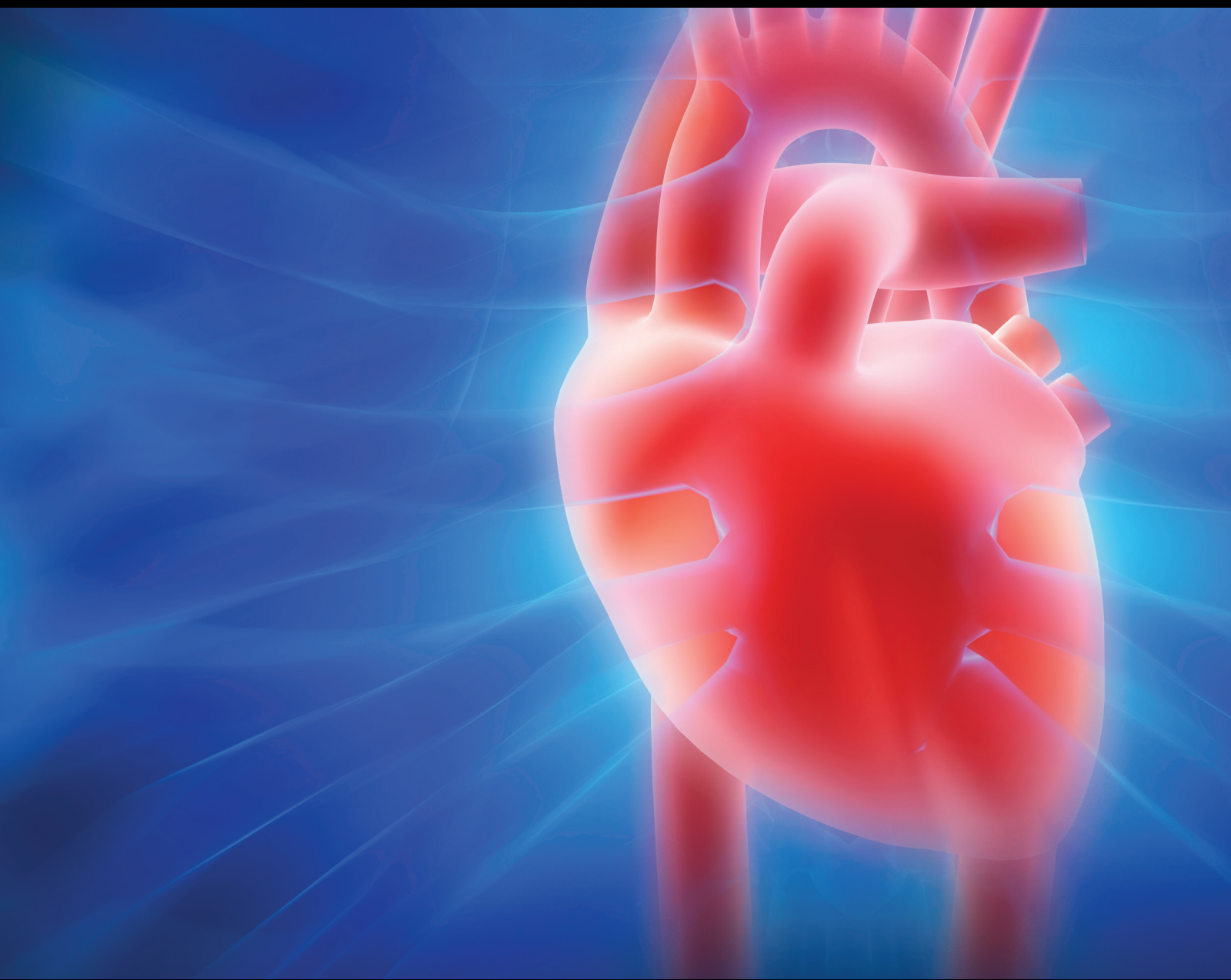
Cardiology Research and Practice

Cardiac Arrhythmias: Update on Mechanisms and Clinical Managements

Guest Editors: Yi-Gang Li, David G. Benditt, Thomas Klingenheben, Kai Hu, and Dali Feng



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Editorial Board

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Editorial

Cardiac Arrhythmias: Update on Mechanisms and Clinical Managements

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Atrial fibrillation (AF) is one of the most common arrhythmias in adults and is associated with a high incidence of stroke and heart failure (HF). Despite the advance of AF catheter ablation during the past decades, the high reoccurrence rate of AF after catheter ablation urges improvements of diagnostic approaches, therapies, and technologies. P. D. Dallaglio et al. reviewed the role of adenosine in pulmonary vein isolation in a meta-analysis of 11 studies. The analysis revealed that adenosine is useful to unmask dormant connection (DC) after a first ablation procedure and further ablation at sites of DC would reduce the rate of redo procedures for postablation AF recurrence. The authors also suggested that the use of adenosine should be accompanied by sufficient waiting time.

Cryoablation is an equivalent alternative to radiofrequency ablation for paroxysmal atrial fibrillation. According to the work of S. Conti et al., the use of second-generation cryoballoon was associated with lower procedure duration and fluoroscopy time and comparable procedural success as compared to first-generation cryoballoon.

Cardioversion is used widely in AF patients. The researches of Y. M. Rochlani et al. and D. Wang et al. showed the safety and efficiency of cardioversion for AF patients. Y. M. Rochlani and colleagues found that external electrical cardioversion during an AF related hospitalization could significantly reduce in-hospital stroke, mortality, length of stay, and cost for hospitalization. D. Wang et al. revealed that cardioversion during AF catheter ablation does not impair the maintenance of sinus rate or the recovery of cardiac function.

Even though digoxin has been used in clinical practice to treat heart diseases for decades, the proof of benefit is scarce. The new meta-analysis by S. Chamaria et al. confirms recent evidence that the use of digoxin increases all-cause mortality in all atrial fibrillation patients. In contrast, digoxin is not associated with higher mortality in the important subgroup of patients with AF and HF.

Reactive oxygen species (ROS) are well known to play a role in ischemic heart diseases, whereas their significance in arrhythmias is less well established. A. A. Sovari reviewed several possible ways for ROS to induce arrhythmia including causing focal activity and reentry, altering multiple cardiac ionic currents, promoting cardiac fibrosis, and impairing gap junction function.

Malignant ventricular arrhythmia is always on the top of the list of heart diseases. There is a continuous need of research with regard to revealing the pathogenetic mechanisms and identifying new targets of treatment. Activation of sympathetic nerves after myocardial infarction plays an important role in sudden cardiac death and left ventricular remodeling. C.-Y. Li and Y.-G. Li reviewed the sympathetic rejuvenation after MI. Degeneration and death of sympathetic fibers occur within and around the infarction zones.

After MI, sympathetic fibers would regenerate back to the myocytes around the infarcted area. The authors point out that, as part of sympathetic remodeling after MI, excessive sympathetic nerve sprouting might be a potential mechanism for fatal arrhythmia in chronic MI.

Most of heart diseases have multiple causes of which genetics represent a major determinant. Along with the rapid development of gene sequencing and genome-wide association study, single nucleotide polymorphism (SNP) is associated with detection of high-risk population, diagnosis of diseases, and sensitivity to targeted treatment. The study of F. Galati et al. suggests that RyR2 QQ2958 genotype might identify a subgroup of ICD implanted patients at particular high risk of malignant ventricular arrhythmias.

Commotio Cordis is defined as the mechanical stimulation of the heart which induces ventricular fibrillation and sudden cardiac death. It happens mainly in sports. D. H. Wolbrom et al. reviewed the mechanisms and clinical management of ventricular arrhythmias under this situation. In the acute event, rapid defibrillation with AED could save life; however, catheter ablation might be the option for the few patients who develop ventricular arrhythmias during chronic stage. The mechanism of Commotio Cordis is still unclear. In an experimental swine model of chest blow trauma, C. Madias et al. found that stretch activation of the L-type calcium channel and intracellular calcium overload do not seem to play a key role in preventing ventricular arrhythmias.

Skin burns at the site of an indifferent electrode patch are a rare complication after radiofrequency catheter ablation. In a study of H. Ibrahim et al., the incidence of significant skin burns was 0.28%. Higher BMI, procedure time, and postprocedure pain were the factors predicting skin burn.

Historically, a prolonged PR interval by itself does not necessarily imply clinical consequences. Interestingly, using data from a large population-based study, M. P. Husby et al. found that a long PR interval was associated with higher LV mass, LV stroke volume, and LV end-systolic and end-diastolic volumes. Increased ventricular volume and wall stress might lead to arrhythmias.

We hope you enjoy this issue which succinctly explores the depth and breadth of currently available clinical management and mechanisms of atrial fibrillation and ventricular arrhythmias.

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Research Article

Incidence and Factors Predicting Skin Burns at the Site of Indifferent Electrode during Radiofrequency Catheter Ablation of Cardiac Arrhythmias

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Radiofrequency catheter ablation (RFA) has become a mainstay for treatment of cardiac arrhythmias. Skin burns at the site of an indifferent electrode patch have been a rare, serious, and likely an underreported complication of RFA. The purpose of this study was to determine the incidence of skin burns in cardiac RFA procedures performed at one institution. Also, we wanted to determine the factors predicting skin burns after cardiac RFA procedures at the indifferent electrode skin pad site. *Methods*. A retrospective case control study was performed to compare the characteristics in patients who developed skin burns in a 2-year period. *Results*. Incidence of significant skin burns after RFA was 0.28% (6/2167). Four of the six patients were female and all were Caucasians. Four controls for every case were age and sex matched. Burn patients had significantly higher BMI, procedure time, and postprocedure pain, relative to control subjects ($p < 0.05$, one-tailed testing). No one in either group had evidence of dispersive pad malattachment. *Conclusions*. Our results indicate that burn patients had higher BMI and longer procedure times compared to control subjects. These findings warrant further larger studies on this topic.

1. Introduction

Radiofrequency catheter ablation (RFA) has become a mainstay for treatment of cardiac arrhythmias. It is considered a highly effective treatment modality with a low complication rate [1, 2]. Up to 3% of patients undergoing radiofrequency catheter ablation develop major complications from the procedure. These complications include AV block, cardiac tamponade, coronary artery spasm, thrombosis, pericarditis, vascular injury, thromboembolism, TIA or stroke, pulmonary hypertension, pneumothorax, left atrial-esophageal fistula, and phrenic nerve paralysis [3]. Skin burns at the site of an indifferent electrode patch have been a rare, serious, and likely an underreported complication of RFA [4, 5]. Although different studies have looked at incidence and factors that have led to skin burns at the site of skin pad attachment while performing hepatic tumor ablative procedures, [6, 7] literature related to this complication is scant in cardiac arrhythmia ablative procedures. Incidence of skin burns at the site of the indifferent electrode is currently low but it is likely going to increase in future as higher power settings and multiple ablations are more frequently used for ablation of cardiac arrhythmias [8]. Only case reports and case series have been published in the past. This study aims to determine the incidence and different factors predicting possible skin burns at the site of an indifferent electrode patch while performing these cardiac arrhythmia ablative procedures.

2. Methods

A retrospective case control study design was used to examine the characteristics in patients who developed skin burns related to the radiofrequency ablation, compared to those who did not develop this complication during the procedure. All patients ≥18 years of age who underwent cardiac RFA procedures from 4/1/2012 to 3/31/2014 that developed skin

Table 1: Comparisons for nominal variables between the cases (burn patients) and the controls[1].

Characteristic	Cases (%)	Controls (%)	p value
Hypertension	5/6 (83.3%)	20/24 (83.3%)	0.746
Diabetes	2/6 (33.3%)	5/24 (20.8%)	0.433
Postprocedure pain	4/6 (67.7%)	0/24 (0%)	0.001
Type of skin patch			0.545
3M stockert skin patch	4/6 (66.7%)	14/24 (58.3%)	
Valley Lab skin patch	2/6 (33.3%)	10/24 (41.7%)	
General anesthesia	4/6 (66.7%)	13/24 (54.2%)	0.469

[1]The one-tailed Fisher's exact test was used for the analyses.

Table 2: Comparisons for quantitative variables between the cases (burn patients) and the controls[1].

Characteristic	Cases (%)	Controls (%)	p value
BMI	36.6 (27.7–65.0)	30.6 (17.6–52.6)	0.044
Procedure time (min)	224.5 (63–332)	122.5 (23–357)	0.035
Maximum temperature (°C)	55.0 (50.0–65.0)	50.0 (40.0–70.0)	0.078
Maximum current (watts)	60.0 (35.0–70.0)	50.0 (35.0–100.0)	0.325

BMI: body mass index.
[1]The one-tailed Mann-Whitney U test was used for the analyses.

burns at the site of the indifferent electrode skin patch were included in the review. Controls, defined as patients >18 years of age who underwent cardiac RFA procedures and did not develop skin burns, were randomly selected from the same time frame and matched on age and sex.

Data collected included patient demographics, patient's past medical history (e.g., hypertension and diabetes), height, weight, and BMI. Procedure details (including diagnosis for ablation, type of sedation used, total procedure time, type of generator, maximum temperature reached, and maximum power in watts) were also obtained. Impedance data were not recorded for this patient population and were not included in our study. Indifferent electrode skin patch characteristics (e.g., type, area of attachment, and evidence of malattachment of skin pad at the end of procedure) were collected, as were data related to the characteristics of the burns (e.g., patient complains of pain or not and burn degree (redness, second-degree skin burns, and third-degree skin burns)). Length of hospital management for the burn was also recorded.

Incidence of skin burns from RFA was determined using a query of all patients who underwent cardiac RFA procedures. There were a total of six patients who developed a burn during the study time frame. For the sample size determination, we assumed an odds ratio of 6.0 as clinically important, with 20% of the controls exposed to the risk factor and with $\alpha = 0.05$ and $\beta = 0.20$. We planned to be able to detect a statistically significant effect with four control patients for every burn patient, using a one-tailed test. The records from 24 control patients and six burn patients were reviewed for this study.

Data were analyzed using IBM SPSS Statistics v 21.0. (Armonk, NY). Quantitative data were analyzed using the Mann-Whitney U test and are shown as the mean ± SD. Nominal data were analyzed using Fisher's exact test and are shown as percentages. Significance was assessed at $p < 0.05$, using one-tailed testing.

3. Results

Incidence of the significant skin burns after the RFA ablation procedure was found to be 0.28% (6/2167) during the study period. Two of our six burn subjects were males and all were Caucasians. Eight of the 16 control subjects were males, 22/24 were Caucasian, and their age (63.7 ± 8.6 years, mean ± SD) was similar to that of the burn patients (63.7 ± 8.1 years). No significant difference was present between cases and controls with regard to hypertension, diabetes, skin pad, or type of sedation (Table 1). Postprocedure pain was predictably present in significantly more cases compared to controls.

No patient in either group had evidence of dispersive pad malattachment and none of the patients' hair was removed at the site of attachment. Subjects with burns had significantly greater BMI and total procedure time, relative to the control subjects (Table 2). There were no statistically significant differences between the cases and controls with regard to maximum temperature reached or maximum current in watts.

4. Discussion

Radiofrequency ablation of cardiac arrhythmias uses low voltage and high frequency electrical energy. During the management of nonarrhythmic conditions like radiofrequency ablation of hepatic tumors, increased level of radiofrequency energy is frequently used, causing higher incidence of potential complications including skin burns at the site of the indifferent electrode patch [9]. Severe skin burns occur in 0.1%–3% of patients undergoing RF ablation of solid abdominal tumors while mild skin burns occur in up to 33% of such patients [6, 10]. This high rate is postulated to be secondary to high power settings and prolonged procedure times during these ablations [11].

Tissue temperature increases with the passage of electric current, with the greatest increase in temperature being at the site of catheter tip, while the temperature increase is attenuated at the site of indifferent electrode site with the help of dispersive skin patches. Increase of temperature at the catheter tip is the foundation of the therapy with RFA as it causes the local destruction of the tissue. Dispersive skin pads at the site of the indifferent electrode function to disperse the electrical energy exiting the body and thus prevent the occurrence of skin burns by spreading the energy over a larger surface area [3]. In prior studies, it has been predicted that the temperature rises to 45–47°C. At the indifferent electrode site, there is a risk factor for development of skin burns [12].

Malattachment of dispersive pads, presence of hair at the site of pad attachment, and increased amount of subdermal fat have been described as known risk factors for development of skin burns. Fat tissue acts like an insulator and increases the temperature secondary to increase in the resistance [9]. Dysfunction of the skin pad by either malattachment or physical damage concentrates the exiting current's available area, resulting in increased tissue temperature and higher risk for development of skin burns.

Our results showed that there was a low incidence of burns at the indifferent electrode skin pad during RFA ablation procedures for cardiac arrhythmias. Nevertheless, this can be a potentially serious complication, as two of our patients developed third-degree burns requiring increased burn care. All of our six burn patients were Caucasians, indicating that there might be a predisposition to develop the skin burns secondary to skin characteristics. However, 22 of our 24 controls were also Caucasians, thus making this association weaker as Caucasian patients seem to be the predominant ethnic group who underwent the RFA procedures at our institution.

BMI is an important factor which can be helpful in predicting the patients' risk of developing skin burns. It is expected that, with increased body weight, there would be more impedance during the RFA procedure, resulting in an increased incidence of the skin burns at the site of the indifferent electrode. Our results indicated that burn patients had significantly higher BMI relative to our control subjects. We suggest that care should be taken in patients who have increased BMI while performing the RFA procedures.

Hair was not removed at the site of the indifferent electrode in our patients, as this might lead to the malattachment of the dispersive skin pad, increased resistance, and thus increased incidence of skin burns which can be associated with it. There was no evidence of malattachment of the indifferent electrode in either the cases or the controls. Another interesting finding was that all patients who had burns had the indifferent electrode applied to the left flank. Among the controls, 12 out of 24 patients had the indifferent electrode attached in the left flank. One patient from the controls had the placement on the left leg, five had it on the right leg, and six controls had the indifferent electrode placed in the right flank region. In a previous study, risk of development of skin burns was found to be lower in patients with the dispersive skin pad attached to the thigh as compared to other body parts [13]. Optimal position of the dispersive skin pad needs to be studied further as it seems to be an important risk factor that is easily modifiable.

Four out of our six cases with the skin burns complained of the pain at the site of indifferent electrode placement. Further, these patients had developed second- and third-degree skin burns. Pain assessment at the end of the procedure for the patients who are under conscious sedation and at the time of becoming conscious for those who undergo the procedure under general anesthesia can be helpful for actively looking for the skin lesions in a timely manner.

Total procedure time was significantly higher in burn patients relative to control subjects, suggesting that it may be a clinically important factor of predicting skin burns. Increased total procedure time indicates technical difficulty of the procedure, patient characteristics that are unfavorable, and/or a difficult to treat arrhythmia requiring increased duration of the procedure to achieve adequate ablation.

Maximum temperature reached during the procedure did not seem to have a major impact on our study sample. This points towards the fact that sustained ablation for longer period of time is more likely to cause the skin burns than higher temperature for shorter periods of time. Thus, one of the important steps in reduction of the post-RFA ablation skin burns is to not prolong the ablation procedure.

Impedance is an important factor in radiofrequency ablation procedures and its monitoring can be helpful in predicting development of skin burns. It is the weighted average of electrical resistivity of all the tissues between the ablation catheter and the indifferent electrode patch. Regions closer to the radiofrequency ablation catheter have the highest weightage in determination of impedance because of high electrical density [14].

Important determinants of impedance include increased body surface area, blood flow to the tissues, coagulum, and char formation. Volume of resistive medium between the two electrodes is proportional to the impedance. Thus, obesity and larger body surface area result in high impedance as subdermal fat acts as an insulator. Power used in radiofrequency ablation of cardiac arrhythmias is proportional to the voltage and inversely proportional to the system impedance [15]. Thus, if impedance increases, in order to deliver the same amount of energy to the cardiac tissue, higher power settings are needed. As studies have found that high power and prolonged periods of cardiac ablation are associated with higher incidence of skin burns, [9] it can be concluded that the high impedance is a risk factor for development of skin burns and would be interesting factor to look at in future studies.

Steam popping is another important phenomenon. During the generation of resistive heat during radiofrequency ablation of the cardiac arrhythmias, cardiac tissue fluid can vaporize, forming steam bubbles which can potentially burst open with an audible pop with continued ablation (generally occurs at tissue temperatures above 100°C). An important potential complication associated with this phenomenon is cardiac perforation. Steam popping and vaporization have been associated with a drop in the impedance [16, 17]. The frequency of the steam pops has been noted to be high with higher power [18]. Theoretically, steam popping can be used

as a predictor of skin burns which also can occur at high power settings. This has not been described in the literature before and can be looked at in future studies as a potential predictor.

Further studies are needed to assess other possible predictors including impedance and voltage and for confirmation of our results. Establishment of these predictors would help decrease this possible complication, which can be a major issue in many patients.

5. Limitations

An important limitation of the study is its small sample size. Another important limitation is the fact that we did not have the complete records of the important possible predictor of voltage used during the procedure. Therefore, we could not assess any association with it. We used a case control study design, which could raise concerns as to whether there might be other differences between the cases and controls which could be driving the significant differences seen in this study. Finally, a decision was made at the time of writing the protocol to use one-tailed testing for all of the statistical analyses.

6. Conclusion

This is the first comparative study reporting on skin burns following RFA. Although the sample size was small, burn patients had significantly higher BMI, procedure times, and postprocedure pain relative to control subjects. While larger studies are needed to confirm these findings, these results should be kept in mind when planning to perform RFA.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

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Research Article

Comparison between First- and Second-Generation Cryoballoon for Paroxysmal Atrial Fibrillation Ablation

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Introduction. Cryoballoon (CB) ablation has emerged as a novel treatment for pulmonary vein isolation (PVI) for patients with paroxysmal atrial fibrillation (PAF). The second-generation Arctic Front Advance (ADV) was redesigned with technical modifications aiming at procedural and outcome improvements. We aimed to compare the efficacy of the two different technologies over a long-term follow-up. *Methods*. A total of 120 patients with PAF were enrolled. Sixty patients underwent PVI using the first-generation CB and 60 patients with the ADV catheter. All patients were evaluated over a follow-up period of 2 years. *Results*. There were no significant differences between the two groups of patients. Procedures performed with the first-generation CB showed longer fluoroscopy time (36.3 ± 16.8 versus 14.2 ± 13.5 min, resp.; $p = 0.00016$) and longer procedure times as well (153.1 ± 32 versus 102±24.8 min, resp.; $p = 0.019$). The overall long-term success was significantly different between the two groups (68.3 versus 86.7%, resp.; $p = 0.017$). No differences were found in the lesion areas of left and right PV between the two groups (resp., $p = 0.61$ and 0.57). There were no significant differences in procedural-related complications. *Conclusion*. The ADV catheter compared to the first-generation balloon allows obtaining a significantly higher success rate after a single PVI procedure during the long-term follow-up. Fluoroscopy and procedural times were significantly shortened using the ADV catheter.

1. Introduction

Pulmonary vein isolation (PVI) is the cornerstone of any catheter-based treatment for patients with paroxysmal atrial fibrillation (PAF) [1, 2]. Electrical isolation is commonly performed by a circumferential lesion set around the pulmonary veins [1–3]. The standard "point-by-point" technique remains challenging and time-consuming. Cryoballoon (CB) technology would theoretically allow PVI with a single application [4–8]. The first-generation CB, Arctic Front™ (Medtronic, Inc., Minneapolis, MN, USA), has been available since 2006 in Europe [7, 8]. With respect to the first-generation CB, the second-generation, Arctic Front Advance™ (ADV), version was designed with technical modifications aiming at procedural outcome improvement [9–11]. The number of injection ports has been doubled and these have been placed more distally on the catheters shaft resulting in a larger and more uniform zone of freezing on the balloons surface if compared with the previous version [12]. Aim of the study was to compare the acute and long-term success of these two different technologies.

2. Methods

2.1. Patient Population. We retrospectively analyzed 120 patients undergoing PVI using the CB technology who completed at least 2 years of follow-up. All patients had symptomatic and drug-resistant PAF according to the current ESC and HRS/EHRA/ECAS guidelines [1, 2]. Data were

accurately collected for each patient from medical notes after discharge and included basic demographic, clinical information, pharmacological therapy, date of hospitalization and discharge, presence of comorbidities, and cardiovascular events during hospitalization. From June 2011 to June 2013 sixty patients underwent PVI using the Arctic Front™ CB catheter and 60 patients using the ADV ablation catheter. The 28 mm CB was used in all procedures. In addition, electroanatomical mapping using NavX Velocity 3.0 system (St. Jude Medical, Minneapolis, MN, USA) was performed in a subgroup of patients. The study protocol was approved by the local Ethics Committee.

2.2. Pulmonary Vein Isolation. All patients underwent preprocedural transthoracic echocardiography to asses left ventricular ejection fraction and left atrial dimension. To exclude the presence of thrombi in the left atrium or in the left atrial appendage a transesophageal echocardiography was performed the day before the procedure. Moreover, a preprocedure magnetic resonance imaging or computed tomography with segmentation of the left atrium was performed to assess left atrial anatomy in detail. Procedures were performed either with continued oral anticoagulation using warfarin and therapeutic INR (2.0 to 3.0) or using low-molecular weight heparin bridging. All PVI procedures were performed by experienced operators beyond the learning curve. Briefly, all procedures were carried out in conscious sedation using propofol infusion. A deflectable decapolar catheter was inserted through right femoral vein and positioned into the coronary sinus to guide the transseptal puncture and to pace the left atrium during treatment of the left PVs and was subsequently moved to the superior vena cava where it was used to stimulate the right phrenic nerve during treatment of the right PVs. A single transseptal puncture was performed using a needle system (BRK, St. Jude Medical, St. Paul, MN, USA) and a standard transseptal sheath (SL0 8F or 8.5F, St. Jude Medical, St. Paul, MN, USA), subsequently exchanged with a steerable 15F sheath (FlexCath™, 15F, Medtronic, Inc., Minneapolis, MN, USA). Before transseptal puncture, heparin was administered intravenously as bolus (10000 U) followed by a continuous infusion (1000 U/hr) reaching ACT level >350 sec. The FlexCath was continuously irrigated with heparinized saline (2 mL/hr). An esophageal temperature probe was used in all patients (Esotherm Plus, FIAB) to monitor intraesophageal temperature increase. The probe was adjusted during the procedure to stay as close as possible to the ablation catheter. Cryotherapy was interrupted if the endoluminal esophageal temperature dropped below 18°C. Two cryotherapy applications were delivered to each PV, 240–300 seconds each, aiming for a minimum temperature of less than −40°C. After treatment of all PVs, entrance block was confirmed with high-output pacing (12 V, 2.9 ms) using the Lasso™ (Biosense Webster, Diamond Bar, CA, USA), Afocus (St. Jude Medical, Minneapolis, MN, USA), or Achieve™ mapping catheter (Medtronic, Inc., Minneapolis, MN, USA). "Far field" capture and sensing were ruled out using differential pacing maneuvers. Any residual conduction into the PVs was treated by further cryotherapy applications.

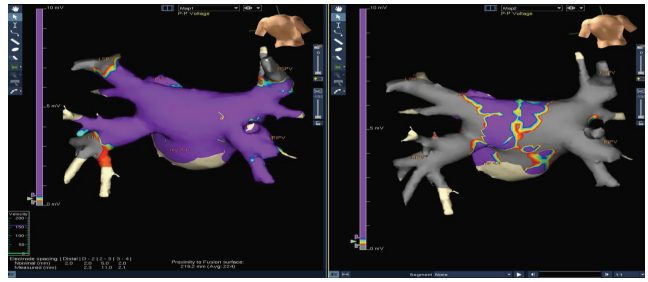

Figure 1: High-density voltage map of the left atrium using electroanatomic mapping, NavX Velocity 3.0, before and after the procedure.

Successful PVI was confirmed when all PV potentials were abolished or were dissociated at least 20 minutes after the last cryotherapy application to that vein.

2.3. Lesion Area Comparison. In each patient who underwent PVI using the electroanatomic mapping system NavX Velocity 3.0, a high-density voltage map of the left atrium was performed, before and after the procedure, using the mapping catheter Afocus. After cryotherapy, the border between the scar area and healthy atrial tissue was defined using a 0.1–0.5 mV as offset (0.1 mV was defined as scar or absolutely silent tissue). The border between scar and normal tissue was defined including both ipsilateral PVs. Using an implemented tool in the NavX Velocity 3.0, the lesion area (cm^2) was automatically calculated by the system (Figure 1).

2.4. Follow-Up. Patients were followed up in the outpatient clinic 3 months after the procedure and every 3 months during the first year after ablation and every 6 months thereafter. At each visit, a standard 12-lead ECG was obtained in all patients. All patients were followed up with Holter-ECG monitoring at 6 and 12 months and annually after the PVI procedure. After 90 days of blanking period, any documented episode of AF or atrial arrhythmias lasting >30 seconds was considered a recurrence. All antiarrhythmic agents were withdrawn at 3 months after ablation. Clinical events occurring during the follow-up and documentation of the events were carefully checked. Clinical success was defined as complete freedom from symptomatic arrhythmia and the absence of any atrial arrhythmia during Holter monitoring.

2.5. Statistical Analysis. This was an observational, retrospective, single-center study. Continuous variables are reported as mean ± standard deviation. Comparison of continuous variables was performed using independent sample Student's *t*-test and categorical data with Fisher's exact test. Arrhythmia-free survival curves were generated by the Kaplan-Meier method and compared with the Log Rank test. Statistical significance was considered with a p value of <0.05. SPSS 20.0 statistical software (SPSS Italia, Inc., Florence, Italy) was used for statistical analysis.

Table 1: Baseline patient characteristics.

	CB, 1st ($n = 60$)	CB, 2nd ($n = 60$)	p
Male sex, n (%)	41 (68.3)	50 (83.3)	0.14
Mean age, years (mean ± SD)	59.1 ± 12.2	57.2 ± 10.9	0.37
Body mass index, Kg/m^2 (mean ± SD)	26 ± 2	26 ± 3	0.59
Paroxysmal atrial fibrillation, n (%)	60 (100)	60 (100)	1
Left atrial diameter, mm (mean ± SD)	22.9 ± 5.1	22.5 ± 4.7	0.60
Left ventricular ejection fraction, (mean ± SD)	62.5 ± 6.1	60.9 ± 7.4	0.72
Hypertension, n (%)	25 (41.6)	23 (38.3)	0.63
Hypercholesterolemia, n (%)	12 (20)	14 (23.3)	0.61
Diabetes mellitus, n (%)	4 (6.6)	5 (8.3)	0.73
Hypertriglyceridemia, n (%)	5 (8.3)	6 (10)	0.71
Active smoking, n (%)	8 (13.3)	9 (15)	0.69
Coronary artery disease, n (%)	4 (6.6)	5 (8.3)	0.73
Dilated cardiomyopathy, n (%)	0	0	—
Valve disease, n (%)	4 (6.6)	3 (5)	0.40
Previous cardiac surgery, n (%)	3 (5)	2 (3.3)	0.46
Previous ischemic stroke, n (%)	—	1 (1.6)	0.53
Chronic renal failure, n (%)	4 (6.6)	3 (5)	0.40
Previous ablation procedures for AF, n (%)	0	0	—

Table 2: Fluoroscopy time and procedure time comparison between the first- and second-generation CryoBalloon catheter.

	CB, 1st	CB, 2nd	p
Procedure time, min (mean ± SD)	153.1 ± 32	102 ± 24.8	0.019
Fluoroscopy time, min (mean ± SD)	36.3 ± 16.8	14.2 ± 13.5	<0.001

3. Results

Baseline clinical characteristics of patients are reported in Table 1. There were no significant differences between the 2 study groups regarding age, gender, cardiovascular risk factors, left ventricular ejection fraction, left atrial dimension, and medical therapy. No significant differences were found between the two study groups regarding CHA2DS2-VASc and HAS-BLED scores.

No patients had evidence of left atrial thrombosis during transesophageal echocardiography. Acute success rate and procedural-related complications are reported in Table 3. Procedures performed with the first generation CB showed longer fluoroscopy time (36.3 ± 16.8 versus 14.2 ± 13.5 min, resp.; $p < 0.001$) and longer procedure times as well (153.1 ± 32 versus 102 ± 24.8 min, resp.; $p = 0.019$) compared to the second-generation ADV catheter (Table 2). Interestingly, no statistically significant differences were found in the lesion area of left and right PVc between the two groups (resp., $p = 0.61$ and 0.57, Table 4). The overall success rate after single PVI procedure including both first- and second-generation CB was 77.5%. The long-term freedom-from-AF as showed in the Kaplan-Meier survival analysis was significantly different between the two different CB (68.3% with the first-generation CB versus 86.7% with the second-generation ADV catheter, resp.; Log Rank $p = 0.017$, Figure 2).

4. Discussion

This retrospective analysis provides data on long-term efficacy of CB ablation performed in a single high-volume center. The main findings of this study are that the use of the second-generation ADV catheter significantly improved the long-term procedural success after single PVI procedure and reduced procedure duration and fluoroscopy exposure time.

Our results in terms of procedural success using the first generation CB are in line with those coming from the North American Arctic Front STOP AF Pivotal Trial (68.3% versus 69.9% resp.) [13]. Several reports have shown that CB ablation with the new ADV catheter is associated with higher success rate of PVI and better outcome. In a first report of Fürnkranz et al. comparing the first-generation CB with the ADV, single-shot PVI rate increased from 51% to 84% ($p < 0.001$) [12]. Procedure duration and fluoroscopy exposure time were also significantly decreased using the novel CB catheter. In a retrospective analysis, Aryana et al. confirmed that ADV catheter significantly reduced procedure time and fluoroscopy time. Freedom from AF at 6, 9, and 12 months was 89, 86, and 82%, respectively, during a mean follow-up of 16 ± 8 months [14]. Giovanni and coworkers recently reported a significantly higher freedom from AF at 1-year follow-up with the second-generation ADV catheter with respect to the first-generation CB. Freedom from AF

Table 3: Acute success and procedure-related complications.

