic group (24-26). Therefore, hypertension is widely accepted to predispose individuals to AF (5), and so are CAD and HF (5, 7). Patients with ESRD had

significantly increased risk of AF, which even increased further together with other risk factors. The reported incidences of AF in patients with ESRD ranged from 1 to 14.8% depending on the co-existence of other risk factors (27). Risk factors other than age, sex, and comorbidities included race, height, personal habits, hemodynamic parameters, cardiac murmurs, electrocardiogram or echocardiographic parameters, but inconsistency remains between different stratification schemes (7-9). Nevertheless, observation from FHS and CHARG-AF scores may imply that a risk stratification scheme incorporating clinical factors but not electrocardiogram and echocardiographic parameters might provide satisfactory accuracy for predicting incident AF (7, 9).

The Role of Alcoholism in Risk of AF Development

Alcohol consumption is ubiquitous in Western countries (28), and has been defined as light (<7 standard drinks/week), moderate (7-21 standard drinks/week), and heavy (>21 standard drinks/week) alcohol consumption, where 1 standard drink is approximately 12 g of alcohol (28). The association of alcohol and AF was first brought into attention as “holiday heart syndrome”

in patients hospitalized with AF following a weekend binge (29), and more studies have been conducted on their association since then. Alcohol has complex effects to both cardiac structures and electrophysiological remodeling, and possible pathophysiological mechanisms underlying the association between alcohol and AF include direct toxicity and alcohol's contribution to obesity, sleep-disordered breathing, and hypertension (28). Alcohol could be a trigger for AF and facilitate progressive atrial remodeling with regular long-term consumption, leading to an arrhythmogenic substrate (28).

Moderate habitual consumption increases the incidence of AF in a dose-dependent but non-linearly manner, with relative risks of AF 1.08 for 7 standard drinks/week, 1.17 for 14 standard drinks/week, 1.26 for 21 standard drinks/week, 1.36 for 28 standard drinks/week, and 1.47 for 35 standard drinks/week (30–33). Heavy habitual consumptions with ≥ 40 standard drinks/week might be a more important risk factor than hypertension or obesity (34). Despite all the findings about alcohol and AF in real-world observations, many of the individual studies were underpowered to demonstrate a strong relationship, and some disagreements about details of alcoholism exist. For example, one meta-analysis showed that only wine and liquor, but not beer, were associated with incident AF in those consuming >14 standard drinks/week, while a community-based pooled cohort reported similar associations across different types of alcohol (33). There remains a conflict whether men and women were equally affected with alcohol consumptions (31, 35). Furthermore, most studies determined the quantity of alcohol consumption by self-reporting, rather than objective blood or urine samples, which raises the concern of precise quantification. Besides, the pattern and amount of alcohol consumption might be variable from time to time and thus it's difficult to determine the presence and degrees of habitual consumption. All conditions mentioned above highlighted the difficulty in defining "alcoholism" in clinical practice.

For easier and more extensive clinical application, we tested the accuracy of mTaiwan AF score which excluded alcoholism from the original Taiwan AF score for the prediction of incident AF in the present study. The AUCs of the mTaiwan AF score were 0.861 for 1-year follow up, 0.829 for 5-year follow up, 0.795 for 10-year follow up and 0.751 for 16-year follow up, which were quite similar to that of Taiwan AF score (0.857 for 1-year follow up, 0.825 for 5-year follow up, 0.797 for 10-year follow up, and 0.756 for 16-year follow up). Therefore, we demonstrated that the mTaiwan AF score without the consideration of alcoholism still provides reliable accuracy for the prediction of incident AF and could be easily applied in the clinical practice to replace Taiwan AF score when accurate information regarding alcoholism was not available.

Study Limitations

In the present study, we validated the use of the mTaiwan AF score, derived from the original Taiwan AF score, in the prediction of incident AF. Since both the Taiwan AF score and mTaiwan AF score were based on the same cohort, it is

expectable that the performance of mTaiwan AF score would not differ significantly from that of the original score. More studies are necessary to further validate the mTaiwan AF score in external cohorts.

CONCLUSION

Based on our prior publication (10), we developed a modified clinical risk scoring scheme, the mTaiwan AF score (–2 to 14), to stratify individual risk of new-onset AF. This modified scheme is feasible and reliable for clinical assessment and can easily identify high-risk population in whom a more proactive screening strategy for AF should be taken into consideration.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board, Taipei Veterans General Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

J-NL is responsible for manuscript drafting. S-SL is responsible for creation of tables and figures. T-JC is responsible for the resources of database. T-CT is responsible for critical revision. S-AC is responsible for study idea and conceptualization. T-FC is responsible for study idea and organizing the whole article. All authors contributed to the article and approved the submitted version.

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Ischemic Stroke in Non-Gender-Related CHA₂DS₂-VA Score 0~1 Is Associated With H₂FPEF Score Among the Patients With Atrial Fibrillation

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Background: Ischemic strokes (ISs) can appear even in non-gender-related CHA₂DS₂-VA scores 0~1 patients with atrial fibrillation (AF). We explored the determinants associated with IS development among the patients with non-gender-related CHA₂DS₂-VA score 0~1 AF.

Methods and Results: In this single-center retrospective registry data for AF catheter ablation (AFCA), we included 1,353 patients with AF (24.7% female, median age 56 years, and paroxysmal AF 72.6%) who had non-gender-related CHA₂DS₂-VA score 0~1, normal left ventricular (LV) systolic function, and available H₂FPEF score. Among those patients, 113 experienced IS despite a non-gender-related CHA₂DS₂-VA score of 0~1. All included patients underwent AFCA, and we evaluated the associated factors with IS in non-gender-related CHA₂DS₂-VA score 0~1 AF. Patients with ISs in this study had a lower estimated glomerular filtration rate (eGFR) ($p < 0.001$) and LV ejection fraction (LVEF; $p = 0.017$), larger LA diameter ($p < 0.001$), reduced LA appendage peak velocity ($p < 0.001$), and a higher baseline H₂FPEF score ($p = 0.018$) relative to those without ISs. Age [odds ratio (OR) 1.11 (1.07–1.17), $p < 0.001$, Model 1] and H₂FPEF score as continuous [OR 1.31 (1.03–1.67), $p = 0.028$, Model 2] variable were independently associated with ISs by multivariate analysis. Moreover, the eGFR was independently associated with IS at low CHA₂DS₂-VA scores in both Models 1 and 2. AF recurrence was significantly higher in patients with IS (log-rank $p < 0.001$) but not in those with high H₂FPEF scores (log-rank $p = 0.079$), respectively.

Conclusions: Among the patients with normal LVEF and non-gender-related CHA₂DS₂-VA score 0~1 AF, the high H₂FPEF score, and increasing age were independently associated with IS development (ClinicalTrials.gov Identifier: NCT02138695).

Keywords: atrial fibrillation, CHA₂DS₂-VA score, H₂FPEF score, stroke, atrial myopathy

INTRODUCTION

Atrial fibrillation (AF) is a significant risk factor for ischemic strokes (ISs), and the CHA₂DS₂-VASc score has been suggested to be the most reliable parameter for the IS risk stratification (1). The current guidelines recommend introducing oral anticoagulant therapy for stroke prevention in non-valvular AF patients with CHA₂DS₂-VASc scores of 2 points or higher. In contrast, no antithrombotic therapy is advantageous for men with scores of 0 or 1 point and women with scores of 1 or 2 points, respectively, in terms of the risk-benefit profile (2, 3). Nevertheless, AF patients with low CHA₂DS₂-VASc scores who are not recommended to undergo anticoagulant therapy exhibit an annual IS risk of 1.15% per year (4–7). This level of IS risk is similar to the annual IS risk in AF patients with higher CHA₂DS₂-VASc scores who are on anticoagulant therapy (5, 8). Therefore, identifying IS-related factors or clinical predictors of ISs in AF patients with low CHA₂DS₂-VASc scores might be valuable for stroke prevention in relatively young and active low-risk patients with AF (9–11). However, the mechanisms of IS in patients with AF are heterogeneous and the CHA₂DS₂-VASc score comprehensively evaluates both cardioembolic and non-cardioembolic risks, such as complex aortic plaque and carotid and intracranial arteriosclerosis (12). For this reason, IS risk-assessment studies in patients with low CHA₂DS₂-VASc scores and low numbers of comorbidities have been limited by epidemiologic data dependent on the International Classification of Diseases code (13). In this study, we explored the potential risk factors of ISs among patients with non-gender-related CHA₂DS₂-VA score 0~1 AF depending on the H₂FPEF score (14), which is a recently developed risk score for heart failure with preserved ejection fraction (HFpEF) and known to be related with atrial myopathy (15). Additionally, we evaluated the outcome of AF catheter ablation (AFCA). The purpose of this study was to compare and assess the risk factors of ISs in the patients with non-gender-related CHA₂DS₂-VA score 0~1 AF.

MATERIALS AND METHODS

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

Study Subjects

The study is conducted in compliance with the ethical rules of the Declaration of Helsinki (2013) as a statement of ethical principles for medical research involving human subjects by The World Medical Association and approved by the Institutional Review Board of Yonsei University Health System. From January 2009 to April 2020, 1,353 patients with a diagnosis of AF were identified as having a normal left ventricular (LV) systolic function and low non-gender-related low CHA₂DS₂-VA score (0–1 points both in men and women) in the Yonsei AF Ablation Cohort Database (ClinicalTrials.gov Identifier: NCT02138695) and underwent AFCA for symptomatic and drug-refractory non-valvular AF. Written informed consent was obtained from all patients before the study inclusion. The exclusion criteria for AFCA were as follows: (1) permanent AF refractory to

electrical cardioversion; (2) presence of a left atrial (LA) or LA appendage thrombus on transesophageal echocardiography; (3) no measurements of the left ventricular (LV) diameter, LV end-diastolic dimension (LVEDD), or ratio between the early mitral inflow velocity and mitral annular early diastolic velocity (EEm) by transthoracic echocardiography; (4) significant structural heart disease other than LV hypertrophy, such as significant valvular heart disease of grade two or greater, hypertrophic/ischemic/dilated cardiomyopathy, or congenital heart disease; (5) a history of a prior AFCA or cardiac surgery; and (6) a left ventricular ejection fraction (LVEF) \leq 50%. Among those patients, 113 experienced IS despite non-gender-related CHA₂DS₂-VA score 0~1, and divided into groups at high risk of and medium/low risk of a cardioembolic stroke, respectively, based on the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification scheme (16) for the analysis of stroke subtype differences. All included patients underwent AFCA, and the time difference between the previous stroke event and AFCA and comorbidities was confirmed by the electrical medical record review. The CHA₂DS₂-VA score was recalculated immediately before the IS event by medical record review in patients with a prior IS.

Calculating the H₂FPEF Score

The H₂FPEF score has six variables based on clinical and echocardiographic values: heaviness (body mass index (BMI) $>$ 30 kg/m², 2 points), hypertension (on two or more antihypertensive medicines, 1 point), atrial fibrillation (paroxysmal or persistent, 3 points), pulmonary hypertension (Doppler echocardiographic estimated pulmonary artery systolic pressure $>$ 35 mmHg, 1 point), elderly status (age $>$ 60 years, 1 point), and filling pressure (Doppler echocardiographic E/Em $>$ 9, 1 point). The baseline H₂FPEF score was calculated through medical record review same as the CHA₂DS₂-VA score recalculation in patients with a prior IS except echocardiographic parameters. In patients without a previous IS, the baseline H₂FPEF score was calculated with the variables obtained within 3 months before the AFCA.

Echocardiographic Measurement

Transthoracic echocardiography was conducted in all patients using commercially available devices (Vivid 7 or Vivid E9 from GE Healthcare, Chicago, IL, USA or iE 33 from Philips, Amsterdam, the Netherlands) as recommended by the American Society of Echocardiography (17). Standard images were obtained in the parasternal and apical views through two-dimensional, Doppler, and M-mode images, such as the LA anteroposterior diameter and LV end-systolic and LVEDD dimensions. The early Doppler mitral inflow (E) was recorded by the pulsed wave from the apical window, with a 1- to 3-mm pulsed Doppler sample volume placed between the tips and mitral leaflets during diastole. The early diastolic mitral annular velocity (Em) was recorded as the peak early diastolic tissue velocity using color Doppler tissue imaging of the septal mitral annulus. The early diastolic mitral inflow velocity ratio to the early diastolic mitral annular velocity (E/Em) was calculated. Tricuspid regurgitation (TR) and estimated right atrial (RA)

pressure were evaluated using the recommended methods, and the right ventricular systolic pressure (RVSP) was calculated as $4 \times (\text{TR jet})^2 + \text{estimated RA pressure}$ (18). For Doppler-derived parameters, at least 3 consecutive beats were measured and averaged (19).

Electrophysiologic Characterization and Radiofrequency Catheter Ablation

Intracardiac electrograms were obtained using the Prucka CardioLab electrophysiology system (GE Healthcare, Chicago, IL, USA). A 3D electroanatomical map (Ensite NavX; Abbott Laboratories, Chicago, IL, USA; CARTO3; Johnson & Johnson Inc., NJ, USA.) was generated using a circumferential pulmonary-vein mapping catheter through a long sheath (Schwartz left 1; Abbott Laboratories, Chicago, IL, USA) through merging the 3D geometry generated by the electroanatomic mapping system with the corresponding 3D spiral CT images. Separately, a 3D LA voltage mapping was performed by obtaining the contact bipolar electrograms from 350 to 500 points on the LA endocardium during atrial pacing (high RA; pacing cycle length: 500 ms). The bipolar electrograms were filtered at 32–300 Hz. Color-coded voltage maps were generated by using the bipolar electrograms, and the peak-to-peak voltage was generated as previously described (20).

Statistical Analysis

The baseline characteristics of patients were compared using descriptive statistics, presented as median (interquartile interval) values for continuous variables and as numbers (percentages) for categorical variables. With reference to a previous study (14), we set a 5 point H₂FPEF score as the cut-off value, which has shown the probability of HFpEF >80%. To identify factors associated with the presence of a stroke, we performed univariate and multivariable logistic regression analyses. We conducted three models of multivariable logistic regression analyses because of the multicollinearity among the H₂FPEF score and age or E/Em. Model 1 was analyzed by adding variables that were having $p < 0.10$ of the univariate models. Model 2 was analyzed by treating the H₂FPEF score as a continuous variable, and model 3 was analyzed by treating it as a categorical variable. To compare the effect of individual H₂FPEF score variables, we performed multivariable logistic regression based on individual H₂FPEF score variables. A subgroup analysis was performed based on the comorbidities not included in the H₂FPEF score variables. Two-sided p -values of <0.05 were considered to be statistically significant. Statistical analyses were conducted using SAS version 9.4 (SAS Institute, Cary, NC, USA) and R version 4.0.0 (R Foundation for Statistical Computing, Vienna, Austria) software.

RESULTS

Baseline Patient Characteristics

Among 3,648 consecutive patients in this single-center prospective registry, we included 1,353 patients with AF (24.7% female, median age 56 years, paroxysmal AF 72.6%) who had non-gender-related CHA₂DS₂-VA score 0–1 at the times of enrollment ($n = 1,240$) or previous IS events ($n = 113$), normal

LV systolic function, and available H₂FPEF score. The time difference between the previous stroke events and inclusion was a median of 1.0 [interquartile range (IQR):1.0–4.0] year in 113

TABLE 1 | Comparison of the baseline characteristics between non-gender-related CHA₂DS₂-VA score 0–1 AF patients with strokes and those without strokes.