	CB, 1st ($n = 60$)	CB, 2nd ($n = 60$)	p
PVI achieved, (%)	95	98	ns
Catheter failure, n (%)	3* (5)	1# (1.6)	ns
Need of touch-up, n (%)	3 (5)	1 (1.6)	ns
Acute PNP, n (%)	2 (3.3)	1 (1.6)	ns
Chronic PNP, n (%)	0	0	—
Cerebral embolization, n (%)	0	0	—
Pericardial effusion, n (%)	1 (1.6)	0	ns
Cardiac tamponade, n (%)	0	0	—
PV stenosis, n (%)	0	0	—
Atrioesophageal fistula, n (%)	0	0	—
Vascular injury, n (%)	3 (5)	2 (3.3)	ns

PVI: pulmonary vein isolation; PNP: phrenic nerve palsy; #: FlexCath failure; ∗: 2/3 FlexCath failure, 1/3 Cryoballoon failure.

Table 4: Comparison of lesion area between the first- and second-generation CryoBalloon catheter. Data obtained from electroanatomic mapping performed after cryoablation using the NavX system (St. Jude Medical, St. Paul, MN, USA).

Lesion area	CB, 1st	CB, 2nd	p
LPVs, cm^2 (mean ± SD)	68.2 ± 44	75.3 ± 26	0.61
RPVs, cm^2 (mean ± SD)	73.1 ± 33	79.4 ± 22	0.57

LPVs: left pulmonary veins; RPVs: right pulmonary veins.

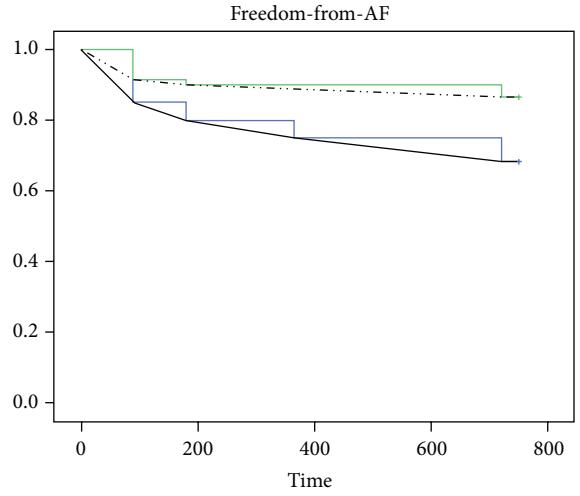

Figure 2: The Kaplan-Meier survival analysis shows a significant difference in freedom-from-AF recurrence between patients undergoing atrial fibrillation ablation using the first-generation Cryoballoon (CB1) and the second-generation Cryoballoon (CB2) catheter (Log Rank $p = 0.017$).

off antiarrhythmic drugs (AAD) therapy was achieved in 84% of patients treated with the ADV catheter, compared to 66% of success rate obtained with first-generation CB ($p = 0.038$). In their experience, procedural and fluoroscopy times were also significantly decreased by the use of ADV catheter [15]. Similar findings were reported by Fürnkranz et al. The authors found freedom from AF after a single procedure without AAD therapy after 1 year in 63.9% of patients treated with the first generation of CB versus 83.6% ($p = 0.008$) of patients with the ADV catheter [16]. Liu et al. during a mean follow-up of 12 ± 4 months found an overall 76.0% of CB success rate, respectively, 89.7% with ADV catheter versus 59.7% with the first-generation CB ($p < 0.001$) [17]. In addition to previous published paper, we performed for the first time a comparison between lesion areas created by the two different CB. Despite the redesign of the ADV catheter, in our experience the improved acute and long-term procedural success seems not to be related to an increased area of lesion. The main technical limitation of the first generation CB was the temperature gradient from the equator to the distal pole of the CB. More specifically, the first-generation CB had four injection ports positioned just distal to the equator, cooling the balloon surface with a temperature gradient with relatively higher temperatures at the distal pole. As a result, continuous lesions are created if the balloon is centered in the PV antrum. Differently, eccentric CB positions may lead to incomplete lesion formation of tissue, resulting in reconnection gap. Thus, repeated freezing with different CB positions were often necessary to achieve PVI prolonging both procedural and fluoroscopy time. The ADV catheter was redesigned doubling the injection ports and placing themselves more distally on the catheters shaft creating a larger and more uniform zone of freezing on the CB surface. Together, these modifications have been shown to improve procedural and early clinical efficacy during short-term follow-up. Notably, we report a very low incidence of procedure-related complications. It could be related to the size of CB used at our center; indeed we only use the 28 mm balloon due to safety reasons. Creation of proximal lesions at the antrum of PVs should prevent or at least reduce complications such as PV stenosis and phrenic nerve palsy.

5. Study Limitations

This study has some limitations: it is a single-center retrospective analysis in a highly selected population. In order to

complete at least 2 years of follow-up, we excluded patients in which the follow-up was not fully available. Finally, the follow-up was performed for the majority of patients with 12-lead ECG and Holter-ECG monitoring; unfortunately, an event recorder was not available for all patients.

6. Conclusion

On the long-term follow-up, PVI using the ADV performs significantly better when compared to the first-generation CB. Procedure duration and fluoroscopy exposure time were significantly shortened with the ADV catheter. Based on electroanatomical mapping, lesion areas created by the two CB were not statistically different.

Abbreviations

PVI: Pulmonary vein isolation
PAF: Paroxysmal atrial fibrillation
CB: Cryoballoon
ADV: Arctic front advance
PV: Pulmonary vein
AAD: Antiarrhythmic drug.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Impact of Additional Transthoracic Electrical Cardioversion on Cardiac Function and Atrial Fibrillation Recurrence in Patients with Persistent Atrial Fibrillation Who Underwent Radiofrequency Catheter Ablation

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Backgrounds and Objective. During the procession of radiofrequency catheter ablation (RFCA) in persistent atrial fibrillation (AF), transthoracic electrical cardioversion (ECV) is required to terminate AF. The purpose of this study was to determine the impact of additional ECV on cardiac function and recurrence of AF. *Methods and Results*. Persistent AF patients received extensive encircling pulmonary vein isolation (PVI) and additional line ablation. Patients were divided into two groups based on whether they need transthoracic electrical cardioversion to terminate AF: electrical cardioversion (ECV group) and nonelectrical cardioversion (NECV group). Among 111 subjects, 35 patients were returned to sinus rhythm after ablation by ECV (ECV group) and 76 patients had AF termination after the ablation processions (NECV group). During the 12-month follow-ups, the recurrence ratio of patients was comparable in ECV group (15/35) and NECV group (34/76) (44.14% versus 44.74%, $P = 0.853$). Although left atrial diameters (LAD) decreased significantly in both groups, there were no significant differences in LAD and left ventricular cardiac function between ECV group and NECV group. *Conclusions*. This study revealed that ECV has no significant impact on the maintenance of SR and the recovery of cardiac function. Therefore, ECV could be applied safely to recover SR during the procedure of catheter ablation of persistent atrial fibrillation.

1. Introduction

Atrial fibrillation (AF) is the most supraventricular arrhythmias which involved 0.4–1% of people in the general population [1]. AF lead to a low quality of life and high risk of heart failure, stroke, mortality, and rehospitalization [2–4]. Drug therapy is less effective in maintaining sinus rhythm in 40% of all patients [5] with high adverse effects. Nowadays, left atrial catheter ablation is widely used to treat AF [6, 7]. Pulmonary vein isolation (PVI) and complex fractionated atrial electrograms (CFAE) ablation are two common strategies to eliminate triggers and arrhythmogenic substrate of AF [8, 9]. Moreover, additional linear ablation lines, for example, at the left atrial roof and mitral isthmus, may abolish more substrate. However, there are considerable amounts of people who need to receive transthoracic electrical cardioversion (ECV) to terminate persistent AF even after ablation. It is not clear whether ECV affect the recovering of cardiac function and reoccurrence of AF after radiofrequency catheter ablation (RFCA). Therefore, the purpose of this study was to determine the impact of additional ECV on cardiac function after RFCA.

2. Methods and Materials

Patients with symptomatic drug-resistant persistent AF who underwent catheter ablation at our hospitals were included in this study. Persistent AF is defined as AF which is sustained beyond seven days, or lasting less than seven days but necessitating pharmacologic or electrical cardioversion

[10]. Transthoracic echocardiography (TTE) was performed 3 times (before and 6 and 12 months after ablation) to measure conventional parameters and LA function. Ethics approval of the present study was obtained from the local review committee, and all patients provided written informed consent.

Echocardiographic study was performed by an observer who was blinded to the study design using an IE33 ultrasound machine (PHILIP, USA) with a 2.5 MHz transducer. Echocardiograms were recorded and analyzed offline using a customized software package (EchoPAC Systems, PHILIP, USA).

Extensive encircling pulmonary vein isolation (PVI) was performed at the atrial interface of the PV-left atrium [11]. A 7.5-Fr irrigation catheter with a 3.5 mm distal electrode (ThermoCool, Biosense Webster, USA) was used for ablation. An electroanatomical mapping system (Carto™, Biosense Webster, Diamond Bar, CA, USA) was used to validate that linear lines were continuous. The endpoint of the extensive PVI was creation of extensive bidirectional conduction block from the atrium to the PVs. If AF was sustained after PVI, additional ablation consisting of linear ablation of the LA roof, superior vena cava isolation, and/or ablation of continuous fractionated atrial electrograms was performed. If AF did not terminate after that additional ablation, SR was restored by transthoracic electrical cardioversion (100–200 J). Patients who did not restore SR were excluded from this study. Patients were then divided into two groups on the basis of transthoracic electrical cardioversion: electrical cardioversion (ECV group) and none electrical cardioversion (NECV group).

After ablation, patients were followed up for 12 months. At each outpatient visit, a 12-lead electrocardiogram (ECG), 24 hours' Holter, and echocardiographic study were performed. ECG and Holter also were done any time the patients reported palpitations. If the ECG showed any episodes of AF or any other atrial tachyarrhythmias lasting >30 s during follow-up, recurrence of AF was diagnosed.

Continuous data are expressed as mean ± SD. Categorical data are expressed as absolute numbers or percentages. Comparisons between groups were performed using independent samples t-test, and χ^2 test as appropriate. Two-sided $P < 0.05$ was considered significant for all analyses.

3. Results

A total of 111 patients (89 men; age 56±11 years) were included in this study. Among them, 35 patients were returned to sinus rhythm after ablation by ECV (ECV group) and 76 patients had AF termination after the ablation processions (NECV group). As shown in Table 1, the clinical characteristics of the patients in the ECV and NECV groups were comparable. During the 12-month follow-ups, the recurrence ratio of patients was comparable in ECV group (15/35) and NECV group (34/76) (44.14% versus 44.74%, $P = 0.853$).

As shown in Figure 1(b), left atrial diameters (LAD) tent to decrease significantly compared with preablation in both ECV and NECV groups during the 6 and 12 months' follow-ups. There were no significant changes of LVEDs, LVEDd, and LVEF in both groups during follow-ups. Importantly, there were no significant differences in these parameters between ECV group and NECV group which reveal that ECV did retard the recovery of cardiac function (Figure 1(b)).

Table 1: Clinical characteristics and recurrence.

	ECV (35)	NECV (76)	P value
Demographics			
Age (years)	56 ± 12	56 ± 10	0.987
Male (%)	28 (80)	61 (77.6)	0.974
BMI (kg/m^2)	26.3 ± 3.8	24.9 ± 2.7	0.647
Comorbidity, n (%)			
Hypertension (%)	12 (34.3)	23 (30.1)	0.672
Diabetes mellitus (%)	2 (5.7)	3 (3.9)	0.677
CHD (%)	2 (5.7)	4 (5.3)	0.922
Drugs			
ACE/ARB	6 (17.1)	13 (17.1)	0.996
β-blocker	13 (37.1)	29 (38.2)	0.509
AADs, class I	11 (31.4)	24 (31.6)	0.987
AADs, class III	23 (65.7)	50 (65.8)	0.993
Duration (years)	7.2 ± 6.1	5.5 ± 5.4	0.129
Recurrence (%)	15 (42.86)	34 (44.74)	0.853

4. Discussions

This study had revealed that ECV during RFCA in patients with persistent AF did not affect recurrence of AF and LA and LV function in the long term follow-ups although LAD reduced significantly after ablation.

Recovering to SR was expected to achieve better outcome of persistent AF ablation [12]. However, it is controversial to use ECV to terminating AF [13, 14]. Faustino et al. [13] reported that termination of AF through atrial tachycardia during catheter ablation was more effective than both ECV and direct SR in maintaining stable SR. In contrast, Wang et al. [14] observed that long-term SR maintenance is not associated with the style of AF termination. Mont et al. [7] had revealed that repeatedly ECV could act as a predictor factor for ablation failure for long time. In this study, we found that the recurrence ratio was similar in ECV group (15/35) and NECV group (34/76) during the 12 months' follow-ups (Table 1). This finding suggested that the requirement of ECV to terminate AF was not a good indicator for high AF recurrence.

Different results had been reported about the changes of cardiac function after ablation. Previous study revealed that CA can reduce left atrial (LA) volume without a deleterious impact on contractile function [15]. In contrast, a recent study based on MRI imaging reported that LA contractility and compliance are markedly impaired years after successful AF ablation which is closely related to scar burden [16]. ECV causes a so-called phenomenon of "left atrial stunning" [17] which characterized that left atrial function does not recover and even decrease further in patients with AF or atrial flutter (AFL). Similar phenomena were reported in drug cardioversion and spontaneous termination of AF [18, 19]. In

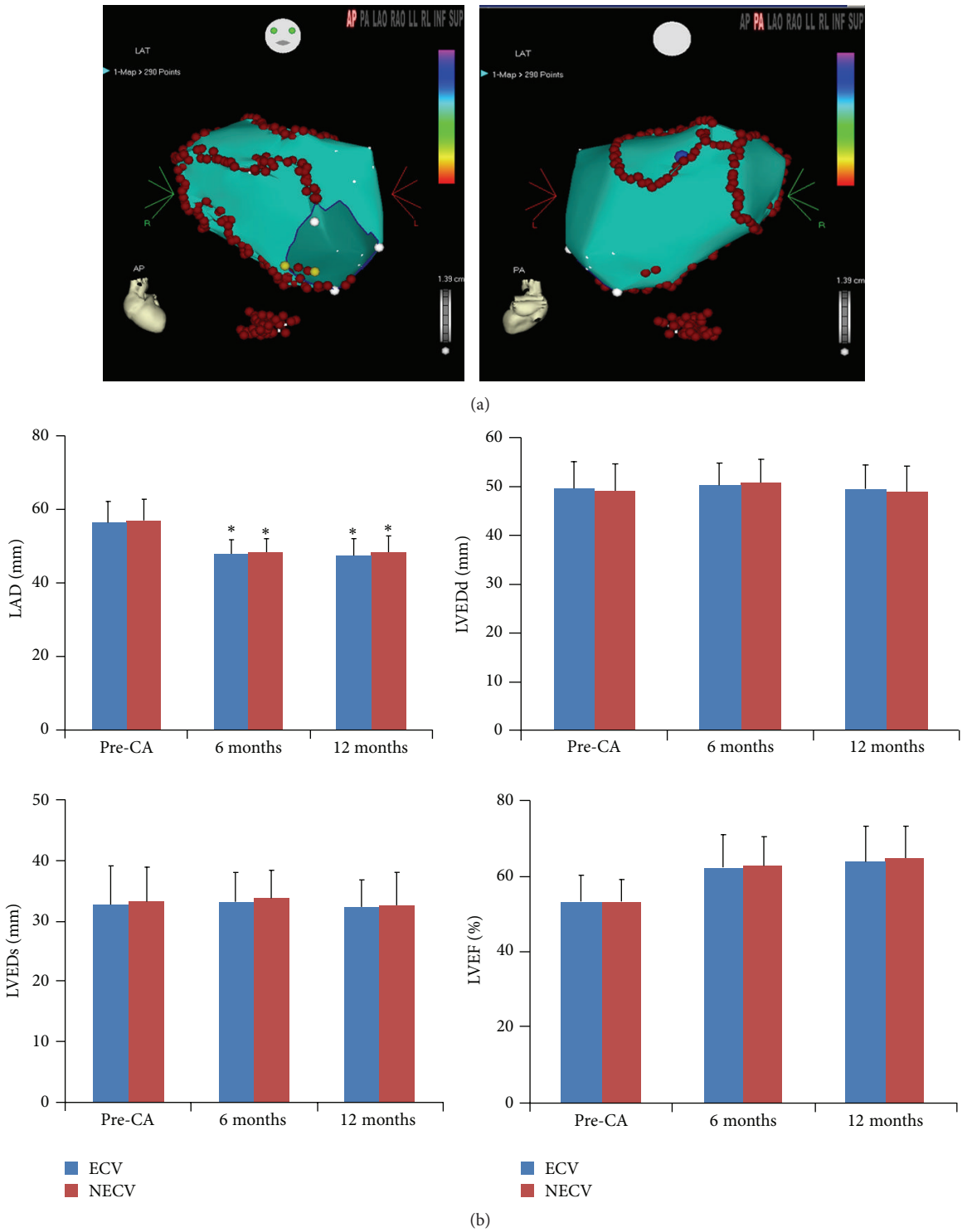

Figure 1: The three-dimensional diagram of catheter ablation persistent atrial fibrillation and myocardial biomarkers. Representative circumferential pulmonary vein isolation and additional ablation line on an electroanatomic map (a). Cardiac function by echocardiography (b). LAD: left atrial diameter. LVEDd: left ventricle diastolic end diameter. LVEDs: left ventricle systolic end diameter. LVEF: left ventricle ejection fraction. $^{*}P < 0.01$ versus pre-CA (before catheter ablation).

this study, LAD tent to reduction in both groups. Furthermore, ECV has no further and directed impact on cardiac function and LAD.

Taken together, our findings revealed that ECV has no significant impact on the maintenance of SR and the recovery of cardiac function. Therefore, ECV could be applied safely to recover SR during the procedure of catheter ablation of persistent atrial fibrillation.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

The Role of Adenosine in Pulmonary Vein Isolation: A Critical Review

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The cornerstone of atrial fibrillation (AF) ablation is pulmonary vein isolation (PVI), which can be achieved in more than 95% of patients at the end of the procedure. However, AF recurrence rates remain high and are related to recovery of PV conduction. Adenosine testing is used to unmask dormant pulmonary vein conduction (DC). The aim of this study is to review the available literature addressing the role of adenosine testing and determine the impact of ablation at sites of PV reconnection on freedom from AF. Adenosine infusion, by restoring the excitability threshold, unmasks reversible injury that could lead to recovery of PV conduction. The studies included in this review suggest that adenosine is useful to unmask nontransmural lesions at risk of reconnection and that further ablation at sites of DC is associated with improvement in freedom from AF. Nevertheless it has been demonstrated that adenosine is not able to predict all veins at risk of later reconnection, which means that veins without DC are not necessarily at low risk. The role of the waiting period in the setting of adenosine testing has also been analyzed, suggesting that in the acute phase adenosine use should be accompanied by enough waiting time.

1. Introduction

Catheter ablation is an effective treatment for atrial fibrillation (AF); the cornerstone of this procedure is durable and effective pulmonary vein isolation (PVI). This is particularly important for paroxysmal AF, whose pathophysiological basis resides in the electrical properties of the strands of muscular tissue that propagate from the left atrium (LA) into the pulmonary veins [1]. These "transition" zones show anisotropic conduction and can have altered excitability that may be able to initiate AF. Following the recommendation of the Heart Rhythm Society and the European Heart Rhythm Association Guidelines in many centers, the first step of AF ablation procedure is PVI, which can be achieved in more than 95% of patients at the end of the procedure [2]. These results are obtained with similar success rates with radiofrequency ablation (RF), cryoablation, and laser ablation [2].

However, AF recurrence rates remain high and substantially unchanged in recent years [2]. AF recurrences are related to recovery of PV-LA conduction; according to some studies, in paroxysmal and persistent AF, the recovery of the PV-LA conduction is associated with high recurrence rates. Conversely, in paroxysmal AF patients, recurrences are extremely rare in patients who maintain permanent PVI [3, 4]. These concepts can be translated, at least partially, to persistent AF, in which the importance of the recovery of the PV-LA conduction has also been observed [3, 5].

It seems therefore essential to ensure, in the acute setting, the effectiveness and durability of the PVI, as this has a direct impact on the long-term outcome. When performing PVI, two main issues have to be addressed: first, how to discern whether permanent isolation (scar tissue) or a reversible injury (oedema) has been obtained?; second, how to know if acutely permanent isolation is going to predict absence of reconnection in the long term?

More than ten years ago, the use of adenosine in PVI was first described [6]. It was administered following initial PVI to unmask dormant pulmonary vein conduction (DC),

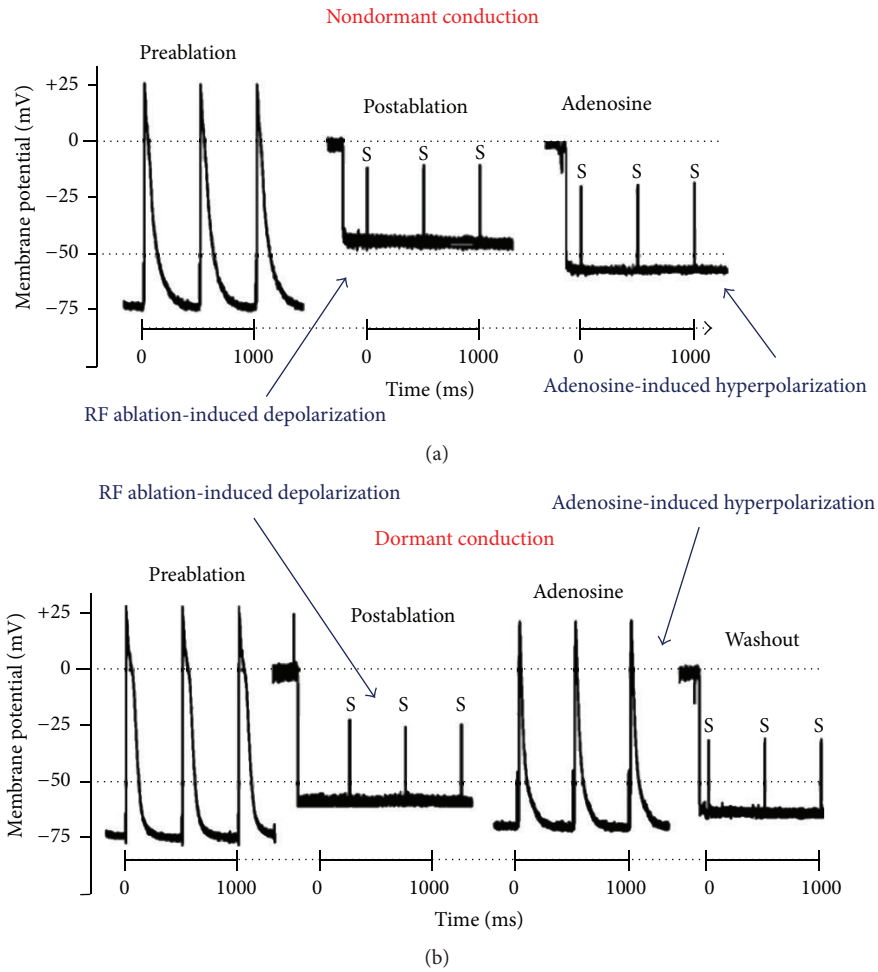


Figure 1: Microelectrode recordings before and after pulmonary vein isolation in a PV without dormant conduction (upper panel) and in a PV with dormant conduction (lower panel). S: stimulus artifacts with no response. Dotted line: excitability threshold at −50 mV (adapted from Datino et al. [7]).

reversible injury, and identify vein reconnection. Adenosine is now commonly used in clinical practice to assess DC with the assumption that additional ablation at sites of reconnection may improve long-term freedom from AF. The aim of this paper is to review the available literature addressing the role of adenosine testing and determine the impact of ablation at sites of PV reconnection on freedom from AF following PVI.

2. Mechanism of Adenosine

First described by Arentz et al. in 2004 [6], the infusion of adenosine after PVI was shown to be able to unmask incomplete lesions. After this observation, several studies found similar results but it was only in 2010 that Datino et al. [7] elegantly elucidated the mechanism of action of adenosine.

To understand adenosine's action, it is necessary to review the effect of RF energy on the LA myocardial cells (assuming the same net effect for other energy sources). After RF ablation, the cell membrane is damaged and is unable to maintain the resting potential, which is the ability to hyperpolarize the cell membrane compared to the extracellular space. This damage changes the resting potential to a level that is above the excitability threshold, making the cell unable to depolarize and conduct.

Potassium currents, activated during the recovery phase of the action potential (AP), contribute to the membrane hyperpolarization and are also damaged by RF lesions. Transient outward potassium currents (IK_{ado}) are especially present in PV cells and in the LA-PV junction.

Adenosine is able to increase these currents facilitating the membrane hyperpolarization and restoring the excitability threshold (Figure 1). Adenosine has a differential effect on PV cells and LA cells. It is able to shorten the action potential duration in both PV and LA cells but significantly hyperpolarizes the resting membrane potential and increases dV/dt_{max} only in PV cells. After radiofrequency ablation, if the membrane damage is not complete and permanent, adenosine infusion can favour recovery of the resting potential and restore excitability. Those cells that have suffered complete and irreversible damage will not be able to respond to adenosine infusion and their membrane will remain depolarized and unexcitable.

Adenosine is also active on the Na channel of the PV cells by removing the voltage-dependent I_{Na} inactivation and increasing the dV/dt_{max} (maximum velocity of phase 0 of the AP). An interesting study by Cheung et al. [8] noted that the PV reconnection occurs during the bradycardia phase of adenosine infusion, confirming the hyperpolarization mechanism underlying the adenosine effect.

The common final pathway of adenosine infusion is to recover excitability and conduction capacity of those cells with partial damage, thus unmasking reversible injury that could lead to recovery of PV-LA conduction and favour AF recurrences.

3. Studies Investigating the Use of Adenosine in PVI

Eleven studies addressing the use of adenosine in radiofrequency PVI were identified (Table 1). In total, 3775 patients were included in 4 retrospective studies ($n = 845$), 5 prospective nonrandomized studies ($n = 283$), and two prospective randomized studies ($n = 2650$): the ADVICE trial (Adenosine Following Pulmonary Vein Isolation to Target Dormant Conduction Elimination, $n = 534$) and the UNDER-ATP trial (UNmasking Dormant Electrical Reconduction by Adenosine Triphosphate, $n = 2113$), which will be analyzed separately [9–11].

These studies have mainly followed two algorithms to assess the utility of adenosine in PVI.

3.1. Adenosine Given versus Adenosine Not Given. Three retrospective studies addressed the impact of adenosine administration after PVI on freedom from AF [12–14] (Table 1). Each study compared a group of patients undergoing adenosine infusion after PVI (and additional ablation in case of DC) with a historical cohort from the same center of PVI patients not receiving adenosine. These studies included 612 patients, 242 of whom received adenosine after PVI. PV reconnection after adenosine infusion was observed in 190 PVs (31%) in 118 patients (49%). The time at which adenosine was given was 20 minutes after PVI in 1 study [13] and not specified in 2 studies [12, 14]. Additional ablation was performed at sites of reconnection until complete isolation of PVs after further adenosine testing. After an average follow-up of 14 months, cohorts of patients who had received routine adenosine administration after PVI showed better outcome with lower AF recurrence rate (Figure 2). The mean overall freedom from AF in patients given adenosine was 76% (73%–80%) versus 61% (60%–62%) in patients not tested.

3.2. Reconnection and Reablation versus No Reconnection. Seven studies, 5 prospective, involved 598 patients divided in two cohorts: an adenosine infusion was used in all patients, and those with DC were reablated until complete isolation of PVs and were compared to patients without DC [6, 12, 15–19] (Table 1). In two studies (101 patients) reablation was not performed in cases of reconnection [6, 16]. 452 patients had paroxysmal AF (76%). The timing of adenosine administration was variable, ranging from immediately after PVI to 30 minutes after PVI. In total, patients with DC after adenosine testing were 282 (47%) and out of 1569 veins tested, 23.7% demonstrated DC (1 study did not specify the total number of veins tested [15]). After an average follow-up of 13 months, patients with DC and reablation did not show better outcome in terms of freedom from AF, but in fact they had an overall nonsignificant trend to worse outcome: 61% for patients with reconnection (38%–76%) and 71.5% in cases with no DC (44%–89%) [20] (Figure 3). Excluding those studies that did not perform further ablation in cases of DC [6, 16], the same outcome was observed (61% versus 72% AF freedom).

3.3. Interpretation of Study Results. Due to the many differences observed between these studies, it seems particularly difficult to definitively interpret the results presented. Most studies are retrospective with a historical cohort as the comparator, which involves limitations related to the period effect, the technological advances, and innovations. In addition, ablation techniques differ substantially between studies: segmental PVI was the technique of choice in the earliest studies while circumferential antral ablation or wide area encirclement was mostly used in more recently published ones. The differences in ablation techniques may influence the results in terms of AF recurrence and could introduce a bias when evaluating the role of adenosine. In some studies, the PVI was performed by antral ablation and electroanatomical mapping followed by careful mapping of the DC sites after adenosine administration [15, 18]. This technique allowed accurate mapping of the earliest adenosine induced PV activation and could help in guiding further ablation lesions. In comparison with purely anatomical PV encirclement, this electrophysiological approach may help in optimizing the adenosine test, perhaps resulting in better outcome in terms of AF recurrences.

Furthermore, adenosine dose and the presence of concurrent isoproterenol infusion show large variations across the studies, and not all of them are specified which was the endpoint of adenosine administration, with the presence of temporary heart block as only a surrogate goal. Finally, the wide range in timing of adenosine administration after PVI could be an important issue when evaluating early conduction recovery after ablation.

Despite these differences, the results seem to suggest that the presence of dormant PV conduction revealed by adenosine could be a marker for the absence of transmurality of ablation encircling lesions in a vein at especially high risk of reconnection. This could be secondary to anatomical challenges or poor catheter contact and adenosine may help to unmask the presence of ineffectively ablated areas [21]. In this setting, the use of the adenosine test, even if in retrospective studies, has appeared to improve outcomes compared to patients not tested.

On the other hand, it could be expected that further ablation at sites of reconnection should lead to higher freedom from AF recurrences but all studies analyzed failed to demonstrate this assumption.

Generally, in the studies described, the additional ablation is limited to the immediate site of PV reconnection and no further extension of the waiting period or more

Table 1: Radiofrequency ablation studies addressing adenosine use in PVI.

Study	N	Comparison groups	n (%)	n veins	pAF (%)	Waiting time (min)	Adenosine dose (mg)	Tested veins	Reconnected veins (%)	Follow-up (months)	AF-free (%)	p	Redo procedure	Reconnected veins in redo	
Adenosine given versus adenosine not given															
Hachiya et al. (2007) [12]† Retrospective	252	Adenosine given Adenosine not given	82 170	164 —	62 (76) 135 (79)	— —	30 —	164 —	41 (25%) in 34 patients (41%) —	6.1 ± 3.3	60 (73%) 102 (60%)	0.04	—	—	
Matsuo et al. (2007) [13]† Retrospective	148	Adenosine given Adenosine not given	54 94	224 —	36 (67) 60 (64)	20 —	20 —	224 —	59 (26%) in 30 patients (56%) —	19.9 ± 6	43 (80%) 56 (60%)	<0.05	9 (17%) 36 (38%)	DC+ DC−	8/14 (57%) 12/22 (55%)
Kumagai et al. (2010) [14]† Retrospective	212	Adenosine given Adenosine not given	106 106	216 —	94 (89) 86 (81)	— —	10 —	216 —	90 (42%) in 54 patients (51%) —	16 ± 5 16 ± 7	81 (76%) 66 (62%)	0.03	11 (10%) 10 (9.4%)		— —
Kobori et al. (2015) [11]* Randomized Prospective	2113	Adenosine given Adenosine not given	1112 1001	—	1420 (67%)	43 —	0.4 mg/kg —	— —	307 in 1112 patients (27.6%) —	12	68.7% 67.1%	0.25	—		—
Reconnection versus no reconnection															
Tritto et al. (2004) [19] Prospective	29	Reconnection No reconnection	16 (55) 13 (45)	74	21 (72)	10	12	62	22 (35%) 0	6.3 ± 2.4	11 (69%) 9 (69%)	1	6	DC+ DC−	6/8 (75%) 4/5 (80%)
Arentz et al. (2004) [6]¶ Prospective	29	Reconnection No reconnection	13 (45) 16 (55)	83	20 (69)	0	12–18	53	13 (24%) 0	12	5 (38%) 7 (44%)	1	14	DC+ DC−	5/7 (71%) 7/20 (35%)
Hachiya et al. (2007) [12]* Retrospective	82	Reconnection No reconnection	34 (41) 48 (59)	164	62 (76)	0	30	164	41 (25%) 0	6.1 ± 3.3	23 (68%) 37 (77%)	—	—		—
Matsuo et al. (2010) [17] Retrospective	233	Reconnection No reconnection	139 (60) 94 (40)	930	144 (62)	20	20	928	225 0	29 ± 13	87 (62.6%) 62 (66%)	0.69	43 (31%) 28 (30%)	DC+ DC−	42/78 (54%) 119/202 (59%)
Gula et al. (2011) [16]*¶ Prospective	72	Reconnection No reconnection	25 (35) 47 (65)	50 94	25 (100) 47 (100)	30	12	50 94	29 (58%) 0	12	19 (76%) 35 (74%)	1	6 (24%) 12 (26%)	DC+ DC−	9/10 (90%) 22/26 (85%)
Miyazaki et al. (2012) [18]* Prospective	109	Reconnection No reconnection	39 (36) 70 (64)	78 140	39 (100) 70 (100)	0	40	78 140	42 (54%) 0	12	20 (51%) 51 (73%)	0.03	10 (26%) 22 (31%)	DC+ DC−	6/10 (60%) 27/54 (50%)
Anter et al. (2014) [15] Prospective	44	Reconnection No reconnection	16 (36) 28 (64)	—	8 (50) 16 (57)	30	12–48	—	26 0	12	8 (50%) 25 (89%)	0.009	3 (7%)		— —
Macle et al. (2015) [10]# Randomized Prospective	401	Reconnection and no ablation Reconnection and ablation No reconnection	147 (28) 137 (26) 117‡	— — —	147 (100) 137 (100) 117 (100)	20	12–18	2085	— — 0	12	42.3% 69.4% 55.7%	<0.001 0.019	110		88% 48% 55%

*Veins considered and tested per pair; ¶no further ablation in case of dormant conduction; †historical cohort as comparator; ‡out of 250 patients without dormant conduction 117 were randomized to intense follow-up registry. #This study specified the freedom from any atrial tachycardia as the primary endpoint. DC+: veins with dormant conduction at first procedure; DC−: veins without dormant conduction at first procedure; pAF: paroxysmal atrial fibrillation.

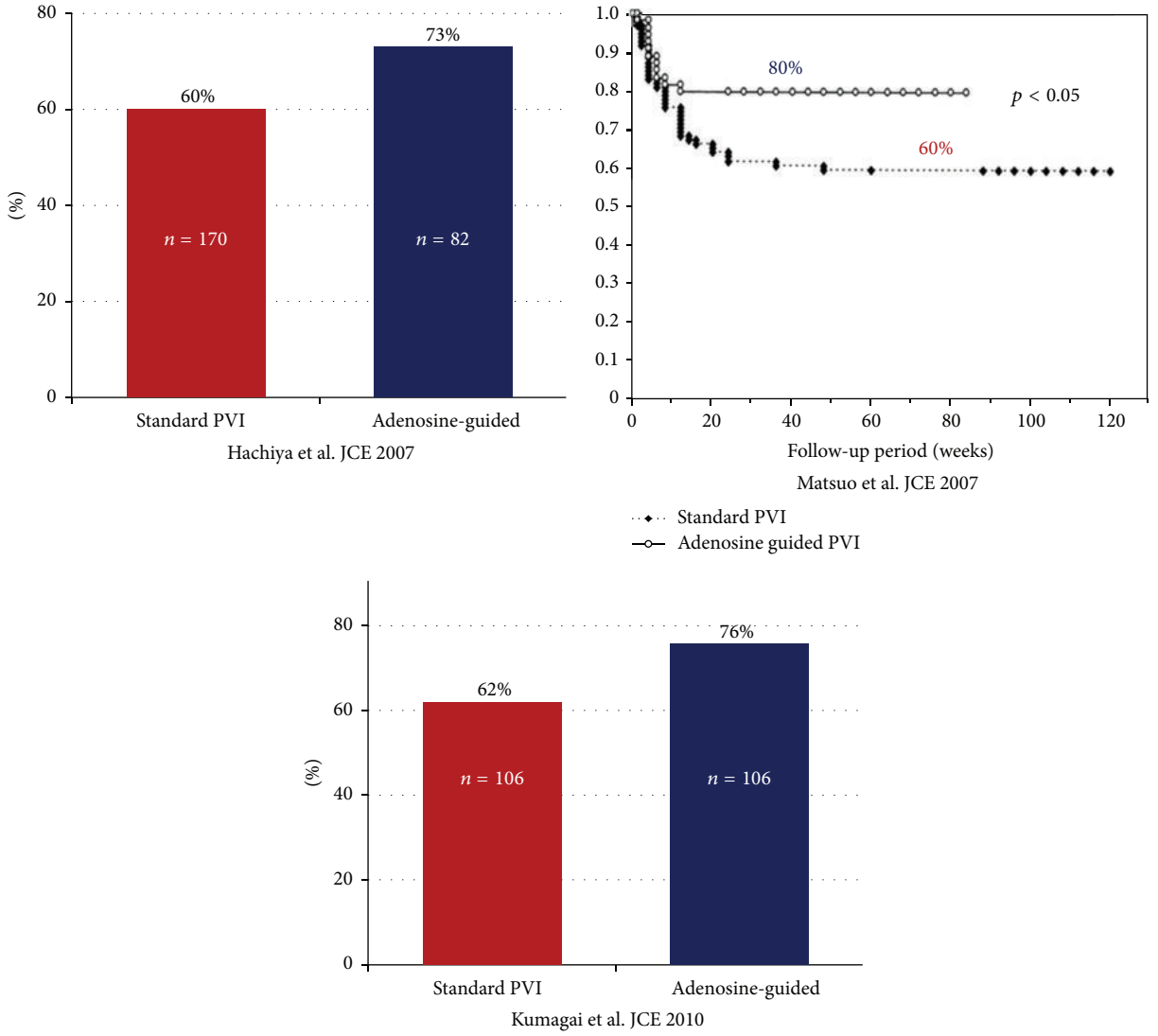


Figure 2: Freedom from AF in three nonrandomized retrospective studies comparing adenosine given versus adenosine not given [12–14]. PVI: pulmonary vein isolation.

extensive reinforcement of the circumferential ablation line was performed. So the overall picture is that it is important to unmask veins at high risk but the usefulness of further ablation is yet to be determined.

3.4. The ADVICE Trial. The ADVICE trial is first prospective multicenter randomized study addressing the role of adenosine in PVI [9, 10]. The aim of the study was to determine whether an adenosine-guided ablation strategy improves the long-term efficacy of PVI for the treatment of paroxysmal AF. Of the 534 patients undergoing adenosine infusion, 284 (53%) had acute reconnection; 147 of them were randomized to receive no further ablation while 137 received additional RF pulses (over 95% success in eliminating DC). Out of the 250 patients without reconnection, 117 were randomized to an intense follow-up registry.

It is important to note that all patients underwent a 20-minute waiting period after PVI, and after that spontaneous vein reconnection (27% of patients, 9% of PVs) was eliminated and then adenosine was administered. Every vein was tested separately by means of a circular catheter inserted into the vein at the moment of the adenosine infusion, allowing the exact localization of the reconnection point.

The primary endpoint was the first documented symptomatic atrial tachyarrhythmia recurrence (AF, atrial flutter, and atrial tachycardia) after the blanking period. After 12 months of follow-up, patients showing DC and receiving additional ablation had a better outcome in terms of freedom from atrial tachyarrhythmias (69.4%) than patients with DC but without further ablation (42.3%, HR 0.44, $p < 0.001$) and also than those without reconnection (55.7%, HR 0.64, $p = 0.019$) included in the intense follow-up registry (Figure 4).

Need for repeated ablation during follow-up was higher in cases of acute reconnection without additional ablation (35%) compared to patients undergoing further ablation (20.4%, OR 0.48, $p = 0.006$). The authors concluded that

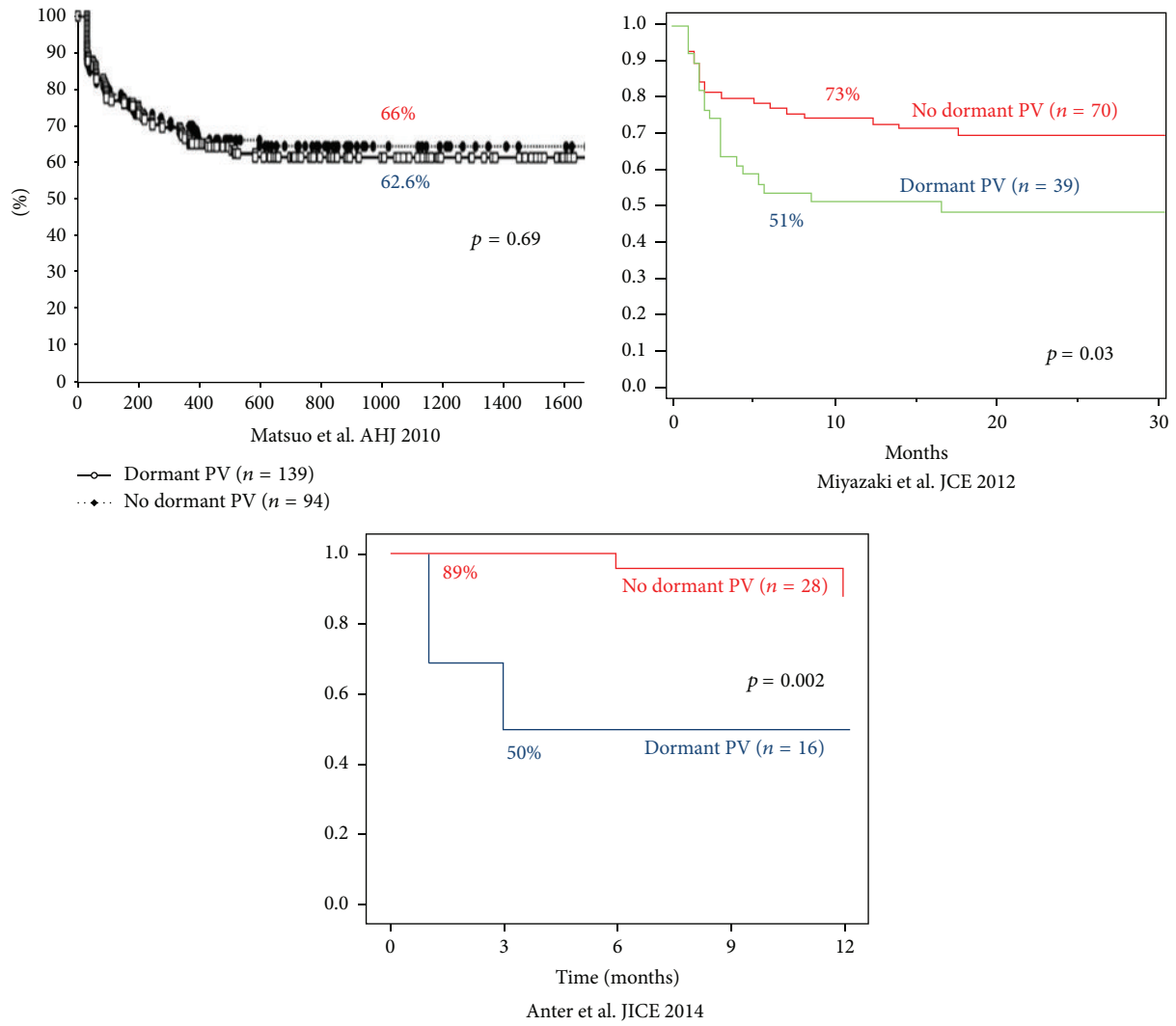


Figure 3: Freedom from AF in three nonrandomized studies comparing patients with adenosine induced reconnection and reablation (dormant PV) versus no reconnection [15, 17, 18]. PV: pulmonary vein.

DC is associated with increased risk of atrial tachyarrhythmia recurrence. Elimination of dormant pulmonary vein conduction reduces recurrent atrial tachyarrhythmias by >50%. They consider that these results support the routine use of adenosine for identification and elimination of DC during PVI procedures for paroxysmal AF.

The ADVICE trial adds important information on the role of adenosine infusion in PVI. On the one hand, it shows that patients with DC not receiving further ablation are extremely prone to AF recurrences. This result had been suggested to a point by the nonrandomized studies involving patients not tested with adenosine; the ADVICE trial confirms that speculation and shows the magnitude of the recurrence rate among those high risk patients. It clearly underlines the importance of looking for reconnection after an appropriate waiting time. Furthermore by clearly separating the effect of waiting time on spontaneous reconnection from the adenosine administration, the study may be able to evaluate the net effect of adenosine testing on the presence of DC.

The second important finding of the ADVICE trial is that further ablation of reconnected veins led to 12-month freedom from symptomatic atrial tachyarrhythmia after a single ablation procedure that was significantly higher than that in patients with DC not reablated. This seems to indicate that, after unmasking DC, it is useful to perform additional ablation to improve long-term outcome.

The third group of patients, those without DC, were intensively followed during 12 months and demonstrated higher atrial tachyarrhythmia recurrence rates than patients with DC and reablation. This finding may be considered quite surprising as this group of patients could be classified as being at low risk of vein reconnection, after a waiting period, ablation of spontaneous reconnection, and negative adenosine test.

As presented above, previous studies were unable to identify a benefit in further ablation, and on the contrary there was a trend, although not significant, towards a higher rate of recurrence compared to patients without reconnection.

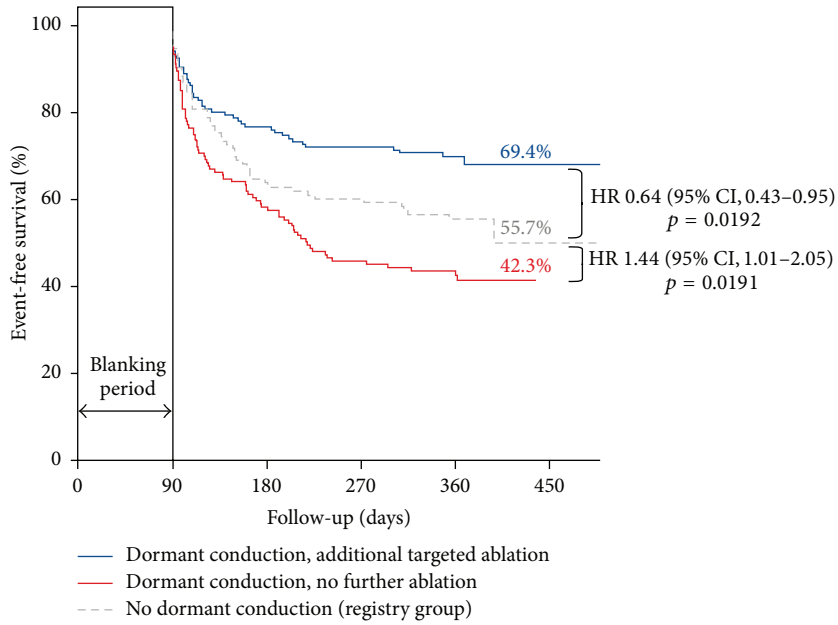


Figure 4: Freedom from symptomatic atrial tachyarrhythmia after a single ablation procedure in the ADVICE trial [9, 10].

One may speculate that negativity to adenosine test is not, per se, an indicator of low risk of recurrence but instead a response that leaves the door open to a possible reconnection, perhaps occurring not so early. In fact adenosine response is not an all-or-nothing phenomenon, but it is strictly related to the degree of cell damage, and its ability to restore the excitability threshold depends on the degree of depolarization the cell has suffered. In view of these considerations, the results of the ADVICE trial may suggest that patients without adenosine unmasked DC should be checked very carefully and perhaps, as discussed later, given more time during a waiting period.

3.5. The UNDER-ATP Trial. The UNDER-ATP trial is a recently published prospective multicenter randomized study addressing the role of adenosine in PVI [11].

The aim of the study was to determine whether an adenosine-guided ablation strategy improves the long-term efficacy of PVI for the treatment of paroxysmal, persistent (22.7%), and long lasting (10.1%) AF. Of the 2113 enrolled patients, 1001 were randomized to standard PVI and subsequent follow-up while 1112 underwent adenosine infusion at a fixed dose of 0.4 mg/Kg body weight after a variable waiting time following PVI (median time 43 min). After waiting time, 42% of patients in both groups had spontaneous reconnection that was targeted with further ablation. Thereafter, adenosine test was performed in the adenosine-guided PVI group, unmasking dormant conduction in 489 PVs among 307 patients (27.6%). Additional RF was delivered until dormant conduction was completely eliminated (98% of success). Almost 74% of patients were ablated with double circular catheter technique; consequently, PVs were tested with a single adenosine infusion for each pair of veins. A considerable number of patients received additional lines, specifically complex fractionated electrogram ablation (12%), mitral isthmus line (6.7%), roof line (18.2%), and superior vena cava isolation (13.9%).

The primary endpoint was recurrent atrial tachyarrhythmias at 1 year with the blanking period of 90 days after PVI.

After 12 months of follow-up, no differences were observed between groups, and 68.7% of patients in the adenosine-guided PVI group and 67.1% of patients in the conventional PVI group were free from the primary endpoint. Subgroup analysis showed no differences in terms of primary endpoint between patients with paroxysmal AF versus persistent/long standing and between patients with PVI alone versus PVI + additional lines.

The results of this study are clearly divergent from those obtained by the ADVICE trial. To understand these differences, it may be helpful to carefully analyze methodology and treatment approach. The ADVICE trial allowed a fixed waiting time of 20 minutes after completion of PVI and the dose of adenosine used depended on the ability to produce AV block or sinus pause. In the UNDER-ATP trial, the dose of adenosine was predetermined and the same for every patient, regardless of the effect produced. Moreover, there was no protocol regarding timing of adenosine administration, which was infused after the left atrial ablation was complete, resulting in a median time from PVI to adenosine more than double that of the ADVICE trial. On this basis, it is not surprising that in the UNDER-ATP trial the spontaneous reconnection was higher and the adenosine induced dormant conduction was lower than the ADVICE trial. Next, the ADVICE trial examined only patients with paroxysmal AF treated with PVI alone. In the UNDER-ATP trial, 32.8% of the patients had persistent or long standing AF and PVI procedure was often accompanied by additional linear lesions or complex electrogram ablation. This is remarkable since it

is known that PVI in paroxysmal AF targets pulmonary vein triggers and adenosine test may help in assuring complete PVs isolation but it lacks any effect in case of additional linear lesions or non-PVs triggers. Moreover, patients with persistent or long standing AF develop more advanced disease with other driving mechanisms that may increase the risk of recurrence irrespective of the adenosine test results or the PVs reconnection.

There is another aspect to consider when analyzing the differences between the two studies. The UNDER-ATP trial used an anatomical approach to PVI by extensively encircling ipsilateral PVs mostly with the use of double circular catheters. The double circular catheter was also used for adenosine testing, preventing from accurate mapping of the DC site and tagging of the reconnection points before further RF delivery. Conversely, the ADVICE trial followed a different approach, based on the EP mapping of the DC sites for each vein that were tagged and targeted with further ablation.

These considerations may help in understanding the differences observed between both studies in order to extract concepts that can be useful in clinical practice. It can be suggested that adenosine test should probably be confined to patients with paroxysmal AF treated with PVI alone and that the accurate EP mapping of the DC site is a requisite for a reliable adenosine test.

3.5.1. Other Energy Sources. The use of adenosine in PVI has also been studied in cases of ablation with other energy sources. Although with less amount of evidence, cryoablation and laser ablation already have some studies that have examined the role of adenosine (Table 2). There are 5 cryoablation studies, 3 of them with a control group [22–26], that analyzed the presence of DC after adenosine infusion. In cases of appearance of DC, additional applications were performed by cryoballoon or with a conventional cryothermal catheter until disappearance of DC after further adenosine infusion. The lack of homogeneity between these studies, as seen for RF ablation papers, prevents a reliable generalization of the results. The differences in the methodology used, the limited number of patients included, the presence of historical cohorts as a comparator, the varying dose of adenosine, and the presence or nonpresence of waiting time require extremely careful interpretation of the results. The overall picture is that the transmurality of the lesions is clearly related to positivity to adenosine: these studies revealed that the isolation of the veins with the first freeze, the time to isolation, and the nadir temperature are factors that identify veins being permanently isolated and less prone to DC and long-term recurrence. Two studies [23, 26] observed that freedom from AF recurrences was higher in the adenosine and reablation group compared to patients that did not receive adenosine. In one study, it has been observed that ablation with additional DC evaluation and treatment was found to independently reduce the risk of AF recurrence at follow-up [26]. Furthermore, the best results were obtained when the waiting time was combined with adenosine infusion. Finally, it can be noted (Table 2) that the percentage of patients with DC (and therefore the percentage of veins) seems less in cryoablation than in RF studies. The reason for this finding is unclear; however, it has been suggested that one of the strengths of cryoablation is the ability to create continuous and contiguous injuries, whose absence could be related to higher positivity to adenosine [29]. On the other hand, this finding could be related to a different time dependency of the adenosine response in cryoablation compared to RF, as it could be suggested by the similar success rates of both techniques in published studies in terms of freedom from AF. Probably the different mechanism of lesion formation implies a different time course of the adenosine response. Nevertheless, the absence of direct comparison and the paucity of evidence on this subject make every interpretation merely speculative.

Regarding laser ablation, evidence about the use of adenosine is more limited; one study [27] compared cryoablation with laser ablation and found higher rates of DC in laser ablation, but with the same AF recurrence rates after 1 year. Another study [28] observed that DC is associated with poor vein occlusion, longer duration of laser application, lower mean power of the applied laser energy, and higher overall number of 5.5 W applications; targeting veins with dormant conduction seems to improve rates of freedom from AF.

4. Waiting Time

Waiting time is a key variable when assessing PV reconnection after isolation. The mechanism of injury, based on the cell membrane damage and subsequent death, suggests that ensuring a permanent lesion requires some waiting time. How much time should be waited after PVI is a matter of discussion as there is a wide range of waiting times in the studies analyzed, and in the daily setting it could be difficult to allow more than 20 minutes after PVI. It has to be taken into account that the waiting time alone has a very important value because it allows those transient and nontransmural injuries to recover and restore LA-PV conduction.

The recovery of conduction is greater when increasing the waiting time and in some cases LA-PV conduction has been observed 60 minutes or more after ablation [30]. Cheema and colleagues [31] showed that patients with 60 minutes of waiting time had reconnection in >30% of the PVs and such patients, after additional ablation, had significantly higher freedom from AF than patients without any waiting time after PVI.

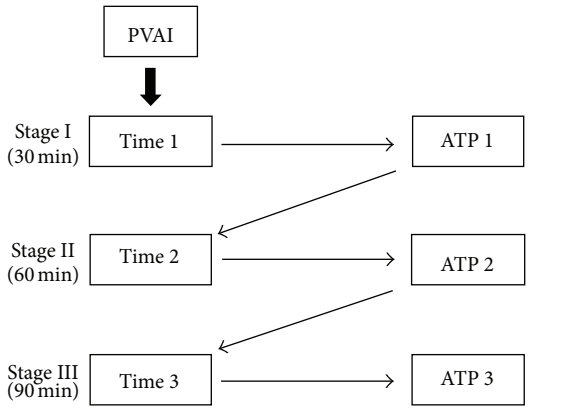
Yamane and colleagues [32] combined the use of waiting time and adenosine: the study algorithm had 3 subsequent stages, each of them constituted by 30 minutes of waiting time and adenosine infusion (Figure 5(a)), followed by additional ablation at each step in case of reconnection. They proved that reconnection can be observed until 90 minutes after PVI and after 2 separated adenosine administrations. Thanks to this protocol, which obviously prolonged procedure times, they obtained 92% of freedom from AF after 1 year. These studies suggest that both waiting time and adenosine are useful when assessing permanent lesions after PVI but it remains unclear whether they provide the same information.

Jiang et al. [33] studied the relationship between waiting time and adenosine: immediately after PVI adenosine (20 mg) was infused, a waiting period of 30 minutes was

Table 2: Cryoablation and laser ablation studies addressing adenosine use in PVI.

Study	*N*	Comparison groups	*n*	*n* veins	Energy	pAF	Waiting time (minutes)	Adenosine dose (mg)	Reconnected patients (%)	Reconnected veins (%)	Follow-up (months)	AF freedom (%)	*p*	Recurrence in patients with DC (%)
Chierchia et al. 2009 [22]	39	—	—	149	Cryo	100%	15	20	5 (13%)	7 (4.6%)	6	77%	—	2/5 (40%)
Van Belle et al. 2012 [23]	99	A given	34	132	Cryo	100%	0	25	7 (21%)	9 (8%)	17 ± 5	68%	0.04	—
		A not given	65	—	Cryo	100%	—	—	—	—	17 ± 5	46%		—
Ciconte et al. 2014 [24]	50	A given	—	200	Cryo	82%	30	18–30	6 (12%)	8 (4%)	7 ± 1.7	86%	—	0 (0%)
Kumar et al. 2015 [25]	90	A given	45	179	Cryo	79	30	15 ± 3	—	8 (4.5%)	13 ± 1	84%	NS	0 (0%)
		A not given	45	179	Cryo		—		—	—	12 ± 2	79%		—
Compier et al. 2015 [26]	98	A given	36	143	Cryo	86%	30	17 ± 5	15 (42%)	20 (14%)	12 ± 1	83%	0.02	—
		A not given (historical cohort)	62	—	Cryo	90%	—	—	—	—	11 ± 1	60%		—
Kumar et al. 2014 [27]	60	A given	40	151	Cryo	87.5%	30	12 ± 3	4 (2.5%)	4 (5%)	11 ± 3	85%	NS	—
		A given	20	80	Laser	90%	30	12 ± 3	7 (35%)	11 (13.8%)	9 ± 2	85%		—
Üçer et al. 2015 [28]	26	A given	—	102	Laser	100%	30	18	5 (20%)	6 (6.7%)	6	81%	—	2/5 (40%)

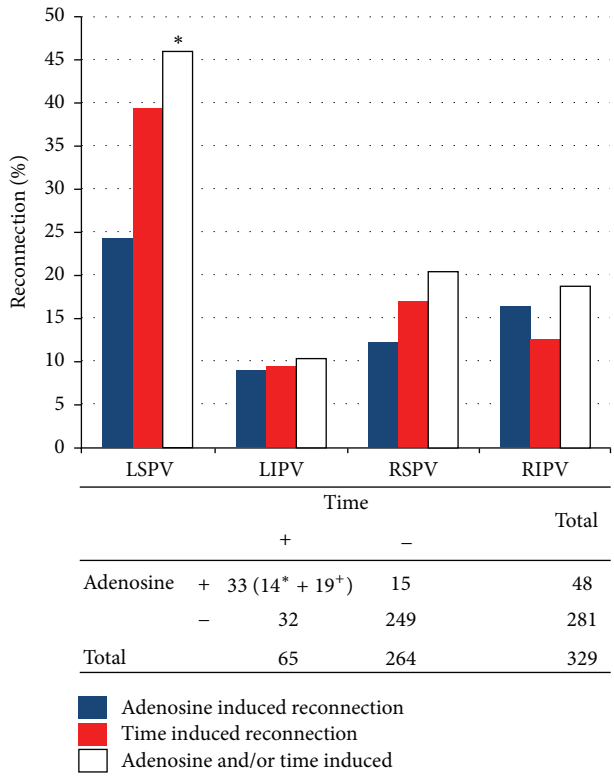
A: adenosine; AF: atrial fibrillation; DC: dormant conduction; pAF: paroxysmal atrial fibrillation.



Acute PV reconnections after PVI after each step

Time 1 (30 min)	ATP 1	Time 2 (60 min)	ATP 2	Time 3 (90 min)	ATP 3	Total
29%	29%	25%	14%	3%	0%	100%

(a)



		Time		Total
		+	–	
Adenosine	+	33 (14* + 19+)	15	48
	–	32	249	281
Total		65	264	329

(b)

Figure 5: (a) Study algorithm and results from Yamane et al. [32]; PVAI: pulmonary vein isolation, ATP: Adenosine Triphosphate. (b) Incidence of reconnection in individual pulmonary veins mediated by adenosine and/or time, adapted from Jiang et al. [33] (see text for details).

allowed before assessment of reconnection (Figure 5(b)). Out of 329 PVs, 80 presented reconnection: 15 were adenosine positive but did not have permanent reconnection after the waiting time, while, out of 65 time-positive reconnections, 32 were adenosine-negative. They also noted that most of the PVs that reconnected with both adenosine-induction and waiting time after 30′ after PVI were found to be conducted at the same gap. This study concluded that the agreement between the two techniques is only moderate ($K = 0.5$) and that adenosine infusion may facilitate the process of time dependent reconnection.

In conclusion, it could be claimed that adenosine's effect may vary according to the degree of cell damage and membrane depolarization. In case of greater damage, adenosine may not be immediately able to restore the hyperpolarization required to reach the excitability threshold and it may be possible that only after enough waiting time its facilitating action could lead to the recovery of the LA-PV conduction. Therefore, the role of adenosine and waiting time can be considered as interdependent and it is advisable to use both techniques to ensure permanent injuries.

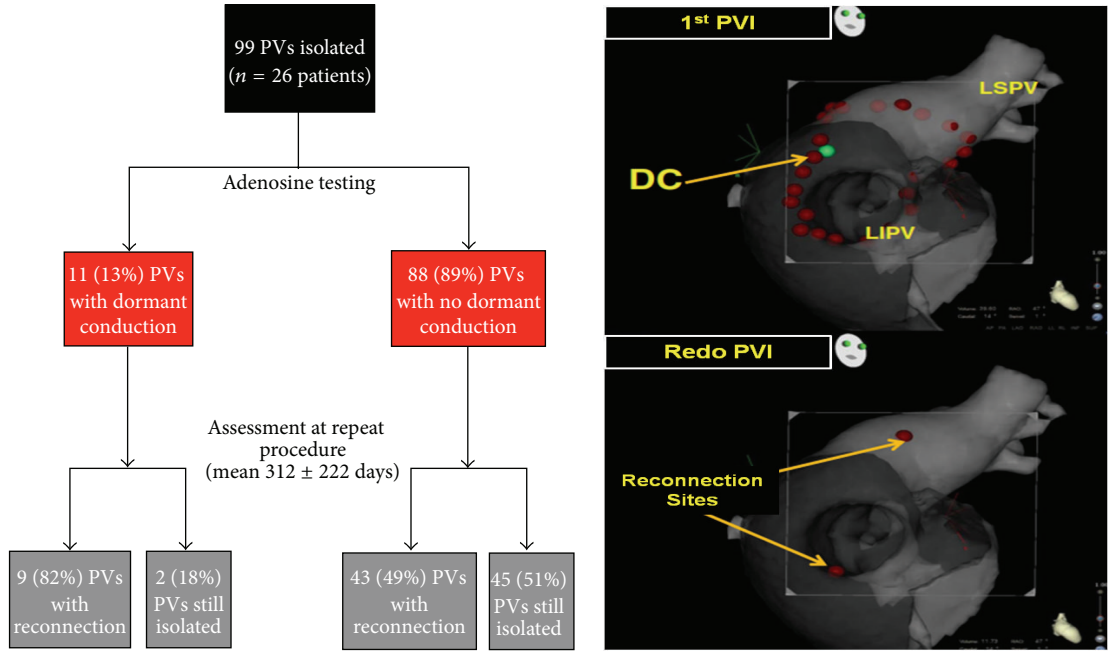
5. Prediction of Reconnection

As discussed above, adenosine seems quite useful to predict acute reconnection and possibly additional ablation may help in reducing the risk of AF recurrence. Nevertheless, it has still to be clarified if long-term PV reconnection, by far the most important cause of AF recurrence after PVI, can be predicted by adenosine induced dormant conduction.

Gula and coworkers [16] suggested that adenosine testing for the assessment of transient conduction recovery does not appear to predict recurrence of clinical AF. In 18 redo procedures, they observed 31 reconnected veins: 9 had been positive to adenosine and 22 negative at initial ablation. In this study, adenosine correctly predicted 13 out of 36 veins, resulting in positive predictive value (PPV) of 90% and negative predictive value (NPV) of 15%.

Lin et al. [34] described similar results in their study designed to address the ability of DC to predict AF recurrence and PV reconnection. In 26 redo procedures, they observed 53% rate of chronic PV reconnection (52 out of 99 PVs). DC had a PPV of 82% and NPV of 51% (Figure 6(a)). In these 2 studies, adenosine was shown to have good specificity and predictive value for future PV reconnection, especially if DC was left unablated [16]. Sensitivity was low and adenosine was unable to rule out chronic reconnection in case of absence of DC.

These studies analyzed each vein as a whole and did not focus on the precise spot of reconnection compared to the spot of DC at initial ablation procedure. In order to have reliable information about adenosine's ability to predict



(a) (b)

Figure 6: (a) Adenosine testing results and PV reconnection rate at redo procedure in Lin et al. [34]. (b) Example of redo procedure reconnection sites that totally differed from DC sites at first procedure [35]. DC: dormant conduction. PVI: pulmonary vein isolation (see text for details).

outcome, it appears to be extremely important to understand if a vein reconnects in the same spot with DC. Okishige and coworkers [35] performed a prospective study to assess the predictive value of the response to adenosine in terms of identifying the reconnection sites associated with AF recurrence. In their study, sites of DC were tagged using a three-dimensional mapping system and left unablated. DC was observed in 56 out of 91 patients (62%). After an average follow-up of 15 months, 62 patients were free from AF recurrences; 32 of them (52%) had DC during the first PVI. A second ablation procedure was performed in 29 patients (32%), all of them with DC during the first PVI.

Nineteen (66%) patients had reconnections sites that all differed from those of the DC sites, whilst only 3 patients (10%) had all reconnections sites identical to the DC sites. The remaining patients (24%) had reconnection sites that involved not only DC sites but also different sites (Figure 6(b)). The kappa test showed a poor agreement between the DC sites and reconnection sites in all PVs ($\kappa = 0.157$). The overall result is that the vast majority of the reconnection sites differed from the DC sites that were detected by an adenosine injection in the first session. Finally, the ADVICE trial showed that, in 110 redo procedures, the reconnection rate was 48% for PVs with DC and reablation ($n = 40$), 55% for PVs without DC ($n = 229$), and 88% for PVs with DC without reablation ($n = 82$).

In conclusion, there seems to be enough evidence to affirm that, despite a good specificity and PPV, the high number of false negative results (very low sensitivity) substantially decreases the diagnostic capacity of adenosine in terms of predicting long-term PV reconnection. These findings strengthen the idea that the overall benefit of adenosine testing and targeted ablation of DC is mostly related to the elimination of those lesions that appear to be nontransmural in the acute phase, with possible positive prognostic effect, according to the ADVICE trial results. On the contrary, the usefulness of the adenosine test as a predictor of chronic reconnection is likely to be modest at best.

6. Other Techniques

New techniques have been described in order to improve ability of identifying incomplete lesion after RF ablation. Among new technologies, the use of Pace-capture PVI could be useful in facilitating identification of residual gaps [36]. Andrade et al. compared 40 patients that received additional ablation in case of atrial capture at high output pacing on the ablation line with a group of patients with additional ablation in case of adenosine revealed DC. Rates of pace capture in the pace-capture group and rates of DC in the adenosine group were found to be similar but patients in the pace-capture group who underwent adenosine testing showed significant reduction in the incidence of DC [37].

Recently the use of contact force catheters has been introduced in RF ablation with the aim to obtain true permanent lesions more efficaciously than standard catheters [38]. It has been shown that the use of contact forces helps in improving AF ablation effectiveness [39] and may lead to better long-term results in terms of freedom from AF [40]. One study evaluated adenosine response with the use

of contact force catheters and observed that the presence of DC is significantly reduced compared to standard catheters, suggesting that this could be related to better AF freedom in the long term [41].

7. Conclusion

This critical review tries to offer a complete overview on the role of adenosine in PVI based on the available literature.