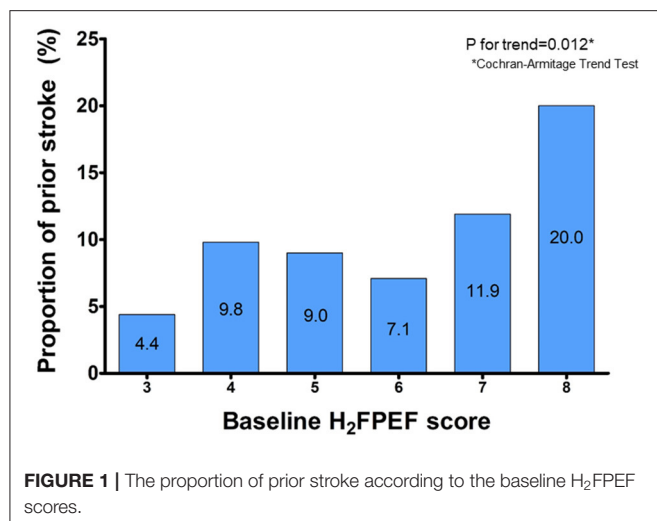
Variables	Overall, N = 1,353	Low CHA ₂ DS ₂ -VA prior stroke (+), N = 113	Low CHA ₂ DS ₂ -VA prior stroke (-), N = 1,240	p-value
Age (years)	56 (50, 62)	62 (58, 67)	56 (49, 61)	<0.001
Female, n (%)	334 (24.7)	28 (24.8)	306 (24.7)	1.000
Smoking, n (%)	513 (38.0)	45 (39.8)	468 (37.8)	0.104
Alcohol, n (%)	688 (50.9)	52 (46.0)	636 (51.3)	0.508
Paroxysmal AF, n (%)	978 (72.6)	77 (68.1)	901 (73.0)	0.317
Heart failure, n (%) [*]	35 (2.6)	5 (4.4)	30 (2.4)	0.329
Hypertension, n (%)	383 (28.3)	31 (27.4)	352 (28.4)	0.915
Diabetes, n (%)	44 (3.3)	6 (5.3)	38 (3.1)	0.312
Vascular disease, n (%)	22 (1.6)	4 (3.5)	18 (1.5)	0.196
BMI (kg/m ²)	24.7 (22.9, 26.6)	24.3 (23.1, 26.1)	24.7 (22.9, 26.6)	0.252
H ₂ FPEF score, baseline	5 (4, 6)	6 (4, 6)	5 (4, 6)	0.018
eGFR, (mL/min/1.73 m ²)	89 (78, 103)	84 (74, 94)	90 (79, 103)	0.001
Medication				
Beta blocker, n (%)	493 (35.0)	42 (37.2)	431 (34.8)	0.685
ACEI/ARB, n (%)	283 (20.9)	22 (19.5)	261 (21.1)	0.781
Statin, n (%)	324 (24.0)	62 (54.9)	262 (21.1)	<0.001
Echocardiography				
LA diameter (mm)	40 (36, 44)	42 (39, 45)	40 (36, 44)	0.001
LVEF (%)	64 (60, 69)	63 (59, 67)	65 (60, 69)	0.017
E/Em	8.5 (7.0, 11.0)	10.0 (8.0, 12.1)	8.4 (7.0, 10.7)	<0.001
DT (ms)	175 (155, 205)	171 (153, 208)	177 (155, 205)	0.444
TR jet (m/s)	2.2 (2.0, 2.4)	2.3 (2.1, 2.5)	2.2 (2.0, 2.4)	0.006
RVSP (mmHg)	25 (22, 28)	26 (23, 29)	25 (21, 28)	0.007
LAA peak velocity (cm/s) [†]	49 (34, 67)	37 (25, 58)	51 (36, 67)	<0.001
LA pressure (mmHg)				
Peak	21 (15, 27)	20 (14, 27)	21 (15, 27)	0.423
Mean	11 (8, 16)	11 (7, 15)	11 (8, 16)	0.430
Nadir	4 (0, 8)	4 (1, 7)	4 (0, 8)	0.983
3D bipolar mean voltage (mV)				
LA voltage	1.4 (0.9, 1.9)	1.3 (0.8, 1.7)	1.4 (0.9, 1.9)	0.161
LAA voltage	2.2 (1.3, 3.3)	2.1 (1.2, 3.1)	2.3 (1.3, 3.3)	0.261

The data are presented as the number (%), and median (interquartile interval). Non-parametric continuous variables as assessed by the Kolmogorov–Smirnov method, were analyzed by Kruskal–Wallis H test.

ACEI, angiotensin-converting-enzyme inhibitor; AF, atrial fibrillation; ARB, angiotensin receptor blocker; BMI, body mass index; DT, deceleration time; E/Em, the ratio of the early diastolic mitral inflow velocity (E) to the early diastolic mitral annular velocity (Em); GFR, glomerular filtration rate; LA, left atrium; LAA, left atrium appendage; LVEF, left ventricular ejection fraction; RVSP, right ventricular systolic pressure; TR, tricuspid regurgitation.

^{*}Defined as conventional HFpEF diagnosis criteria: left ventricular ejection fraction $\geq 50\%$ with exertional dyspnea that was not caused by extracardiac causes.

[†]Velocity was measured at transesophageal echocardiography.



patients with previous stroke events, and 99 of them had a high risk for cardioembolism to the TOAST classification. About 84% of previous stroke events (95/113) had occurred within a year.

Factors Associated With ISs in Patients With Non-Gender-Related CHA₂DS₂-VA Score 0~1

We compared the AF patients with non-gender-related CHA₂DS₂-VA score 0~1 and those at the time of the IS in Table 1. Patients who experienced IS at the time of non-gender-related CHA₂DS₂-VA score 0~1 were older ($p < 0.001$) and had a higher baseline H₂FPEF score ($p = 0.018$), E/Em values ($p < 0.001$), and RVSP ($p = 0.007$), larger LA dimension ($p = 0.003$), lower eGFR ($p = 0.001$), and left atrium appendage (LAA) peak velocity ($p < 0.001$) than those without IS.

LV Diastolic Dysfunction and IS at Non-Gender-Related CHA₂DS₂-VA Score 0~1

Figure 1 shows a linear relationship trend between the baseline H₂FPEF score and non-gender-related CHA₂DS₂-VA score 0~1. Patients with the baseline H₂FPEF score ≥ 5 were generally older ($p < 0.001$) and had higher proportions of hypertension ($p < 0.001$). They had an increased BMI value ($p < 0.001$), larger LA diameters ($p < 0.001$), higher E/Em values ($p < 0.001$) and RVSP ($p < 0.002$), and higher prescription rate of renin-angiotensin-aldosterone system blockers ($p < 0.001$) and statins ($p = 0.004$, Table 2).

The univariate and multivariate analysis for IS in patients with non-gender-related CHA₂DS₂-VA score 0~1 is listed in Table 3. For the multivariate logistic regression analyses, we tested 2 models because of collinearity between age and H₂FPEF score. Age [OR 1.11 (1.07–1.17), $p < 0.001$, Model 1] and H₂FPEF score as continuous [OR 1.31 (1.03–1.67), $p = 0.028$, Model 2] variables were independently associated with ISs by multivariate analysis. The eGFR was also independently associated with IS at low CHA₂DS₂-VA scores in both Models 1 and 2.

TABLE 2 | Comparison of the baseline characteristics based on H₂FPEF score 5 in non-gender-related CHA₂DS₂-VA score 0~1 patients with AF.

Variables	H ₂ FPEF score, baseline		
	<5 ($n = 566$)	≥ 5 ($n = 787$)	p -value
Age (years)	55 (50, 60)	58 (50, 63)	<0.001
Female, n (%)	146 (25.8)	188 (23.9)	0.460
Smoking, n (%)	215 (38.0)	298 (37.9)	0.858
Alcohol, n (%)	281 (49.7)	407 (51.8)	0.642
Paroxysmal AF, n (%)	420 (74.6)	558 (71.2)	0.184
Heart failure, n (%)*	15 (2.7)	20 (2.5)	1.000
Hypertension, n (%)	106 (18.7)	277 (35.2)	<0.001
Diabetes, n (%)	19 (3.4)	25 (3.2)	0.977
Vascular disease, n (%)	8 (1.4)	14 (1.8)	0.759
Prior stroke, n (%)	40 (7.1)	73 (9.3)	0.177
BMI (kg/m ²)	23.2 (21.7, 24.2)	26.2 (25.1, 27.7)	<0.001
eGFR (mL/min)	92 (80, 104)	88 (77, 101)	0.021
Medication			
Beta blocker, n (%)	181 (32.0)	292 (37.2)	0.056
ACEi/ARB, n (%)	68 (12.0)	215 (27.4)	<0.001
Statin, n (%)	113 (20.0)	211 (26.8)	0.004
Echocardiography			
LA diameter (mm)	38 (35, 42)	41 (37, 45)	<0.001
LVEF (%)	64 (60, 69)	65 (60, 69)	0.960
E/Em	7.9 (6.7, 9.0)	9.4 (8.0, 11.8)	<0.001
DT (ms)	179 (157, 206)	175 (154, 204)	0.330
TR jet (m/s)	2.2 (2.0, 2.4)	2.2 (2.0, 2.4)	<0.001
RVSP (mmHg)	24 (21, 28)	25 (22, 29)	0.002
LAA peak velocity (cm/s) [†]	48 (36, 67)	50 (33, 67)	0.550
LA pressure (mmHg)			
Peak	21 (16, 27)	20 (15, 27)	0.255
Mean	12 (8, 16)	11 (8, 16)	0.256
Nadir	4 (1, 8)	4 (0, 8)	0.471
3D bipolar mean voltage (mV)			
LA voltage	1.4 (0.9, 1.8)	1.4 (0.9, 1.9)	0.903
LAA voltage	2.3 (1.4, 3.3)	2.2 (1.3, 3.3)	0.469

The data are presented as the number (%), and median (interquartile interval). Non-parametric continuous variables as assessed by the Kolmogorov-Smirnov method were analyzed by the Kruskal-Wallis H test.

ACEi, angiotensin-converting-enzyme inhibitor; AF, atrial fibrillation; ARB, angiotensin receptor blocker; BMI, body mass index; DT, deceleration time; E/Em, the ratio of the early diastolic mitral inflow velocity (E) to the early diastolic mitral annular velocity (Em); GFR, glomerular filtration rate; LA, left atrium; LAA, left atrium appendage; LVEF, left ventricular ejection fraction; RVSP, right ventricular systolic pressure; TR, tricuspid regurgitation.

*Defined as conventional HFpEF diagnosis criteria: left ventricular ejection fraction $\geq 50\%$ with exertional dyspnea that was not caused by extracardiac causes.

[†]Velocity was measured at transesophageal echocardiography.

The Effect of Individual Factors of H₂FPEF Score and Subgroup Analysis

We compared the effect of individual variables of the H₂FPEF score on IS at non-gender-related CHA₂DS₂-VA score 0~1 in the logistic regression models (Figure 2). Among six variables, age ≥ 60 years [OR 4.34 (2.34–8.20), $p < 0.001$] and E/Em ≥ 9 [OR 2.28 (1.24–4.23), $p = 0.008$] were independently associated with ISs in this low-risk group. In the subgroup analysis, the

TABLE 3 | Univariate and multivariate logistic regression analysis for the predictors of prior ISs in patients with non-gender-related CHA₂DS₂-VA score 0~1 AF.

	Univariate analysis		Multivariable model 1 [†]		Multivariable model 2 [‡]	
	Unadjusted OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Age	1.10 (1.08–1.14)	<0.001	1.11 (1.07–1.17)	<0.001		
Female	1.01 (0.63–1.55)	0.981				
Smoking	1.09 (0.73–1.61)	0.672				
Alcohol	0.81 (0.55–1.19)	0.280				
Paroxysmal AF	0.79 (0.53–1.21)	0.267				
Heart failure*	1.87 (0.63–4.52)	0.206				
Hypertension	0.95 (0.61–1.45)	0.829				
Diabetes	1.77 (0.66–4.00)	0.204				
Vascular disease	2.49 (0.71–6.82)	0.104				
BMI	0.96 (0.90–1.02)	0.203				
H ₂ FPEF score, baseline (continuous variable)	1.21 (1.05–1.40)	0.008			1.31 (1.03–1.67)	0.028
eGFR	0.98 (0.97–0.99)	0.002	0.98 (0.96–1.00)	0.021	0.98 (0.96–0.99)	0.004
Echocardiography						
LA diameter	1.06 (1.03–1.10)	0.001	0.97 (0.92–1.03)	0.387	0.98 (0.93–1.04)	0.575
LVEF	0.96 (0.93–0.99)	0.023	0.93 (0.88–0.98)	0.013	0.96 (0.91–1.02)	0.408
E/Em	1.09 (1.05–1.13)	<0.001	1.12 (1.04–1.19)	0.001		
DT	1.00 (0.99–1.00)	0.604				
TR jet	1.13 (0.86–1.45)	0.243				
RVSP	1.05 (1.02–1.08)	0.001	0.99 (0.94–1.05)	0.823		
LAA peak velocity	0.98 (0.97–0.99)	0.001	1.00 (0.99–1.02)	0.906	0.99 (0.98–1.01)	0.296
LA pressure						
Peak	1.00 (0.97–1.02)	0.684				
Mean	1.00 (0.97–1.03)	0.902				
Nadir	1.00 (0.97–1.04)	0.702				
3D bipolar mean voltage						
LA voltage	0.98 (0.58–1.64)	0.935	0.98 (0.58–1.64)	0.935	0.78 (0.47–1.26)	0.321
LAA voltage	0.90 (0.75–1.07)	0.254				

CI, confidence interval; OR, Odds ratio.

AF, atrial fibrillation; BMI, body mass index; DT, deceleration time; E/Em, the ratio of the early diastolic mitral inflow velocity (E) to the early diastolic mitral annular velocity (Em); GFR, glomerular filtration rate; LA, left atrium; LAA, left atrium appendage; LVEF, left ventricular ejection fraction; RVSP, right ventricular systolic pressure; TR, tricuspid regurgitation.

*Defined as conventional HFpEF diagnosis criteria: LVEF \geq 50% with exertional dyspnea that was not caused by extracardiac causes.

[†]Model 1: age, eGFR, LA diameter, LVEF, E/Em, RVSP, LAA peak velocity, and LA mean voltage.

[‡]Model 2: H₂FPEF score (continuous variable), eGFR, LA diameter, LVEF, LAA peak velocity, and LA mean voltage.

H₂FPEF score was consistently related to the risk of ISs at non-gender-related CHA₂DS₂-VA score 0~1, regardless of sex, AF types, diabetes, vascular disease, and renal function (Figure 3).

Rhythm Outcome of AFCA

Over median 28 months of follow-up, the cumulative AF recurrence rate after AFCA was significantly higher in the patients with IS under non-gender-related CHA₂DS₂-VA score 0~1 (log-rank $p = 0.001$, Figure 4A), but not high H₂FPEF score ≥ 5 (log-rank $p = 0.079$, Figure 4B), E/Em > 9 (log-rank $p = 0.241$, Figure 4C), or eGFR ≤ 60 ml/min/1.73 m² (log-rank $p = 0.250$, Figure 4D).

DISCUSSION

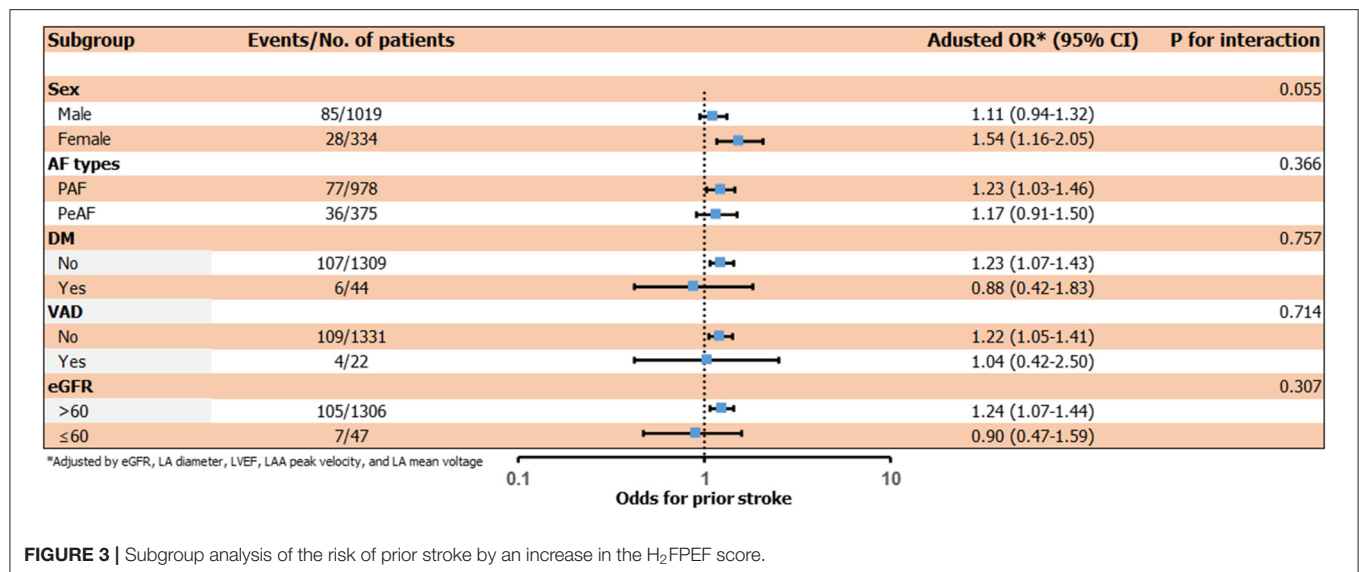
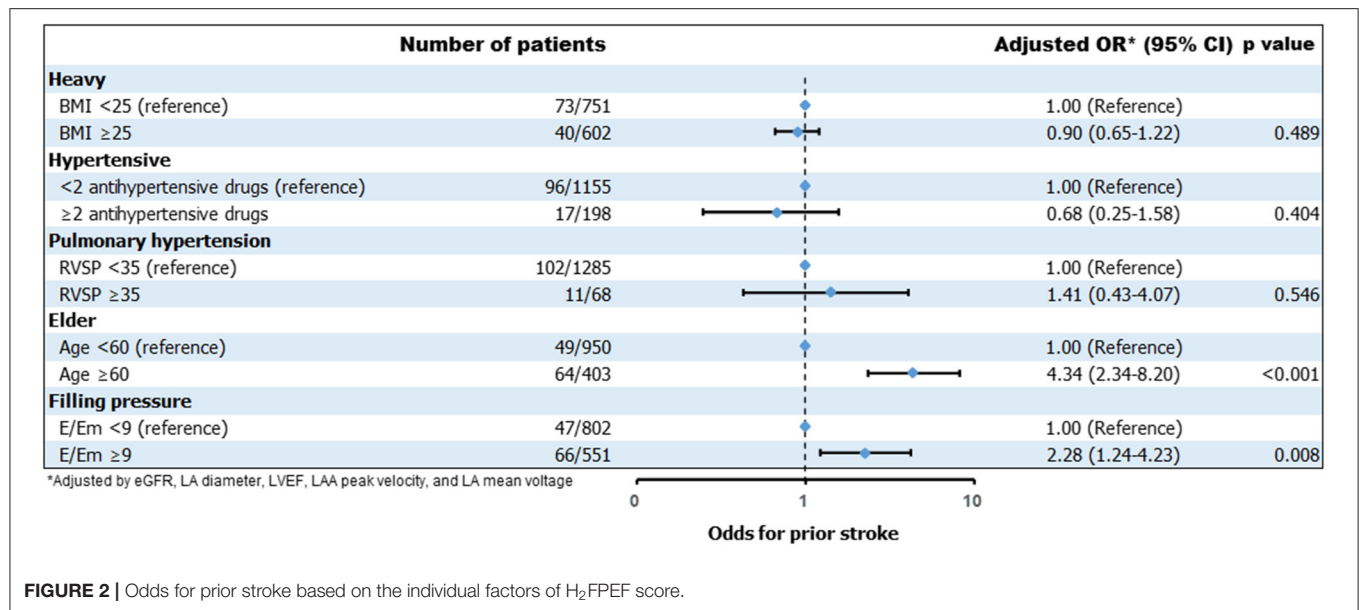
Main Findings

This study explored the risk factors for ISs based on the H₂FPEF score in AF patients with non-gender-related CHA₂DS₂-VA

score 0~1. Among these low-risk patient groups, increased age, the high H₂FPEF score, and low eGFR were independently associated with ISs. Among six variables of the H₂FPEF score, age over 60 years, and E/Em ≥ 9 showed a significantly greater risk of ISs at non-gender-related CHA₂DS₂-VA score 0~1 among the patients with AF who were referred for catheter ablation.