Data presented suggests that adenosine is useful to unmask nontransmural lesions at high risk of reconnection. This means that further ablation at sites of DC is associated with improvement in freedom from AF after PVI. Therefore, based on these considerations, adenosine should be used routinely in clinical practice. Nevertheless, it is fundamental to keep in mind the limitations of the adenosine testing. In the acute phase, adenosine use should be accompanied by enough waiting time, as the adenosine response immediately after RF lesion may lack real value. Moreover, it has been demonstrated that adenosine is not able to predict all veins at risk of reconnection, mostly due to the very low negative predictive value, which means that veins without DC are not necessarily at low risk of reconnection.

Risks of PV reconnection and AF recurrence are related to the ability of creating reliable permanent scar. Among new technologies, contact force catheters improve lesion transmurality and significantly reduce the incidence of DC.

In conclusion, the objective of PVI should be trying to obtain complete and permanent lesions from the beginning, and the combination of adenosine, waiting time, and contact force assessment seems at the moment the best strategy to achieve this goal.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

Cellular and Molecular Mechanisms of Arrhythmia by Oxidative Stress

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Current therapies for arrhythmia using ion channel blockade, catheter ablation, or an implantable cardioverter defibrillator have limitations, and it is important to search for new antiarrhythmic therapeutic targets. Both atrial fibrillation and heart failure, a condition with increased arrhythmic risk, are associated with excess amount of reactive oxygen species (ROS). There are several possible ways for ROS to induce arrhythmia. ROS can cause focal activity and reentry. ROS alter multiple cardiac ionic currents. ROS promote cardiac fibrosis and impair gap junction function, resulting in reduced myocyte coupling and facilitation of reentry. In order to design effective antioxidant drugs for treatment of arrhythmia, it is essential to explore the molecular mechanisms by which ROS exert these arrhythmic effects. Activation of Ca^{2+}/CaM-dependent kinase II, c-Src tyrosine kinase, protein kinase C, and abnormal splicing of cardiac sodium channels are among the recently discovered molecular mechanisms of ROS-induced arrhythmia.

1. Scope of the Problem in Treatment of Arrhythmias

Cardiovascular disorders are the most common cause of death in the United States and most of the developed countries [1]. Ventricular fibrillation (VF) and ventricular tachycardia (VT) are the most common cause of sudden cardiac death (SCD) [2]. Atrial fibrillation (AF), although usually not life threatening, is associated with higher thromboembolism, increased mortality, and high healthcare cost and its incidence is increasing [3–8].

Current therapies for treatment of arrhythmias are antiarrhythmic drugs, catheter ablation, and implantable cardioverter defibrillators (ICDs) for VT/VF. Although these therapies have had some success, they have limitations. Ion channel blockade has important limitations for chronic treatment and prevention of those arrhythmias. For example, the Cardiac Arrhythmia Suppression Trial (CAST) showed that treatment of premature ventricular contractions (PVCs) with class IC antiarrhythmic drugs may increase cardiovascular mortality in patients with myocardial infarction (MI) [9]. Chronic treatment of AF with current antiarrhythmic drugs has not been more effective than a rate control strategy in reducing thromboembolism [10] which may suggest the ineffectiveness of the antiarrhythmic drugs in maintaining the sinus rhythm. Paradoxically, a common adverse effect of all currently available antiarrhythmic drugs is proarrhythmia [11, 12]. Catheter ablation therapy is based on creating an anatomically fixed lesion in the heart in order to block the reentrant circuit or propagation of the focal activity. Studies using optical mapping of the heart have shown ectopic foci and reentrant circuits are usually multiple and dynamic in complex arrhythmias such as VF and AF [13–15]. Catheter ablation for VT is primarily an adjuvant therapy for reduction of symptoms in patients with ICD [16] and cannot provide a reliable method for prevention of SCD. Defibrillation has shown success in terminating VT/VF. Nevertheless, it does not prevent the occurrence of arrhythmia, and frequent ICD shocks worsen the quality of life and may even increase mortality [16]. ICDs are relatively expensive. A study that evaluated data from multiple randomized clinical trials estimated that the cost of the ICD-related primary prevention of SCD ranged from $34,000 to $70,200 for each life-year [17]. In addition, about 70% of the patients who receive an ICD never

have any appropriate defibrillation and only 30% of patients with sudden cardiac arrest (SCA) meet current criteria for implantation of ICD [18].

Many of the aforementioned limitations of current therapies for arrhythmia arise from the fact that these therapies do not address the underlying pathophysiology of arrhythmia. A more successful therapeutic approach may arise from targeting upstream pathologies that result in abnormalities in ionic currents and emergence of reentry and focal activity. Oxidative stress, which is an imbalance between production and neutralization of reactive oxygen species (ROS), is an example of a possible upstream therapeutic target. Most clinical risk factors of AF such as hypertension, age, and cardiothoracic surgeries are conditions that are associated with oxidative stress [19]. Serum markers of oxidative stress have been shown to be elevated in patients with AF [20–22]. AF in human is associated with a significant reduction in the expression of antioxidant genes as well as a significant increase in the expression of five genes related to ROS, supporting a shift toward prooxidation state in AF [23]. Cardiomyopathy, which is associated with significantly higher risk of VT/VF, is associated with oxidative stress and increased oxidation and carbonylation of proteins [24]. Perfusion of H_2O_2 of hearts in the Langendorff setting induces VT/VF and AF [13–15, 25], providing evidence that ROS elevation can be a cause of arrhythmia.

Despite considerable evidence that ROS play an important role in the genesis of arrhythmia, limited clinical studies using conventional antioxidants have shown conflicting results [26]. The biology of ROS is complex and designing an effective antioxidant therapy for arrhythmia requires an in-depth understanding of the cardiac sources of ROS, the ROS molecules structures and properties, triggers of ROS production, and the downstream effects of ROS which result in arrhythmia. The study of mechanisms by which ROS elevation may result in arrhythmia may lead to the discovery of novel therapeutic targets for treatment of arrhythmia.

2. Oxidative Stress Facilitating Focal Activity and Reentry

ROS can lead to focal activity. It has been shown that ROS prolong action potential duration (APD) in rat and guinea pig myocytes and induce early afterdepolarizations (EADs) and delayed afterdepolarization (DADs) [27]. Consistent with that finding, ROS has been shown to facilitate ventricular arrhythmia in aged and hypertensive rat hearts mainly via an EAD mechanism [25, 28].

ROS can also provide substrate for reentry. One possible mechanism for reentry in oxidative stress is via heterogeneous APD prolongation in which at a moment one area with shorter APD is excitable while the area with prolonged APD is not excitable [15, 25, 29]. In an angiotensin II activation mouse model with elevated ROS levels and in a mitochondrial oxidative stress mouse model conduction velocity (CV) is decreased and inducible VTs are mainly caused by reentry [30–32].

The results from mathematical modeling studies have supported both reentry via decreased in conduction CV and focal activity via EAD/DAD mechanisms for ROS-mediated arrhythmias [13, 33]. It is not clear in which conditions and at what levels ROS may promote one of the two mechanisms: focal activity and reentry. Nevertheless, ROS can promote both.

3. Arrhythmogenic Ionic Effects of Oxidative Stress

ROS elevation affects several ionic currents in cardiomyocytes. The effect of ROS on total and late Na^+ current is important and can be arrhythmogenic. One of the mechanisms of H_2O_2-induced APD prolongation and EAD formation is by promoting an enhanced late sodium (Na^+) current [34]. Ranolazine, a late Na^+ current blocker, can suppress ROS-mediated EADs and arrhythmia, which suggests a role for the increased late Na^+ current in the pathogenesis of ROS-mediated arrhythmia [25]. Treatments with H_2O_2 and angiotensin II enhance the late Na^+ current; however, those treatments decrease the overall Na^+ current in isolated myocytes partially through the downregulation of SCN5A transcription [35]. While an increase in late Na^+ current may result in arrhythmia via an EAD mechanism, the reduction in total Na^+ current seen by ROS may cause a reduction in CV and provide substrate for reentry. ROS can downregulate cardiac sodium channels and mitochondrial antioxidants can reverse this effect [36]. This can also provide a substrate for arrhythmia in a similar fashion that happens in Brugada syndrome [37–39].

ROS may also alter intracellular Ca^{2+} handling in a way that generates arrhythmia. ROS may stimulate the L-type Ca^{2+} current, which can facilitate EAD [40]. In addition, hydroxyl radicals increase the open probability of cardiac ryanodine receptors, which control the Ca^{2+} release from the sarcoplasmic reticulum (SR) to the cytoplasm [41]. Abnormal Ca^{2+} release from SR during diastole may result in formation and propagation of DADs [42, 43]. Even a brief exposure to OH^- significantly decreases SR Ca^{2+} uptake, which leads to an increased Ca^{2+} level in myocytes during diastole [44]. This short-term effect on Ca^{2+} transport is likely due to the OH^--mediated peroxidation of lipid membranes and protein sulfhydryl formation, which leads to an indirect effect on the SR Ca^{2+} transporter [44]. Increase in intracellular levels of Ca^{2+} can then translate to an inward current by sodium-calcium exchanger (NCX) action. In addition ROS may increase directly NCX activity [43].

In addition to the cardiac Na^+ current and intracellular Ca^{2+} handling, oxidative stress affects also cardiac potassium currents. ROS may inhibit K_{ATP} channels [45] and downregulates I_{to} [46, 47]. These effects of ROS and its possible suppression of I_{Kr} and I_{Ks} [48] can potentially decrease the repolarization reserve, prolong the APD, and facilitate triggered activity.

In summary, ROS can affect all major ionic currents with increases in the late Na^+ current, L-type Ca^{2+} current, leak of Ca^{2+} from SR, and NCX activity. ROS decrease peak sodium current and SERCA-mediated SR Ca^{2+} uptake. All these changes are likely to increase intracellular Ca^{2+} levels,

prolong the APD, reduce of CV, and facilitate triggered activity and reentry.

4. Oxidative Stress and Cell Coupling

Oxidative stress promotes myocardial fibrosis [49, 50]. Biophysical properties of the extracellular matrix (ECM) are important factors that can affect the propagation of the action potential in the heart. For example, increased collagen deposition in the ECM, which is seen in increased myocardial fibrosis and scar tissue formation after myocardial infarction, may provide a barrier to the AP propagation and contribute to reentry [51]. In addition, collagen deposition may reduce electrical coupling between myocytes and facilitate focal activities by reducing the sink-to-source effect in the region of the heart [14]. Proliferation of fibroblasts and their transformation to myofibroblasts may be associated with myocyte-fibroblast coupling, which is potentially arrhythmogenic via increased ectopic activity [52].

Another important factor that affects electrical coupling of myocytes is gap junction function, and oxidative stress impairs gap junction conduction [30, 53, 54]. Gap junctions are channels in all compartments of the heart that form the conduction pathways between cells allowing for an electrical syncytium. Connexin 43 (Cx43) is the major component of gap junctions in the ventricular myocytes, and it is one of the important connexins in the atria. Cx43 is decreased in human heart failure, a condition that is associated with significantly increased ROS levels and increased risk of arrhythmia [24, 55–57]. In cardiac angiotensin II activation and mitochondrial oxidative stress mouse models, ROS elevation significantly decreases Cx43 levels, impairs gap junction conduction, and results in spontaneous and pacing induced arrhythmia [31, 32, 58, 59]. Treatments with a mitochondria-targeted antioxidant and angiotensin receptor blockers prevent the gap junctional remodeling and arrhythmias, supporting a key role for oxidative stress in Cx43 remodeling and impairing electrical coupling between myocytes [30–32].

5. Molecular Mechanisms of ROS-Induced Arrhythmia

While ROS seems to be able to cause arrhythmias, at least when externally supplied, and there are some possible alterations that can explain the arrhythmogenic substrate created by ROS, more effort and focus are required to explore the molecular mechanisms by which ROS result in those abnormalities. These mechanisms may involve the effect of ROS on genes, transcriptional regulation, protein trafficking, and posttranslational modifications. There are examples of studies that described novel molecular mechanisms for ROS-induced arrhythmia, which may result in discovering potentially new antiarrhythmic targets; however, there are much more research needed in this area.

The molecular mechanism by which ROS affect the cardiac Na^+ current and how the Na^+ current reduction can be prevented will be important steps toward designing new and effective antiarrhythmic drugs. Abnormal splicing of cardiac sodium channel mRNA is a possible mechanism for the decreased sodium current in arrhythmia. The splicing factors RBM25 and LUC7L3 are elevated in human heart failure tissue and mediate truncation of SCN5A mRNA in both Jurkat cells and human embryonic stem cell-derived cardiomyocytes [60]. RBM25/LUC7L3-mediated abnormal SCN5A mRNA splicing reduces Na^+ channel current to a range known to cause sudden cardiac death [61]. It has been shown that angiotensin II and hypoxia, known to cause elevation in ROS levels [62–64], are associated with the aforementioned splicing factor abnormalities resulting in a Na^+ current reduction in the heart [60]. Another mechanism by which ROS may affect cardiac sodium current is probably via protein kinase C (PKC). It has been shown that mitochondrial ROS causes a reduction in cardiac sodium current and that effect can be prevented by inhibition of PKC [36]. In addition, c-Src inhibition can recover cardiac Na^+ current to normal in a mouse model of manganese superoxide dismutase deficient with elevated mitochondrial ROS, which suggests that c-Src partially mediates the effect of ROS on cardiac Na^+ current reduction [59].

Ca^{2+}/CaM-dependent kinase II (CaMKII) can be activated by ROS, and its activation probably mediates several of the ROS-induced arrhythmogenic effects [65]. If CaMKII is activated under prooxidant conditions, two methionine residues become oxidized, and the sustained activation of CaMKII, independent of its binding to Ca^{2+}/CaM, occurs [66]. Pretreatment of hypertensive rat hearts with a CaMKII-inhibitor KN-93 prevents VT inducibility during oxidative stress [67]. These findings are supported by both patch-clamp and Ca^{2+}-imaging studies, which demonstrate that the oxidative-stress-induced activation of CaMKII causes arrhythmias [68]. Several recent studies have linked the use of CaMKII inhibitors to a decrease in catecholaminergic polymorphic ventricular tachycardia [69, 70].

Other possible arrhythmogenic mechanisms by which CaMKII activation exerts arrhythmic effects include RyR phosphorylation and activation under oxidative stress [71, 72]. An increase in the open probability of RyR is thought to be mediated by CaMKII activation [42]. In addition, CaMKII has been shown to shift the voltage dependence of Na^+ channel availability by approximately +5 mV, to hasten recovery from inactivation, and to increase late Na^+ current in cardiomyocytes [73]. CaMKII plays an important role in L-type Ca^{2+} channel facilitation, the Ca^{2+}-dependent augmentation of Ca^{2+} current (I_{CaL}) exhibited during rapid repeated depolarization [74]. In addition, CaMKII has been identified recently as one of the mediators of fibroblast proliferation in response to angiotensin II [75]. Because CaMKII is a relatively indiscriminate kinase, it is very possible that other mechanisms are involved in the genesis of arrhythmia by CaMKII activation under oxidative stress.

6. Conclusion

Ventricular and atrial arrhythmias place considerable burden on the healthcare system. Currently, available therapies for arrhythmia have certain limitations. In order to identify

Table 1: A summary of mechanisms of oxidative stress induced arrhythmia and potential therapeutic targets.

Affected ion channels	Na^+ current reduction (via PKC and c-Src, also via abnormal splicing) ⇒ reduction in conduction velocity
	KATP inhibition ⇒ repolarization abnormality
	NCX activation ⇒ increasing inward current and facilitating afterdepolarization
	I_{to}, I_{Kr}, I_{Ks} inhibition ⇒ abnormal repolarization
	Increase in inward Ca^{++} current (direct or via CaMKII activation) ⇒ facilitating afterdepolarization
	Increase in late Na^+current ⇒ facilitating afterdepolarization
Effect on intracellular Ca^{++} handling	Impairment of SERCA ⇒ increase intracellular Ca^{++} levels ⇒ facilitating afterdepolarization
	Affecting RyR receptor (via CaMKII activation) ⇒ leakiness of SR ⇒ increase intracellular Ca^{++} levels ⇒ facilitating afterdepolarization
Effect on myocyte-myocyte coupling	Affecting assembling of Cx43 at gap junctions ⇒ reduction in conduction velocity
Effect on extracellular matrix	Activating fibrotic process (via TGF-β) ⇒ reduction in conduction velocity and impaired myocyte-myocyte coupling due to collagen deposition

CaMKII: Ca^{2+}/calmodulin-dependent protein kinases II; Cx43: connexin 43; NCX: Na^+/Ca^{2+} exchanger; RyR: ryanodine receptor; SERCA: sarco-/endoplasmic reticulum Ca^{++} - ATPase; TGF-β: Transforming Growth Factor-β.

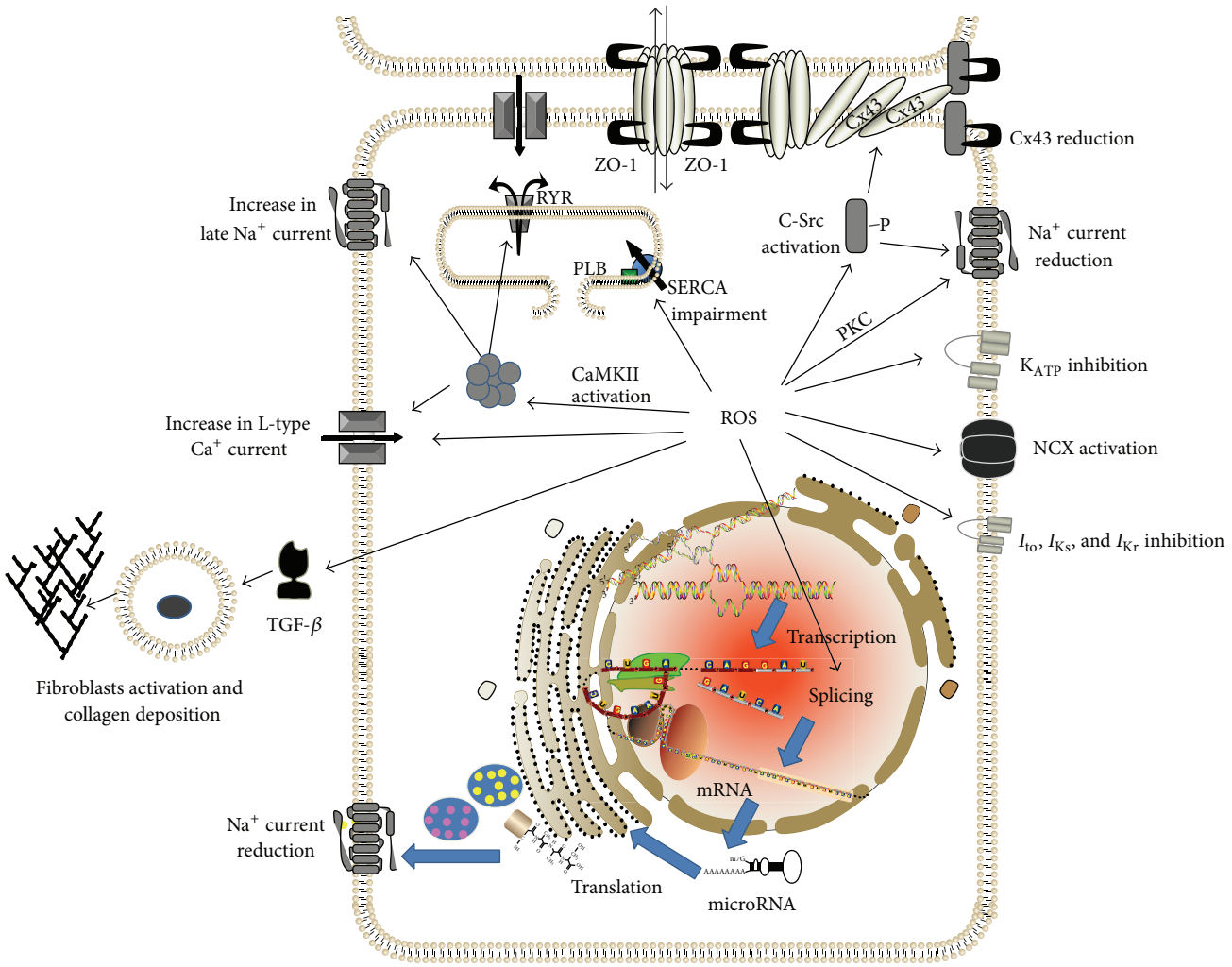

Figure 1: Schematic review of some of the important known mechanisms by which excess ROS may induce arrhythmia. Activation of CaMKII, c-Src, and PKC may mediate several important effects of ROS on ionic currents resulting in arrhythmia. In addition, ROS adversely affect splicing of mRNA of cardiac sodium channels resulting in abnormal truncated cardiac sodium channel proteins and a reduction in normal sodium channels. ROS also increase fibrosis and impair gap junction conduction, resulting in reduced myocyte coupling. Abnormal splicing, activation of CaMKII, c-Src, and PKC are among emerging new antiarrhythmic therapeutic targets. CaMKII: Ca^{2+}/calmodulin-dependent protein kinases II; CX43: connexin 43; NCX: Na^+/Ca^{2+} exchanger; PLB: phospholamban; ROS: reactive oxygen species; RYR: ryanodine receptor; SERCA: sarco-/endoplasmic reticulum Ca^{2+}-ATPase; TGF-β: Transforming Growth Factor-β; ZO-1: Zonula Occludens-1.

new, effective therapeutic targets for treatment of arrhythmia, mechanisms of the genesis of arrhythmia should be further explored. One possible upstream therapeutic target for treatment of arrhythmia is oxidative stress. It has been shown that excess amount of ROS can result in both reentry and focal activity by modifying many of the ionic currents in cardiomyocytes, cardiomyocyte coupling, and important elements of the extracellular matrix (Figure 1 and Table 1). Molecular mechanisms by which ROS exert those effects are the key areas under investigation. Activation of CaMKII, c-Src, PKC, and abnormal splicing of cardiac sodium channels are among the emerging new therapeutic targets.

List of Abbreviations

AF: Atrial fibrillation
APD: Action potential duration
CaMKII: Ca^{2+}/calmodulin-dependent protein kinases II
Cx43: Connexin 43
CV: Conduction velocity
DAD: Delayed afterdepolarization
EAD: Early afterdepolarization
ICD: Implantable cardioverter defibrillator
NCX: Na^{+}/Ca^{2+} exchanger
PLB: Phospholamban
ROS: Reactive oxygen species
RyR: Ryanodine receptor
SERCA: Sarco-/endoplasmic reticulum Ca^{2+}-ATPase
SCA: Sudden cardiac arrest
SCD: Sudden cardiac death
TGF-β: Transforming Growth Factor-β
VT: Ventricular tachycardia
VF: Ventricular fibrillation
ZO-1: Zonula Occludens-1.

Conflict of Interests

Ali A. Sovari has the pending patent: Mitochondria Antioxidants for Prevention of Sudden Death by Raising Connexin 43 Levels (Application no. 61/503,096).

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Review Article

Mechanisms and Clinical Management of Ventricular Arrhythmias following Blunt Chest Trauma

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Nonpenetrating, blunt chest trauma is a serious medical condition with varied clinical presentations and implications. This can be the result of a dense projectile during competitive and recreational sports but may also include other etiologies such as motor vehicle accidents or traumatic falls. In this setting, the manifestation of ventricular arrhythmias has been observed both acutely and chronically. This is based on two entirely separate mechanisms and etiologies requiring different treatments. Ventricular fibrillation can occur immediately after chest wall injury (commotio cordis) and requires rapid defibrillation. Monomorphic ventricular tachycardia can develop in the chronic stage due to underlying structural heart disease long after blunt chest injury. The associated arrhythmogenic tissue may be complex and provides the necessary substrate to form a reentrant VT circuit. Ventricular tachycardia in the absence of overt structural heart disease appears to be focal in nature with rapid termination during ablation. Regardless of the VT mechanism, patients with recurrent episodes, despite antiarrhythmic medication in the chronic stage following blunt chest injury, are likely to require ablation to achieve VT control. This review article will describe the mechanisms, pathophysiology, and treatment of ventricular arrhythmias that occur in both the acute and chronic stages following blunt chest trauma.

1. Introduction

Under certain conditions, sustained ventricular arrhythmias can develop immediately or late in the setting of nonpenetrating, blunt chest trauma. The manifestation of ventricular arrhythmias in this setting is a rare, but potentially fatal complication that can include triggered, automatic, and reentrant ventricular arrhythmias. The acute manifestation of ventricular fibrillation (VF) in this setting, often referred to as commotio cordis, has been well described and occurs in the absence of cardiac structural injury [1]. The development of monomorphic ventricular tachycardia (VT) and/or ventricular premature depolarizations (VPDs) beyond the acute phase following blunt chest trauma is more commonly associated with right or left ventricular structural abnormalities but with little understanding of the underlying mechanisms or recommended therapies. We will discuss both of these phenomena in this review along with current treatment and available outcome data.

2. Review of Ventricular Arrhythmia Mechanisms in Blunt Chest Trauma

2.1. Commotio Cordis, Ventricular Fibrillation. Since the mid-1800s, nonpenetrating, blunt chest trauma, with no structural cardiac damage, resulting in sudden cardiac death has been described in the literature using the Latin term, *commotio cordis*. Although case studies have been published describing isolated incidents since the 19th century, it was not until the establishment of the United States Commotio Cordis Registry in 1996 that we began to understand the significance of this phenomenon. Since the inception of the registry, about 10 to 20 cases are reported annually to the database and have

allowed for a better understanding of the prevalence and public health implications necessary to increase survival rates [2, 3].

Commotio cordis is defined as the "mechanical stimulation of the heart by non-penetrating, impulse-like impact to the precordium that, through intrinsic cardiac mechanisms, gives rise to disturbances of cardiac rhythm of varying type, duration, and severity, including sudden cardiac death, in the absence of structural damage that would explain any observed effects" [4]. In this condition, mechanical stimulation of the heart by nonpenetrating impact to the precordium gives rise to VF (Figure 1). Since the establishment of the United States Commotio Cordis Registry, more than 220 cases have been reported [1]. From this database, we can see that 75% of these cases took place during either competitive or recreational sporting event. Typically, the patient is a young male competing or participating in a sporting activity who is struck with a small, dense projectile like a baseball or hockey puck at a high velocity over the cardiac silhouette [5–7]. It is not known why young males experience this rare phenomenon more often, but it is suspected that younger individuals have higher chest compliance compared with older adults, and males, who typically play more compact sports, are important risk factors for this condition [1].

This is a rare phenomenon due to the required synchronization of certain pathophysiological variables. During impact-related animal model studies, commotio cordis inducing VF can be prompted with impacts at a certain critical time of the cardiac cycle. Specifically, this time period includes the upslope of the T-wave, about 10 to 40 milliseconds prior to the T-wave peak. Typically, as a result of this impact, the morphology appears initially uneven and undulating, described as either a polymorphic ventricular tachycardia (PVT) or Torsade de Pointes. While PVTs are typically observed at first, they will progress into VF if sustained for over 10 beats. Outside this time frame, VF was rarely seen. In addition to the narrow time period where the cardiac rhythm is susceptible to electrical abnormalities secondary to a direct blow over the cardiac silhouette, successively higher speeds of impact increased the likelihood of VF in swine models up until 80 km/h (50 mph). Between 80 and 112 km/h (50–70 mph), the likelihood of VF dropped and increased the likelihood of cardiac rupture. Other determinants that play a key role in VF production include location, energy, shape, and hardness of the object [1, 8].

The mechanisms in in vivo studies indicate that a high speed impact with a dense projectile to the chest causes premature ventricular depolarization, coupled with elevated ventricular stretch-related activation of mechanosensitive ion channels such as ATP-dependent K^+ channels. This mechanism can result in the ventricular arrhythmias described above and, occasionally, will result in sudden cardiac death. Additional investigations of the characteristics of mechanical stretch in commotio cordis indicate that Ca^{+2} waves are initiated by damaged myocardial tissue, whereby nonuniform muscles are stretched during contraction triggering VF [9, 10]. Furthermore, several additional individual variables have been noted to increase VF incidence in multiple animal studies and patient case reports [11, 12]. In general, variations of wider QRS and longer QT periods at baseline (possibly influenced by genetic differences in genotypes of repolarization channels) have higher susceptibility to VF.

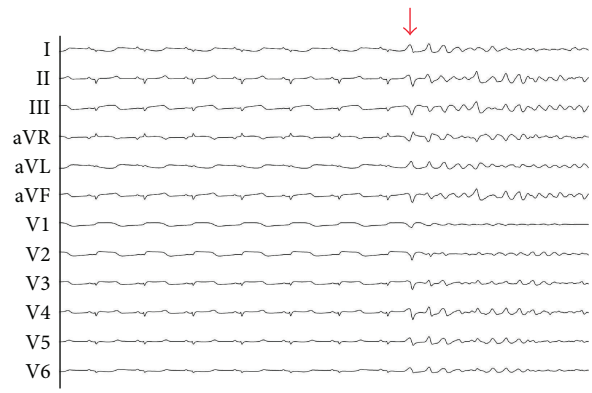

Figure 1: Ventricular fibrillation initiated by premature ventricular depolarization.

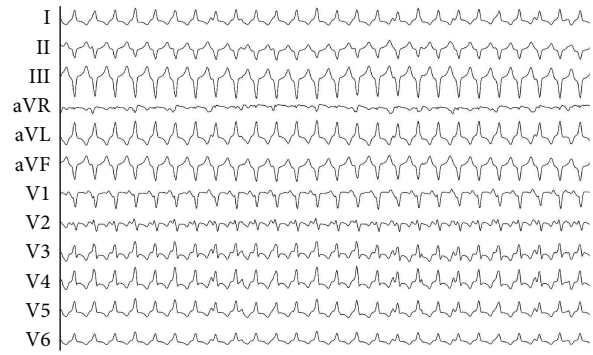

Figure 2: Reentrant ventricular tachycardia.

2.2. Monomorphic Ventricular Tachycardia due to Electrical and Structural Ventricular Remodeling Long after Blunt Chest Injury. A second manifestation of ventricular arrhythmias following blunt chest trauma is the development of ventricular tachycardia (Figure 2) due to ventricular aneurysm and/or tissue fibrosis that develops long after the initial injury. Very rarely, blunt chest injury leads to electrical and structural ventricular remodeling that can serve as the underlying substrate for the development of sustained monomorphic VT well beyond the acute setting [13, 14]. This phenomenon is not well described and is poorly understood as opposed to commotio cordis, and a review of the literature has revealed no long-term epidemiological studies of this specific phenomenon.

In this setting, the traumatic event results in permanent structural damage to the heart muscle most commonly involving the right ventricle (RV) and may result in RV aneurysmal development. The RV is more prone to cardiac contusion and subsequent thinning of the ventricular wall because of its anterior location within the chest. While traumatic RV aneurysms are uncommon, these rare cases

usually present outside the acute setting of the injury, generally found incidentally during autopsy, or following the manifestation of clinical symptoms of ventricular tachycardia (i.e., palpitations, near-syncope, etc.) [13, 15]. We performed a systematic literature review for such cases and only identified 7 instances of chronic sustained VT development from a remote blunt chest wall injury. Nonetheless, it is important to note that RV aneurysm development rarely results in the development of ventricular tachycardia [13, 14, 16]. Patients that do develop abnormal ventricular substrate leading to VT beyond the acute injury phase appear to require an aggressive management and treatment approach. Of note, 2/7 of these patients did not have evidence of any overt cardiac structural changes and the electrophysiology studies were highly suggestive of a focal mechanism in both cases.

The other 5 patients did have evidence of an underlying abnormal structural pathophysiology including aneurysm and/or scar development that was involved in the clinical VT. This is highly suggestive of a causal relationship of chest wall injury to the development of cardiac structural changes and most likely a reentrant VT likely due to inhomogeneous scarring with a variable degree of surviving myocardial tissue. The resulting arrhythmogenic substrate is characterized by zones of slow conduction due to nonuniform anisotropy, resulting in fixed and/or functional regions of conduction block. This facilitates reentry as it generates enough time for tissue in the circuit to recover its excitability to allow the excitation wave front to reenter the initial site of the block, thereby creating a reentrant circuit [17, 18].

3. Implications and Clinical Management

3.1. Immediate and Acute Ventricular Arrhythmia Manifestation. In a study by Link et al., juvenile swine were used to demonstrate the effectiveness of defibrillation for ventricular arrhythmias as a result of nonpenetrating, blunt chest trauma in the absence of structural injury. In the 2003 study, swine were analyzed with automated external defibrillators (AEDs) 30 seconds after receiving a blow to the chest by a baseball at 64 km/h (40 mph), inducing VF. The animals were defibrillated with a 200 J biphasic waveform, escalated to 300 J and then 360 J if needed, and were randomly assigned to groups and defibrillated at either 1-, 2-, 4-, or 6-minute intervals without prior cardiopulmonary resuscitation (CPR). The study demonstrated that the AED device correctly analyzed the VF in the 50 baseball induced ventricular arrhythmias in all cases and was able to successfully terminate the ventricular rhythm with single 200 J defibrillation in 94% cases. The other three events required additional defibrillation to terminate the arrhythmia but were ultimately successful with 3 or less shocks 100% of the time [8, 23].

The time from the traumatic event to initial shock was a significant predictor in the survival of the swine, according to the 2003 study by Link et al. The swine defibrillated at a one-minute interval had a 100% (13 of 13) survival rate; the swine defibrillated at 2 minutes had a 92% survival rate (11 of 12); survival at 4 minutes was 46% (6 of 13); and, finally, initial defibrillation at 6 minutes had a survival rate of 25% (3 of 12). This clearly demonstrates the importance in early recognition and defibrillation as critical factors to ensure effective management of commotio cordis patients.

Although the animal studies have shown the effectiveness of early defibrillation for acute ventricular arrhythmias secondary to blunt chest trauma, data over the last 15 to 20 years has demonstrated that the outcomes are generally poor. The survival rate from 1996 to 2014 is approximately 25% [1]. However, as public awareness of commotio cordis and AED access has increased over the past few years, the survival rate has begun to increase. A study analyzing the survival rates from 2006 to 2012 demonstrated that the survival rate has more than doubled to 58% (31 of 53 events) [3].