CHA₂DS₂-VASc Score and Low IS Risk Patients With AF

We have been using the CHA₂DS₂-VASc score as an epidemiologically reasonable stroke prevention index (21). In spite of the 1.15% annual risk of ISs, the existing guidelines do not recommend antithrombotic therapy to AF patients with non-gender related CH₂DS₂-VASc scores of <2 -point (≤ 1 point in men and ≤ 2 points in women) (2, 3, 5–7). However, it remains clinically important to predict and prevent ISs in these low-risk patients, primarily young and active individuals. Another weak point is that the CH₂DS₂-VASc score components include not



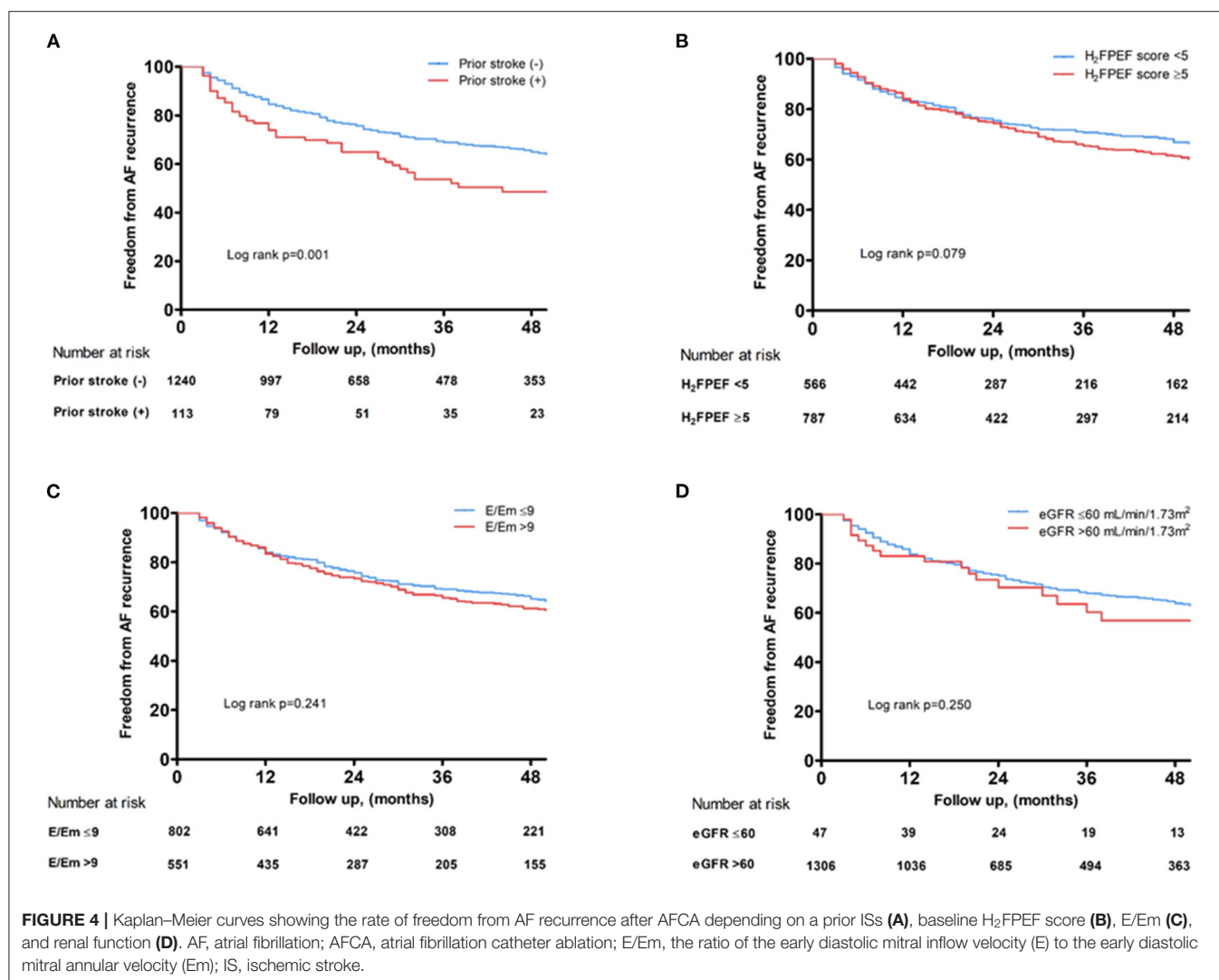
only cardioembolic but also non-cardioembolic risk factors (12) and do not encompass pathophysiological mechanisms, such as atrial myopathy, hemodynamic factors, the AF burden, or hypercoagulability (22, 23).

H₂FPEF Score and Risk of ISs in AF Patients With Low CHA₂DS₂-VASc Scores

Although the associated comorbidities tend to be less, hemodynamic factors are more likely to contribute to the mechanism of IS in AF patients with non-gender-related CHA₂DS₂-VA score 0~1 in this study. Recently, growing interest in the potential for an atrial myopathy that leads to AF progression and contributes to systemic thromboembolism has emerged (24). King et al. (25) discerned the relationship

between atrial fibrosis and the risk of a stroke in patients with AF using late-gadolinium enhanced cardiac MRI. Leong et al. (23) reported LA dysfunction contributes to the mechanism of ISs by analyzing the LA strain. Atrial myopathy generates the condition vulnerable to atrial dysfunction, fibrosis, structural remodeling, and blood stasis, increasing the risk of thrombus development.

Although genetic factors may contribute to atrial myopathy in certain specific low-risk patients with AF (26, 27), the LV diastolic dysfunction could be a key contributing factor. LV diastolic dysfunction increases the atrial filling pressures and triggers progressive atrial enlargement, dysfunction, and atrial myopathy, eventually leading to ISs (28). Kim et al. (29) and Yu et al. (30) reported that LV diastolic dysfunction represented by the E/Em is associated with a greater risk for ISs and LA



remodeling, especially in female patients with AF. The recently developed integrated scoring system, the H₂FPEF score (14), which estimates an adverse effect on hemodynamics, may help to identify LA myopathy (15, 31). Furthermore, we proved the baseline H₂FPEF score is independently associated with ISs in AF patients with non-gender-related CHA₂DS₂-VA score 0~1.

Clinical Implications

Based on the results of this study, physicians should consider the potential risk of ISs in AF patients with non-gender-related CHA₂DS₂-VA score 0~1, especially in patients with increased age, a high H₂FPEF score, or renal dysfunction. Recently, we demonstrated that the active rhythm control of AF by AFCA is superior to medical therapy in the risk reduction of ISs (32) and AFCA reduces the H₂FPEF score a year after the procedure in AF patients with underlying LV diastolic dysfunction (33). Therefore, despite a non-gender-related CHA₂DS₂-VA score of 0~1, we have to pay more attention to the risk of IS or rhythm control status for

those patients with old age, the high H₂FPEF score, or renal dysfunction.

Limitations

Our study had several limitations that should be noted. First, the population of this study was a single-center AFCA cohort with detailed clinical and imaging data. As these patients were referred for AFCA, the results of this study cannot be generalized. Second, ISs were classified and diagnosed by neurologists, and silent ISs were not excluded in the study. Third, because this study is retrospectively designed including the AF population with detailed imaging and physiological data, the timing of the IS event was not accurately reflected in the baseline characteristics. However, most of the prior strokes (84%) occurred within 1 year of the study time point. Furthermore, we checked precise age at the time of stroke events in patients with ISs and adjusted the non-gender-related CHA₂DS₂-VA score. Fourth, although their proportion was small, we did not exclude the patients with mitral annular

calcification or mitral regurgitation whose E/Em could not represent LV filling pressure.

CONCLUSION

Among AF patients with normal LV systolic function and non-gender-related CHA₂DS₂-VA score 0~1, age, renal function, and the high H₂FPEF score, that may allow for LA myopathy identification, were significantly associated with the ISs.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board of Yonsei University Health System. The patients/participants provided their written informed consent to participate in this study.

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AUTHOR CONTRIBUTIONS

H-NP and MK: conceptualization, validation, writing—original draft, and writing—review and editing. H-NP, HY, T-HK, J-SU, YK, HN, BJ, M-HL, and JH: data curation. MK: formal analysis and Software. H-NP, MK, HY, T-HK, D-IL, BJ, and M-HL: methodology. H-NP, MK, HY, T-HK, D-IL, J-SU, YK, HN, BJ, M-HL, and JH: investigation. All authors contributed to the article and approved the submitted version.

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The Beneficial Effects of Beta Blockers on the Long-Term Prognosis of Patients With Premature Atrial Complexes

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Aims: Premature atrial complexes (PACs) have been reported to increase the risk of adverse cardiovascular outcomes. Beta blockers at low dosages may help to reduce PAC symptoms, but it is unclear whether they can improve long-term outcomes.

Methods: Patients enrolled from a Holter cohort in a medical referral center were stratified into high-burden (≥ 100 beats/24 h) and low-burden (< 100 beats/24 h) sub-cohorts, and propensity score matching between treatment groups and non-treatment groups was conducted for each sub-cohort.

Results: In the high-burden sub-cohort, after propensity score matching, the treatment group and non-treatment group respectively had 208 and 832 patients. The treatment group had significantly lower mortality rates than the non-treatment group [hazard ratio (HR) = 0.521, 95% confidence interval (CI) = 0.294–0.923, $p = 0.025$], but there was no difference in new stroke (HR = 0.830, 95% CI = 0.341–2.020, $p = 0.681$), and new atrial fibrillation (HR = 1.410, 95% CI = 0.867–2.292, $p = 0.167$) events. In the low-burden sub-cohort, after propensity score matching, there were 614 patients in the treatment group and 1,228 patients in the non-treatment group. Compared to the non-treatment group, up to 40% risk reduction in mortality was found in the treatment group (HR = 0.601, 95% CI = 0.396–0.913, $p = 0.017$), but no differences in new stroke (HR = 0.969, 95% CI = 0.562–1.670, $p = 0.910$) or atrial fibrillation (HR = 1.074, 95% CI = 0.619–1.863, $p = 0.800$) were found.

Conclusions: Beta blockers consistently decreased long-term mortality in high-burden and low-burden patients. Interestingly, this effect was not achieved through reduction of new-onset stroke or AF, and further research is warranted.

Keywords: premature atrial complex (PAC), arrhythmia, beta blocker, prognosis, atrial fibrillation, stroke

INTRODUCTION

Premature atrial complexes (PACs) are a common type of arrhythmic disturbance in the general population (1). PAC burden is known to be age-dependent, but is not associated with sex (2). Patients with PACs are at increased risk of stroke and mortality over the long term, either due to the burden of PAC itself, or to subsequent atrial fibrillation (AF) (3–6). We have previously revealed an association between higher PAC burden and higher risk of all-cause mortality and cardiovascular death (2), and PACs have been reported to be an important factor in the initiation and perpetuation of AF (7). The main focal mechanisms of PACs, including enhanced automaticity, early afterdepolarization, or delayed afterdepolarization, are highly related to adrenergic activation (7). As sympathetic antagonists, beta blockers are known to bring long-term benefits to a number of cardiovascular conditions, particularly heart failure, which involves a vicious cycle of sympathetic overactivation. Patients with high PAC burden are commonly prescribed with beta blockers, especially for symptomatic sufferers, but the long-term effects of beta blockers on cardiovascular outcomes remain unclear as yet. Recently, we found that a threshold of ≥ 100 beats/24 h could serve as a cutoff for the prediction of adverse cardiovascular outcomes in patients with PACs (2). Therefore, in this study, we aimed to investigate the effects of beta blockers on the long-term prognosis of patients with a high (≥ 100 beats/24 h) or low (< 100 beats/24 h) burden of PACs by using propensity score matched treatment and non-treatment groups.

METHODS

Databank

We conducted a retrospective cohort study, using the Cardiovascular Disease Databank from National Cheng-Kung University Hospital (NCKU) to enroll consecutive patients who had undergone 24-h Holter monitoring. The Databank has previously been validated (2), and contains the complete anonymized inpatient and outpatient electronic medical records of the NCKU cardiovascular department, including patients who had undergone invasive or non-invasive cardiovascular studies. Longitudinal data regarding patient demographics, symptoms, laboratory data, medications, and imaging studies from January 1, 2009 to July 1, 2020 were collected. The Databank was established upon data collected under the Artificial Intelligence with Deep Learning and Genes on Cardiovascular Disease study, which is registered in ClinicalTrials.gov (NCT03877614). This study was approved by an independent ethics committee (IEC) at NCKU (A-ER-107-149, A-ER-108-381), and was conducted in accordance with institutional and local regulations, Good Clinical Practice (GCP), and the Declaration of Helsinki. Patient informed consent was waived due to the retrospective nature of this study and the anonymization of patient data.

Study Cohort

The Databank were consecutively analyzed from January 1, 2010 to August 31, 2019, and 29,851 Holter monitoring records from 36,553 patients were identified (**Figure 1**). Patients aged less than

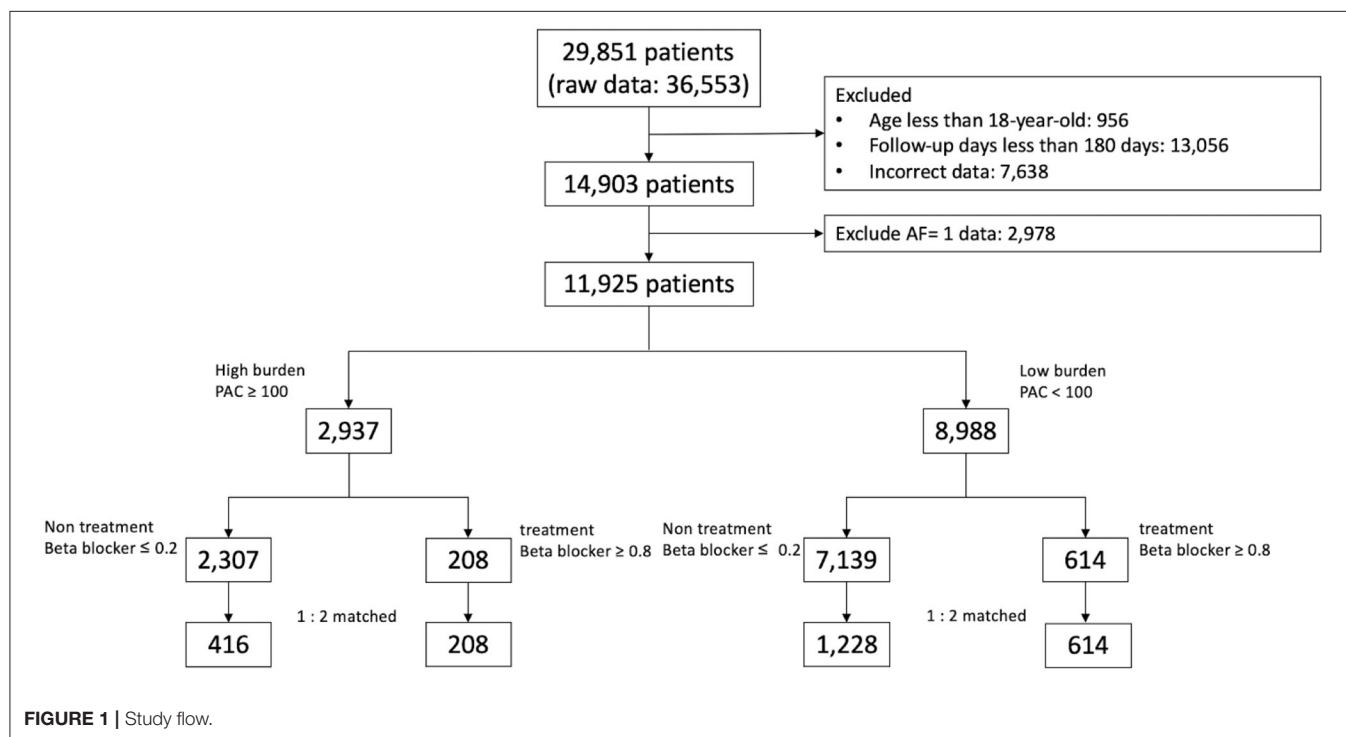


TABLE 1 | Demographic and clinical characteristics of patients with a high burden of PACs in the treatment and non-treatment groups before and after propensity score matching.