The American Heart Association has recommended through various public awareness campaigns and training courses that any sudden collapse of an athlete after a blow to the chest from a solid projectile should be considered commotio cordis with rapid initiation of CPR and defibrillation. Immediate recognition is key to ensuring early resuscitation is initiated and remains a significant factor influencing survival from commotio cordis. Increased awareness has led to the wide distribution of AEDs in both public places, like parks, as well as many sporting venues [24]. These widely distributed AEDs, which are now available for laypersons to use when they recognize commotio cordis, can be used immediately after the onset of the ventricular arrhythmias. The combination of recognizing commotio cordis and initiating resuscitative efforts and the wide availability of AEDs are the most significant changes in the past few years which have likely led to the increased survival rates [5].

3.2. Chronic Ventricular Arrhythmia Manifestation. The management of chronic ventricular arrhythmias is less clear due to its infrequent nature and associated structural changes. A comprehensive literature review suggests that the incidence of ventricular arrhythmias due to ventricular structural changes is exceptionally rare. We identified only 7 such cases published between 1998 and 2014 and have outlined the clinical presentation, treatment, and follow-up in Table 1 and in further detail below. All patients presented with palpitations and recurrent episodes of monomorphic VT. Four patients (67%) presented with a left bundle branch block VT morphology consistent with a right ventricular origin and 3 patients had right bundle branch block morphologies suggestive of LV origin. Three of the 4 patients with RV VT had structural abnormalities identified during noninvasive imaging in the form of RV free wall thinning and/or aneurysm. One patient with LV VT did not have overt structural changes identified and the other LV VT patient had evidence of LV epicardial lateral wall contact with a previous fractured rib that had experienced blunt chest injury 2 years priorly. Three cases had documented ventricular arrhythmias acutely following the initial chest injury which continued during long-term follow-up. The other 3 cases manifested symptoms and monomorphic VT for the first time at variable time points and ranged from 6 months to 20 years after the initial chest wall injury. Importantly, 6/7 (86%) of patients continued to have VT despite pharmacologic and antiarrhythmic drug therapy requiring electrophysiology study, cardiac mapping, and ablation therapy. Epicardial radiofrequency ablation or

Table 1: Ventricular tachycardia long after BCI case findings.

Case	Age/gender	Symptoms	Time from BCI to Tx	SHD	VT morph and SOO	Tx	Success	Follow-up
(1) Mera et al., 1998 [19]	36 M	Palpitations Syncope	"Several months"	N	LB, inferior axis RVOT	CA	Y	24 months
(2) Martínez et al., 2003 [20]	24 F	Palpitations	20 years	Y	RBBB 205 bpm LV apex	Aneurysm resection	Y	12 months
(3) Schaer et al., 2007 [16]	33 M	Palpitations	22 months	Y	LB, superior axis 300 bpm RV free wall	ICD	—	24 months NSVT only
(4) Horduna et al., 2011 [21]	10 M	Palpitations	3 years	N	RB, superior axis 110 bpm LV anterolateral PM	CA	Y	9 months
(5) Michowitz et al., 2012 [14]	59 M	ICD therapy	20 years	Y	LB, inferior axis 130 bpm RV free wall	CA	Y	6 months
(6) Casado-Arroyo et al., 2012 [22]	62 M	Palpitations Dizziness	2 years	Y	RB, superior axis 160 bpm LV lateral wall	SA	Y	12 months
(7) Shakil et al., 2012 [13]	33 M	Palpitations	6 months	Y	LBBB RV free wall	SA	Y	8 months

M, male; F, female; BCI, blunt chest injury; VT, ventricular tachycardia; Tx, treatment; SOO, site of origin; CA, catheter ablation; SA, surgical ablation; NSVT, nonsustained ventricular tachycardia; LB, left bundle; RB, right bundle; ICD, internal cardioverter-defibrillator.

surgical cryoablation was performed in 3 cases and 1 patient underwent aneurysm repair. All 6 case reports undergoing ablative/surgical treatment indicated success at eliminating the patients' arrhythmia without recurrence during an average follow-up of 11.8 ± 7.2 months (range: 6–24 months) off antiarrhythmic medications. Only 1 patient in this series with a left bundle, superior axis VT and inferolateral RV thinning on CMR was implanted with an internal cardioverter-defibrillator (ICD) and did not undergo ablation as there was only NSVT and there were no detected ICD events during 22-month follow-up.

Case 1. Mera et al. report their experience in a 36-year-old previously healthy man that presented following a freight train collision that involved blunt chest wall injury [19]. The patient complained of palpitations associated with frequent monomorphic VPDs identified on telemetry. Several hours later while in the hospital, he developed symptomatic left bundle, inferior axis monomorphic VT which self-terminated after the patient had syncopized. Transthoracic echocardiogram and coronary angiography were normal. The patient continued to complain of palpitations and dizziness several months later with frequent VPDs and nonsustained VT despite β-blockers and sotalol. Subsequent electrophysiology study localized the VPD site of origin to the RVOT. Radiofrequency ablation at this site successfully eliminated the ectopic beats and remained asymptomatic during a 2-year follow-up. Although the ventricular arrhythmias appear to have manifested immediately after chest wall injury and continued during follow-up, the focal mechanism of the arrhythmia, absence of significant cardiac contusion, and normal cardiac function on echocardiogram suggest that this patient may have had idiopathic RVOT VPDs/VT unrelated or further elucidated after the blunt chest injury.

Case 2. Martínez et al. describe the case of a 24-year-old female with ongoing palpitations and sustained right bundle branch block VT at 205 bpm suspected to involve the LV apical region [20]. The patient had suffered a severe blunt chest injury involving a rock 20 years priorly. She did not develop any acute complications after this event, but ventriculography demonstrated apical aneurysm with normal coronary angiography indicating myocardial contusion and injury eventually resulting in LV apical scar and aneurysm. The patient underwent aneurysm resection with Teflon and pericardial patch insertion. Repeat EP study with RV programmed extrastimuli at three cycle lengths failed to induce any arrhythmias. The patient remained asymptomatic without VT recurrence during 12-month follow-up. Characterization of the VT mechanism was not performed, but the underlying aneurysm and elimination of VT following resection are suggestive of reentry. This case is similar to the more common scenario of patients that develop LV aneurysms and monomorphic VT following myocardial infarction. Subendocardial resection has been shown to be a highly effective treatment for recurrent VT in a majority of post-MI patients [25].

Case 3. Schaer et al. describe a patient who is a 33-year-old male hockey player who began experiencing palpations after a body-check during a professional ice hockey game [16]. A left bundle superior axis monomorphic VT was induced but not targeted with ablation therapy. The patient discontinued his professional sports activities but did not receive any further treatment. A CMR performed a year after the injury showed a small area of myocardial thinning in the inferolateral wall of the RV. It was suspected that the body-check that resulted in palpations acutely after the chest trauma was the cause of the subsequent RV structural defect. Nearly 2 years later, the patient reported similar symptoms after another body-check during a recreational ice hockey game. The patient demonstrated VT with the same morphology as the original incident and underwent cardioversion and ICD implantation. A 2-year follow-up revealed no additional episodes of palpations or ICD detected events.

Case 4. Horduna et al. report a pediatric case in a 10-year-old boy referred to VT management 3 years after being struck by a car [21]. The patient developed monomorphic VT shortly after the traumatic injury during prolonged hospitalization. The patient continued to develop sustained VT with a right bundle, superior axis VT despite verapamil and sotalol therapy. CMR and echocardiogram were normal. The electrophysiology study and endocardial left ventricular electroanatomic activation mapping were performed during VT and identified a focal source of early activation at the mid-portion of the anterolateral LV consistent with an anterolateral papillary muscle VT. Radiofrequency ablation was delivered at this site and resulted in VT termination without recurrence after 9 months off all antiarrhythmic medications. Although this patient did not have evidence of mitral valve injury or ventricular scar on CMR imaging and endocardial bipolar voltage mapping, this VT site of origin is highly suggestive of papillary muscle injury that occurred during the initial blunt chest traumatic event. The pathophysiologic result from this injury and its role in the manifestation of VT are unclear. It is possible that tissue changes in this region causing the suspected nonautomatic focal VT mechanism are subtle and beyond the detectable resolution of CMR imaging and bipolar voltage mapping.

Case 5. Michowitz et al. report a case of recurrent VT in a 59-year-old man that had been kicked in the chest by a horse 20 years earlier [14]. No acute arrhythmias or abnormal clinical manifestations were observed initially, but subsequent RV dilation was seen on serial echocardiography. Due to amiodarone toxicity, it was decided that the patient would discontinue use of the antiarrhythmic medication and undergo electrophysiology study and ablation. Imaging prior to the procedure revealed a dilated RV and ECG showed right bundle branch block. Endocardial RV electroanatomic mapping demonstrated free wall and septal bipolar low voltage indicative of tissue fibrosis. Mid-diastolic potentials were seen during VT activation mapping more likely indicative of a reentrant versus focal mechanism. Endocardial ablation was performed but was unsuccessful in terminating the VT, prompting epicardial access, mapping, and ablation. However, epicardial ablation did not terminate the VT. Only after further RV endocardial lesions were delivered was late VT termination seen. Late termination from the RV endocardium after

endocardial and epicardial ablation may have been due to a mid-myocardial RV circuit. The patient remained free of any VT recurrence during 6-month follow-up.

Case 6. Casado-Arroyo et al. report a case of recurrent right bundle, superior axis sustained monomorphic VT in a 62-year-old man that had a traumatic blunt chest wall injury from a fall 2 years priorly [22]. The patient developed palpitations, dizziness, and depressed left ventricular function. Computer tomographic (CT) imaging identified LV lateral wall contract with prior rib fractures consistent with the VT electrocardiographic morphology. Epicardial surgical mapping was performed and identified a signal 60 ms pre-QRS at the LV lateral wall. Cryoablation at this site resulted in immediate VT termination and elimination. The presystolic electrogram and the absence of overt structural heart disease, despite the particularly unusual CT finding, are suggestive of a focal VT mechanism. The patient denied any further symptoms and left ventricular function returned to normal during a 12-month follow-up.

Case 7. Shakil et al. report a case of left bundle branch block NSVT morphology in a 33-year-old man who presented with palpations for 2 weeks [13]. The patient experienced a blunt chest injury during a skiing accident 6 months prior to the symptoms and, subsequently, it was hypothesized that the injury was the impetus behind the NSVT and related symptoms. An exercise tolerance test was attempted multiple times but was discontinued each time because of sustained VT that resolved with rest. The patient was admitted to the hospital and underwent a contrast-enhanced CT angiography. The imaging revealed myocardial dilatation in the RV free wall near the annulus of the tricuspid valve consistent with aneurysm and fibrosis. Endocardial RV mapping and ablation of the VT were attempted but were unsuccessful due to anatomic limitations and challenges reaching the inferior RV aneurysm. The patient was initiated on amiodarone and β-blocker therapy but ultimately refused long-term antiarrhythmic drug use. The patient elected to undergo a hybrid surgical procedure involving RV epicardial mapping and cryoablation of the RV aneurysm. The patient was discharged 4 days later after an uneventful postoperative stay and remained free of any further VT off antiarrhythmic medications during 8-month follow-up.

In the past, medical management with antiarrhythmic drugs was the standard choice of treatment among clinicians to control ventricular arrhythmias. Today, we know that 40% of patients do not respond to this type of treatment for VT [13, 26]. For patients who do not respond to the antiarrhythmic drugs or have some other contraindications, such as toxemia, other options are available. As we described in the case reports presented in this review, these other options are endocardial resection, repair of the aneurysm, and radiofrequency/cryoablation [27].

4. Conclusions

Nonpenetrating, blunt chest trauma is a serious medical condition with multiple types of clinical presentations and variable prognoses. Typically, blunt chest trauma is a result of dense projectiles used during competitive and recreational sports, but various other etiologies have been described, including body-checks during ice hockey, horse kicks to the chest, and ski related injuries. Ventricular arrhythmias can develop acutely or long after the event, each occurring based on two entirely separate pathophysiologies with different treatment requirements. Ventricular fibrillation can occur immediately after chest wall injury (commotio cordis) and requires rapid defibrillation. Reentrant ventricular tachycardia can develop in the chronic stage due to underlying structural heart disease long after blunt chest injury. The associated arrhythmogenic tissue may be complex and provides the necessary substrate to form a reentrant VT circuit. Ventricular tachycardia in the absence of overt structural heart disease appears to be focal in nature with rapid termination during ablation. Regardless of the VT mechanism, patients with recurrent episodes despite antiarrhythmic medication in the chronic stage following blunt chest injury are likely to require ablation to achieve VT control. This was associated with a high degree of success during clinical follow-up off antiarrhythmic drugs.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Utilization and Predictors of Electrical Cardioversion in Patients Hospitalized for Atrial Fibrillation

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Atrial fibrillation (AF) is a common arrhythmia in adults associated with thromboembolic complications. External electrical cardioversion (DCCV) is a safe procedure used to convert AF to normal sinus rhythm. We sought to study factors that affect utilization of DCCV in hospitalized patients with AF. The study sample was drawn from the Nationwide Inpatient Sample (NIS) of the Healthcare Cost and Utilization Project in the United States. Patients with a primary discharge diagnosis of AF that received DCCV during hospitalization in the years 2000–2010 were included. An estimated 2,810,530 patients with a primary diagnosis of AF were hospitalized between 2001 and 2010, of which 1,19,840 (4.26%) received DCCV. The likelihood of receiving DCCV was higher in patients who were males, whites, privately insured, and aged < 40 years and those with fewer comorbid conditions. Higher CHADS2 score was found to have an inverse association with DCCV use. In-hospital stroke, in-hospital mortality, length of stay, and cost for hospitalization were significantly lower for patients undergoing DCCV during AF related hospitalization. Further research is required to study the contribution of other disease and patient related factors affecting the use of this procedure as well as postprocedure outcomes.

1. Introduction

Atrial fibrillation (AF) is the most common clinically encountered arrhythmia in adults. The estimated prevalence of AF is expected to increase from 5.2 million in 2010 to 12.1 million by 2030 [1]. AF related hospitalization has increased by 23% between 2000 and 2010, and the cost of care associated with this diagnosis is also noted to be on the rise [2]. Electrical cardioversion (DCCV) with concomitant antiarrhythmic therapy is a safe and effective technique to achieve conversion to normal sinus rhythm in patients with AF [3]. Age, gender, and race related differences in the prevalence and outcomes of AF have been studied. There is limited literature on the impact of these demographic factors on the treatments and procedures offered to patients with AF. Data regarding utilization of DCCV as a procedural treatment for AF in the United States is scarce. In this study, we describe trends in utilization of DCCV as a procedural treatment for AF in the United States over a 10-year period, explore demographics related disparities in utilization of DCCV for AF, identify predictors for DCCV use in a real world setting, and assess the impact of DCCV use on outcomes.

2. Methods

The Nationwide Inpatient Sample (NIS) contains data on hospital inpatient stays from states participating in the Healthcare Cost and Utilization Project (HCUP). Data for a single year includes information on approximately 8 million inpatient stays from about 1,000 hospitals. We used data from the years 2001–2010, a ten-year-long period. We identified adult patients hospitalized for AF using the primary discharge diagnosis and further identified those that underwent DCCV during the hospital stay using ICD-9 codes. For the demographic characteristics, proc surveyfreq and proc surveymeans were used taking the sample design into account. For logistic regression, we used proc surveylogistic. Demographic variables used for our analyses include gender

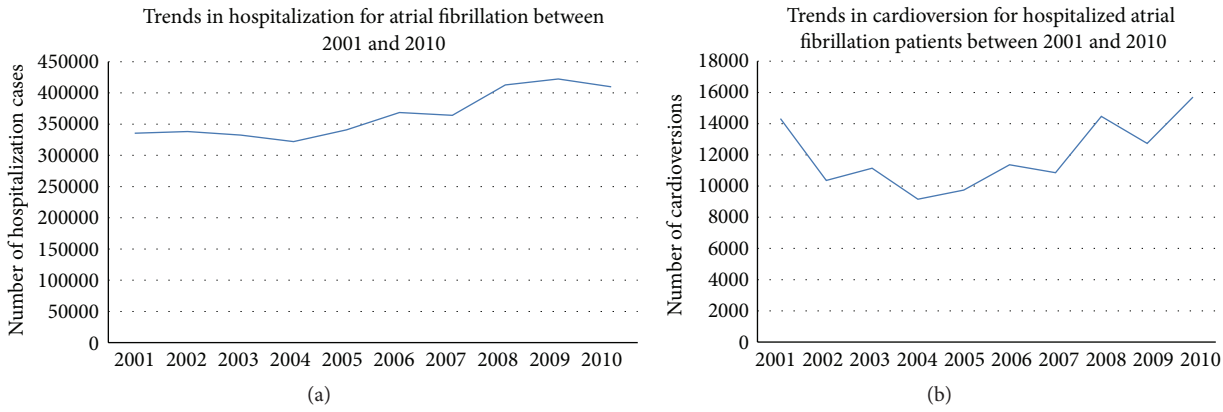

Figure 1

(female (reference group)), race (whites (reference group), blacks, and others), and age (<40 years (reference group), 40–64 years, 65–74 years, and 75 years and above). Comorbidities such as diabetes, congestive heart failure, and hypertension were identified using appropriate ICD-9 codes and accounted for. Outcomes with regard to length of stay [LOS], in-hospital mortality, cost of hospitalization, and postoperative stroke were measured. Descriptive statistics and outcome measurements were calculated using univariate analysis. Multivariate logistic regression was used for Table 2 to describe predictors for utilization of DCCV and backward elimination method was used to derive the final model for multivariate logistic regression. All statistical analyses were conducted using SAS version 9.3 (SAS, Cary, North Carolina). A p value of less than 0.05 was considered statistically significant.

3. Results

There were a total of 2,810,530 patients hospitalized with a primary diagnosis of AF between 2001 and 2010, of which 119,840 (4.26%) patients underwent inpatient DCCV. Though AF related hospitalization has been increasing, trends in utilization of DCCV have remained stable (Figure 1). There were significant age related disparities in the utilization of DCCV with the procedure being performed the most in patients between 41 and 64 years of age (Table 1) and least commonly in patients over 75 years of age. DCCV rates were higher in men as compared with women (58.4% versus 41.6%). Among patients who received DCCCV, 86.4% were Caucasian, 5.9% were African American, and 7.7% belonged to other races. 37% of patients receiving DCCV were privately insured. 53.2% had Medicare, 5.1% had Medicaid, and 4.6% were uninsured. Patients who underwent DCCV had significantly lower prevalence of diabetes (17.8% versus 20.9%, $p < 0.001$), hypertension (59.1% versus 61.7%, $p < 0.001$), and congestive heart failure (13.4% versus 14.7%, $p < 0.001$) when compared with those who did not. A third of the DCCV procedures were elective (29.6%) and teaching hospitals performed significantly more DCCV than nonteaching hospitals (52.9% versus 47.1%, $p < 0.001$). Volume of DCCV procedures was distributed equally across the different regions of the United States. Incidence of in-hospital stroke was significantly lower in patients who underwent DCCV during AF related hospitalization (1% versus 2.3%,

Table 1: Demographics, comorbidities, payer status, and hospital data for patients hospitalized for atrial fibrillation.

	No cardioversion	Cardioversion	p value
Demographics			
Age (years)			<0.0001
≤40	262580 (9.6%)	1275 (1%)	
40–64	752172 (27.6%)	42332 (34.7%)	
65–74	605721 (22.2%)	30118 (24.7%)	
≥75	1108150 (40.6%)	36644 (30.1%)	
Sex			<0.001
Men	1282442 (46.9%)	70983 (58.4%)	
Women	1453591 (53.1%)	50611 (41.6%)	
Race			<0.0001
White	2223231 (81.5%)	105241 (86.4%)	
Black	220210 (8.1%)	7225 (5.9%)	
Others	285236 (10.5%)	9374 (7.7%)	
Comorbidities			
Hypertension	1043744 (38.3%)	49886 (40.9%)	<0.0001
Diabetes	571796 (20.9%)	21596 (17.8%)	<0.001
Congestive heart failure	403164 (14.7%)	16330 (13.4%)	0.0012
CHADS2 score			<0.0001
0	345294	14603	
1	799072	30766	
>1	1584312	76471	
Payer status			<0.0001
Private	737885 (27.1%)	45040 (37%)	
Medicare	1662845 (61%)	64805 (53.2%)	
Medicaid	173315 (6.4%)	6175 (5.1%)	
Others	151140 (5.5%)	5660 (4.6%)	
Hospital type			<0.001
Teaching center	1064453 (39.2%)	57191 (47.1%)	
Nonteaching center	1651500 (60.8%)	64177 (52.9%)	

Table 2: Differences in clinical outcomes between patients hospitalized for AF based on use of cardioversion.

Outcomes	No cardioversion	Cardioversion	p value
Postop stroke	67897 (2.3%)	1287 (1%)	<0.0001
Cost of hospitalization ($ ± SD)	7780.22 (106.66)	5297.89 (114.21)	<0.0001
Length of stay (days ± SD)	3.85 (0.02)	3.53 (0.05)	<0.0001
In hospital mortality	30408 (1.1%)	412 (0.3%)	<0.0001

Table 3: Multivariate regression analysis for predictors of cardioversion in patients hospitalized for atrial fibrillation.

	Odds ratio	p value
Age (years)		<0.0001
<40	1.00 (Ref)	
40–64	1.07 (0.99–1.15)	
65–74	1.03 (0.94–1.12)	
≥75	0.7 (0.64–0.76)	
Sex		<0.0001
Men	1.26 (1.23–1.31)	
Women	1.00 (Ref)	
Race		<0.0001
White	1.00 (Ref)	
Black	0.66 (0.64–0.74)	
Others	0.62 (0.57–0.67)	
Hospital type		<0.0001
Teaching	1.00 (Ref)	
Nonteaching	0.58 (0.52–0.64)	
CHADS2 score		0.0027
CHADS = 0	1.00 (Ref)	
CHADS = 1	0.91 (0.86–0.97)	
CHADS > 1	0.92 (0.88–0.97)	
Diabetic	1.00 (Ref)	<0.0001
Nondiabetic	1.18 (1.14–1.23)	
Insurance		<0.0001
Private	1.00 (Ref)	
Medicare	0.84 (0.81–0.89)	
Medicaid	0.69 (0.64–0.74)	
Others	0.66 (0.59–0.74)	

$p < 0.0001$). In-hospital mortality was also significantly lower in the AF cohort that received DCCV (0.3% versus 1.1%, $p < 0.001$). Median length of stay was 3.53 days in the DCCV group compared with 3.85 days in the non-DCCV cohort ($p < 0.0001$). Cost of hospitalization was significantly lower in patients that received DCCV as compared with those who did not (5297.89$ versus 7780.22$, $p < 0.0001$) (Table 2). On multivariate logistic regression analysis, age < 40 years, male sex, Caucasian race, private payer status, and lower CHADS2 score were independent predictors of utilization of DCCV in AF (Table 3).

4. Discussion

Our study has several important findings. First, using a large real world population sample, we found significant disparities in the utilization of DCCV in the United States based on race, sex, insurance status, and comorbidities. These findings are consistent with results from other studies that identify race and gender as factors contributing to disparate utilization of procedural treatments in patients with AF [4, 5]. An important finding in our study is that use of DCCV is associated with a significant lower rate of in-hospital stroke and mortality, along with decreased length of hospital stay and hospitalization costs.

Maintenance of sinus rhythm is a highly debated area in the management of AF. Data from large randomized controlled trials has shown that rhythm control does not offer any additional survival benefit over rate control in the management of AF [6, 7]. In patients with AF and congestive heart failure, the AF-CHF trial showed that restoration of sinus rhythm was not significantly more beneficial than rate control [8]. Results from these trials led to a movement promoting rate control; however, contrarians to this idea believe that factors such as poor antiarrhythmic efficacy, high risk of toxicity, and older age of the patients included in the trials override the benefits of rhythm control. With the discovery of more effective antiarrhythmic agents and procedural techniques such as AF ablation, superiority of rhythm control over rate control is postulated. An exploratory analysis in patients less than 65 years of age has shown lower all-cause mortality with rhythm control when compared with rate control [9]. Most trials comparing the two treatment strategies have followed up patients for up to 5 years showing no survival benefit with rhythm control. A Canadian study included 26130 participants with newly diagnosed AF and followed them up for up to 9 years. They discovered that mortality was higher in the rhythm control group in the first 6 months, was equal in both groups for about 4 years, and trended down in the rhythm control group as compared to rate control group after the fifth year [10]. This data predicts that, with readvent of rhythm control, DCCV and AF ablation rates are expected to trend up in the next decade [11].

Race and gender related disparity in AF therapies has been studied previously. Naderi et al. explored racial differences in AF management in hospitalized patients and found that black men were less likely to receive an ablation or DCCV procedure [5]. Bhave et al. analyzed Medicare encounter data for 517,94 patients with newly diagnosed AF and discovered that white men receive the most aggressive care [4]. In an observational study of 5333 AF patients, Dagres et al. found that, in patients with atypical or no AF related symptoms, women underwent rhythm control less frequently as compared to men [12]. While all the reasons for these disparities are not entirely clear, differences in the clinical manifestations of AF, comorbidities, and patient and provider preferences have been shown to contribute. Data from the Euro Observational Research Programme Pilot Survey on AF [12] indicates that women are oftentimes older and more symptomatic with AF than men and have a higher risk of stroke, making them more likely to receive rate control. Men, on the other hand,

are more likely to develop tachycardia-induced cardiomyopathy [13], prompting more frequent use of a rhythm control strategy. Rienstra et al., in the Rate Control versus Electrical Cardioversion Study, found that women with persistent AF have greater cardiovascular morbidity and mortality when randomized to a rhythm control strategy as compared to rate control, while maintaining a similar quality of life [14]. Other factors related to treatment, access to healthcare, and affordability also play a part. African American patients have been shown to have lower rates of anticoagulation [15], prompting the use of a rate control strategy. Patients who do not have access to tertiary care centers or specialists who perform this procedure on a frequent basis miss out on the opportunity to get DCCV. Results from our study show that patients admitted to teaching hospitals are more likely to get DCCV. This could be partly explained by the availability of cardiologists and electrophysiologists with a higher level of experience. Insurance (payer) status was found to be a significant predictor for DCCV in our analysis. This is consistent with data from other studies that describe disparities in utilization and outcomes based on payer status [16, 17]. An interesting finding in our study was the relationship between comorbidities and DCCV utilization. Patients with less comorbidity such as hypertension, diabetes, congestive heart failure, and an overall lower CHADS2 score were found to be more likely to receive DCCV. Intuitively, these patients have a lower risk of stroke and greater chances of reversion as well maintenance of normal sinus rhythm, explaining our findings. Patients who underwent DCCV were found to have better outcomes in terms of fewer complications, mortality, and economic burden of hospitalization, as previously reported by Deshmukh et al. [18]. The lower rate of post-DCCV stroke in the nationwide sample is encouraging. Our results show that DCCV is an effective yet simple strategy to convert to normal sinus rhythm that can be employed in patients with AF with additional benefits of reduction in hospital mortality and hospitalization cost.

5. Limitations

Our study has several limitations that are inherent to large nationwide databases. NIS records discharge diagnoses and billing level data, which has potential to be miscoded leading to misrepresentation of the procedure volume. Inaccurate coding or lack of coding for comorbidities could lead to erroneous interpretations related to overall level of sickness or health. This database lacks information about clinical characteristics of the disease such as type and duration of AF, etiology, symptomatology, anticoagulant use, and imaging data, all of which are important factors considered while making treatment decisions. Lack of this data creates scope for unmatched variables between the two groups that could be contributing to the results. Due to lack of data regarding the type of AF, patients have not been substratified into groups to identify those who were not candidates for cardioversion such as paroxysmal AF or long-standing persistent atrial fibrillation. These limitations may confound study results and the improved outcomes with cardioversion may not be causal due to underlying conditions influencing its application rather than the procedure itself. Lack of hospital course and follow-up data makes it impossible to assess the degree of success or failure of the procedure in the longer term. The postprocedure complications recorded in this database are those that occur during the same admission and lack of postdischarge complications questions the long-term implications; for example, AF patients undergoing DCCV have a high risk for stroke in the first 4 weeks after the procedure and we do not have data about this beyond the in-hospital stay. A large number of patients with AF are managed in the outpatient setting, while the current study provides information pertaining to AF related hospitalization.

6. Conclusion

In a large nationally representative sample of patients hospitalized with AF, only a small fraction were found to undergo DCCV. Procedure trend for DCCV in AF has been fairly stable over the last 10 years while the number of hospitalizations for AF has been rising steadily. Younger patients, white patients, men, and those with fewer comorbid conditions are most likely to receive DCCV during AF related hospitalization. Patients who undergo DCCV were found to have lower rates of complications and lower length of hospital stay as well as cost of hospitalization. These findings need to be investigated in further detail at a more granular level in order to create solutions to eliminate these disparities.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

L-Type Calcium Channels Do Not Play a Critical Role in Chest Blow Induced Ventricular Fibrillation: Commotio Cordis

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Background. In a commotio cordis swine model, ventricular fibrillation (VF) can be induced by a ball blow to the chest believed secondary to activation of mechanosensitive ion channels. The purpose of the current study is to evaluate whether stretch induced activation of the L-type calcium channel may cause intracellular calcium overload and underlie the VF in commotio cordis. *Method and Results*. Anesthetized juvenile swine received 6 chest wall strikes with a 17.9 m/s lacrosse ball timed to the vulnerable period for VF induction. Animals were randomized to IV verapamil ($n = 6$) or placebo ($n = 6$). There was no difference in the observed frequency of VF between verapamil (19/26: 73%) and placebo (20/36: 56%) treated animals ($p = 0.16$). There was also no significant difference in the combined endpoint of VF or nonsustained VF (21/26: 81% in verapamil versus 24/36: 67% in controls, $p = 0.22$). *Conclusions*. In this experimental model of commotio cordis, verapamil did not prevent VF induction. Thus, in commotio cordis it is unlikely that stretch activation of the L-type calcium channel with resultant intracellular calcium overload plays a prominent role.

1. Introduction

Sudden cardiac death due to the induction of ventricular fibrillation (VF) by blunt chest wall blows is defined as commotio cordis. Most commonly occurring in the setting of sport, commotio cordis has been reported with increasing frequency [1, 2]. Commotio cordis is a primary electrical event in which both the timing of impact and a rapid rise in left ventricular (LV) pressure are critical [3–6]. The rapid rise in LV pressure likely results in myocardial cell membrane stretch and deformation of the cell membrane with resultant activation of mechanosensitive ion channels. The K^+_{ATP} channel is among the stretch sensitive ion channels that have been implicated in the pathophysiology of commotio cordis [7].

The L-type calcium channel ($I_{Ca,L}$) may also be activated by cell membrane stretch with resultant calcium influx into the cell (Figure 1) [8, 9]. Intracellular calcium overload underlies the mechanism of VF in catecholaminergic polymorphic ventricular tachycardia (CPVT). Intracellular calcium overload may also contribute to arrhythmogenesis in the Long QT Syndrome (LQTS), particularly in the Timothy syndrome [10].

In the current experiment we test the hypothesis that chest wall impact activates $I_{Ca,L}$, causing an intracellular calcium spike, which can result in early-after-depolarizations (EADs) or delayed-after-depolarizations (DADs) and induce VF. If this is the case, then blocking $I_{Ca,L}$ prior to chest wall impact should reduce the incidence of VF in commotio cordis. Verapamil, a synthetic papaverine derivative, primarily blocks $I_{Ca,L}$ but also may have some blocking effect on the ryanodine receptor, both of which may contribute to intracellular calcium overload.

2. Methods

In conformity with the regulations of the Association for Assessment and Accreditation of Laboratory Animal Care, the Institutional Animal Care and Use Committee of the Tufts Medical Center (Boston, MA, USA) approved the research protocol. Twelve male domesticated swine with a weight from

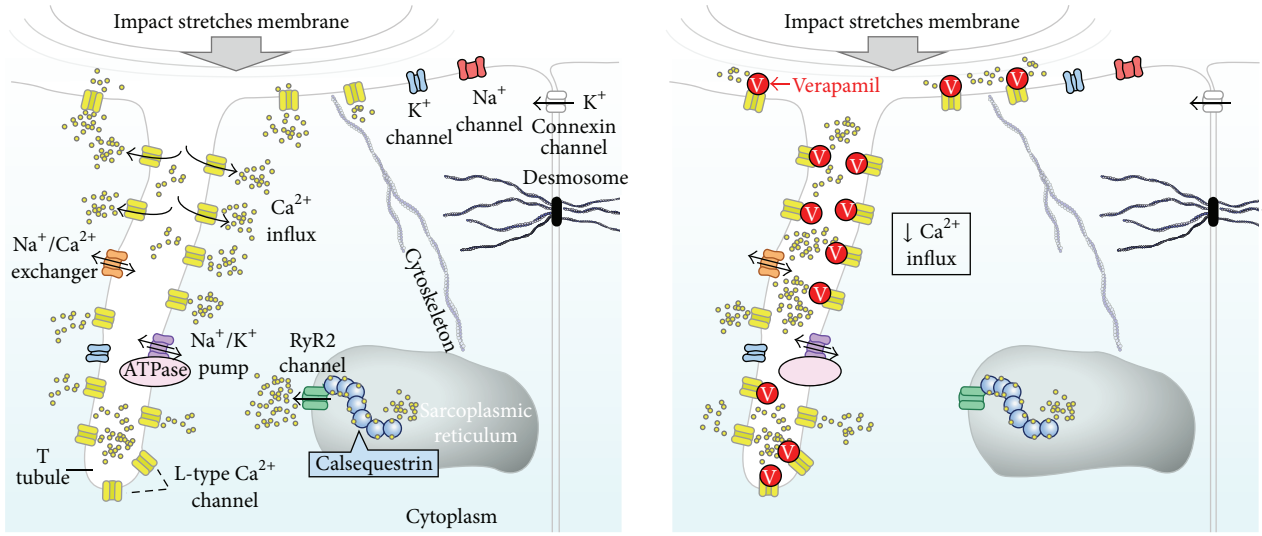

FIGURE 1: Impact of the chest wall marked increases left ventricular intracavitary pressure which in turn amplifies left ventricular wall strain and myocyte membrane stretch. This cell membrane deformation may activate stretch activated ion channels, which may in part cause the ventricular fibrillation in commotio cordis. In the left panel, a placebo animal, there is an increased influx of calcium due to stretch activation of the $I_{Ca,L}$ channel. If the $I_{Ca,L}$ channel is involved in commotio cordis, blockade of the channel (right panel) should reduce the ventricular fibrillation seen in our model.

15 to 20 kg were anesthetized with ketamine and inhaled isoflurane. Animals were intubated and placed on a respirator, and general anesthesia was maintained throughout the experiment with isoflurane (2% isoflurane gas in 100% oxygen gas). Millar Mikrotip® (Houston, TX, USA) pressure catheters were introduced into the left ventricle via the femoral artery. Electrocardiographic tracings and left ventricular pressures were recorded continuously utilizing an analogue-to-digital converter (Chart® software; AD Instruments, Mountain View, CA, USA). Recordings were sampled at 2,000 Hz, not filtered, and saved on a laptop computer.

Each animal was placed prone in a sling to approximate physiologic cardiac anatomy and hemodynamics. Chest blows were directed toward the anatomic center of the left ventricle as identified by transthoracic echocardiography. Chest blows were delivered by a lacrosse ball propelled at 17.9 m/s. Speed of the impact object was assessed using a chronograph (Oehler Research, Austin, TX, USA) modified for low velocities. Triggered from the surface electrocardiograms of the swine, the release and subsequent impact of the ball were gated to the cardiac cycle using a commercially available cardiac stimulator (EP-2, EP Medical, Inc., Budd Lake, NJ, USA). All impacts were timed to the vulnerable window for VF induction in commotio cordis (10 to 40 msec prior to the peak of the T-wave) [3].

Prior to impact, swine were randomly assigned to either calcium channel blockade with a verapamil infusion (0.4 mg/kg) or placebo (normal saline, 100 ml) [11]. Five minutes prior to the first chest wall impact, a technician not involved in the subsequent portions of the study administered the solutions intravenously. Investigators performing the chest blows remained blinded to the solutions (verapamil or control) each animal received. Measurements and interpretations of all electrocardiographic data were performed by a single investigator blinded to the solution administered. Animals given verapamil had a similar heart rate (106 ± 23 versus 108 ± 13 bpm; $p = 0.89$) compared to control animals but had a lower left ventricular systolic pressure (63 ± 16 versus 89 ± 12 mmHg; $p = 0.01$).

Ventricular fibrillation was defined as polymorphic ventricular arrhythmia requiring defibrillation. Nonsustained VF was defined as ≥3 beats of VF that terminated spontaneously. If VF occurred after a chest blow, the animal was immediately defibrillated. After each episode of VF, the animal's blood pressure, heart rate, and left ventricular ejection fraction (by echocardiography) were monitored. If these parameters returned to the baseline levels, repeat impacts were delivered for a total of 6 impacts per animal.

Continuous data were reported as mean ± SD. Differences between the groups were analyzed by chi-square (or Fisher's exact test, where appropriate), linear regression for continuous outcome variables, and logistic regression for dichotomous outcome variables. Analysis was performed in SAS statistical software (Version 8, Cary, NC, USA).

3. Results

Twelve domesticated swine received a total of 62 impacts within the vulnerable time window for VF induction. Initiation of VF was observed in 19 of 26 (73%) impacts in animals given verapamil, compared with 20 of 36 (56%) in control animals ($p = 0.16$) (Figures 2 and 3). Ventricular fibrillation occurred immediately following the chest blow. Despite defibrillation, two animals in the verapamil group could not be resuscitated after induction of VF from the initial chest impact. Defibrillation terminated all other VF episodes with normalization of blood pressure and LV systolic function.

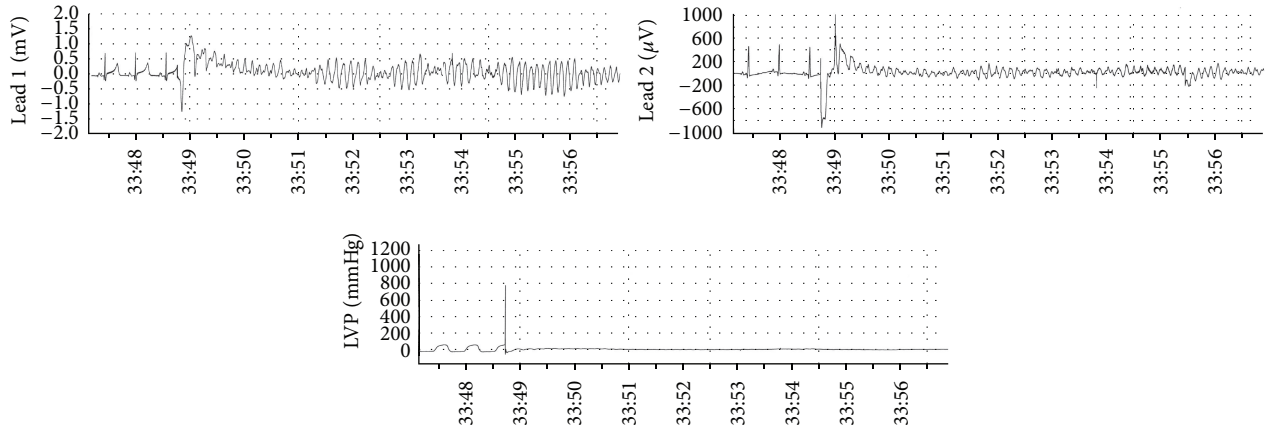

Figure 2: Initiation of ventricular fibrillation with a chest wall impact of a 40 mph lacrosse ball directly over the cardiac silhouette of an 18 kg swine. The impact occurs on the upslope of the T-wave and produces an immediate left ventricular pressure (LVP) rise to 800 mmHg and ventricular fibrillation.

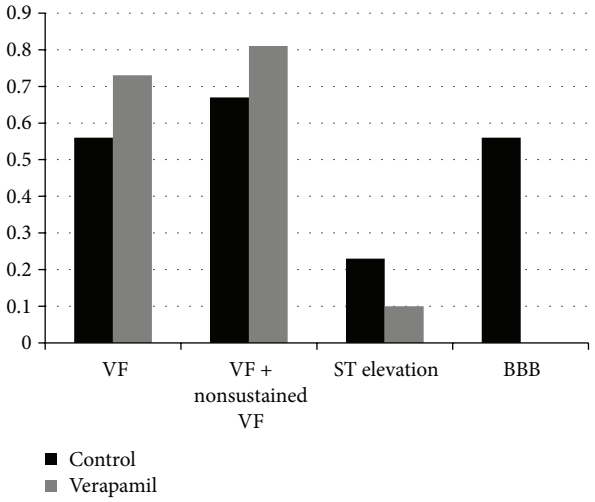

Figure 3: Bar graph of the results of 62 impacts to 12 animals with 36 impacts in 6 control animals and 26 impacts in 6 animals given verapamil. There was no difference in the endpoints of ventricular fibrillation (VF) or the combined endpoint of VF + nonsustained VF. Verapamil administration did decrease the severity of ST segment elevation and the induction of a bundle branch block (BBB).

Nonsustained VF was observed in 2 of 7 (28%) verapamil strikes compared with 4 of 16 (25%) controls. Thus, the combined endpoint of VF or nonsustained VF was met in 21 of 26 (81%) verapamil strikes compared with 24 of 36 (67%) controls ($p = 0.22$).

In strikes that did not induce VF, the frequency of categorical ST elevation did not differ between groups. However, pretreatment with verapamil did result in a diminished magnitude of ST elevation (100 ± 65 mV in the verapamil animals compared with 233 ± 105 mV in the controls; $p = 0.01$). Verapamil also reduced the frequency of chest blow induced bundle branch block (BBB), 0 of 7 verapamil strikes compared to 9 of 16 (56%) placebo strikes ($p = 0.03$). Peak left ventricular pressure after impact was slightly greater for animals pretreated with verapamil. Impact in verapamil animals caused a mean left ventricular peak pressure of 689 ± 53 mmHg versus 628 ± 123 mmHg in control animals ($p = 0.02$).

4. Discussion

In the current experiment, prestrike administration of verapamil did not prevent VF in a well-established commotio cordis model. Thus, intracellular calcium overload caused by activation of the $I_{Ca,L}$ channel does not appear to be involved in the mechanism of VF in commotio cordis. Yet, intracellular calcium overload may contribute to the ST elevation and BBB observed in clinical cases and experimental models of chest wall trauma [3, 12].

Mechanical stimulation of the myocardium resulting in electrical events is well described, occurring in such circumstances as catheter-induced ectopy during intracardiac procedures and in the production of premature ventricular depolarizations by thumping the chest during asystole [13]. This phenomenon, termed mechanoelectric coupling, has been attributed to the presence of mechanosensitive ion channels that are activated by stretch or pressure changes within the myocardium. We have hypothesized that the inciting event in commotio cordis is the activation of mechanosensitive ion channels as a result of the rapid rise in left ventricular pressure and ensuing myocardial stretch [14]. We have previously shown that the initiation of VF in commotio cordis is related to the peak left ventricular pressure generated by the chest wall blow [3–6]. However, the cellular mechanisms that underlie the initiation of VF in commotio cordis remain incompletely understood and the identification of the potential mechanosensitive ion channels remains unresolved.

In ventricular myocytes, the L-type calcium current regulates excitation contraction coupling and influences the plateau height of the action potential, as well as total action potential duration [15]. During the plateau phase of the action potential, extracellular calcium enters the myocyte through

the L-type calcium channel and elicits calcium release from the sarcoplasmic reticulum in a process termed calcium-induced calcium release [16, 17]. However, an excess of intracellular calcium may lead to EADs, DADs, and VF. Excessive intracellular calcium is the hallmark of several arrhythmogenic diseases caused by mutations in calcium regulatory genes.

Catecholaminergic polymorphic ventricular tachycardia is an arrhythmogenic disorder caused by mutations in one of the calcium regulatory genes [16]. Dominant mutations in the ryanodine receptor gene and recessive mutations in the calsequestrin-2 gene result in increased calcium current leak from the sarcoplasmic reticulum through the ryanodine receptor, resulting in intracellular calcium overload [17]. In CPVT, exercise or stress results in stimulation of beta adrenergic receptors, promoting cyclic AMP mediated phosphorylation of ryanodine receptors, leading to further intracellular calcium overload. The resultant excess calcium current can depolarize the myocyte at the end of the action potential, creating DADs. Delayed afterdepolarization reaching a critical threshold can trigger clinical arrhythmias, including ventricular extrasystoles, bidirectional ventricular tachycardia, and VF [17]. The mainstay of medical therapy for CPVT has been beta blockade, but there is evidence that treatment with verapamil may result in additional benefit [18]. It has been theorized that the effects of verapamil in CPVT are due to a reduction in the primary calcium signal through blockade of the L-type calcium channel [18, 19]. Verapamil might further reduce the calcium current through the ryanodine channel by indirectly reducing cyclic AMP or possibly by directly binding and blocking the channel [18, 20].

In Timothy syndrome, a "gain of function" mutation in the L-type calcium channel leads to reduced voltage dependent calcium channel inactivation [10]. The resulting inward calcium currents induce intracellular calcium overload that results in action potential prolongation and is associated with onset of DADs and triggered activity. Cardiac manifestations of Timothy syndrome include QT prolongation, torsades de pointes, and VF [10]. Blockade of the L-type calcium channel with verapamil can decrease the incidence of ventricular tachyarrhythmias in Timothy syndrome [21].

Calcium channels are known to exhibit mechanosensitive properties, including the cation nonselective stretch activated channel (SAC) and the more selective L-type calcium channels [8]. Mechanical stretch can result in increased intracellular calcium concentrations by direct activation of these channels, which further triggers the release of stored calcium in the sarcoplasmic reticulum [8]. In this study, administration of verapamil did not have a significant effect on the induction of VF in a commotio cordis model. The observation of a nonsignificant increased frequency of VF induction in the verapamil treated group likely relates to the higher maximum left ventricular pressures achieved from chest wall blows in this group. Although verapamil can have potent antifibrillatory effects in ischemia models [11, 22–25], presumably by decreasing myocardial ischemia, the instantaneous nature of VF in commotio cordis argues against ischemia as a cause of VF. Interestingly, verapamil pretreatment in our model did result in diminution of ST elevation and reduction in bundle branch block from chest wall impacts, suggesting that these effects may be mediated via stretch induced $I_{Ca,L}$ channel activation.

The SAC has been shown to be activated by stretch in Langendorff models [26, 27] causing intracellular calcium increase and thus could also perhaps be involved in commotio cordis. However, a previous experiment in our model that targeted blockade of the SAC calcium channel did not show a reduction in the initiation of VF [28]. Interestingly, similar to verapamil pretreatment, blockade of SAC reduced the frequency and magnitude of ST elevation compared with controls. Taken in summation, these data suggest that although calcium channels, including SAC and the L-type calcium channel, exhibit mechanosensitive properties and are activated by forceful chest impact, they do not appear to play an integral role in the initiation of chest blow induced VF.

Despite the negative results with regard to VF shown in this current study and the previous SAC study, we do believe that commotio cordis is caused by ion channel activation produced by mechanical stretch of the cell membrane. The mechanosensitive K^+_{ATP} channel has been identified among the ion channels activated by chest wall blow in commotio cordis [7]. We have previously shown that the infusion of glibenclamide, a sulfonylurea that acts primarily by inhibiting the K^+_{ATP} channel, reduced the magnitude of ST elevation and the incidence of VF in our model. Further supporting the role of mechanoelectric coupling as the inciting event in commotio cordis are data revealing that initiation of VF by chest blows is significantly increased by selective disruption of the cytoskeleton [14]. These data suggest that mechanical deformation of the cell membrane is fundamental to the activation of ion channels and underlies the mechanism of VF in commotio cordis.

5. Limitations

Plasma verapamil levels were not measured; however, the dosing administered in this study was previously used in other experiments in whole animals of similar size [11]. Verapamil also inhibits vascular smooth muscle contraction and can result in vasodilation and hypotension. This is the likely reason that two of the animals given verapamil were not able to be resuscitated after successful defibrillation. The failure to resuscitate these animals reduced the number of impacts in swine, but we do not feel that this materially would have altered the outcome of this study. Even if all additional impacts in these animals failed to induce VF there would remain no significant difference in VF initiated by chest blows. In addition, we limited the velocity of impact to a single velocity of 40 mph, based on our studies that show that this is the velocity most likely to initiate VF in our experimental model [6].

6. Conclusion

In this study, infusion of verapamil did not alter the frequency of VF induction in our commotio cordis model. Verapamil did reduce the amount of ST elevation and the frequency

of bundle branch block following chest impact. Our data indicate that although L-type calcium channels may exhibit mechanosensitive properties and may be activated by forceful chest wall impact, they do not play an integral role in the initiation of VF in commotio cordis. It is unlikely that myocardial intracellular calcium overload plays a vital role in the induction and maintenance of chest blow induced VF.

Abbreviations

VF: Ventricular fibrillation
LV: Left ventricular
CPVT: Catecholaminergic polymorphic ventricular tachycardia
LQTS: Long QT syndrome
EAD: Early-after-depolarization
DAD: Delayed-after-depolarization
BBB: Bundle branch block.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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Clinical Study

RyR2 QQ2958 Genotype and Risk of Malignant Ventricular Arrhythmias

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Ventricular arrhythmias are one of the most common causes of death in developed countries. The use of implantable cardiac defibrillators is the most effective treatment to prevent sudden cardiac death. To date, the ejection fraction is the only approved clinical variable used to determine suitability for defibrillator placement in subjects with heart failure. The purpose of this study was to assess whether genetic polymorphisms found in the ryanodine receptor type 2 (Q2958R) and histidine-rich calcium-binding protein (S96A) might serve as markers for arrhythmias. Genotyping was performed in 235 patients treated with defibrillator for primary and secondary prevention of arrhythmias. No significant association was found between the S96A polymorphism and arrhythmia onset, whereas the QQ2958 genotype in the ryanodine receptor gene was correlated with an increased risk of life-threatening arrhythmias. Concurrent stressor conditions, such as hypertension, seem to increase this effect. Our findings might help to better identify patients who could benefit from defibrillator implantation.

1. Introduction

Sudden cardiac death (SCD) is one of the most frequent causes of death in industrialized countries. SCD is commonly the result of ventricular tachycardia (VT) and/or ventricular fibrillation (VF) that occur secondary to a complex interplay between a susceptible myocardial substrate typically affected by cardiomyopathy and a transient trigger. The use of implantable cardioverter-defibrillator (ICD) is the most effective treatment to prevent this disease, because it may terminate the arrhythmia by low-voltage antitachycardia pacing or high-energy cardioversion. However, only a minority of patients benefit from these devices, because the majority of patients with an ICD have never received a shock appropriate for VT or VF. Furthermore, a substantial number of patients, who die suddenly, are not identified as high risk prior to death and do not receive an ICD implantation [1]. Although numerous clinical and serum biomarkers have been investigated for use in the risk stratification of SCD [2], the ejection fraction (EF) remains the only approved clinical variable that is used to determine suitability for ICD placement in subjects with heart failure (HF). As a result, there is a substantial interest in identifying more reliable predictors that could help to discriminate which patients are most likely to benefit from an ICD implant. The identification of genetic alterations responsible for rare hereditary arrhythmic diseases, such as Brugada syndrome, long QT, and catecholaminergic polymorphic ventricular tachycardia (CPVT), has focused the attention on the molecular basis of arrhythmias, particularly on the role of the calcium channels and associated proteins. The ryanodine receptor type 2 (RyR2), which is expressed primarily in cardiac muscle [3], is one of the three isoforms of the family of ryanodine receptors that regulate the duration and amplitude of the Ca^{2+} flow from the sarcoplasmic reticulum (SR). It is well known that aberrant diastolic Ca^{2+} release via RyR2 leads to contractile dysfunction by reducing the SR Ca^{2+} content. This provides a substrate for delayed after depolarisation (DAD), which ultimately leads to lethal arrhythmias [4]. Recently, Ran et al. [5] found that the G1886S variant (rs3766871) of the *RyR2* gene was associated with an increased risk of ventricular arrhythmias and sudden cardiac death. The histidine-rich Ca-binding protein (HRC),

expressed predominantly in striated muscle, is another SR component involved in the regulation of the Ca^{2+} uptake [6, 7], accumulation [8], and release from the sarcoplasmic reticulum [7, 9]. As reported in previous studies [10–12], ion channels polymorphisms have the potential to modify the clinical phenotype. These findings suggested the idea that also polymorphisms in RyR2 and HRC genes might have the same potential, thus representing an important factor in determining the risk of arrhythmia in HF patients who could benefit from an ICD implantation. The most common RyR2 polymorphism is RyR2-Q2958R (rs34967813, A/G), described for the first time by Tiso et al. [13], with a heterozygous prevalence of 34% in Caucasians and 10% in African Americans. It is localized within the area of interaction with the RyR2 modulator [14] and has remained highly conserved during the evolution of the ryanodine receptors [14–17]. Therefore, it will be of interest to study the functional consequences of this variation. HRC is known as an effective regulator of RyR2 activity and SR Ca^{2+} release. A genetic variant of HRC, Ser96Ala (rs3745297, G/T), disrupts the Ca^{2+} microdomain around the RyR2, as it alters the Ca^{2+} dependent association of RyR2 and HRC [18] and may enhance RyR2 activity from the SR luminal side, increasing uncontrolled Ca^{2+} release and induced Ca^{2+} instability. Arvanitis et al. [19] identified an association between HRC-S96A and malignant ventricular arrhythmias in patients with idiopathic dilated cardiomyopathy. In this study, we investigated whether these two polymorphisms (RyR2-Q2958R and HRC-S96A) are associated with the occurrence of spontaneous ventricular arrhythmias in patients receiving an ICD for primary and secondary prevention of SCD.

2. Materials and Methods

2.1. Patients and Procedures. We enrolled 235 unrelated Caucasian patients, from Salento (Southern Italy), who were consecutively admitted between January 2009 and September 2012 at the Department of Cardiology of Hospital "Card. G. Panico," and treated with an ICD, according to class I or class II indications of ACC/AHA/HRS guidelines [20, 21]. Patients with long QT syndrome, Brugada syndrome, or CPVT were excluded from the study. 157 subjects (66.8%) had an ICD implantation for primary prevention and 78 patients (33.2%) for secondary prevention. Reversible causes of ventricular arrhythmias, such as acute ischemia, electrolyte abnormalities, and QT prolonging medication use, were ruled out. During a mean follow-up time of 44 ± 13 months, 23 patients of the 157 experienced at least one episode of ventricular arrhythmia and they formed group I with the 78 patients of the secondary prevention. The remaining 134 patients of the 157, who did not develop ventricular arrhythmias, formed group II. Ventricular tachycardia was defined by the following characteristics: (i) a regular wide QRS complex (>120 milliseconds) tachycardia at a rate of more than 100 beats per minute and with a uniform and stable QRS morphology of the consecutive beats and (ii) the arrhythmia lasting ≥30 seconds or causing hemodynamic collapse in <30 seconds.

Demographic, clinical, and routine laboratory data were collected from all patients using a structured data form. Before implantation, a transthoracic echocardiogram and a coronary angiography were performed for all patients.

The presence of ischemic heart disease was defined as prior myocardial infarction and/or angina with hospitalization and/or an infarct and/or major ischemia patterns on electrocardiogram or angiographically documented coronary artery disease (>50% stenosis in ≥1 coronary artery). Dilated cardiomyopathy was defined as enlargement of the heart cavity and systolic dysfunction of one or both ventricles in the absence of congenital, coronary, hypertensive, valvular, or pericardial heart disease. The diagnostic criteria included the identification of an ejection fraction (EF) ≤35% and/or a fractional shortening <25%, in association with a left ventricular (LV) end-diastolic dimension >112% of the predicted value corrected for age and body surface area. LV ejection fraction was categorized as ≤35% or >35% according to the ACC/AHA/HRS guidelines [20] for ICD implantation. Patients were divided into the four NYHA (New York Heart Association) classes, based on how much they were limited during physical activity.

Hypertension was defined as a systolic blood pressure ≥140 mmHg or a diastolic blood pressure ≥90 mmHg or the use of antihypertensive medications. Diabetes was defined as a fasting plasma glucose level >7.0 mmol/L or a nonfasting plasma glucose level >11.1 mmol/L or the use of antidiabetic medications. Dyslipidemia was defined as elevated total (>240 mg/dL) or low-density lipoprotein (LDL >130 mg/dL) cholesterol levels or low levels of high-density lipoprotein cholesterol (HDL <40 mg/dL) or elevated triglycerides levels (>150 mg/dL). Smoking was categorized as nonsmoking or current smoking (currently smoking or stopped <1 year ago).

All patients had an ICD with a VT and VF programming which allowed the analysis of stored intracardiac electrograms and/or RR-intervals of ventricular tachyarrhythmias with a cycle length (CL) ≤330 ms. ICDs employed a stepwise analysis of morphology, rate, stability, atrioventricular association, and onset (ventricular acceleration, atrial acceleration or nonacceleration). All patients were followed up in our outpatient clinic at six-month intervals.

100 healthy subjects from the same population were also enrolled. We ruled out any disease by studying patients' medical history, by performing a physical examination, or by performing an echocardiogram and a chest radiograph.

The study was approved by the local ethics committee and conducted in accordance with the guidelines of the declaration of Helsinki. Informed consent was obtained from all subjects prior to participation.

2.2. DNA Analysis. DNA extraction was carried out on total blood using Archive Pure DNA Blood Kit (5-PRIME, Hamburg, Germany) according to the manufacturer's recommended protocol.

We genotyped our patients for the following two variants:

(i) *RyR2* Q2958R (rs34967813, A/G), involving a substitution of adenine with guanine within exon 61, which results in the substitution of arginine for glutamine;

Table 1: Primers sequences, annealing temperatures, and fragment size.

Gene	SNP	Allele	Primer sequence $5' \rightarrow 3'$	Fragment size (bp)	T_a
RyR2	Q2958R (A>G)	A	*Upper*: GGAGAACATTTCCCTTATGAC**A** *Lower*: GCAGGACTAAGGTCCCACAA	476	60°C
		G	*Upper*: GAGAACATTTCCCTTATGAC**G** *Lower*: GCAGGACTAAGGTCCCACAA	476	60°C
HRC	S96A (G>T)	G	*Upper*: AAGGAGGATGAAGATGC**G** *Lower*: TCCTCTTCCTCCTCCTCCTC	347	62°C
		T	*Upper*: AAAAGGAGGATGAAGATGC**T** *Lower*: TCCTCTTCCTCCTCCTCCTC	347	62°C

Bold letters in primer sequences highlight the allele-specific nucleotide, while underlined letters highlight mismatch nucleotide.

(ii) *HRC* S96A (rs3745297, G/T), involving a substitution of guanine with thymine within exon 1, which results in the substitution of alanine for serine.

The DNA polymorphism analyses were performed using AS-PCR. The primers were designed with the Primer3 software [22]. Primer sequences, annealing temperature, and amplification product sizes are shown in Table 1. PCR amplifications were carried out in a total reaction volume of 25 μL, with each reaction containing 100 ng of gDNA, 5 pmol of each primer, 10 mM dNTPs, 2.5 U Taq 5-Prime Eppendorf (5-PRIME, Hamburg, Germany), and 1x reaction buffer. The reaction cycle conditions consisted of an initial denaturation step at 94°C for 5 min, followed by 35 cycles of 30 s denaturation at 94°C, 30 s annealing at varying temperatures (see Table 1 for specific annealing temperatures), and 30 s extension at 68°C, with a final extension at 68°C for 5 min.

Allele-specific primers were constructed by introducing a one-base mismatch sequence before the SNP site. After agarose-gel electrophoresis, the PCR product was visualized with ethidium bromide, photographed, and genotyped.

Due to deviation from Hardy-Weinberg equilibrium (HWE), to exclude genotyping errors, all genomic DNAs genotyped for *RyR2* Q2958R were subjected to direct sequencing. Primers used for direct sequencing were CTACAGATGGTGGCAGCAGA (upper primer) and GCAGGACTAAGGTCCCACAA (lower primer).

2.3. Statistical Analysis. Continuous data are expressed as the mean ± standard deviation; categorical data are expressed as a percentage. A goodness of fit test for normality and a Brown-Forsythe or Levene test for homogeneity of variances were used to assess the applicability of parametric tests. Differences between mean data were compared by Student's t-test for the normally distributed continuous variables or by the Mann-Whitney test for nonnormally distributed variables. Differences in genotype frequencies and other categorical data between cases and controls were compared with Fisher's exact test (mid-p exact p value) and, in Hardy-Weinberg disequilibrium (HWD), with Armitage trend test. The consistency of the genotype frequencies with the HWE was tested using a chi-squared goodness-of-fit test on a contingency table of observed versus expected genotypic frequencies in cases and controls. Post hoc evaluations, where necessary, were performed by means of the Bonferroni correction. The MedCalc Statistical Software version 13.3 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2014) was used for the multivariate logistic regression analysis. A two-sided p value <0.05 was considered significant for all tests.

3. Results

Table 2 provides a summary of the characteristics of our study population. Overall, 83 (35.3%) patients had idiopathic dilated cardiomyopathy (IDCM), 108 (46.0%) patients had dilated ischemic heart disease (IHD), 29 (12.3%) patients exhibited nondilated IHD, and 15 (6.4%) patients had other heart diseases (HDs). Of the total patients in our study, simultaneous cardiac resynchronization therapy was used in 64 (27.2%) patients, 18 (17.8%) in group I and 46 (34.3%) in group II.

Although more male patients were included in group I than in group II, no other significant differences were observed between the two groups with regard to demographic data. With respect to HF aetiology, the difference in the distribution of cardiac pathologies was due to the different ACC/AHA/HRS guidelines indications (in nondilated IHD, an ICD is implanted only for secondary prevention, while in dilated cardiomyopathy, it is indicated in primary prevention). Comparable values for the clinical (NYHA classification) and echocardiographic (EF and ventricular size) prognostic markers were obtained in both groups. Over 45.5% of the total population was hypertensive, 38.3% was diabetic and 40.8% was dyslipidemic; however, hypertension, diabetes, and dyslipidemia were distributed in a uniform manner between the two groups (49.5% versus 42.5%, 39.6% versus 37.3%, and 41.6% versus 40.3%, resp.). Atrial fibrillation was present in 19.1% of our patients and was equally distributed between the two groups (14.9% versus 22.4%; $p = 0.1807$). No significant difference in pharmacological treatment was observed between the groups, with the exception of the antiarrhythmic drug amiodarone, which was taken by 60.4% of patients with VT/VF versus 25.4% of patients in the other group, because it was always given after the first episode of VT/VF documented by the ICD to prevent further arrhythmic episodes and ICD discharge.

No patient was lost to follow-up, during which 2 patients (2.0%) in group I and 2 patients (1.5%) in group II died, all for refractory heart failure. 11 patients (10.9%) in group I and 26 patients (19.4%) in group II were hospitalized for heart failure ($p = 0.1028$).

Table 2: Demographic data and clinical features of the study population according to arrhythmias occurrence.

	Total $n = 235$	Group I $n = 101$	Group II $n = 134$	p value
Demographic				
Male sex, n (%)	182 (77.4%)	86 (85.1%)	96 (71.6%)	0.0177
Age (years)	73 ± 8	73 ± 8	73 ± 8	1.0000
BMI (kg/m^2)	27 ± 5	28 ± 5	27 ± 4	0.0887
Current smoking, n (%)	122 (51.5%)	53 (52.5%)	69 (50.7%)	0.5958
HF etiology				
IDCM, n (%)	83 (35.3%)	19 (18.8%)	64 (47.8%)	0.0001
Dilated IHD, n (%)	108 (46.0%)	42 (41.6%)	66 (49.2%)	
Nondilated IHD, n (%)	29 (12.3%)	29 (28.7%)	0 (0%)	
Other HD, n (%)	15 (6.4%)	11 (10.9%)	4 (3.0%)	
CRT, n (%)	64 (27.2%)	18 (17.8%)	46 (34.3%)	0.0051
NYHA class, n (%)				
I	34 (14.5%)	16 (15.8%)	18 (13.4%)	0.4548
II	106 (45.1%)	50 (49.5%)	56 (41.8%)	
III	92 (39.1%)	34 (33.7%)	58 (43.3%)	
IV	3 (1.3%)	1 (1%)	2 (1.5%)	
Comorbidities				
Hypertension, n (%)	107 (45.5%)	50 (49.5%)	57 (42.5%)	0.2936
Diabetes, n (%)	90 (38.3%)	40 (39.6%)	50 (37.3%)	0.7866
Dyslipidemia, n (%)	96 (40.8%)	42 (41.6%)	54 (40.3%)	0.8937
Atrial fibrillation, n (%)	45 (19.1%)	15 (14.9%)	30 (22.4%)	0.1807
Echo				
LVEDD, (mm)	59 ± 10	60 ± 12	57 ± 13	0.0716
LVESD, (mm)	47 ± 9	48 ± 8	46 ± 10	0.1002
LVEF, (%)	33 ± 10	32 ± 9	34 ± 11	0.1377
Drug therapy				
Beta-blockers, n (%)	200 (85.1%)	86 (85.1%)	114 (85.1%)	1.0000
ACE-inhibitors or ARB, n (%)	184 (78.3%)	82 (81.2%)	102 (76.1%)	0.4247
Antialdosterone, n (%)	150 (63.8%)	61 (60.3%)	89 (66.4%)	0.4107
Diuretics, n (%)	210 (89.4%)	85 (84.1%)	123 (93.3%)	0.0971
Amiodarone, n (%)	95 (40.4%)	61 (60.4%)	34 (25.4%)	0.0001

ARB: angiotensin receptor blockers; BMI: body mass index; HD: heart disease; HF: heart failure; IDCM: idiopathic dilated cardiomyopathy; IHD: ischemic heart disease; CRT: cardiac resynchronization therapy; LVEDD: left ventricular end-diastolic diameter; LVEF: left ventricular ejection fraction; LVESD: left ventricular end-systolic diameter.

The genotypic distribution of the two polymorphisms in the overall cohort and with respect to arrhythmia occurrence is reported in Table 3.

The genotype distribution of the *HRC* S96A polymorphism is in HWE in the total population as in the two study groups. Conversely, the genotype distribution of the *RyR2* Q2958R variant showed deviation from HWE with a high percentage of heterozygotes in the total population and in the two groups.

However, the *RyR2* polymorphism genotypic distribution was not significantly different than that of a control population of 100 healthy subjects recruited within the same medical centre (4% QQ, 94% QR, and RR 2%).

When we compared our group of 101 subjects with ventricular arrhythmias (group I) to 134 patients without documented arrhythmias (group II), we found no significant difference in the distribution of the *HRC* S96A genotypes between them. Conversely, the distribution of *RyR2* Q2958R genotypes was significantly different between the two groups ($p = 0.0040$ with Fisher's exact test and $p = 0.0086$ using Armitage trend test), with a higher percentage of the QQ genotype in group I (13.9%) compared to group II (3.0%). A post hoc analysis with Bonferroni's correction for pairwise comparisons confirmed the significant difference. According to these data, the subjects with the QQ genotype seem to be more susceptible to VT/VF development (RR 1.95; IC 95% 1.45–2.62; $p = 0.0018$).

Multiple logistic regression analysis revealed that the correlation between the QQ genotype and the risk of VT/VF (OR 2.5559; 1.1394 to 5.7334; $p = 0.0228$) is independent from other clinical characteristics such as age, smoking, BMI, hypertension, diabetes, dyslipidemia, and *HRC* S96A polymorphism (Table 4).

Table 3: Genotypic frequency of the two analyzed polymorphisms in the study population and according to arrhythmias occurrence.

	Total	Group I	Group II	p value
RyR2 Q2958R (A>G) (n = 235)				
QQ	18 (7.7%)	14 (13.9%)	4 (3.0%)	
QR	213 (90.6%)	85 (84.2%)	128 (95.5%)	0.0040*
RR	4 (1.7%)	2 (1.9%)	2 (1.5%)	
HRC S96A (G>T) (n = 235)				
SS	38 (16.2%)	12 (11.9%)	26 (19.4%)	
SA	145 (61.7%)	69 (68.3%)	76 (56.7%)	0.1750
AA	52 (22.1%)	20 (19.8%)	32 (23.9%)	

∗ with Fisher's exact test, while $p = 0.0086$ using Armitage trend test.