Variables	Before matching				After matching			
	Non-Tx (N = 2,307)	Tx (N = 208)	p	SMD	Non-Tx (N = 416)	Tx (N = 208)	p	SMD
Age, y, mean (SD)	70.65 (13.90)	69.18 (12.37)	0.143	0.111	68.71 (14.55)	69.18 (12.37)	0.689	0.035
Male, N (%)	1,147 (49.7)	98 (47.1)	0.518	0.052	181 (43.5)	98 (47.1)	0.442	0.072
Follow-up days, mean (SD)	1,270.56 (923.82)	1,087.02 (935.48)	0.006	0.197	1,057.33 (849.33)	1,087.02 (935.48)	0.691	0.033
PACs, mean (SD)	2,540.70 (5,961.64)	3,202.32 (6,886.76)	0.131	0.103	3,293.86 (8,308.50)	3,202.32 (6,886.76)	0.891	0.012
HTN, N (%)	1,402 (60.8)	164 (78.8)	<0.001	0.402	323 (77.6)	164 (78.8)	0.811	0.029
DM, N (%)	608 (26.4)	69 (33.2)	0.041	0.150	119 (28.6)	69 (33.2)	0.280	0.099
Dyslipidemia, N (%)	1,078 (46.7)	131 (63.0)	<0.001	0.331	267 (64.2)	131 (63.0)	0.837	0.025
HF, N (%)	340 (14.7)	29 (13.9)	0.835	0.023	41 (9.9)	29 (13.9)	0.164	0.126
CAD, N (%)	341 (14.8)	41 (19.7)	0.072	0.131	73 (17.5)	41 (19.7)	0.583	0.056
PAOD, N (%)	73 (3.2)	2 (1.0)	0.115	0.155	3 (0.7)	2 (1.0)	>0.999	0.026
Stroke, N (%)	237 (10.3)	20 (9.6)	0.857	0.022	34 (8.2)	20 (9.6)	0.651	0.051
CKD, N (%)	729 (31.6)	45 (21.6)	0.004	0.227	73 (17.5)	45 (21.6)	0.263	0.103
HCM, N (%)	48 (2.1)	9 (4.3)	0.066	0.128	13 (3.1)	9 (4.3)	0.591	0.063
Aspirin, N (%)	259 (11.2)	50 (24.0)	<0.001	0.341	93 (22.4)	50 (24.0)	0.711	0.040
P ₂ Y ₁₂ inhibitor, N (%)	95 (4.1)	27 (13.0)	<0.001	0.321	44 (10.6)	27 (13.0)	0.449	0.075
Warfarin, N (%)	18 (0.8)	2 (1.0)	1.000	0.020	5 (1.2)	2 (1.0)	>0.999	0.023
NOAC, N (%)	21 (0.9)	4 (1.9)	0.296	0.086	7 (1.7)	4 (1.9)	>0.999	0.018
ACEI/ARB, N (%)	207 (9.0)	39 (18.8)	<0.001	0.286	69 (16.6)	39 (18.8)	0.575	0.057

AAD, antiarrhythmic drug; ACEI/ARB, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker; AF, atrial fibrillation; CAD, coronary artery disease; CKD, chronic kidney disease; DM, diabetes mellitus; HCM, hypertrophic cardiomyopathy; HF, heart failure; HTN, hypertension; NOAC, novel oral anticoagulant; PAOD, peripheral arterial occlusive disease; PAC, premature atrial complex; SMD, standardized mean difference; Tx, treatment.

18 years and who were followed up for less than 180 days were excluded. In total 2,978 patients who had a history of AF, as documented by electrocardiography or 24-h Holter recording before the indexed Holter examination were also excluded. For patients with repeated examinations, the earliest PAC burden and clinical information were used for analysis. The final study cohort included 11,925 patients, who were divided into high-burden (≥ 100 beats/24h; $n = 2,937$) or low-burden (< 100 beats/24h; $n = 8,988$) sub-cohorts. In each sub-cohort, patients prescribed with regular beta blockers during $\geq 80\%$ of the entire follow-up period were designated as the treatment group, while patients who never or seldomly ($\leq 20\%$ of the follow-up period) used beta blockers were designated as the non-treatment group, and were selected for analysis. The follow-up period was defined as 3 months before the index Holter examination until the last date on the hospital electronic medical record.

Definition of Clinical Characteristics and Endpoints

The endpoints of this study respectively included all-cause mortality, new onset of stroke or transient ischemic accident (TIA), and new onset of AF. Baseline characteristics, comorbidities, and medications were all recorded on the date of enrollment. To ensure the accuracy of patient diagnoses, each variable was determined comprehensively based on the attending physician's manual input, laboratory results, corresponding treatment, and International Classification of Diseases (ICD) codes (2). All used medications were defined as regular prescriptions if they were given for more than 70%

of the follow-up period. Mortality data was retrieved from the Collaboration Center of Health Information Application, Ministry of Health and Welfare in Taiwan, and further confirmed through linkage with the National Death Registry.

24-h Holter Monitoring

All patients were asked to follow their daily routines without any limitations during the recording period. A DR200/HE Holter (NorthEast Monitoring, Inc., Maynard MA, USA) with a frequency response of 0.05 to 70 hertz in 180 samples/second mode was used (2), with 7-lead placements to acquire triple-channel information: V5 (–, right manubrium; +, left anterior axillary line on the 5th rib), V1 (–, left of the manubrium; +, 2 cm right of the xiphoid process), and lead III (–, centered on the manubrium; +, left of the mid-clavicular line on the 5th rib). All recordings were analyzed using Holter LX Analysis (NorthEast Monitoring, Inc.), with the system programmed to automatically capture all ectopic beats or rhythmic disturbances. The recordings were reviewed by experienced technicians. PAC and PVC (premature ventricular complex) were defined as coupling interval < 90 and $< 80\%$ of the last coupling interval, respectively. Supraventricular and ventricular tachycardia episodes were defined as three or more consecutive supraventricular or ventricular beats, respectively, at a speed of more than 120 beats per minute. A PAC or a supraventricular event was considered when QRS duration was less than 120 milliseconds, unless aberrant morphology of QRS was detected, whereupon this would be considered as a PVC or ventricular tachycardia event. The cumulative number of

TABLE 2 | Demographic and clinical characteristics of patients with a low burden of PACs in the treatment and non-treatment groups before and after matching.

Variables	Before matching				After matching			
	Non-Tx (N = 7,139)	Tx (N = 614)	p	SMD	Non-Tx (N = 1,228)	Tx (N = 614)	p	SMD
Age, y, mean (SD)	56.55 (15.99)	61.01 (12.72)	<0.001	0.309	61.70 (14.49)	61.01 (12.72)	0.317	0.051
Male, N (%)	3,218 (45.1)	296 (48.2)	0.146	0.063	608 (49.5)	296 (48.2)	0.633	0.026
Follow-up days, mean (SD)	1,344.04 (995.22)	1,095.01 (1,033.18)	<0.001	0.246	1,045.11 (900.22)	1,095.01 (1,033.18)	0.286	0.051
PACs, mean (SD)	19.45 (22.67)	21.36 (23.18)	0.046	0.083	22.14 (23.49)	21.36 (23.18)	0.499	0.034
HTN, N (%)	3,042 (42.6)	441 (71.8)	<0.001	0.618	942 (76.7)	441 (71.8)	0.026	0.112
DM, N (%)	1,446 (20.3)	173 (28.2)	<0.001	0.186	359 (29.2)	173 (28.2)	0.676	0.023
Dyslipidemia, N (%)	3,208 (44.9)	408 (66.4)	<0.001	0.444	828 (67.4)	408 (66.4)	0.713	0.021
HF, N (%)	549 (7.7)	64 (10.4)	0.020	0.095	131 (10.7)	64 (10.4)	0.936	0.008
CAD, N (%)	623 (8.7)	114 (18.6)	<0.001	0.290	216 (17.6)	114 (18.6)	0.652	0.025
PAOD, N (%)	100 (1.4)	11 (1.8)	0.545	0.031	24 (2.0)	11 (1.8)	0.952	0.012
Stroke, N (%)	452 (6.3)	46 (7.5)	0.298	0.046	111 (9.0)	46 (7.5)	0.302	0.056
CKD, N (%)	1,103 (15.5)	107 (17.4)	0.216	0.053	236 (19.2)	107 (17.4)	0.386	0.046
HCM, N (%)	97 (1.4)	9 (1.5)	0.970	0.009	18 (1.5)	9 (1.5)	>0.999	<0.001
Aspirin, N (%)	501 (7.0)	197 (32.1)	<0.001	0.666	374 (30.5)	197 (32.1)	0.510	0.035
P ₂ Y ₁₂ inhibitor, N (%)	191 (2.7)	74 (12.1)	<0.001	0.365	126 (10.3)	74 (12.1)	0.278	0.057
Warfarin, N (%)	29 (0.4)	5 (0.8)	0.250	0.052	11 (0.9)	5 (0.8)	>0.999	0.009
NOAC, N (%)	13 (0.2)	1 (0.2)	1.000	0.005	3 (0.2)	1 (0.2)	>0.999	0.018
ACEI/ARB, N (%)	442 (6.2)	151 (24.6)	<0.001	0.527	299 (24.3)	151 (24.6)	0.954	0.006

AAD, antiarrhythmic drug; ACEI/ARB, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker; AF, atrial fibrillation; CAD, coronary artery disease; CKD, chronic kidney disease; DM, diabetes mellitus; HCM, hypertrophic cardiomyopathy; HF, heart failure; HTN, hypertension; NOAC, novel oral anticoagulant; PAOD, peripheral arterial occlusive disease; PAC, premature atrial complex; SMD, standardized mean difference; Tx, treatment.

PACs during the monitoring period was presented as beats/24 h. All arrhythmic episodes, unknown strips, and final formal 24-h Holter reports were reviewed and confirmed by qualified senior cardiologists.

Statistical Analysis

Categorical variables were presented as frequencies and percentages, while continuous variables were reported as means with standard deviations (SD). Chi-squared test with two-tailed Fisher's exact test and Student's *t*-test were respectively used for intergroup comparison of categorical and continuous variables. In order to exclude all possible confounders in each group, propensity score matching between patients who regularly took beta blockers (treatment group) with patients who never or seldomly took beta blockers (non-treatment group) was conducted at a ratio of 1:2 for both high-burden and low-burden sub-cohorts, using a near-neighbor matching algorithm with caliper width of 0.2. The matched variables included age, sex, PAC burden, follow-up days, medical history (diabetes mellitus, dyslipidemia, hypertension, stroke, coronary artery disease, chronic kidney disease, heart failure, peripheral arterial disease, hypertrophic cardiomyopathy), and drug history (aspirin, angiotensin converting enzyme inhibitor/angiotensin receptor blocker, diuretics, P₂Y₁₂ inhibitor, warfarin, and non-vitamin K antagonist oral anticoagulants). Analysis of survival data was then conducted to evaluate the effect of beta blocker treatment. The primary and secondary endpoints were analyzed using the Cox-proportional hazard model. Univariate analysis of beta

blocker use against endpoints was performed, and hazard ratios (HR) with 95% confidence intervals (CI) were calculated. In addition, cumulative event-rate curves were plotted, and the log-rank test was used to compare the survival distributions of treatment and non-treatment groups in each sub-cohort. All statistical tests were 2-sided, and *p* value < 0.05 was considered to be statistically significant. All analyses were performed with R statistical software, version 3.6.3 for Windows.

RESULTS

Baseline Characteristics of the Study Cohort

Baseline characteristics of patients in the high-burden and low-burden sub-cohorts are presented by beta blocker treatment in **Tables 1, 2**. In the high-burden sub-cohort, 2,307 and 208 patients were respectively included in the non-treatment and treatment groups. Mean follow-up was respectively 1,270.56 ± 923.82 days and 1,087.02 ± 935.48 days. No differences were noted in age, sex, and medical history, including stroke, coronary artery disease, heart failure, peripheral arterial disease, hypertrophy cardiomyopathy, use of non-vitamin K antagonist oral-anticoagulants or warfarin, PAC burden, and PVC (premature ventricular complex) burden (980.9 ± 3,313.5 in non-treatment group and 1,052.9 ± 3,609.8 in treatment group, *p* = 0.650). Patients in the treatment group had shorter follow-up duration, more comorbidities, including diabetes mellitus, dyslipidemia, hypertension, and chronic kidney disease, and

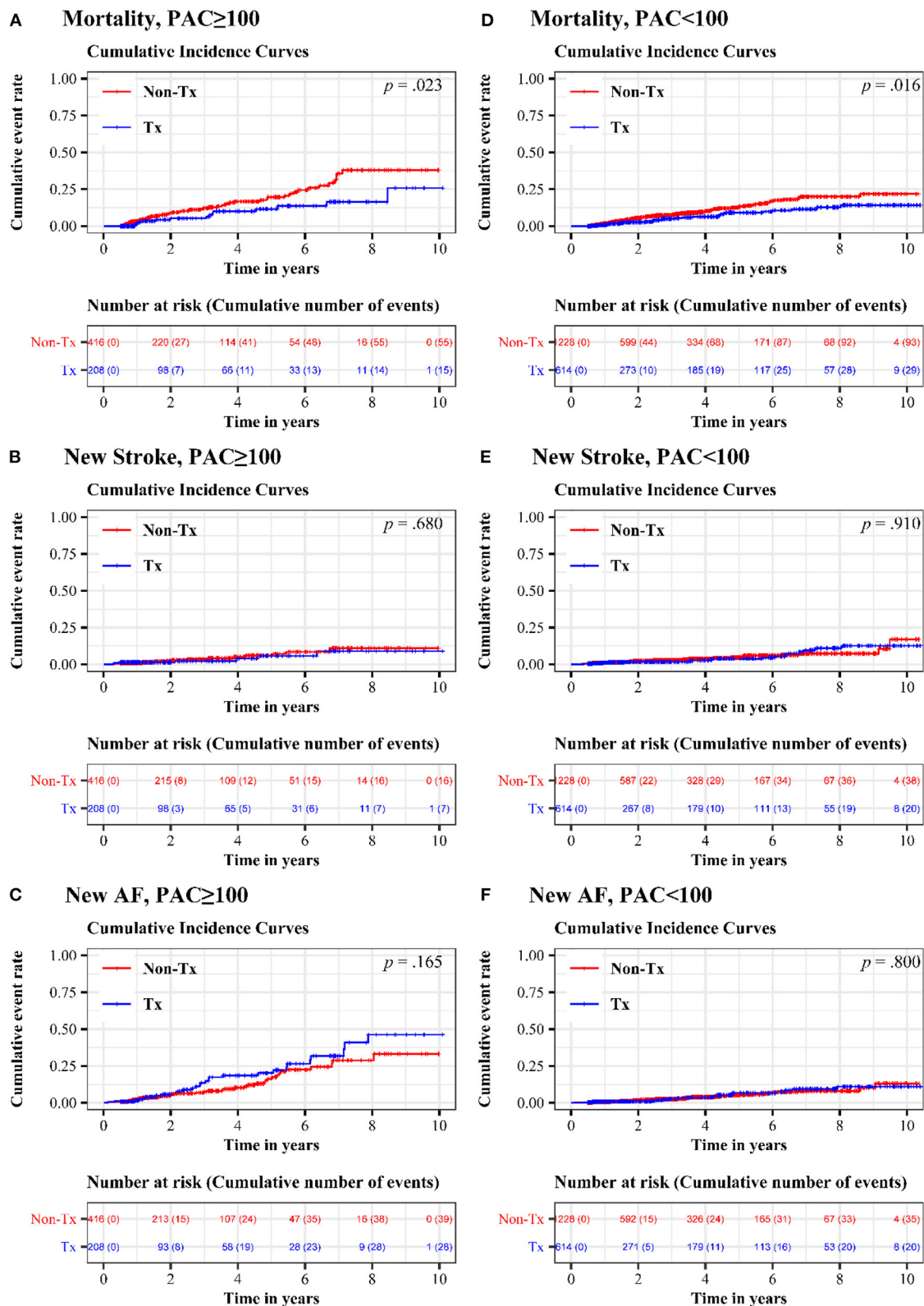


FIGURE 2 | Cumulative incidence of mortality, new stroke, and new AF in treatment and non-treatment groups. In the high-burden sub-cohort, comparison of (A) long-term all-cause mortality (B) long-term cumulative new stroke rate and (C) long-term cumulative new onset rates of AF were exhibited. In the low-burden sub-cohort, (D) long-term all-cause mortality (E) long-term cumulative new stroke rate and (F) long-term cumulative new onset rates of AF were shown. AF, atrial fibrillation; PAC, premature atrial complex.

took more medications (e.g. aspirin, P2Y12 inhibitors, and angiotensin converting enzyme inhibitors/angiotensin receptor blockers) than patients in the non-treatment group before propensity score matching. For patients in the low-burden sub-cohort, the treatment group ($n = 614$) was much older, underwent shorter follow-up ($1,095.01 \pm 1,033.18$ days vs. $1,344.04 \pm 995.22$ days), had more comorbidities, including diabetes mellitus, hyperlipidemia, hypertension, coronary artery disease, heart failure, higher PAC burden, and used more medications (similar to those in the high-burden sub-cohort), than the non-treatment group ($n = 7,139$). However, treatment group had less PVC burdens ($1,017.6 \pm 4,705.9$ vs. $903.0 \pm 3,630.6$, $p < 0.001$). After propensity score matching, no differences in any variables between the treatment and non-treatment groups of each sub-cohort were noted. Although we did not further match PVC burdens, no difference in PVC burden was found among high-burden sub-cohort (non-treatment group vs. treatment group = $980.9 \pm 3,313.5$ vs. $1,052.9 \pm 3,609.8$, $p = 0.059$). Small difference was noted among low-burden sub-cohort ($969.9 \pm 3,666.9$ vs. $903.0 \pm 3,630.6$, $p = 0.049$). The study flow is presented in **Figure 1**.