Table 4: Multiple logistic regression analysis.

	Odds ratio	95% CI	p value
Age	0.9983	0.9643 to 1.0335	0.9235
BMI	0.9926	0.5558 to 1.7724	0.9799
Diabetes	0.9320	0.5275 to 1.6468	0.8085
Dyslipidemia	0.8714	0.4977 to 1.5257	0.6300
Hypertension	0.7225	0.4106 to 1.2715	0.2597
Smoking	0.9118	0.5100 to 1.6302	0.7554
HRC	0.6929	0.4720 to 1.0173	0.0612
RyR2	2.5559	1.1394 to 5.7334	0.0228

BMI: body mass index.

Given the large percentage of hypertensive, dyslipidemic, and diabetic subjects in our cohort, we conducted a stratified association analysis of the genetic variants in the presence or absence of these comorbidities.

Also in the subgroups, Hardy-Weinberg equilibrium was not reached for the *RyR2* Q2958R variant.

The distribution of *RyR2* Q2958R genotypes was not significantly different in dyslipidemic and nondyslipidemic patients between the two groups (Table 5). The same is true in diabetic and nondiabetic individuals, as we believe that the difference apparently emerging is exclusively linked to the low number of patients with diabetes. On the contrary, in hypertensive subjects we found a higher, statistically significant frequency of *RyR2* QQ genotype among VT/VF patients ($p = 0.0001$ with Fisher's exact test and $p = 0.004$ using Armitage trend test), which was associated with an increased risk of malignant ventricular arrhythmias (RR 2.51; IC 95% 1.96–3.23; $p = 0.0001$), whereas in hypertension-free patients the genotypic percentages were the same in the two groups ($p = 1$).

Additionally, in these subgroups, no significant association was observed between the *HRC* S96A genotypes and ventricular arrhythmias (data not shown).

When analyzed by allele status (Table 6), there were no significant differences in baseline clinical characteristics between *RyR2*QQ patients and QR or RR individuals; however, we observed an increase in the percentage of hypertensive subjects in the QQ genotype (66.7%) compared to the other genotypes (43.8%). This analysis indicates that the correlation between the 2958QQ genotype and the history of sustained VT/VF in our patients is independent of other clinical characteristics.

Table 5: Genotypic frequency of *RyR2* Q2958R in the study population subgroups according to arrhythmias occurrence.

	Group I	Group II	p value
Hypertensive patients (n = 107)			
QQ	12 (24.0%)	0 (0%)	
QR	37 (74.0%)	56 (98.2%)	0.0001*
RR	1 (2.0%)	1 (1.8%)	
Not hypertensive patients (n = 118)			
QQ	2 (4.3%)	3 (4.2%)	
QR	43 (93.5%)	68 (94.4%)	1
RR	1 (2.2%)	1 (1.4%)	
Dyslipidemic patients (n = 96)			
QQ	5 (11.9%)	6 (11.1%)	
QR	36 (85.7%)	47 (87.0%)	1
RR	1 (2.4%)	1 (1.9%)	
Not dyslipidemic patients (n = 129)			
QQ	4 (7.4%)	3 (4.0%)	
QR	49 (90.8%)	71 (94.7%)	0.6777
RR	1 (1.8%)	1 (1.3%)	
Diabetic patients (n = 90)			
QQ	6 (15.0%)	2 (4.0%)	
QR	33 (82.5%)	48 (96.0%)	0.0696
RR	1 (2.5%)	0 (0%)	
Not diabetic patients (n = 137)			
QQ	8 (14.3%)	2 (2.5%)	
QR	47 (83.9%)	77 (95.0%)	0.0204
RR	1 (1.8%)	2 (2.5%)	

∗ with Fisher's exact test, while $p = 0.0004$ using Armitage trend test.

4. Discussion

In the present study we found that the *RyR2*QQ genotype seems to be associated with a strong trend towards increased

Table 6: Clinical characteristics and events stratified according to *RyR2* allele status.

	RyR2 QQ $n = 18$	*RyR2* QR + RR $n = 217$	p value
Demographic			
Male sex, n (%)	13 (72.2%)	170 (78.3%)	0.5581
Age (years)	74 ± 7	72 ± 6	0.1811
BMI (kg/m^2)	28 ± 6	28 ± 3	1.0000
Current smoking, n (%)	7 (38.9%)	98 (45.2%)	0.6330
HF etiology			0.6598
IDCM, n (%)	5 (27.8%)	76 (35.0%)	
Dilated IHD, n (%)	8 (44.5%)	100 (46.1%)	
Nondilated IHD, n (%)	3 (16.6%)	28 (12.9%)	
Other HD, n (%)	2 (11.1%)	13 (6.0%)	
NYHA class, n (%)			0.8741
I	3 (16.7%)	30 (13.8%)	
II	9 (50.0%)	98 (45.2%)	
III	6 (33.3%)	86 (39.6%)	
IV	0	3 (1.4%)	
VT/VF, n (%)	14 (77.8%)	87 (40.1%)	0.0025
Comorbidities			
Hypertension, n (%)	12 (66.7%)	95 (43.8%)	0.0837
Diabetes, n (%)	8 (44.4%)	82 (37.8%)	0.6187
Dyslipidemia, n (%)	11 (61.1%)	85 (39.2%)	0.0827
Echo			
LVEDD, (mm)	58 ± 10	61 ± 8	0.1354
LVESD, (mm)	47 ± 8	48 ± 6	0.5093
LVEF (%)	35 ± 10	32 ± 11	0.2643

BMI: body mass index; HD: heart disease; HF: heart failure; IDCM: idiopathic dilated cardiomyopathy; IHD: ischemic heart disease; LVEDD: left ventricular end-diastolic diameter; LVEF: left ventricular ejection fraction; LVESD: left ventricular end-systolic diameter.

susceptibility to life-threatening arrhythmias in patients receiving ICD therapy for primary and secondary prevention. The association was significant in the general population ($p = 0.004$) and was more evident in hypertensive patients ($p = 0.0001$). A marked HWD in the genotypic distribution of Q2958R polymorphism for *RyR2* gene was observed. Moreover, this SNP seems to be an independent risk factor for an increased risk of ventricular arrhythmias, as evidenced by multivariate analysis. Tiso et al. [13] described for the first time the Q2958R polymorphism in *RyR2* gene. As reported in dbSNP (http://www.ncbi.nlm.nih.gov/SNP/), the Q2958 allele frequency was estimated to be 72.1% in the European population (573 subjects) and 64% in the population of North America (120 subjects). In our cohort (335 subjects), the frequency is lowered to 53% with a heterozygous prevalence of 90.6%. Deviation from HWE may be due to biologic or nonbiologic reasons. Genotyping errors are one of the possible sources of HWD, but they are generally small and do not generate sufficient deviations from HWE to be detected. However, to rule out genotyping errors, we reanalyzed this polymorphism by the use of direct sequencing and in all cases sequencing data were consistent with AS-PCR results. To exclude a selection bias (population stratification) due to our inclusion and exclusion criteria, we enrolled and genotyped 100 healthy subjects, obtaining the same genotype distribution.

Another possible cause of deviation from HWE could be a differential survival of subjects with different genotypes: while the newborns of a population may be in HWE, the elderly individuals deviate from HWE. So we think that the prevalence of heterozygosity could be explained by a relatively benign effect of the QR condition on RyR2 channel function. However, this advantage is lacking in the homozygous QQ, becoming risk-conferring in the setting of imposed stress load due to hypertension.

In recent reports, it has been shown that defective interdomain interaction between the IP-domain (putative partner domain of I-domain, which remains to be identified) and the I-domain (amino acids 3722–4610) causes various proarrhythmic states, such as increased frequency of spontaneous Ca^{2+} sparks and the appearance of DAD [23]. We hypothesize that the presence of glutamic acid in position 2958 could weaken the IP-domain/I-domain interaction, keeping RyR2 in a slightly less closed form, resulting in greater Ca^{2+} release. In this context, stress factors such as hypertension can amplify the phenotypic effects of the Q2958 allele [24]. Furthermore, the Q2958R polymorphism lies within the proposed modulator region (MR1, residues 2618–3015) that has been highly conserved during ryanodine receptor evolution [14]. Three potential calmodulin-binding sites, from residues 2774–2806, 2876–2897, and 2997–3015, have been found in this region [25]. The presence of glutamic acid in this region could weaken the interaction of RyR2 with modulators especially in hypertensive patients.

Alternatively, this variant might be in linkage disequilibrium with a gene that contributes to disease susceptibility or affects survival, or with a gene that is associated with the choice of mates, justifying the Hardy-Weinberg disequilibrium.

Different from Arvanitis et al. [19] in our cohort, we did not find any association with the S96A polymorphism in the HRC gene and an increased risk of arrhythmias. The discordant results may be due to the different method of patient recruitment. Only patients with idiopathic dilated cardiomyopathy were analyzed in the work of Arvanitis et al. [19].

5. Conclusions

To date, our study is the first one to analyze the possible role of *RyR2* Q2958R polymorphism in SCD, showing that it might contribute to the onset of malignant cardiac arrhythmias. Stress load due to hypertension seems to modulate this effect. The association that we found may help to determine the arrhythmic risk in HF patients, who could benefit from an ICD implantation according to the ACC/AHA guidelines. The limit of our study is that the genotype distribution of the *RyR2* Q2958R variant showed deviation from HWE. This implies a selected rather than a random sample, invalidating direct comparisons with other populations.

However, we must keep in mind that single-nucleotide polymorphisms are only partial contributors to an individual's risk for developing a disease.

Therefore, our results should be regarded with caution and our findings regarding RyR2 polymorphism should be confirmed in future prospective larger-scale clinical trials specifically designed, comparing similar study groups in primary prevention with the same phenotype (i.e., underlying disease) and adequately powered to detect genotype-specific differences.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

Cardiac Sympathetic Nerve Sprouting and Susceptibility to Ventricular Arrhythmias after Myocardial Infarction

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Ventricular arrhythmogenesis is thought to be a common cause of sudden cardiac death following myocardial infarction (MI). Nerve remodeling as a result of MI is known to be an important genesis of life-threatening arrhythmias. It is hypothesized that neural modulation might serve as a therapeutic option of malignant arrhythmias. In fact, left stellectomy or β-blocker therapy is shown to be effective in the prevention of ventricular tachyarrhythmias (VT), ventricular fibrillation (VF), and sudden cardiac death (SCD) after MI both in patients and in animal models. Results from decades of research already evidenced a positive relationship between abnormal nerve density and ventricular arrhythmias after MI. In this review, we summarized the molecular mechanisms involved in cardiac sympathetic rejuvenation and mechanisms related to sympathetic hyperinnervation and arrhythmogenesis after MI and analyzed the potential therapeutic implications of nerve sprouting modification for ventricular arrhythmias and SCD control.

1. Introduction

The majority of sudden cardiac deaths (SCD) are caused by ventricular tachyarrhythmias (VT) secondary to acute myocardial infarction (MI) [1], which is a major cause of morbidity and mortality in patients with MI. Accumulating evidence from basic and clinical studies has shown a close association between VT, SCD, and sympathetic activity in animals and patients with MI [2]. Following an ischemic insult, sympathetic axon fibers become dysfunctional and infracted, which is followed by neural remodeling characterized by nerve sprouting and heterogeneous patterns of sympathetic innervation [3–5]. Abnormal sympathetic innervation leads to accentuated dispersion of repolarization and increased automaticity and triggers activity, which is underlying the susceptibility to, and initiation of, malignant arrhythmias. Because of this importance, the molecular mechanisms responsible for nerve regeneration after MI, as well as exact mechanisms by which sympathetic hyperinnervation may trigger lethal arrhythmias, have received a great deal of attention from investigators. In this paper, we review current knowledge on regulatory mechanisms implicated in neural sprouting and cardiac arrhythmias following MI, which may provide new valuable therapeutic options to decrease the incidence of VT, VF, and SCD after MI.

2. Normal Autonomic Nervous System of the Heart

The heart is extensively innervated by the autonomic nervous system which is mainly composed of sympathetic and parasympathetic components (Figure 1). Sympathetic nerves come from sympathetic neurons in the superior cervical ganglia, stellate (cervicothoracic) ganglia, and thoracic ganglia which communicate with corresponding cervical or thoracic spinal cord [6]. For these postganglionic cells, axons form the superior, middle, and inferior sympathetic input and then project from the base of the heart into the myocardium along the epicardial vascular structures of the heart [7], whereas parasympathetic innervation to the heart originates predominantly in the parasympathetic neurons in cardiac ganglia whose preganglionic fibers are carried within the vagus nerve. Once entering the pericardial sac, sympathetic and parasympathetic nerve fibers together

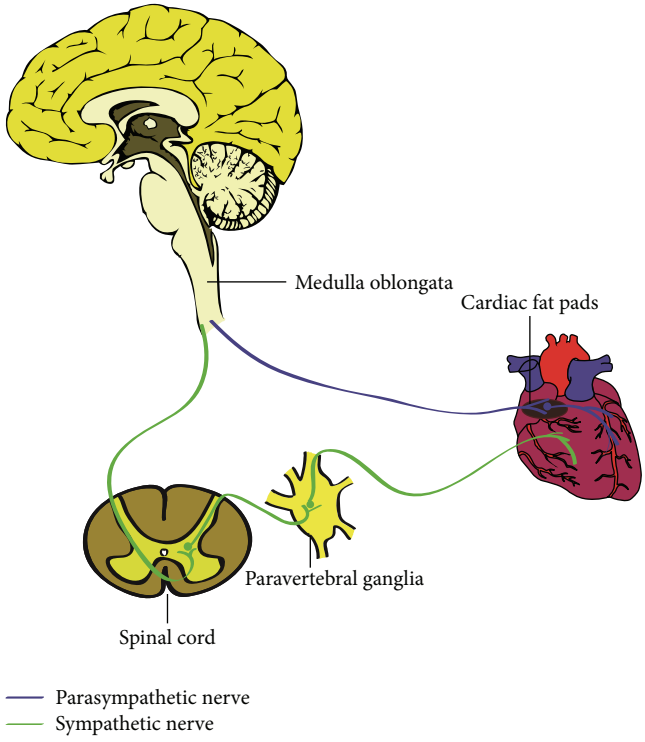

Figure 1: Anatomy and distribution of autonomic innervation of the heart. The cardiac parasympathetic nerves come from parasympathetic neurons located in the cardiac fat pads whose preganglionic fibers are carried within the vagus nerve. The cardiac sympathetic nerves come from the paravertebral ganglia and project from the base of the heart into the myocardium.

with cardiac ganglia form an exquisitely complex network to control of cardiac performance. Throughout the heart, there are multiple ganglionated plexi functions as integration centers, concentrated within fat pads and scattered over the atria and ventricles [8]. It is widely recognized that parasympathetic innervation is sparse in the left ventricle (LV) compared with sympathetic nerves [8]. A recent report, however, suggested that parasympathetic fibers innervate both atria and ventricles [9]. The density of parasympathetic and sympathetic innervation in the special conduction system such as the sinoatrial node and atrioventricular node is considerably higher than working myocardium [10, 11]. In addition to regional difference, autonomic innervation also shows some functional asymmetry. For instance, stimulation of right sympathetic nerves versus left usually results in an increased heart rate versus a prominent increase of blood pressure, respectively, although this functional asymmetry is not absolute. Thus, there might be a theory that ganglionated plexi may serve as a primary site to manage extensive signal inputs ensuing providing output to a specific cardiac structure and presenting exquisitely complex and varying functional significance [12–14].

3. Sympathetic Rejuvenation after MI

It has been well known that MI could result in degeneration and death of sympathetic fibers within the scar [15]. In addition, regions of sympathetic denervation also occur in the viable myocardium beyond the infarcted area [16–19]. After injury, in contrast to central neurons, peripheral neurons typically regenerate back to their targets [20, 21]. Regeneration of sympathetic nerves in the heart after MI has been well characterized in both animals and humans [3, 5, 19, 22–30]. By using growth-associated protein 43 (GAP43), a protein associated with axonal growth cone, to quantify the density of nerve fibers, Zhou and coworkers demonstrated that after MI, nerve sprouting is slow but accelerates to reach an apparent rate within 1 week and then progressively declined by 2 month [26, 27]. Compatible with this finding, ^{123}I-metaiodobenzylguanidine (MIBG) studies presented evidence that sympathetic reinnervation exists in the infracted hearts of patients [3]. Cao et al. performed immunocytochemical staining for S100 protein, neurofilament protein, and tyrosine hydroxylase on explanted hearts to study the distribution and the density of sympathetic nerves. They reported that the density of nerve fibers was significantly higher in the periphery of necrotic tissues of failed hearts [24]. There are inconsistent reports about sympathetic regeneration within cardiac scar. Li et al. found that cardiac infarct was not reinnervated after cardiac ischemia-reperfusion [19], whereas, following chronic cardiac ischemia, there is robust sympathetic regeneration within the infarct [5, 29, 30]. A recent study showed that chondroitin sulfate proteoglycans (CSPGs) presented in the infarct inhibit sympathetic axon outgrowth by acting through Protein Tyrosine Phosphatase Sigma (PTPRS) [31]. Additionally, in the heart postinfarct, sympathetic hyperinnervation is also likely to coexist with denervation [19, 32], a critical contributor to the onset of serious ventricular arrhythmias [33, 34]. Indeed, future studies are warranted to address the important and complex issues area, timing and patterns of sympathetic denervation and reinnervation, and their molecular mechanisms in the infracted heart. In conclusion, the post-MI heterogeneous of sympathetic transmission accounting for a nonuniform electrophysiologic response may create a high-yield substrate for ventricular arrhythmias [1].

3.1. Neurotrophic Factors Are Critical for Regeneration. In fact, the sympathetic efferent regeneration effort is triggered by neurotrophic factors, particularly nerve growth factor (NGF). NGF is a potent neural chemoattractant that exerts critical functions for the survival and differentiation of sympathetic neurons and promotes peripheral nervous axon outgrowth [35, 36]. Following MI, NGF levels significantly increased [26, 30, 37–39] which is likely a major contributor to the causes of sympathetic hyperinnervation [40, 41]. In contrast, infarct-stimulated nerve sprouting was blocked by NGF antibodies in vitro cocultures [30, 31]. Of interest, Zhou et al. observed an upregulation of cardiac NGF with a greater magnitude at the infarcted site than the noninfarcted LV free wall, which was not consistent with patterns of neurilemma proliferation [26]. Transcardiac (difference between coronary sinus and aorta) NGF concentration increased immediately after MI; however, the NGF levels significantly increased in the left stellate ganglion (LSG) from 3 days after MI, without a concomitant increase in mRNA. The authors speculated

that NGF is likely transported retrogradely to LSG [42], which then triggers nerve sprouting in noninfarcted LV sites. Indeed, NGF infusion to the LSG is associated with augmentation of MI-induced sympathetic nerve sprouting [40, 43]. In addition to release from damaged cells within the heart, increased NGF content may be partly due to synthesis from cells such as cardiomyocytes [44], Schwann cells [45], and inflammatory cell [29, 30, 46]. Using transgenic and knockout mouse models, Ieda et al. have shown that ET-1 could facilitate cardiomyocytes' production of NGF during MI [44]. Considering that ET-1 is strongly induced during the MI, the ET-1/NGF pathway may contribute to nerves regeneration following MI [44]. From a mechanistic perspective, NGF might function through the p75 neurotrophin receptor and TrkA receptor in sympathetic neurons to stimulate axon outgrowth [47–50]. Signal transducer and activator of transcription 3 (STAT3) is necessary for NGF-induced sympathetic regeneration in the heart after MI [51]. These would suggest that NGF seems to have a greater impact on sympathetic regeneration in the heart after injury.

3.2. Sema3A Is a Axonal Chemorepellent. Although axon pruning of heart during development is critical for establishment of appropriate neural circuitry which is regulated by a number of factors such as ephrins and semaphorins [52–54], the link between sympathetic hyperinnervation and axon pruning after MI requires further studies to elucidate. Sema3A is a class 3 secreted semaphorin and identified as a potent neural chemorepellent able to cause the retraction and collapse of the neuronal growth cone in the vertebrate [55]. A bulk of evidence documented that sema3A could modulate development of major structure of central nervous system such as the brain and spinal cord and determine peripheral neural patterning and projections, and participate in axon regeneration and neural repair [56, 57]. Ieda and coworkers have shown that sema3A is strongly expressed in the heart during embryogenesis, gradually decreased after birth, which plays a negatively regulatory role in determining the density and patterning of cardiac sympathetic innervations [52]. Importantly, we found cardiac nerve injury caused by MI could not significantly trigger the reexpression of sema3A, but overexpression of sema3A in MI border zone could reduce sympathetic hyperreinnervation accompanied by reduced inducibility of ventricular arrhythmias [58]. Wen et al. demonstrated that sema3A significantly shortens monophasic action potential duration (APD) and effective refractory period at infarct border zones after MI compared with control group [59]. They speculated this alleviation of electrical remodeling after MI may be attributable to suppression of sympathetic nerve sprouting by sema3A. These results indicate that sema3A may play a role in sympathetic pruning in the peri-infarct ventricle.

Overall, as MI results in marked upregulation of neural chemoattractants expression without corresponding elevated chemorepellents expression, it might be that this unbalance in the infracted heart leads to the excessive regeneration behavior of sympathetic nerve axons, thereby contributing to the enhanced risk of ventricular arrhythmias and SCD after MI.

4. Neural and Electrical Remodeling after MI

MI could cause important changes in cellular electrical activity (electrical remodeling) particular of border-zone cells functionally based on ion-channel abnormalities due primarily to ionic loss, membrane breakdown, and intracellular acidosis [60]. For example, a variety of K^+ currents such as I_{to}, I_{K1}, I_{Ks} are downregulated in border-zone cells [61–63]. Generally, sympathetic stimulation leads to shortening of APD and reducing dispersion of refractoriness [64]. In the continued presence of chromanol 293B, a specific I_{Ks} blocker, sympathetic stimulation produces an abbreviation of the epicardial and endocardial cells APD but not that of the M cells, resulting in an accentuated dispersion of repolarization and widening of the T wave [65]. In patients afflicted with LQT1, whose I_{Ks} is also abnormal, β-adrenergic stimulation with epinephrine could result in torsade de pointes (TdP) [66]. Thus marked abnormalities of the electrophysiologic properties of myocardium after infarct most likely distort the patterns of functional myocardial innervations, therefore enhancing the susceptibility to arrhythmias. On the other hand, sympathetic hyperinnervation at scar border zones leads to increased peak Ca^{2+} current and increased repolarization dispersion [67]. In a postinfarct model, nerves sprouting was associated with dispersion of repolarization, along with changes of outward and inward rectifier K^+ currents [68]. Ajijola et al. demonstrated that animals with anteroapical infarcts showed altered epicardial propagation during sympathetic stimulation [69]. These data suggest heightened sympathetic tone likely make a previously dormant channel conductive (or vice versa), consequently altering the electrophysiological properties of the innervated tissues. The coupling between augmented sympathetic remodeling and electrical remodeling provides a plausible explanation for a higher risk of life-threatening arrhythmias after MI.

5. Sympathetic Nerve Sprouting and Ventricular Arrhythmogenesis after MI

It is widely accepted that sympathetic remodeling resulting from MI is strongly associated with the development of VT, VF, and SCD [1, 70, 71]. Indeed, evidence that the role of excessive cardiac sympathetic activity can directly precipitate VT has been provided by studies in patients and animal models with healed MI [72–74]. In contrast, interventions to decrease sympathetic nerve activity have been shown to provide a significant protection from arrhythmias in both patients and animals recovering from MI [75–77]. Importantly, the sympathetic nerve sprouting and compensatory reinnervation following MI have added an interesting dimension to arrhythmogenesis (Figure 2). Specifically, Cao et al. showed that native hearts of transplant recipients exhibit enhanced nerve fibers density around the diseased myocardium [24]. Significantly, an increased density of sympathetic nerves is higher in patients with a history of tachyarrhythmias than in those without tachyarrhythmias. Subsequently, to prove a causal relationship between sympathetic nerve sprouting and arrhythmogenesis, their group augmented myocardial nerve sprouting through chronic

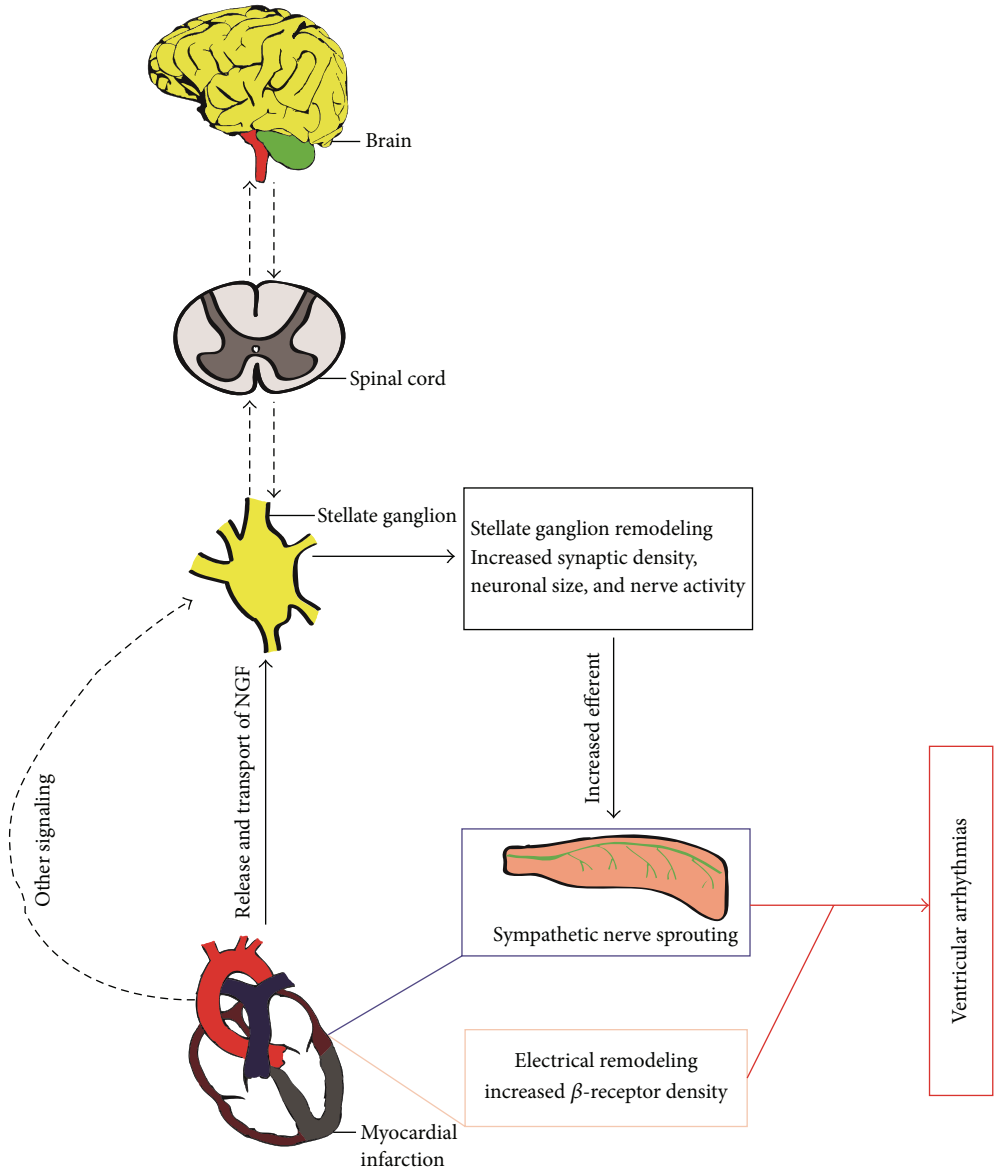

Figure 2: Sympathetic nerve remodeling increases the propensity for cardiac arrhythmias after myocardial infarction. Myocardial infarction induced NGF transported retrogradely to left (and right) stellate ganglion and other injury signals such as increased afferent nerve traffic lead to anatomic remodeling within the stellates. Increased efferent nerve signals back to the heart and promotes nerve sprout which together with electrical as well as β-adrenergic receptor remodeling increases the propensity for malignant ventricular arrhythmias. It is not clear whether higher nervous centers also regulate this process. Solid lines represent known pathways; dotted lines represent unknown.

infusion of NGF [40] or continuous subthreshold electrical stimulation to the LSG [78]. In the experimental group, there was 2-fold increase of sympathetic nerve density and 10-fold increased incidence of VT compared with controls [40]. Moreover, four dogs that underwent NGF infusion died suddenly of spontaneous VF but none in the control group. Similarly, electrical stimulation to the LSG resulted in a dramatic increase of sympathetic nerve density and a much higher incidence of VT in dogs with MI and complete atrioventricular block (AVB) [78]. Cha et al. found that dogs with heart failure induced by rapid pacing exhibited increase of sympathetic nerve density, and dogs that died suddenly had greater nerve density [79], whereas our group found that attenuation of sympathetic nerve regeneration in the MI border zone by sema3A overexpression reduced the susceptibility to post-MI malignant arrhythmias and SCD [58]. All these data indicate a causal correlation between sympathetic nerve sprouting and arrhythmogenesis after MI.

The mechanisms, however, underlying this relationship remain incompletely understood. The excessive sympathetic nerves sprouting was closely correlated to increases of local ventricular transmural dispersion of repolarization [80] and prolongation of the QT interval [43], which may be a potential mechanism for fatal arrhythmia in chronic

MI. In nerve-muscle coculture studies, investigators have reported sympathetic innervation may upregulate expression of functional L-type calcium channels [1]. Increased I_{Ca} may lengthen APD in myocardium. When I_{Ks} was downregulated and induced by MI, sympathetic stimulation tended to accentuate dispersion of repolarization [65]. Furthermore, several studies have shown that after MI, cardiac sympathetic hyperinnervation could modulate the expressions and functions of ion channels including I_{K1}, I_{to} and ionotropic glutamate receptors (iGluRs) [68, 81, 82], leading to lengthening of the QT interval and increased dispersion of refractoriness, thereby resulting in the occurrence of VF and SCD. Thus deleterious sympathetic nerves sprouting might amplify the spatial heterogeneity of myocardial electrophysiological properties and underlie the occurrence of VT, VF, and SCD.

In addition, in ambulatory dogs with NGF to the LSG, AVB, and MI, most of the malignant ventricular arrhythmias were preceded by increased stellate ganglion nerve activity including low-amplitude burst discharge activity (LABDA) and high-amplitude spike discharge activity (HASDA), along with increased nerve sprouting [26]. Han et al. provided a direct physical evidence that structural neural remodeling was associated with increased sympathetic nerve activity after MI [83]. They described a persistent increase in the synaptic density of stellate ganglia accompanied by increased stellate ganglion nerve activity. It is possible that MI induced neurotrophic agents may be transported to the stellate ganglia via nerve tracts and then lead to remodeling of stellate ganglia as well as increase in stellate ganglion nerve activity followed by intramyocardial nerve sprouts [78, 84]. As increased sympathetic nerve discharge facilitates ventricular arrhythmias, hence, increased sympathetic ganglion nerve activity and nerves sprouting jointly contribute significantly to susceptibility to VF and SCD after MI.

It is well established that the sympathetic innervation of the heart functions mainly through the activation of β-adrenergic receptor (β-AR) by the release of norepinephrine (NE). In fact, Zhou and coworkers found that both deleterious nerve sprouting and significant increase in density of β3-AR occur in dogs with MI, AVB, and NGF infusion to the LSG [85]. β3-AR, presents in canine and human cardiac myocytes and exerts profound functions to mediate the membrane ion currents [86–88]. For example, activation of β3-AR could decrease slow delayed rectifier K^+ current and I_{Ks}, contributing to slight prolongation of the APD [87]. Additionally, Billman and coworkers have demonstrated dogs with healing MI susceptible to VF revealed a dramatically enhanced β2-AR response. Interestingly, similar responses did not occur before MI [89]. Presumably, β2-AR activation can provoke increase in intracellular Ca^{2+} transients and after contractions that ultimately trigger VF in the post-MI animal model [89, 90]. Although the nerve densities were not determined in those reports, there is overwhelming evidence MI can provoke nerve sprouting. Considering together these data, it is likely that β-AR remodeling in postinfarcted heart is important for formation of a substrate that triggers malignant ventricular arrhythmias and leads to SCD in chronic MI.

6. Conclusions

Collectively, excessive sympathetic nerve sprouting to reinnervation myocardium in response to MI may be an important element in the arrhythmogenicity of sympathetic nerves remodeling. On the other hand, modest nerve sprouting may contribute to improved hemodynamic performance of the surviving myocardium [91] and potent inhibition of nerve sprouting may also result in abnormal patterns of myocardial innervation and may facilitate propensity for the formation of malignant arrhythmias [52, 92]. In particular, significant advances have been made to understand the causal link between sympathetic nerve sprouting and VT, VF, and SCD after MI. The underlying mechanisms by which sympathetic nerve sprouting together with other changes such as electrical remodeling as a result of MI alter susceptibility to malignant arrhythmias are of considerable interest. Significantly, molecular mechanisms that regulate sympathetic nerves regeneration after MI remain largely to be determined. Better understanding of these complex problems may provide new tools for the prediction, prevention, and therapy of lethal arrhythmias after MI.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

Digoxin Use to Control Ventricular Rate in Patients with Atrial Fibrillation and Heart Failure Is Not Associated with Increased Mortality

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Introduction. Digoxin is used to control ventricular rate in atrial fibrillation (AF). There is conflicting evidence regarding safety of digoxin. We aimed to evaluate the risk of mortality with digoxin use in patients with AF using meta-analyses. *Methods.* PubMed was searched for studies comparing outcomes of patients with AF taking digoxin versus no digoxin, with or without heart failure (HF). Studies were excluded if they reported only a point estimate of mortality, duplicated patient populations, and/or did not report adjusted hazard ratios (HR). The primary endpoint was all-cause mortality. Adjusted HRs were combined using generic inverse variance and log hazard ratios. A multivariate metaregression model was used to explore heterogeneity in studies. *Results.* Twelve studies with 321,944 patients were included in the meta-analysis. In all AF patients, irrespective of heart failure status, digoxin is associated with increased all-cause mortality (HR [1.23], 95% confidence interval [CI] 1.16–1.31). However, digoxin is not associated with increased mortality in patients with AF and HF (HR [1.08], 95% CI 0.99–1.18). In AF patients without HF digoxin is associated with increased all-cause mortality (HR [1.38], 95% CI 1.12–1.71). *Conclusion.* In patients with AF and HF, digoxin use is not associated with an increased risk of all-cause mortality when used for rate control.