Long-Term Prognosis in Patients With High PAC Burden

Figure 2 shows the 10-year cumulative incidence of each endpoint in patients with high and low PAC burdens after propensity score matching. Compared to patients who never or seldomly used beta blockers, patients in the treatment group had 48% risk reduction in long-term all-cause mortality (**Figure 2A** and **Table 3**, HR = 0.521, 95% CI = 0.294–0.923, $p = 0.025$). No significant difference in long-term cumulative new stroke rate was found between these two groups (**Figure 2B** and **Table 3**, HR = 0.830, 95% CI = 0.341–2.020, $p = 0.681$), as was also the case for long-term cumulative new onset rates of AF (**Figure 2C** and **Table 3**, HR = 1.410, 95% CI = 0.867–2.292, $p = 0.167$).

Long-Term Prognosis in Patients With Low Burdens of PACs

Regular beta blocker use was associated with up to 40% risk reduction in long-term all-cause mortality (**Figure 2D** and **Table 3**, HR = 0.601, 95% CI = 0.396–0.913, $p = 0.017$), but no significant differences between the treatment and non-treatment groups in new onset of stroke (**Figure 2E** and **Table 3**, HR = 0.969, 95% CI = 0.562–1.670, $p = 0.910$) and new onset of AF (**Figure 2F** and **Table 3**, HR = 1.074, 95% CI = 0.619–1.863, $p = 0.800$) were noted.

Subgroup Analysis

Figures 3, 4 respectively show the beneficial effects of beta blockers on all-cause mortality in patients with high or low burdens of PACs across the overall sub-cohort and pre-specified subgroups. Regardless of PAC burden in patients, treatment with beta blockers did not provide better outcomes in terms of new-onset stroke or AF over non-treated patients in each pre-specified subgroup (**Supplementary Figures 1–4**).

TABLE 3 | Endpoint hazard ratios in high-burden and low-burden PAC sub-cohorts.

Endpoint	HR (95% CI; p), high burden	HR (95% CI; p), low burden
Mortality	0.521 (0.294, 0.923; $p = 0.025$)	0.601 (0.396, 0.913; $p = 0.017$)
New stroke	0.830 (0.341, 2.020; $p = 0.681$)	0.969 (0.562, 1.670; $p = 0.910$)
New AF	1.410 (0.867, 2.292; $p = 0.167$)	1.074 (0.619, 1.863; $p = 0.800$)

AF, atrial fibrillation; CI, confidence interval; HR, hazard ratio.

Interval Change of PAC Burdens and Beta Blockers Regimen in Treatment Group

In our **Supplementary Table 1**, we presented the mean difference of PAC burdens among patients after PSM undergoing followed up Holter monitoring. Irrespective of treatment with beta blockers, the PAC burden among the high-burden sub-group was reduced, instead of lower-burden sub-group. **Supplementary Figures 5, 6** exhibited respectively the proportion of different beta-blocker prescriptions in treatment group, and the mean daily dosage of beta blockers. The most common prescription was bisoprolol (mean dosage: 3.8 ± 1.6 mg) followed by propranolol (mean dosage: 22.1 ± 13.5 mg).

DISCUSSION

In this long-term follow-up study, we used propensity score matching to show that beta blocker treatment can lower all-cause mortality in patients with high or low PAC burdens. However, no difference in new onset of stroke or AF between treatment and non-treatment groups was found. To the best of our knowledge, this study is the first study to elucidate the beneficial effects of beta blockers on the long-term prognosis of patients with PACs, which are considered to be key risk factors for all-cause mortality and major cardiovascular adverse events (2–6, 8). Although strong evidence is still lacking, the latest consensus document by the European Heart Rhythm Association (9) suggests discussing the initiation of oral anticoagulants for the prevention of stroke with patients that have a high PAC burden (>500 PACs per 24 h or any episode of runs >20 PACs). This suggestion is based on the dose-response effect of PACs on the risk of AF (10). However, besides oral anticoagulation, the treatment of patients with high PAC burdens remains an important unmet clinical need.

Beta blockers targeting the autonomic nervous system are well-known for their efficacy in improving symptoms, reducing hospitalizations, and/or prolonging survival for heart failure patients in randomized controlled studies (11–14). PACs share the same main feature as heart failure (e.g. sympathetic overactivation) (7), and thus in our daily practice, beta blockers are commonly prescribed to treat symptomatic patients with PACs. It is reasonable to explore their effects beyond symptom control. In this study, treatment with beta blockers significantly decreased the risk of all-cause mortality across patients with high or low PAC burdens. Although decreased mortality in the treatment group may be partially due to the well-known benefits of beta blockers in heart failure and sudden cardiac death, our study also demonstrated the consistent benefits in

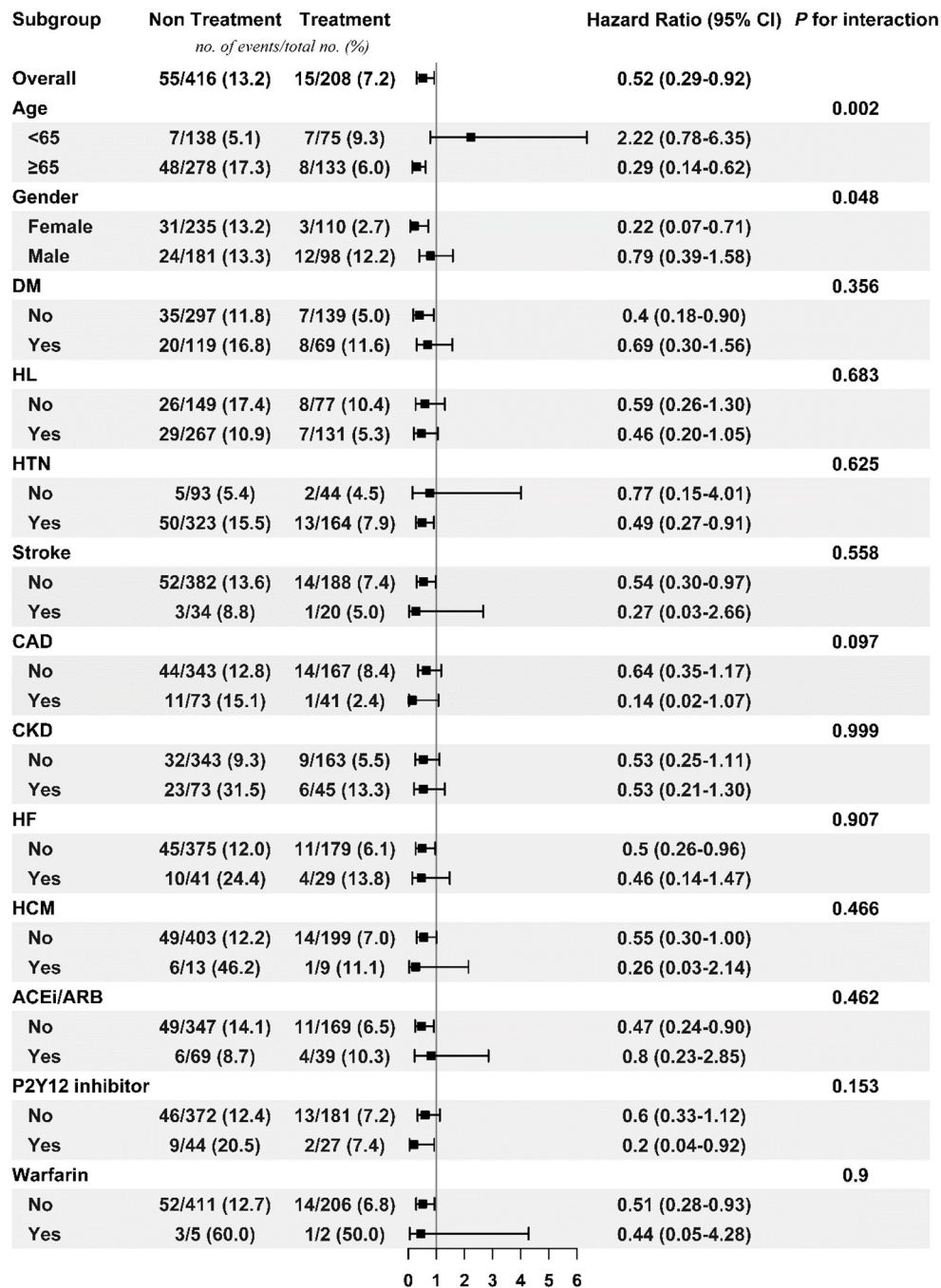


FIGURE 3 | Subgroup analysis of treatment effect on mortality in the high PAC burden sub-cohort. ACEi/ARB, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker; CAD, coronary artery disease; CKD, chronic kidney disease; DM, diabetes mellitus; HCM, hypertrophic cardiomyopathy; HF, heart failure; HL, hyperlipidemia; HTN, hypertension; PAC, premature atrial complex.

lowering mortality rate of patients without heart failure in the treatment group, as shown in **Figures 3, 4**. While concerning similar but statistically significant difference in PVC burdens between treatment and non-treatment groups in low-burden PAC sub-cohort, in our recent data (15), moderate (1,000–10,000 beats per 24 h) and high burdens (>10,000 beats per 24 h) of

PVCs had higher risk of cardiovascular death than low-burden PVC group (Find and Gray's competing risk model adjusted HR = 1.48, 95% CI = 1.09–2.01, $p < 0.05$; HR = 1.70, 95% CI = 1.06–2.71, $p < 0.05$). However, our data showed no difference in all-cause mortality. Our current study focused on the all-cause mortality, but not cardiovascular death in PAC patients taking

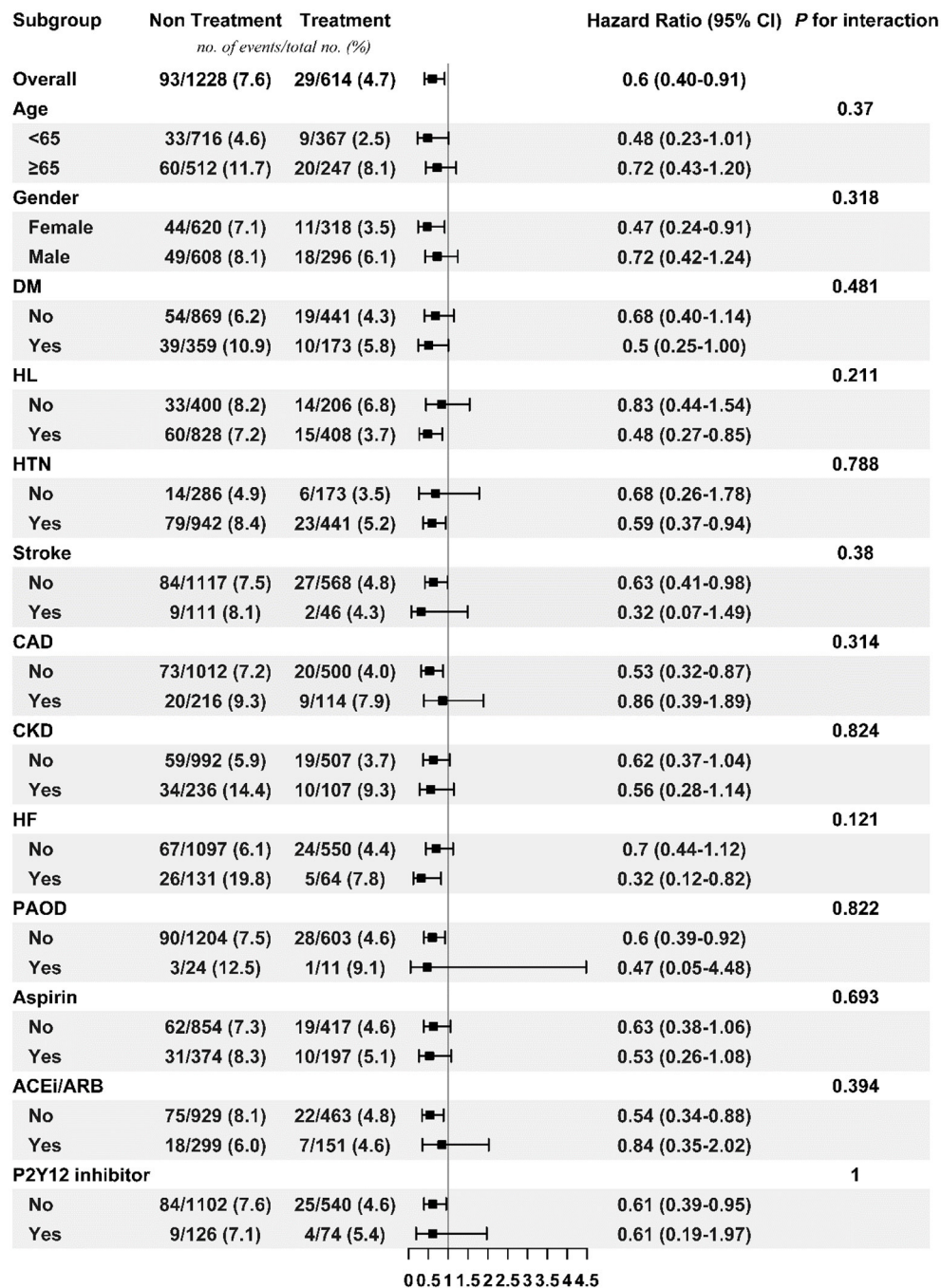


FIGURE 4 | Subgroup analysis of treatment effect on mortality in the low PAC burden sub-cohort. ACEi/ARB, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker; CAD, coronary artery disease; CKD, chronic kidney disease; DM, diabetes mellitus; HF, heart failure; HL, hyperlipidemia; HTN, hypertension; PAC, premature atrial complex; PAOD, peripheral arterial occlusive disease.

beta blockers. We believed that this difference would not affect our main results.

The benefits of beta blockers were not apparent in the prevention of new-onset stroke for both the high-burden and low-burden sub-cohorts, and the existing data regarding this is somewhat mixed; Ziff et al. previously reported that

compared with a placebo group, the risk of stroke decreased in hypertensive patients treated with beta blockers, but beta blockers were less effective than angiotensin converting enzyme inhibitors/angiotensin receptor blockers and calcium channel blockers (16). No benefits of stroke secondary prevention for patients in the acute phase after stroke (17), with coronary artery

disease, or who required cardiac or non-cardiac surgery were noted (16).

The main pathophysiology of PACs as precursors of AF has been attributed to enhanced automaticity or trigger activity related to adrenergic overactivation (7). Regional autonomic modulation to decrease sympathetic outflow may be effective in preventing atrial arrhythmia. Systemic beta blockers have been proven to prevent AF occurrence or recurrence, and continuous treatment with metoprolol CR starting at least one week before direct current cardioversion has been shown to be effective in maintaining sinus rhythm at 6 months after cardioversion (18). Prophylactic beta blockers to prevent postoperative AF is a well-established practice that should be started or continued before cardiac surgery (19). As mentioned in the latest ESC AF guideline (20), some small studies showed the benefits in preventing AF occurrence or recurrence (18, 21); however, most evidence were against a significant role of beta-blockers in preventing AF (22). The observational beneficial effects were derived from clinically significant and symptomatic AF to silent AF, by the effect of rate control from beta-blockers. Similarly, this study showed that regardless of high or low PAC burden, there was no difference in the incidence of new onset AF between treatment and non-treatment groups. Another possibility is that this may be because patients are mostly asymptomatic or already familiar with PAC-related symptoms, and therefore fewer electrocardiography or 24-h Holter monitor tests would be requested, thereby lessening the chance of detecting AF in such patients during follow-up.

In the high burden population younger than 65 years old, beta blockers significantly increased the risk of new-onset AF and were less effective on mortality prevention. In fact, several possibilities were hypothesized: first, these patients were very symptomatic and thus took regular beta blockers and presumed followed up Holter or ECG studies were arranged more frequently, and thus more AF episodes were detected. Another possible reason was that, as mentioned above, beta blockers could not significantly prevent AF occurrence. Finally, although the incidence rates of new-onset AF between high burden and low burden sub-cohort were not directly compared, it was interesting to note that higher event rate in high burden than low burden sub-cohort irrespective of treatment. Adrenergic overactivation was that overwhelmed in this young subgroup, and more AF indeed occurred and worsened the long-term prognosis.

Limitations

Because of the retrospective nature of this study, many patients did not do follow-up Holter monitoring after receiving medication, and thus it is uncertain whether greater reduction in PAC burden or improvement in heart rate variability would be associated with better outcomes. In addition, as mentioned above, new-onset AF may be underestimated in this cohort, perhaps due to a high prevalence of asymptomatic patients or increased familiarity with arrhythmia-related symptoms, resulting in fewer test requests and under-detection of AF. Finally, it was not possible to determine if patients were symptomatic or not in this study, and therefore it is unknown

whether the effects of beta blockers are consistent across symptomatic and asymptomatic patients.

CONCLUSIONS

In this retrospective long-term propensity-matched cohort study, beta blocker usage was associated with lower all-cause mortality in patients with baseline high or low PAC burdens. Interestingly, this effect was not directly associated with reduction of new-onset stroke or AF, and further research to identify the underlying mechanism(s) is warranted.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board, National Cheng Kung University Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

T-CH contributed to conception and design of the study and wrote the first draft of the manuscript. T-CH, P-TL, and M-SH conducted data collection and cleaning from the Databank. P-HC performed the statistical analysis. P-FS conducted statistical validation. T-CH and P-HC prepared the figures and tables. P-FS and P-YL edited and reviewed the manuscript. All authors reviewed, edited, and approved of the final submitted manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcvm.2022.806743/full#supplementary-material>

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Randomized Controlled Trials of Zhigancao Decoction Combined With Metoprolol in the Treatment of Arrhythmia: A Systematic Review and Meta-Analysis

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Objective: Cardiac arrhythmia remains a major public health problem worldwide. Combinations of traditional medicine (TM) and conventional medicine (CM) have been used for arrhythmia treatment, yet the effectiveness and safety of many TM preparations can be controversial. We analyzed the safety and effectiveness of Zhigancao decoction (ZGCD) combined with metoprolol for arrhythmia treatment.

Methods: Systematic searches for randomized clinical trials (RCTs) were conducted in eight databases (January 2010–September 2020) without language restrictions. According to the Cochrane system evaluation method, the overall effectiveness and safety were evaluated by meta-analysis using Review Manager software (version 5.3), and publication bias was qualitatively analyzed using STATA 12.0.

Results: A total of 39 RCTs were incorporated, including 4,260 patients with arrhythmia, with 2,133 patients in the experimental group (ZGCD + metoprolol, ZGCD + BB) and 2,127 patients in the control group (metoprolol only, BB). Meta-analysis revealed that compared with BB, ZGCD + BB could significantly increase the total efficacy (OR = 4.74, 95% CI: 3.78–5.94, $P < 0.01$) and lower the incidences of arrhythmia (MD = -3.39, 95% CI: -4.09 to -2.68, $P < 0.01$). Moreover, mean HR reductions were reported in patients receiving ZGCD + BB the ZGCD + BB group (MD = -8.48, 95% CI: -10.98 to -5.97, $P < 0.01$) and a decrease in TCM symptoms were reported also (MD = -2.92, 95% CI: -3.08 to -2.76, $P < 0.01$). The incidence of adverse events was lower in patients treated with ZGCD + BB (RR = 0.36, 95% CI: 0.26–0.51, $P < 0.01$). These results appeared consistent across common arrhythmias. Nevertheless, the majority of included studies were unable to be formally assessed for bias, and funnel-plot analysis implied a moderate risk of publication bias.

Conclusion: ZGCD + BB appeared to demonstrate good efficacy and fewer adverse reactions compared to BB in the treatment of arrhythmia, and this may represent a useful

complementary therapy. However, our findings must be cautiously evaluated because of the small sample size and low quality of the clinic trials cited in the review. Rigorous and large-scale RCTs are warranted in the future to confirm these results.

Systematic Review Registration: <https://inplasy.com/inplasy-2021-10-0045/>.

Keywords: zhigancao decoction, metoprolol, arrhythmia, meta-analysis, randomized controlled trial

INTRODUCTION

Arrhythmia refers to the abnormal origin or conduction of cardiac activation, resulting in an abnormal heart frequency and/or rhythm. Arrhythmia continues to be a common public health problem worldwide. In China, about 520,000 patients with cardiovascular disease (CVD) die from malignant arrhythmias every year (1). Drugs treating arrhythmia are mainly conventional medicine (CM), but many may cause arrhythmias and even fatal adverse events themselves; thus, their application is limited (2). Metoprolol, slows the heart rate and inhibits cardiac contractility by blocking β -adrenoceptors. Metoprolol is widely used to treat arrhythmias clinically, but has known adverse effects, such as nausea, dizziness, headache, and bradycardia (3).

In China, arrhythmia is frequently treated using a combination of CM and traditional medicine (TM). Zhigancao decoction (ZGCD) recorded in Treatise on Febrile Disease by Zhang Zhongjing in the Han dynasty have been widely used in treating palpitation and irregular pulse for thousands of years in China (4). ZGCD has a unique curative effect in arrhythmia treatment that involves a two-way benign regulatory effect. Its regulatory effects on ion channels, hemodynamics, cardiomyocyte electrophysiology, and related processes have been verified (5). Furthermore, some clinical reports have reported that ZGCD combined with metoprolol has advantages in terms of total efficacy and arrhythmia control. However, because of lack of reliable medical evidence, the effectiveness of this combination remains controversial. Hence, this systematic review and meta-analysis of published randomized clinical trials (RCTs) of ZGCD + BB in arrhythmia treatment was performed.

METHODS

Protocol and Registration

This study protocol was registered and approved by INPLASY (Registration number INPLASY2021100045).

Search Strategy and Selection Criteria

PubMed, Cochrane Library, Web of Science, Clinical Trials, CNKI, VIP, CBM, and Wanfang databases were searched, and the retrieval time was limited to September 2020. The Chinese keywords were xinlvshichang, zhigancaotang, fumaitang, meituoluoe, beitalake, and suijiduihao. Other key words were arrhythmia, arrhythmia, arrhythmias, arrhythmic, cardiac arrhythmia, prepared licorice decoction, roast glycyrrhiza decoction, roasted licorice decoction, zhigancao decoction, metoprolol, and randomized controlled trials. Logical operators were used to formulate retrieval styles using these words as

keywords or free words, and manual retrieval methods were employed. If the reviewers had any questions about the studies, the corresponding author was consulted.

Inclusion Criteria

Participants

The study included patients who conformed to the clinical diagnosis of arrhythmia with recurrent symptoms such as palpitation, shortness of breath, and chest tightness and confirmed clinical diagnosis using electrocardiogram, relevant laboratory findings, and imaging examinations. Patients with severe liver and kidney diseases, hematopoietic system diseases, acute infection, and grade IV heart function were excluded from the study. Only RCTs were included in this meta.

Intervention Measures

The intervention group was treated with ZGCD + BB, while the control group was treated with BB only. Patients in both groups were administered basic treatment for their primary disease, such

TABLE 1 | The outcome indicators.

Outcome indicators	Criteria	Data expression
Total efficacy	(1) Significantly effective events: clinical symptoms and signs essentially disappeared, the number of arrhythmias decreased by more than 90%, and ECG results returned to normal (2) Effective events: clinical symptoms were relieved to a certain extent, the number of arrhythmias was reduced by 50–90%, and ECG results improved (3) Ineffective events: clinical symptoms did not improve or even worsened, the number of arrhythmias decreased by <50%, and there was no significant change in ECG results	The number of (1) + (2) cases
Incidences of arrhythmia	The number of arrhythmias that occurred in the experimental and control groups after treatment was recorded in 24 h	$\bar{x} \pm$
HR of Arrhythmia	Heart rate was measured after wearing the dynamic ECG for 24 h	$\bar{x} \pm s$
TCM syndrome score	According to the main symptoms, such as chest tightness, palpitation, and fatigue sweating, the scores were as follows: asymptomatic, 0; mild, 1; severe, 3; the higher the score, the more serious the condition was.	$\bar{x} \pm s$
Adverse events	Adverse events included nausea, vomiting, dizziness, headache, and bradycardia, among others	The number of cases

as hypotension, lipid lowering, hypoglycemia, anticoagulation, and antiplatelet therapies, and other intervention measures.

Outcome Indicators

We have list the outcome indicators in the **Table 1**.

Literature Exclusion Criteria

① Diagnostic method was not clear; ② Experimental and control groups were not consistent with the above intervention measures or the description of the treatment method was not provided; ③ Outcome index could not be counted; ④ Non-RCTs and non-clinical trial studies; ⑤ Duplicate publications or incomplete studies; ⑥ The full text of the publication was not available.

Data Extraction

Data extraction was independently performed by two researchers, and the relevant studies were extracted. If differences arose during this period, they were resolved through joint discussion, with assistance from a third researcher, if necessary.

Assessment of Trial Quality

The methodology quality evaluation of the included studies was performed using the “bias risk assessment” tool recommended by Cochrane Handbook 5.0. The quality of the included studies was evaluated in terms of the random allocation method, allocation concealment, blinding method, integrity of the results data, and selective reporting of bias of the research results.

We selectively reported the bias of the research results and other aspects of quality evaluation. For each study, the above items were evaluated as “yes” (low bias), “no” (highly biased), or “unclear” (lack of relevant information or uncertainty of bias).

Statistical Analysis

RevMan 5.3 software, provided by the Cochrane collaboration network, was used in the analysis. The two classification variables used OR as the curative effect analysis statistics, and the numerical variables used the mean difference (MD) as the curative effect analysis statistics. Each effect was expressed as a 95% confidence interval (CI). The chi-square test was used to analyze heterogeneity among the studies. When there was a high degree of statistical heterogeneity among the studies ($P < 0.1$, $I^2 > 50\%$), the random effects model was used; otherwise, the fixed effect model was used.

RESULTS

Search Results

A total of 147 studies were retrieved from the search results, and 39 studies (6–44) were included after reading abstracts and full texts. Exclusions comprised duplicate studies, case reports, reviews, retrospective studies, non-randomized controlled trials, and inconsistent trial bases (**Figure 1**).

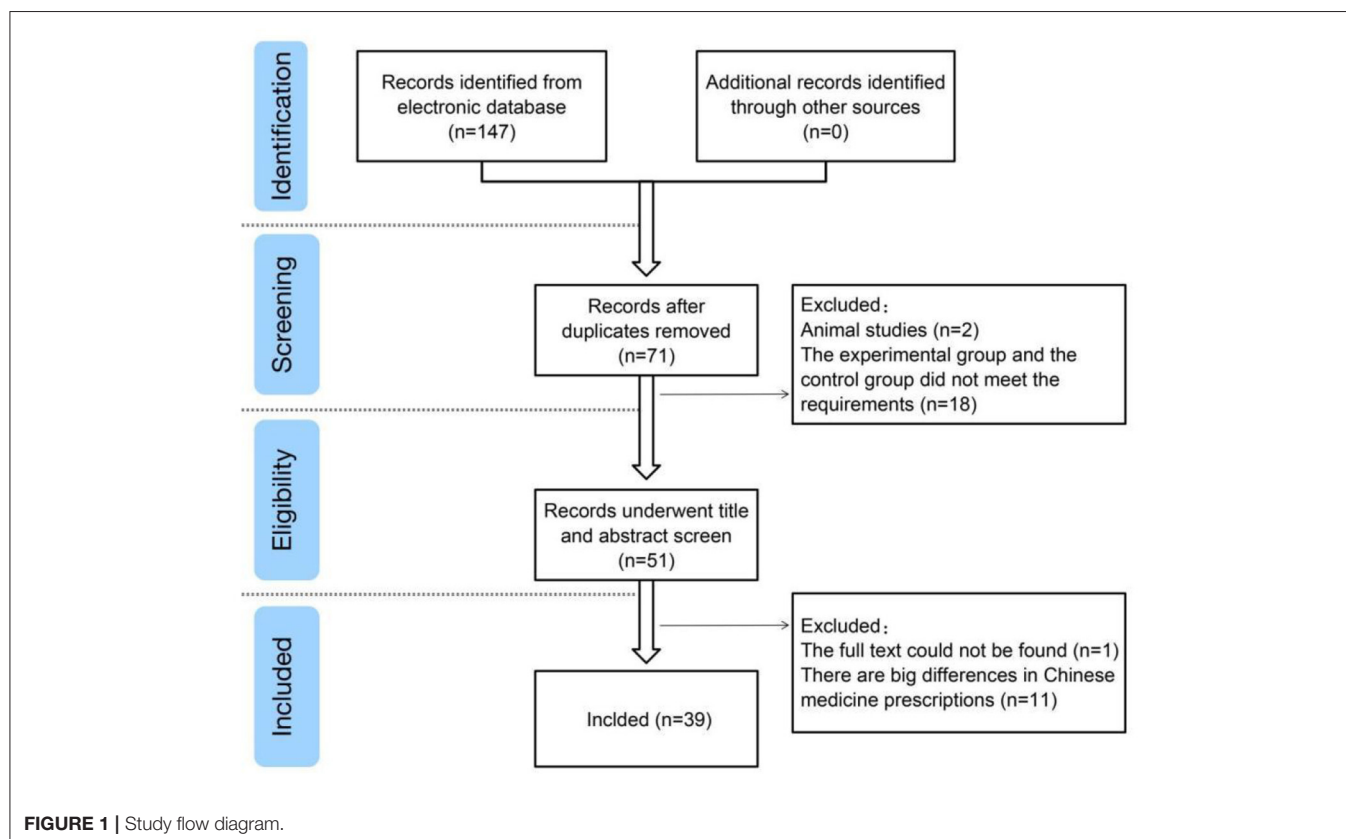


TABLE 2 | Basic characteristics of the included studies.

References	Course of treatment	Control (n)	Trial (n)	Age		Duration		Dosage		Outcome indicators
				C	T	C	T	C	T	
Aidufeng (6)	4 W	39	40	55.8 ± 5.1	56.4 ± 4.8	10.8 ± 1.3 M	11.1 ± 1.7 M	Metoprolol 25 mg/dose, bid	ZGCD bid+Metoprolol 25 mg/dose, bid	①②
Caoyunyan (9)	2 W	57	57	72.5 ± 8.4	73.5 ± 8.9			Metoprolol 50 mg/dose, bid	ZGCD bid+Metoprolol 50 mg/dose, bid	①③
Chenting (8)	12 W	30	30	63.7 ± 9.5	62.1 ± 8.9	3.9 ± 1.7 Y	3.7 ± 1.4 Y	Metoprolol 15 mg/dose→50 mg/dose	ZGCD bid+Metoprolol 15 mg/dose→50 mg/dose	①③⑤
Duanajing (11)	2 W	50	50	62.03 ± 3.74	61.25 ± 3.87			Metoprolol 25 mg→100 mg, bid	ZGCD bid+Metoprolol 25 mg→100 mg, bid	①③④⑤
Guanhui (13)	1 M	40	40	67.61 ± 7.36	67.55 ± 6.78			Metoprolol 6.25 mg/dose, tid; 6.25–12.5 mg/dose, bid, no more than 300–400 mg/d	ZGCD bid + Metoprolol 6.25 mg/dose, tid; 6.25–12.5 mg/dose, bid, no more than 300–400 mg/d	①
Hedeying (14)	4 W	78	78					Metoprolol 12.5 mg, qd	ZGCD tid + Metoprolol 12.5 mg, qd	①②
Linwenzhi (18)	8 W	30	30	56.01 ± 2.11	55.38 ± 2.67	12.63 ± 2.27 M	12.59 ± 2.43 M	Metoprolol 47.5 mg, qd	ZGCD bid + Metoprolol 47.5 mg, qd	①③⑤
Liumengzhen (19)	4 W	71	71	64.3 ± 5.2	64.8 ± 5.1	5.8 ± 2.6 Y	5.7 ± 2.8 Y	Metoprolol 25 mg/dose, bid	ZGCD bid + Metoprolol 25 mg/dose, bid	①③
Suxin (26)	10 D	35	35	49.2 ± 2.3	48.5 ± 2.4			Metoprolol 25 mg/dose, bid	ZGCD bid + Metoprolol 25 mg/dose, bid	①②
Wanzhimin (33)	4 W	46	46	61.63 ± 5.14	61.56 ± 5.25	16.46 ± 6.21 M	16.52 ± 6.26 M	Metoprolol 23.75–47.5 mg, qd	ZGCD bid + Metoprolol 23.75–47.5 mg, qd	①
Yangzhongfen (36)	1 M	400	400	63.9 ± 5.3	64.1 ± 5.4			Metoprolol 23.75–47.5 mg, qd	ZGCD bid + Metoprolol 23.75–47.5 mg, qd	①③
Chenxiaolin (10)	4 W	47	50	61.3 ± 7.4	59.8 ± 6.7			Metoprolol 25–50 mg/dose, bid	ZGCD bid + Metoprolol 25–50 mg/dose, bid	①③
Fanxiuxia (12)	6 W	50	50					Metoprolol 50 mg/dose, bid	ZGCD tid + Metoprolol 50 mg/dose, bid	①②
Huangxiaociang (16)	1 M	48	48	59.8 ± 8.7	61.5 ± 8.2			Metoprolol 25 mg/dose, bid	ZGCD tid + Metoprolol 25 mg/dose, bid	①③
Jiangguo (17)	4 W	47	47					Metoprolol 6.25–25 mg/dose, bid	ZGCD bid + Metoprolol 6.25–25 mg/dose, bid	①②
Peiguoxian (22)	4 W	38	38	41.42 ± 5.18	38.27 ± 5.12	12.58 ± 3.62 M	13.26 ± 2.58 M	Metoprolol 23.75–47.5 mg, qd	ZGCD bid + Metoprolol 23.75–47.5 mg, qd	①
Puqinping (23)		28	28	66 ± 4.3	66 ± 4.7	2.5 ± 1.5 Y	2.5 ± 1.3 Y	Metoprolol iv, 2.5 mg, qd	ZGCD tid + Metoprolol iv, 2.5 mg, qd	①
Sunjunxiong (24)	1 M	40	40	56.1 ± 2.8	56.0 ± 3.5			Metoprolol 6.25–12.5 mg, bid, no more than 300–400 mg/d	ZGCD bid + Metoprolol 6.25–12.5 mg, bid, no more than 300–400 mg/d	①
Tangwansi (27)	10 D	30	30	60.58 ± 4.56	58.83 ± 5.18	5.85 ± 1.34 Y	5.96 ± 1.23 Y	Metoprolol 23.75 mg/dose, qd	ZGCD bid + Metoprolol 23.75 mg/dose, qd	①②③④
Wanglibin (29)	4 W	56	52					Metoprolol 11.875 mg, after 1 week 23.75 mg	ZGCD bid + Metoprolol 11.875 mg, after 1 week 23.75 mg	①⑤

(Continued)

TABLE 2 | Continued

References	Course of treatment	Control (n)	Trial (n)	Age		Duration		Dosage		Outcome indicators
				C	T	C	T	C	T	
Wanglin (30)	4 W	50	50	60.13 ± 7.33	59.85 ± 8.16			Metoprolol 25–50 mg, bid	ZGCD bid + Metoprolol 25–50 mg, bid	①③
Wangshanshan (31)	2 W	47	47	54.38 ± 3.37	55.1 ± 2.06	3.45 ± 2.08 Y	4.82 ± 1.06 Y	Metoprolol 25–100 mg, bid	ZGCD bid + Metoprolol 25–100 mg, bid	①
Wangzhe (32)	4 W	43	45	55.2 ± 5.6	55.0 ± 5.4	11.3 ± 2.7 M	11.1 ± 2.5 M	Metoprolol 25 mg, bid	ZGCD bid + Metoprolol 25 mg, bid	①②③
Wuyanpeng (34)	4 W	54	54	65.22 ± 3.12	65.26 ± 3.14	9.05 ± 1.51 Y	9.06 ± 1.54 Y	Metoprolol 23.75–47.5 mg, qd	ZGCD bid + Metoprolol 23.75–47.5 mg, qd	①③
Xushan (35)	4 W	75	75	49.15 ± 7.93	51.62 ± 8.09			Metoprolol 25 mg, qd	ZGCD bid + Metoprolol 25 mg, qd	①②
Zhangchunyan (37)	4 W	41	42	71.66 ± 5.43	72.17 ± 5.59	8.85 ± 2.38 M	8.92 ± 2.30 M	Metoprolol 6.25 mg, bid→50 mg/dose, bid	ZGCD bid + Metoprolol 6.25 mg, bid→50 mg/dose, bid	①③④
Zhangyanzhen (39)	3 M	60	60	55.0 ± 4.1	58.0 ± 3.7	5 ± 1.7 Y	5 ± 2.9 Y	Metoprolol 100 mg, qd	ZGCD bid + Metoprolol 100 mg, qd	①
Zhangyong (40)	2 W	50	50	54.9 ± 4.5	55.8 ± 2.1	4.5 ± 1.0 Y	4.2 ± 0.8 Y	Metoprolol 25–100 mg, bid	ZGCD bid + Metoprolol 25–100 mg, bid	①③
Zhaobin (41)	4 W	40	40	70.67 ± 6.34	71.64 ± 5.68	7.98 ± 3.45 M	8.23 ± 3.57 M	Metoprolol 6 mg, bid, after 1 week 12 mg, bid, no more than 50 mg/dose	ZGCD bid + Metoprolol 6 mg, bid, after 1 week 12 mg, bid, no more than 50 mg/dose	①③④
Meiyongxian (20)	20 D	32	32	51.8 ± 7.4	52.2 ± 7.5			Metoprolol 25–50 mg, 2–3 times/d	ZGCD bid + Metoprolol 25–50 mg, 2–3 times/d	①
Wangjigang (28)	4 W	45	45					Metoprolol 25 mg, bid	ZGCD bid + Metoprolol 25 mg, bid	①②
Zhangxiaopeng (38)	4 W	44	44					Metoprolol 25 mg, bid	ZGCD bid + Metoprolol 25 mg, bid	①⑤
Huangmianting (15)	4 W	30	30	40.73 ± 13.29	38.97 ± 10.36			Metoprolol 12.5 mg, bid	ZGCD bid + Metoprolol 12.5 mg, bid	①②③
Baiyaping (7)	4 W	44	44	58.22 ± 5.03	58.12 ± 5.23	6.32 ± 1.13 Y	6.52 ± 1.23 Y	Metoprolol 12.5–25 mg, bid, after 1 week 25–50 mg, bid	ZGCD bid + Metoprolol 12.5–25 mg, bid, after 1 week 25–50 mg, bid	①②
Oujianzhao (21)	8 W	17	20	61.15 ± 8.32	60.21 ± 7.13			Metoprolol 12.5–25 mg, bid	ZGCD tid + Metoprolol 12.5–25 mg, bid	①②
Sunyanlin (25)	6 W	45	45	67.21 ± 3.23	65.23 ± 2.98			Metoprolol 50 mg, bid	ZGCD tid + Metoprolol 50 mg, bid	①②
Zhangjilei (42)	2 W	80	80	60.48 ± 4.57	59.95 ± 5.16	5.75 ± 1.08 Y	5.94 ± 1.21 Y	Metoprolol 25 mg, bid	ZGCD bid + Metoprolol 25 mg, bid	②④
Cuixiaoting (43)	8w	40	40			2.6 ± 1.3Y	2.8 ± 1.2	Metoprolol 25 mg, bid	ZGCD tid + Metoprolol 25 mg, bid	⑤

Control (C), metoprolol only; Trial (T), Zhigancao decoction + metoprolol; qd, quaque die; bid, bis in die; tid, ter in die; Outcome indicator: ① Total Efficacy ② Incidences of arrhythmia ③ Adverse Events ④ TCM Syndrome Score ⑤ HR of Arrhythmia.

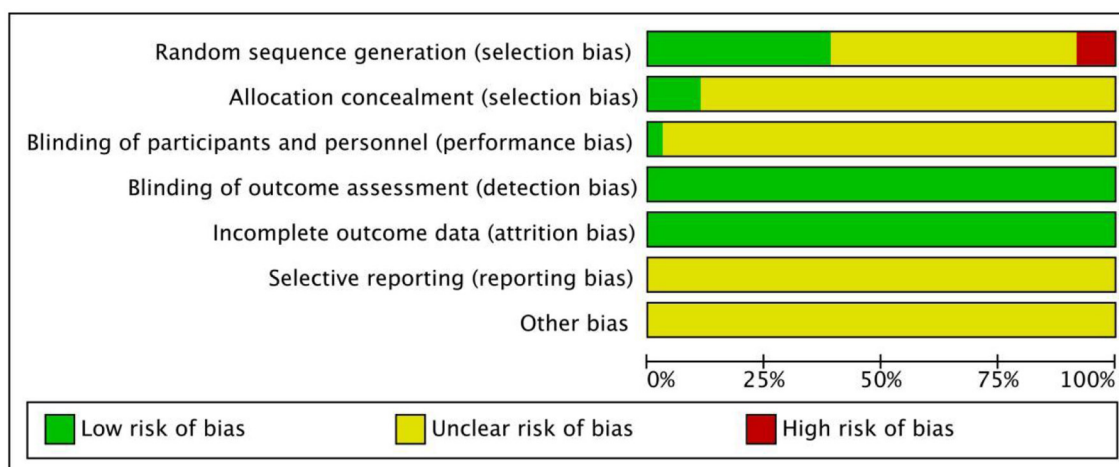


FIGURE 2 | Quality evaluation of the included studies literature.

General Characteristics and Quality Evaluation of the Included Studies

Among the 39 articles included in the general characteristics and quality evaluation of the study, all mentioned that the baseline of the experimental group was similar to that of the control group and was comparable. The terms referencing “random” were mentioned in all studies, but only 11 mentioned the random number table method, and two used allocation concealment. Because of the inconsistent dosage forms of intervention drugs, none of the studies used blind methods; selective reporting results and other sources of bias were not clear. Basic characteristics of the included studies are summarized in **Table 2**, and the quality of the included studies is presented in **Figure 2**.

Meta-Analysis Results

Total Efficacy

A total of 36 studies were included (6–41), and 3,960 patients were analyzed and evaluated, including BB group ($n = 1,977$) and ZGCD + BB group ($n = 1,983$). There was no statistical heterogeneity among the studies ($I^2 = 0\%$), therefore, we used a fixed-effect model. The statistical results revealed that the total effective rate of ZGCD + BB in the treatment of arrhythmias was higher than that of BB, and the difference was statistically significant ($OR = 4.74$, 95% CI: 3.78–5.94, $P < 0.01$). Further subgroup analysis demonstrated that there were 23 cases of coronary heart disease arrhythmia, 5 cases of premature ventricular beats/atrial premature beats, 2 cases of atrial fibrillation, 4 cases of arrhythmia of qi-yin deficiency, and 2 cases of arrhythmia. The results demonstrated that the total efficacy of ZGCD + BB was higher for arrhythmias of different pathological types, as depicted in **Figure 3**.

Incidences of Arrhythmia

A total of 14 studies were included (6, 7, 12, 14, 15, 17, 21, 25–28, 32, 35, 42), and 3,072 patients were analyzed and evaluated, including BB group ($n = 1,530$) and ZGCD + BB group ($n = 1,542$).

There was a large statistical heterogeneity among the studies ($I^2 = 98\%$); therefore, a random-effects model was used. The statistical results revealed that incidences of arrhythmia in the ZGCD + BB group was significantly less than that in the BB group, and the difference was statistically significant ($MD = -3.39$, 95% CI: -4.09 to -2.68 , $P < 0.01$). Further subgroup analysis revealed that premature ventricular beats, atrial premature beats, and junctional dysrhythmias had 14, 10, and 9 studies, respectively. The results indicated that incidence of different pathological arrhythmias in the ZGCD + BB group was significantly lower than that in the BB group, as shown in **Figure 4**.

HR of Arrhythmia

A total of 6 studies were included (8, 11, 18, 29, 38, 43), and 498 patients were analyzed and evaluated, including BB group ($n = 250$) and ZGCD + BB group ($n = 248$). There was a large statistical heterogeneity among the studies ($I^2 = 73\%$), therefore, a random-effects model was used. The statistical results revealed that the HR in the ZGCD + BB group was significantly slowed down than that in the BB group, and the difference was statistically significant ($MD = -8.48$, 95% CI: -10.98 to -5.97 , $P < 0.01$). Subgroup analysis demonstrated that there were 3 studies on atrial premature beats and coronary heart disease. The results proved that HR of different pathological arrhythmias in the ZGCD + BB group was significantly lower than that of the BB, as shown in **Figure 5**.

TCM Syndrome Score

A total of 5 studies (11, 27, 37, 41, 42) were included, and 483 patients were analyzed and evaluated, including BB group ($n = 241$) and an ZGCD + BB group ($n = 242$). There was statistical heterogeneity among them ($I^2 = 0\%$); therefore, a fixed-effects model was used. The statistical results revealed that the TCM syndrome score of the ZGCD + BB group was significantly lower than that of the BB group, and the difference was statistically significant.

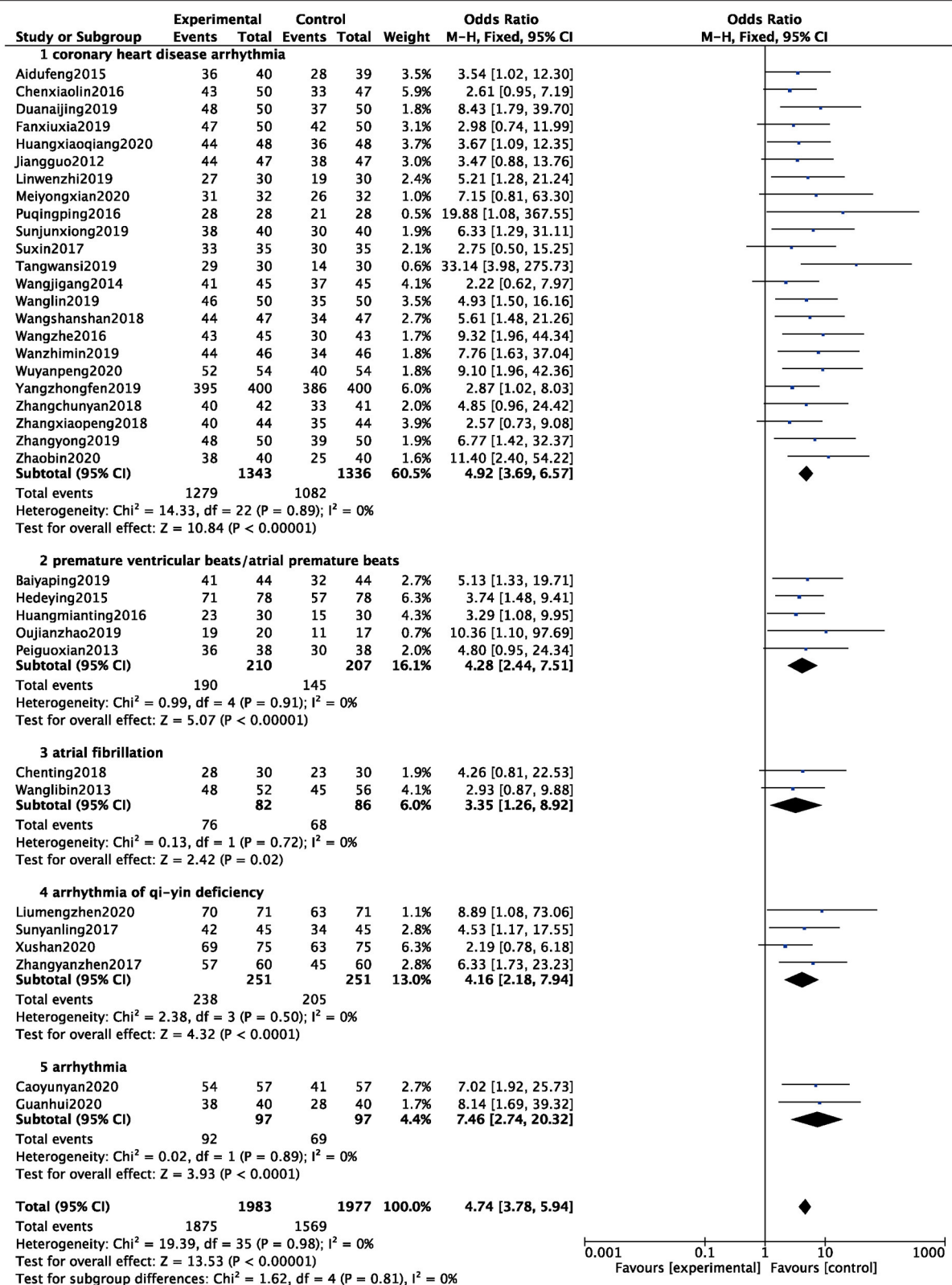


FIGURE 3 | Forest map of total efficacy between experimental group and control group.

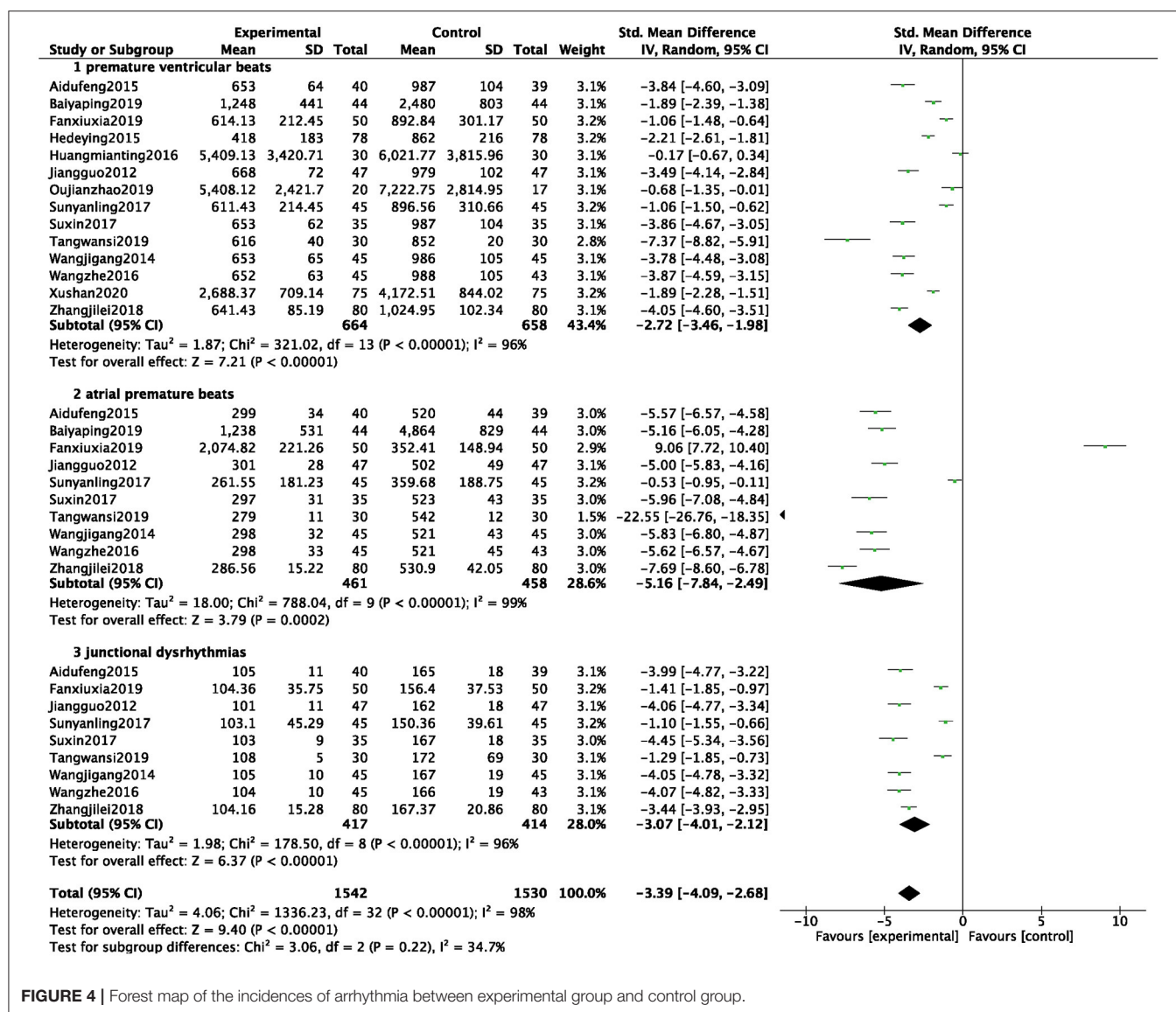


FIGURE 4 | Forest map of the incidences of arrhythmia between experimental group and control group.

significant ($MD = -2.92$, 95% CI: -3.08 to -2.76 , $P < 0.01$), as shown in Figure 6.

Adverse Events

A total of 16 studies counted the occurrence of adverse events (8–11, 15, 16, 18, 19, 27, 30, 32, 34, 36, 37, 40, 41), and 2,208 patients were analyzed and evaluated, including 1,101 cases in the BB group and 1,107 cases in the ZGCD + BB group. There was no statistical heterogeneity among the studies ($I^2 = 0\%$); therefore, a fixed-effect model was used. The results showed that the incidence of adverse events in the BB group was higher than that in the ZGCD + BB group, and the difference was statistically significant ($RR = 0.36$, 95% CI: 0.26 – 0.51 , $P < 0.01$). Subgroup analysis revealed that arrhythmia, atrial fibrillation, premature ventricular beats, arrhythmia of qi-yin deficiency, and coronary heart disease arrhythmia had 1, 1, 1, 2, and 11 items, respectively. The results prompted that adverse events of

different pathological arrhythmias in the ZGCD + BB group were significantly lower than those in the BB only group, as shown in Figure 7.

Publication Bias

An inverted funnel plot was constructed for the total efficacy of the included studies, as illustrated in Figure 8. As shown, the graphic distribution of the graph is not symmetrical and does imply a bias.

DISCUSSION

Metoprolol is a commonly used drug for the treatment of arrhythmias in the clinic, but it has some limitations, including efficacy and safety. Therefore, increasing number of clinical practices are combining CM and TM for arrhythmia treatment, and ZGCD is the most commonly used prescription

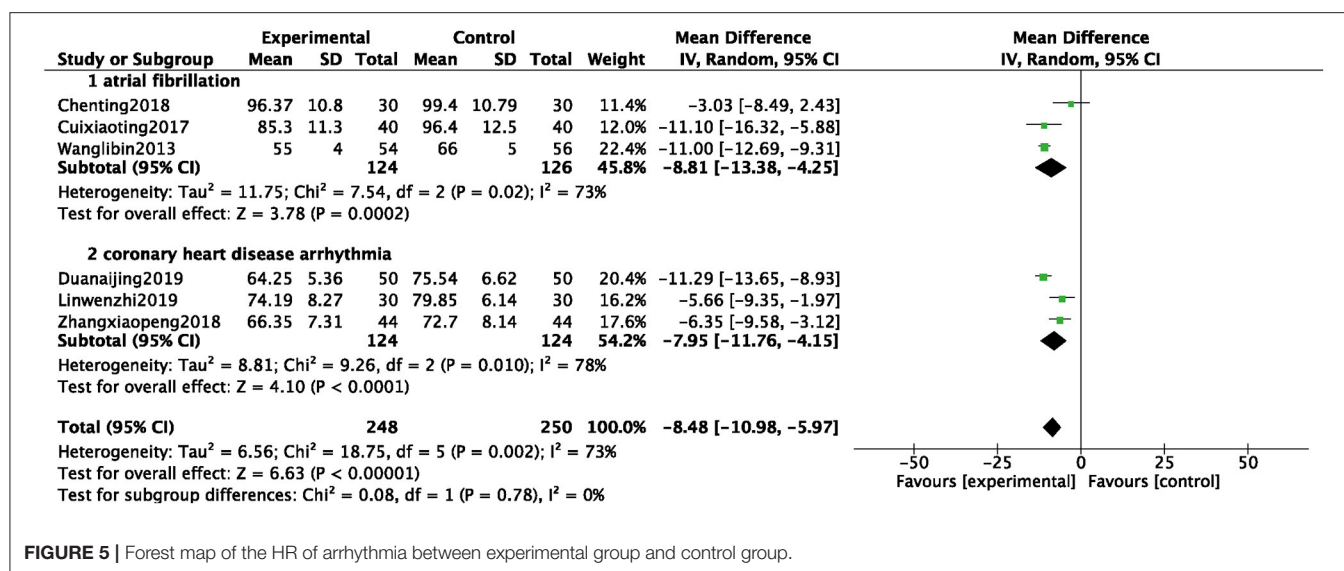


FIGURE 5 | Forest map of the HR of arrhythmia between experimental group and control group.

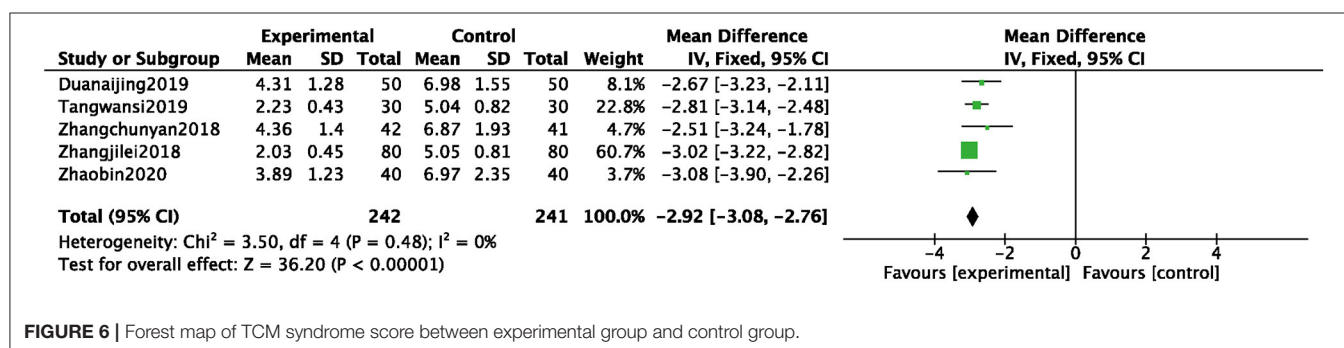


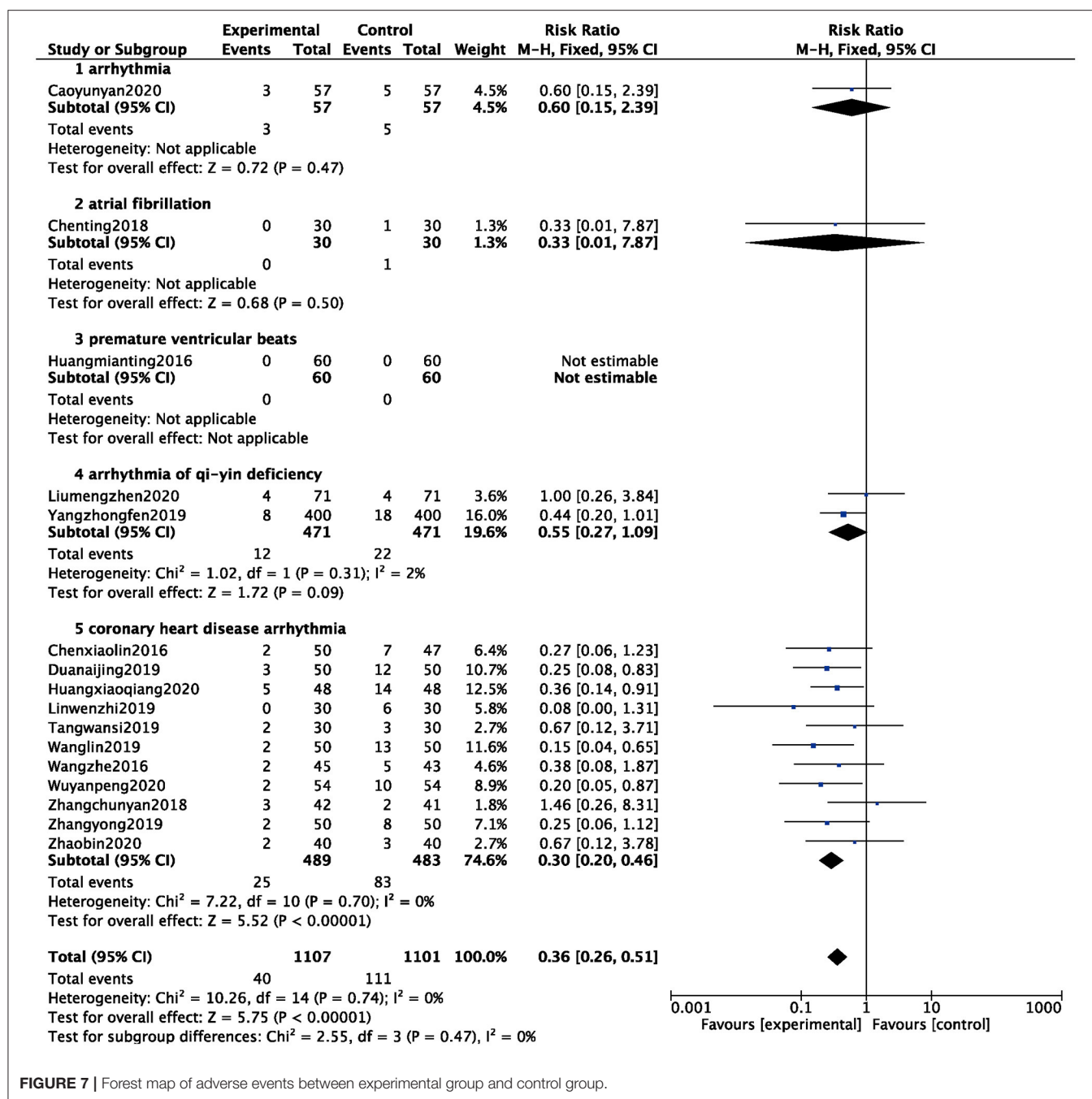
FIGURE 6 | Forest map of TCM syndrome score between experimental group and control group.

among TM. There are also some clinical reports that ZGCD combined with CM has advantages in terms of total efficacy and arrhythmia control. However, availability of high-quality research-based medical evidence remains a challenge.

This is the first meta-analysis investigating ZGCD + BB may exert better effectiveness of arrhythmias. Our systematic evaluation indicated that ZGCD + BB has advantages in the treatment of arrhythmia in terms of total efficacy, arrhythmia control, HR of arrhythmia, and TCM syndrome scores. As a TM prescription, ZGCD has the characteristics of multiple components, targets, and pathways in the treatment of diseases. This may be an important reason why ZGCD supports metoprolol and enhances its effectiveness. The mechanism of ZGCD in the treatment of arrhythmia remains unclear; some reports have shown that ZGCD can reduce HR, prolong MapD, and reduce Tp-e/QT to decrease the occurrence of ventricular arrhythmias (45). ZGCD may be related to the protection of the myocardium by effectively blocking the opening of potassium channels in hypoxic cardiomyocytes (46). Furthermore, modern pharmacological studies have demonstrated that the three main active components of ZGCD, glycyrrhizic acid, total ginsenosides of ginseng, and total saponins of *Ophiopogon*

japonicus, could significantly reduce the automaticity and excitability of isolated rat atrial muscle and prolong the refractory period to inhibit arrhythmia (47). ZGCD has a unique curative effect in the treatment of arrhythmias and a two-way, benign regulatory effect. Its modified and subtracted prescriptions are effective clinically in China, demonstrating the research value and prospects of TM in the treatment of arrhythmias (48).

In addition, our results proved that the ZGCD + BB group had significantly reduced incidence of adverse events compared with the BB. As known, adverse events are an important cause of failure during pre-market clinical trials of drugs and the withdrawal of drugs after marketing. CM, including antiarrhythmic drugs, has more adverse events, which seriously affect drug effectiveness and cause secondary injuries to patients. However, as a TM prescription, ZGCD not only has a good effect on arrhythmias, but is also safe. No obvious ZGCD-related adverse events have been observed in publicly published research reports or in the adverse drug reaction notifications of the China National Medical Products Administration. In addition, it can significantly reduce adverse events, such as nausea, vomiting, constipation, dizziness, headache, and bradycardia. In short, ZGCD combined with metoprolol appears



safe and effective, and is worthy of further consideration for clinical utility.

This study followed the principles of evidence-based medicine. We carefully evaluated the quality of each study, used subgroup analysis to explore heterogeneity, detected publication bias, and discussed possible influencing factors to provide reliable evidence for clinical practice and decision-making. Our meta-analysis is unprecedented and innovative and includes many studies and comprehensive evaluation indicators. However, the following limitations are also present: ① The

studies are published in the Chinese language, exclusively, with low quality, and may be biased; ② All studies refer to terms referencing the word “random,” but only 11 studies referenced the random number table method; ③ Because of the inconsistent dosage forms of intervention drugs, none of the studies used blind methods; ④ Differences between individual patients may also lead to bias, such as gender, age, underlying diseases, and treatment of underlying diseases; ⑤ The plot is not symmetrical and does imply a bias. It is likelihood of bias in both patient selection, and non-blinded nature of an intervention; ⑥

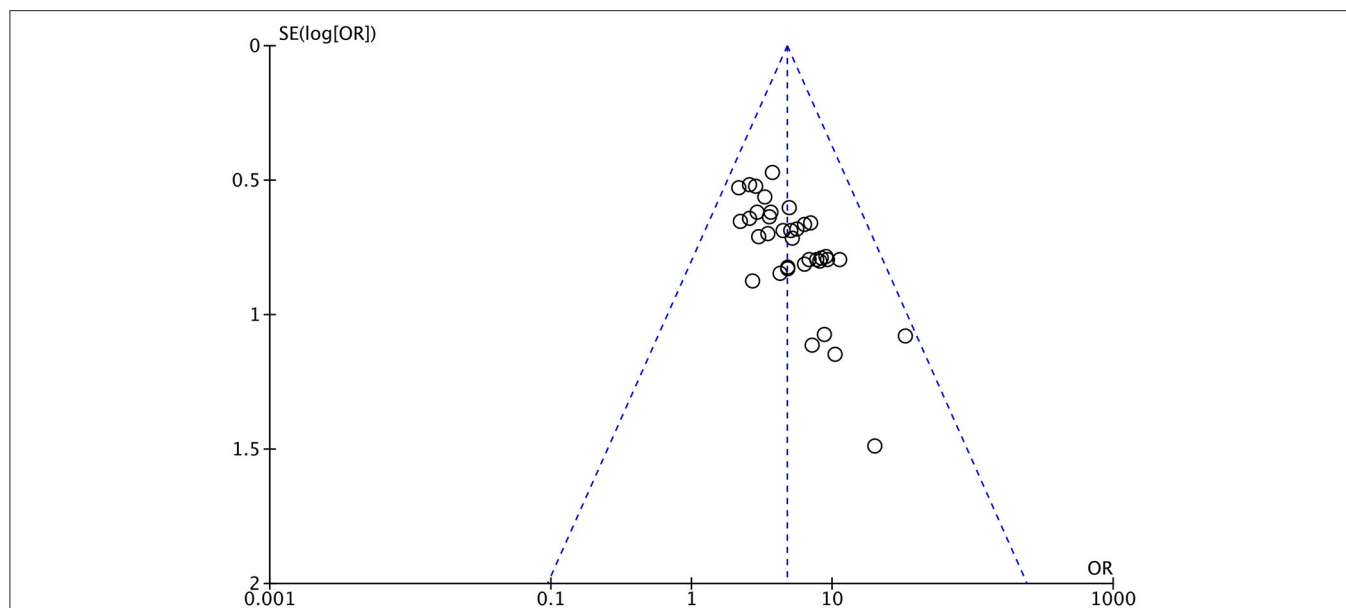


FIGURE 8 | Funnel chart of total efficacy.

In different studies, the compatibility dose composition of TM compounds is different, which may cause bias.

CONCLUSION

ZGCD + BB appeared to demonstrate good efficacy and fewer adverse reactions compared to BB in the treatment of arrhythmia. The addition of this TM may represent a useful complementary therapy to standard approaches. However, our findings must be cautiously evaluated because of the small sample size and the low quality of the cited clinical trials that lack strict clinical design. High-quality, suitably powered and double-blinded RCTs are required to confirm these findings. Further mechanistic investigations on the underlying biology of ZGCD are warranted and may yield important biological insights into arrhythmogenesis.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

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AUTHOR CONTRIBUTIONS

YY contributed to carry out the protocol, drafted the manuscript, and carried out the acquisition of data and analysis. F-LG, QH, RZ, and X-YZ participated in the data extracting. GL and PL were in charge of quality control. QS and S-JY designed and managed this protocol. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcvm.2022.795903/full#supplementary-material>

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