1. Introduction

Digoxin is the oldest cardiac medication currently being used in clinical practice. With its unique mechanism of action, digoxin has traditionally had a role in the management of heart failure and atrial fibrillation. Rigorous prospective trials evaluating digoxin did not exist until the 1980s. Initial clinical trials of digoxin comparing the drug to vasodilators, milrinone, and placebo and the subsequent digoxin withdrawal trials showed substantial evidence that digoxin offered symptomatic benefits to patients with heart failure, but mortality benefits of digoxin remain controversial. The Digitalis Investigator Group (DIG) trial sponsored by the NIH, designed to detect mortality differences with digoxin use in patients with sinus rhythm and systolic dysfunction, failed to show any survival benefit with digoxin use [1]. A post hoc analysis of the study more than a decade later showed that patients who had higher serum digoxin concentrations had an absolute 11.8% increase in all-cause mortality. While recent retrospective and prospective studies show an association of digoxin use with increased mortality in patients with heart failure who are in sinus rhythm, a Cochrane meta-analysis of 13 studies showed a neutral effect on mortality [2]. Recent meta-analysis has shown an association of increased mortality with use of digoxin as a rate-controlling agent in patients with atrial fibrillation only [3, 4]. The safety and benefit of digoxin in patients with atrial fibrillation and heart failure for rate control continue to be controversial. Here we used meta-analytical techniques to assess the risk of mortality with digoxin use in patients with atrial fibrillation and heart failure.

2. Methods

Our analysis is based on the guidelines of the meta-analysis of observational studies in the Epidemiology Group [5].

2.1. Inclusion and Exclusion Criteria. We included prospective or retrospective observational studies with a primary objective to analyze the association between digoxin and all-cause mortality in patients with atrial fibrillation with or without heart failure. Titles and abstracts were evaluated and rejected after initial screening according to the following inclusion and exclusion criteria: studies were included if (1) digoxin was compared to no digoxin or any other rate-controlling drug in patients with atrial fibrillation; (2) the duration of follow-up was at least 6 months; (3) adjusted hazard ratio was reported; (4) all-cause mortality was the endpoint.

Studies were excluded if (1) they included only patients with postoperative atrial fibrillation; (2) there was no control group; (3) they included only patients with heart failure; (4) adjusted hazard ratios were not reported. Abstracts alone were not considered.

2.2. Search Strategies. We searched MEDLINE (1966–2015) and Web of Science (1966–2015) databases to identify relevant studies. We used the following keywords: "digoxin," "atrial fibrillation," "heart failure," and "mortality." In addition, the "Related Articles" feature on PubMed was used and a manual search was conducted using bibliographies of included studies and review articles on this topic. Titles and abstracts were reviewed independently by two reviewers (Surbhi Chamaria and Anand M. Desai). Differences were resolved by consensus.

2.3. Quality Assessment and Data Extraction. The quality of each study was evaluated according to the guidelines developed by the United States Preventive Task Force and the Evidence-Based Medicine Working Group [6, 7] The following characteristics were assessed: (1) inclusion and exclusion criteria; (2) representative study sample; (3) explanation of sample selection; (4) full specification of clinical and demographic variables; (5) follow-up at least 6 months; (6) reported loss of follow-up; (7) definition of outcomes and outcome assessment; and (8) adjustment of possible confounders in multivariate analyses. Studies were graded as poor if they met <3 criteria, fair if they met 3–5 criteria, and good if they met ≥5 criteria.

Two reviewers (Surbhi Chamaria and Anand M. Desai) extracted (1) publication details including first author's last name and year of publication; (2) study design; (3) characteristics of the study population including: gender, race, age, and comorbidities (hypertension, diabetes, previous strokes, ejection fraction, and chronic kidney disease); (4) variables included in the multivariate analyses; and (5) adjusted hazard ratio (HR) with 95% confidence interval (CI) from the multivariate analyses. All studies used a cox proportional hazards analysis to calculate adjusted HR. Wherever the studies used a propensity score matching, HRs for this meta-analysis were extracted from the propensity matched analysis.

2.4. Statistical Analysis. The degree of association between digoxin and all-cause mortality in patients with atrial fibrillation, with and without heart failure, was measured as a HR. All the studies employed Cox proportional hazard models to examine association of digoxin and mortality, thereby enabling the use of one consistent measure throughout. One study was excluded as it reported relative risk and no HR [8]. Risk estimates (HRs) were extracted. These studies reported use of multivariate and propensity score models to adjust for potential confounders including age, sex, heart failure, hypertension, chronic kidney disease, beta-blocker use, aspirin use, warfarin use, and history of previous stroke.

A prespecified subgroup analysis was performed based on whether heart failure population was included and reported in the study. HRs were transformed logarithmically as they did not follow a normal distribution. The standard error was calculated from Log HR and the corresponding 95% confidence interval (CI). The inverse variance method was used to achieve a weighted estimate of the combined overall effect. Results for heterogeneity were examined by the forest plots and calculating a Q statistic, which we compared with the I^2 index [9]. Significant heterogeneity was considered present at the 5% level of significance (for the Q test) and values of I^2 exceeding 56% [9]. Overall analyses (Q test $P < 0.01$; $I^2 = 85\%$) and all subgroups except patients with atrial fibrillation and heart failure only exhibited significant heterogeneity. This prompted us to adopt the random effect model. All primary analyses were performed using Cochrane's review manager 5.2. This model allowed a distribution of the true effect size rather than assuming one true effect size. It took into account within-study and between-study variance.

The underlying heterogeneity further prompted us to perform metaregression analysis to investigate factors contributing to heterogeneity and if our study outcome (all-cause mortality) was affected by factors other than our primary treatment (digoxin) [10, 11]. We adopted a weighted regression random effect model and carried out a multivariant regression using three predetermined factors including hypertension, left ventricular ejection fraction, and prior history of stroke using comprehensive meta-analysis version 3. These factors were selected based on factors shown to increase mortality with digoxin in individual studies and on availability of data for majority of the studies included. A two-sided P value < 0.05 was regarded as significant for all analyses. Data was represented as forest plots for primary analysis. Potential publication bias was assessed with the Egger test and represented graphically with Begg's funnel plots of the natural log of the HR versus its standard error [12].

3. Results

The literature search yielded 17910 potential studies—15038 by key words search and 2872 from other sources (Figure 1). After screening titles and abstracts and removing duplicated studies, 17808 articles were excluded. An additional 87 articles were excluded because they were either review articles or did not satisfy our inclusion criteria. Out of the 15 articles selected for detailed evaluation three were excluded from analysis for one or more of the following reasons: (1) not reporting HR for

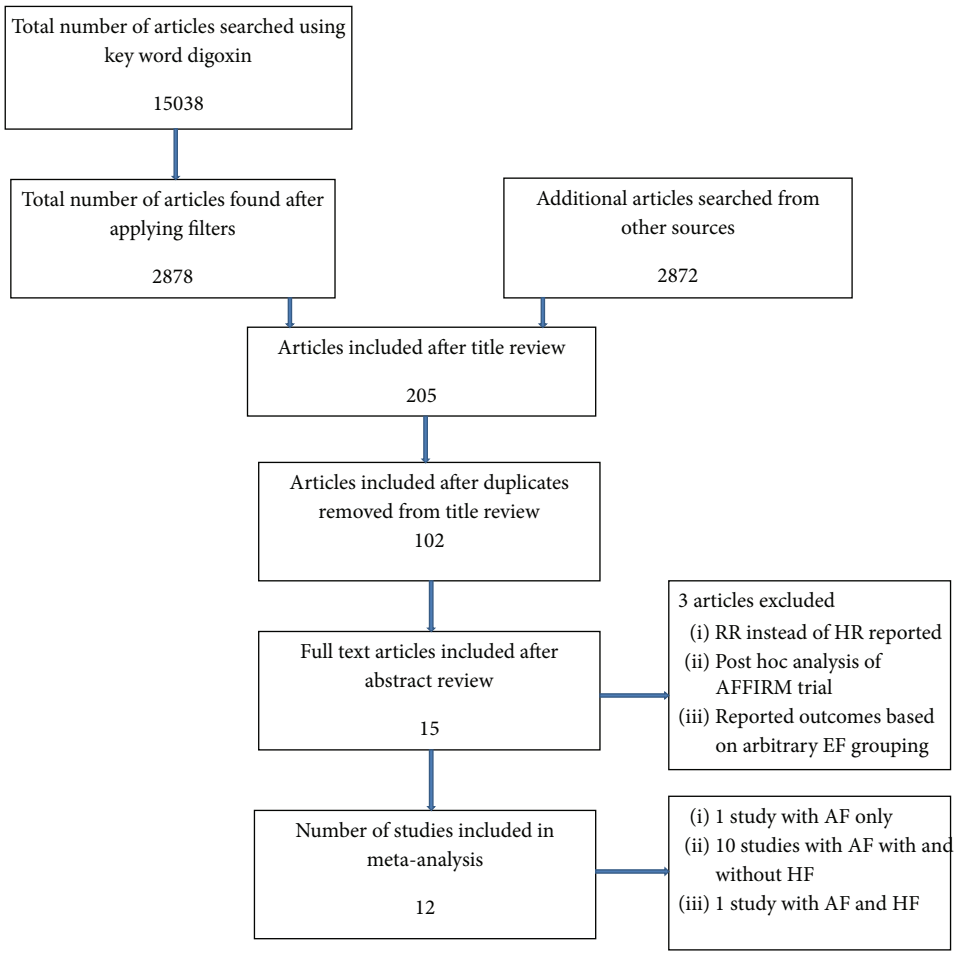

Figure 1: Prisma flow diagram for study selection.

mortality; (2) duplicating patient population from another study; (3) excluding 22% of patients from the AFFIRM trial as data regarding their previous use of digoxin prior to the trial was missing; and (4) reporting outcomes based on left ventricular ejection fraction less than or more than 30%, an arbitrary delineation that differed from the rest of the studies and did not form part of our prespecified analysis. All the studies included in the meta-analyses used digoxin primarily for rate control of atrial fibrillation and not for the management of heart failure.

3.1. Patient Population and General Characteristics of Included Studies. For our primary comparison evaluating effect of digoxin on patients with atrial fibrillation, we included 12 studies with 321,944 patients. Ten of these 12 studies included atrial fibrillation patients both with and without heart failure, but only three of these ten studies reported separate outcomes for patients with and without heart failure and seven did not. Of the remaining two studies, one included only patients with atrial fibrillation without heart failure and one included only patients with atrial fibrillation and heart failure.

Baseline characteristics of the included studies for our primary comparison are shown in Table 1. The baseline characteristics of the patients included in these trials based on the treatment with digoxin are presented in Table 2. The 12 studies varied in size, characteristics of patient populations, ancillary therapy for heart failure, and use of antiplatelet or anticoagulation drugs for stroke prevention.

Importantly, for all studies, treatment with digoxin was not randomized. Majority of the studies did not state the dose of digoxin used and only one study [13] measured the level of digoxin during the follow-up period. Seven out of the twelve studies commented on the number of patients with chronic kidney disease, out of which only one study [14] reported that the use of digoxin in patients with chronic kidney disease correlated significantly with increased mortality.

3.2. Results of Component Studies. In all the four studies that considered the effect of digoxin in patients with AF and no HF there was an increase in all-cause mortality [13, 15–17]. In all the four studies, patients were older and had more baseline comorbidities compared to other studies.

Table 1: Study characteristics evaluating the association of digoxin and the risk of mortality in patients with atrial fibrillation.

First author	Study design	Digoxin group	Control	Age (yrs)	Male (%)	CHF (%)	Follow-up (yrs)	Primary endpoints	Analysis method	Study quality
Chao (2014) [15]	Prospective	829	3,952	70.8 ± 12.5	52.1	23.8	4.3	All-cause mortality	CRM, NPM	Good
Fauchier (2009) [14]	Prospective	402	867	76 ± 13	56.2	100.0	2.4	All-cause mortality	CPHM, NPM	Good
Shah* (2014) [16]	Retrospective	23,200	77,399	79.4 ± 7.2	42.8	NA	4.2	All-cause mortality	CPHM, PM	Good
Shah** (2014) [16]		15,181	24,331	80.1 ± 7.4	50.0	100.0	3.1	All-cause mortality	CPHM, PM	Good
Friberg (2010) [22]	Prospective	802	2,022	78	45.7	63.7	4.6	All-cause mortality	CRM, PM	Good
Whitbeck (2013) [18]	Retrospective	2,153	1,905	NA	NA	12.0	3.5	All-cause mortality	CPHM, PM	Good
Gjesdal (2008) [19]	Retrospective	3,911	3,418	71 ± 9	66.9	45.3	0.8	All-cause mortality	CPHM, NPM	Good
Turakhia (2014) [20]	Prospective	28,679	93,786	71.7 ± 10.2	98.5	21.3	2.8	All-cause mortality	CPHM, PM	Good
Mulder (2014) [24]	Retrospective	284	324	68 ± 8	59.9	8.5	3	All-cause mortality	CPHM, NPM	Good
Rodríguez-Mañero (2014) [23]	Retrospective	212	565	76.9 ± 8.4	46.2	19.8	2.9	All-cause mortality, survival free of admission	CPHM, NPM	Good
Freeman (2014) [13]	Retrospective	4,858	22,430	71.9 ± 11.9	50.2	0.0	1.2	All-cause mortality	CPHM, PM	Good
Pastori (2015) [21]	Prospective	171	644	74.4 ± 7.2	53.2	25.7	2.7	All-cause mortality	CPHM, PM	Good
Allen (2015) [17]	Prospective	2948	6671	76	55.7	21.9	1.8	All-cause mortality	CFM, PM	Good

CHF: congestive heart failure; NA: not available; CRM: Cox regression model; CPHM: Cox proportional hazards model; PM: propensity matching; NPM: not propensity matched; CFM: Cox frailty model.
Shah*: study characteristics who only had atrial fibrillation.
Shah**: study characteristics in patients who had both atrial fibrillation and congestive heart failure.

Table 2: Baseline characteristics of patients included in the component studies.

First author	Sex (%)	Age (yrs)	CKD (%)	BB (%)	ACEi (%)	CAD (%)	DM (%)	CHF (%)	HTN (%)	Stroke (%)	ASA (%)	Coumadin (%)
Chao (2014) [15]	53.4	68.0	8.1	16.2	18.5		32.1	16.0	68.5	19.8		
Fauchier (2009) [14]	60.0	74.3	9.3	50.0	77.1	21.4	17.0	100.0	44.1	4.7	31.2	58.0
Shah* (2014) [16]	45.6	79.0	12.5	41.8	29.0	37.8	22.4	0.0	59.1	7.8	29.9	60.3
Shah** (2014) [16]	50.0	80.2	28.3	50.3	47.4	58.9	31.4	100.0	61.0	4.6	36.8	63.0
Friberg (2010) [22]	52.0	75.5	2.0	51.0	36.0	20.0	19.0	50.4	47.5	17.5	40.5	44.5
Whitbeck (2013) [18]				30.0		38.0		8.0	71.0	13.5		
Gjesdal (2008) [19]	69.0	71.0		5.0		44.8	23.4	36.0	76.9	21.1	16.3	
Turakhia (2014) [20]	98.4	72.0	36.0	60.1	55.2		28.5	17.7	60.7	5.9	15.7	58.6
Mulder (2014) [24]	65.2	68.0		66.4	51.2	17.9	11.2	6.9	60.9			
Rodríguez-Mañero (2014) [23]	52.4	75.7	9.8	31.1		17.8	28.0	16.3	77.4	4.3		
Freeman (2014) [13]	53.5	71.2	35.5	56.1	35.2	5.3	23.5	0.0	77.1	35.8	6.8	33.1
Pastori (2015) [21]	55.9	73.5		40.8	69.5	48.2	21.4	19.7	89.4	16.3	8.1	100.0
Allen P (2015) [17]	56.9	75.5				31.7	31.5	13.0				
Allen I (2015) [17]	57.2	75.5				31.7	31.5	10.0				

CKD: chronic kidney disease, BB: beta-blocker, ACEi: ace inhibitor, CAD: coronary artery disease, DM: diabetes mellitus, CHF: congestive heart failure, HTN: hypertension, and ASA: aspirin.
Shah*: metaregression done on patients who only had atrial fibrillation.
Shah**: metaregression done on patients who had both atrial fibrillation and congestive heart failure
Allen P: prevalent digoxin group; Allen I: incident digoxin group.

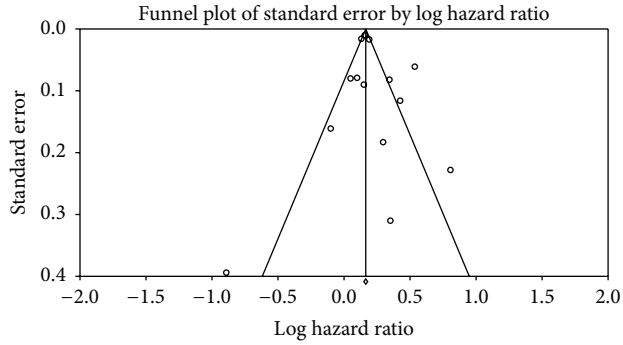

Figure 2: Funnel plot.

In the study by Freeman et al. [13], mean serum digoxin concentration was higher among patients who died in the digoxin group.

Of the seven studies that considered all-cause mortality in patients with atrial fibrillation irrespective of heart failure status, four of them showed an increase in all-cause mortality with the use of digoxin [18–21]. In all of these studies digoxin users were older and had more baseline co-morbidities as compared with non-digoxin users. Three studies in this group did not show an increase in mortality [22–24]. Of the four studies that considered patients with atrial fibrillation and concomitant heart failure, three studies showed that digoxin had no effect on all cause mortality [14, 15]. Analysis of the Begg's funnel plot of the included studies showed no significant publication bias (Figure 2).

3.3. Results of Meta-Analysis and Metaregression. Results of the combined analysis of adjusted HR for all-cause mortality for all patients with atrial fibrillation irrespective of heart failure status showed that patients prescribed digoxin had almost a 25% higher risk of mortality compared to those not on digoxin (HR 1.23, 95% CI 1.16–1.31, Figure 3). However, a prespecified subgroup analysis performed for the purpose of this study showed that in patients with atrial fibrillation and heart failure, there was no increase in all-cause mortality with digoxin use (HR 1.08, 95% CI 0.99–1.18). All-cause mortality was higher with the use of digoxin in patients with atrial fibrillation alone (HR 1.38, 95% CI 1.12–1.71).

We attempted to explore the reasons behind the heterogeneity among the included studies and to investigate factors influencing the effect of digoxin on mortality, by performing a metaregression analysis. Confirming our findings of the subgroup analysis of studies with atrial fibrillation and heart failure, a univariate metaregression analysis showed that the percentage of patients with heart failure in the included studies negatively correlated with the hazard ratio for all-cause mortality ($P = 0.04$). In addition, the number of patients with hypertension and history of previous stroke positively correlated with increased mortality. A multivariant metaregression model including all the three factors, hypertension, heart failure, and history of previous stroke, showed that heart failure ($P = 0.03$, Figure 4) and hypertension ($P < 0.001$, Figure 5) but not previous history of stroke ($P = 0.26$) strongly correlated with increased mortality in the studies and they contributed almost entirely to the heterogeneity between studies (R^2 analog = 1). Of note, mean age and percentage of beta-blocker use in the study population did not have any correlation with the hazard ratios when used as covariates in the metaregression.

4. Discussion

Although digoxin is widely used as a rate-controlling drug in atrial fibrillation, there is a paucity of randomized controlled trials evaluating its safety. The long-term effect of digoxin on mortality and heart failure hospitalization in HF patients was studied in the prospective randomized trial Digitalis Investigators Group (DIG). The study showed that digoxin compared with placebo had no effect on survival when used with angiotensin converting enzyme inhibitors and diuretics. A meta-analysis of 13 studies of digoxin in heart failure confirmed that digoxin had no effect on mortality in heart failure but all the studies in the analysis excluded patients with atrial fibrillation. Additionally, the results of the meta-analysis heavily relied on the DIG trial. Our meta-analysis showed a similar outcome in atrial fibrillation patients with heart failure, that is, no effect on mortality. In patients with atrial fibrillation alone without coexisting heart failure, the combined hazard ratio showed an increased risk of death.

Two recent meta-analyses assessing the effect of digoxin in patients with atrial fibrillation were published. The study by Ouyang et al. [3] concluded that digoxin increases mortality in patients with atrial fibrillation and heart failure. This difference in results stems from key disparities in inclusion criteria and data extraction between the two studies: (1) HR of a subgroup analysis reported by Mulder et al. for AF patients with NT pro-BNP more than 1003 pg/mL was taken as a surrogate for heart failure by Ouyang et al. while we did not make such assumptions. (2) While our study did not include separate outcomes for AF patients with and without heart failure for Rodríguez-Mañero et al. and Whitbeck et al., two studies that self-reported missing data on heart failure and LV systolic dysfunction data in a substantial number of their patients, Ouyang et al. proceeded to include that data in their subgroup analysis. (3) Ouyang et al. included risk estimates from Whitbeck et al. [18] and Gheorghiade et al. [25], two studies that performed post hoc analyses on the AFFIRM trial, thus duplicating the population. We excluded the report by Gheorghiade to avoid duplication and also because the analysis excluded 22% of the AFFIRM study population due to missing data on digoxin use prior to the start of the trial. (4) Further, Ouyang et al. have included outcome estimates from Georgiopoulou et al. [26], a study that reported only a combined end point of time to death or urgent transplantation or left ventricular assist device implantation. We excluded this study from our analysis as the study by Georgiopoulou et al. was primarily a study of the effect of digoxin in patients with heart failure and they did not report a separate hazard ratio for mortality.

The second meta-analysis by Vamos et al. [4] reported increased mortality in patients with HF on digoxin but

Study or subgroup	Log[hazard ratio]	SE	Weight	Hazard ratio IV, random, 95% CI
1.1.1 AF only				
Allen et al., 2015 I	0.688	0.295	1.0%	1.99 [1.12, 3.55]
Allen et al., 2015 P	0.199	0.13	4.0%	1.22 [0.95, 1.57]
Chao et al., 2014-1	0.247	0.104	5.5%	1.28 [1.04, 1.57]
Freeman et al., 2014	0.536	0.061	9.5%	1.71 [1.52, 1.93]
Shah et al., 2014-1	0.157	0.01	15.3%	1.17 [1.15, 1.19]
Subtotal (95% CI)			**35.2%**	**1.38 [1.12, 1.71]**
Heterogeneity: $\tau^2 = 0.04$; $\chi^2 = 41.34$, df = 4 ($P < 0.00001$); $I^2 = 90\%$				
Test for overall effect: $Z = 2.99$ ($P = 0.003$)				
1.1.2 AF + CHF				
Allen et al., 2015 I	0.049	0.234	1.5%	1.05 [0.66, 1.66]
Allen et al., 2015 P	0.04	0.1	5.7%	1.04 [0.86, 1.27]
Chao et al., 2014-2	−0.131	0.174	2.5%	0.88 [0.62, 1.23]
Fauchier et al., 2009	−0.101	0.161	2.9%	0.90 [0.66, 1.24]
Shah et al., 2014-2	0.131	0.016	14.9%	1.14 [1.10, 1.18]
Subtotal (95% CI)			**27.5%**	**1.08 [0.99, 1.18]**
Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 5.10$, df = 4 ($P = 0.28$); $I^2 = 22\%$				
Test for overall effect: $Z = 1.70$ ($P = 0.09$)				
1.1.3 AF with or without CHF				
Friberg et al., 2010	0.049	0.08	7.4%	1.05 [0.90, 1.23]
Gjesdal et al., 2008	0.425	0.116	4.7%	1.53 [1.22, 1.92]
Mulder et al., 2014	−0.892	0.394	0.6%	0.41 [0.19, 0.89]
Pastori et al., 2015	0.806	0.228	1.6%	2.24 [1.43, 3.50]
Rodríguez-Mañero et al., 2014	0.351	0.31	0.9%	1.42 [0.77, 2.61]
Turakhia et al., 2014	0.191	0.017	14.8%	1.21 [1.17, 1.25]
Whitbeck et al., 2013	0.344	0.082	7.2%	1.41 [1.20, 1.66]
Subtotal (95% CI)			**37.2%**	**1.29 [1.10, 1.51]**
Heterogeneity: $\tau^2 = 0.03$; $\chi^2 = 25.64$, df = 6 ($P = 0.0003$); $I^2 = 77\%$				
Test for overall effect: $Z = 3.14$ ($P = 0.002$)				
Total (95% CI)			**100.0%**	**1.23 [1.16, 1.31]**
Heterogeneity: $\tau^2 = 0.01$; $\chi^2 = 83.10$, df = 16 ($P < 0.00001$); $I^2 = 81\%$				
Test for overall effect: $Z = 6.87$ ($P < 0.00001$)				
Test for subgroup differences: $\chi^2 = 6.56$, df = 2 ($P = 0.04$), $I^2 = 69.5\%$				

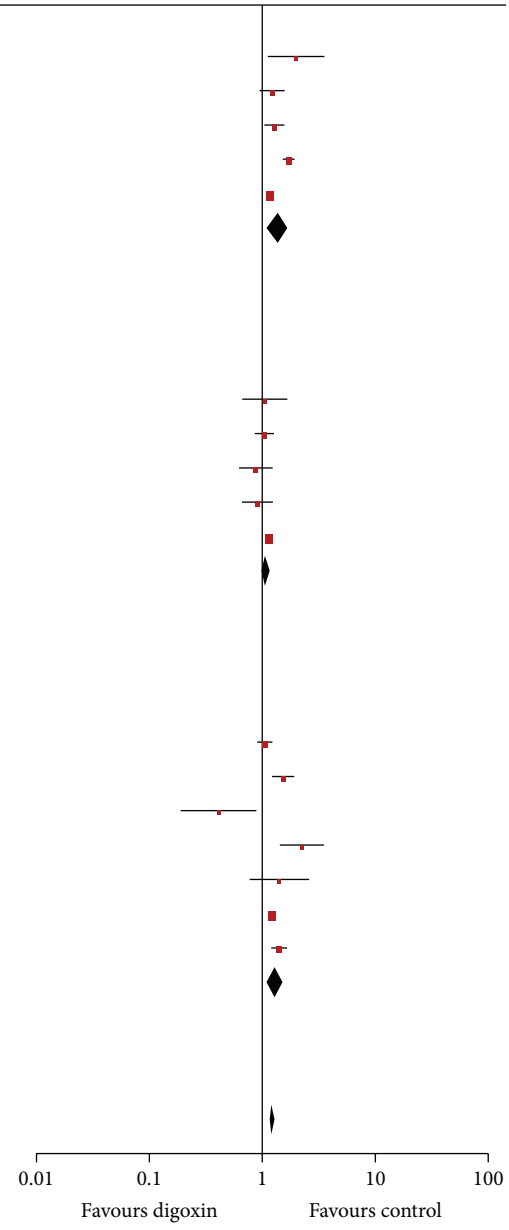

Figure 3: Forest plot showing combined effect of digoxin on all-cause mortality in studies with patients with atrial fibrillation only, atrial fibrillation with heart failure only, and atrial fibrillation with or without heart failure.

a subgroup analysis of studies with HF and AF noted no difference in mortality between the groups, similar to our study. Our analysis is updated with the recently published results from a large AF cohort that was not included in Vamos et al. Furthermore, multiple studies categorized as digoxin use in patients with atrial fibrillation without heart failure by Vamos et al. included up to 50% patients with heart failure (Table 1) making their conclusions unclear. Moreover, the relative risks from the study by Hallberg et al. [8] have been combined with the adjusted HRs from other studies by both Ouyang et al. and Vamos et al., while we excluded Hallberg et al. to avoid combining a cumulative risk measure with an instantaneous risk measure. Finally, our analysis also includes a metaregression showing that the percentage of HF patients reported in all the studies negatively correlated with the hazard ratio for all-cause mortality, corroborating the overall evidence that HF might blunt the effect of digoxin on the all-cause mortality of patients with AF.

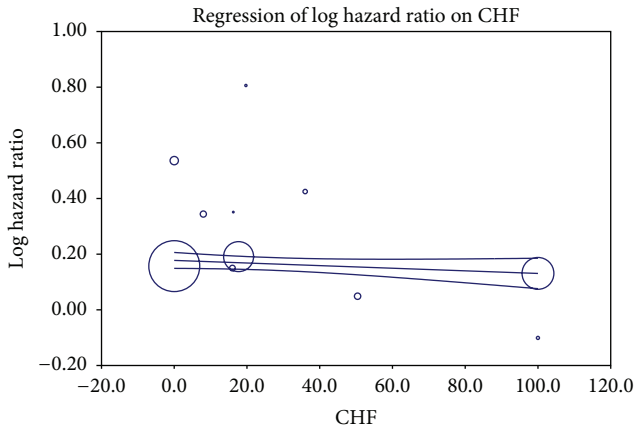

Figure 4: Effect of percentage of patients with CHF in individual studies on mortality risk of digoxin using a multivariate metaregression model; increased percentage of patients with CHF on the x-axis correlates with decreased HR on the y-axis.

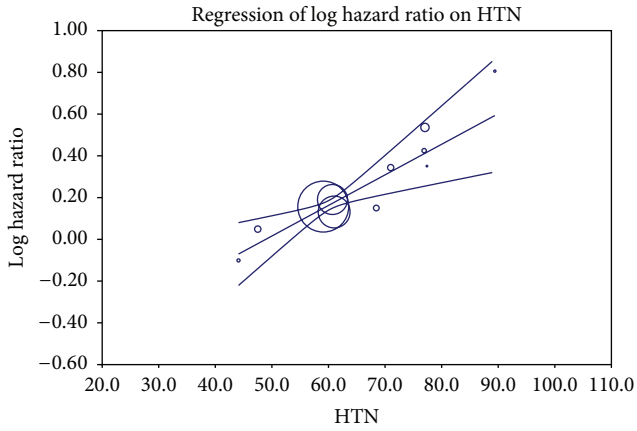

Figure 5: Effect of percentage of hypertensive patients in individual studies on mortality risk of digoxin using a multivariate metaregression model including hypertension, heart failure, and previous stroke; increased percentage of patients with hypertension on the x-axis correlated with increased HR on the y-axis.

The result of our meta-analysis is clinically very relevant. In patients with HF, pharmacologic therapy for rate control in AF is limited due to negative inotropic effects of commonly used drugs that prolong refractoriness of the AV node including beta-blockers and nondihydropyridine calcium channel blockers. In addition, these drugs also cause hypotension in HF patients with severe systolic dysfunction. In this context, digoxin is an essential alternate for rate control and continues to be recommended for patients with HF (Class IC) in the recent guidelines for the management of patients with AF [27]. Therefore, any association of mortality with digoxin use in this patient population has to be proven beyond doubt. A post hoc analysis of the DIG trial showed that the effectiveness of digoxin in patients with heart failure depended on serum digoxin concentration (SDC). Only one component study in the recent meta-analyses including ours reported SDC [13] and none correlated the outcomes with SDC, a potential confounding factor.

The reasons behind the difference in the effect of digoxin on mortality in patients with atrial fibrillation and heart failure and patients with atrial fibrillation alone are unclear. It has been shown that the beneficial effects of digoxin in heart failure in patients with sinus rhythm are due to its neurohormonal modulation effect and inotropic effect which vary with serum digoxin concentration. At low doses, neurohormonal effects provide symptomatic relief with a positive inotropic effect, but at increasing doses, the inotropic effect may increase myocardial oxygen consumption and arrhythmogenicity. In contrast, in patients without heart failure, beneficial effects may be overshadowed by the potential harmful effects of digoxin [28].

Digoxin toxicity can cause every known disturbance of cardiac impulse formation and propagation leading to significant arrhythmias. The positive inotropic action of digoxin is likely due to increased intracellular calcium. This increased intracellular calcium load not only augments contractility, but also can initiate delayed after depolarization and triggered arrhythmias [29]. Digoxin can initiate ectopic activity and produce bradycardia including AV block [30], plausible mechanisms of increased mortality observed with digoxin use in atrial fibrillation in the absence of heart failure.

Limitations. The present meta-analysis is based on retrospective and prospective nonrandomized trials and consequently has limitations in the wider application of the results of our analysis. Specifically, patients who are prescribed digoxin in nonrandomized retrospective studies may be inherently different from patients not prescribed digoxin and this is clear from the comorbidities seen in digoxin patients. While the Cox proportional hazards model (all studies) and propensity score models (all but 3 studies) used by individual studies included in our analysis somewhat mitigate this weakness by adjusting for known variables, they do not completely eliminate it. Also, our meta-analysis includes some large registries and cohort studies that could potentially influence the effect sizes but by employing a random effect model, we expect to moderate such impact. Warfarin use in component studies ranged from 33% to 100% and was not reported in some, thus limiting our ability to analyze and understand the effect of warfarin use as a covariate. Finally, guideline directed medical therapy use specifically in patients with CHF within the study cohort in component studies was not reported. In the two studies with 100% CHF population, a large proportion of patients was not on ACE inhibitors and beta-blockers, limiting the interpretation of our results.

5. Conclusion

This meta-analysis of nonrandomized studies shows that digoxin is not associated with increased all-cause mortality when used as a rate-controlling drug in patients with

atrial fibrillation with coexistent heart failure but it is associated with increased mortality when used in patients with atrial fibrillation alone. Large, well-designed, randomized controlled trials are needed to further address this issue.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

The Association between the PR Interval and Left Ventricular Measurements in the Multiethnic Study of Atherosclerosis

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Introduction. Few studies have examined the association between the PR interval (PRi) and subclinical cardiovascular disease measures. *Methods and Results*. The Multiethnic Study of Atherosclerosis (MESA) is a population-based study of 6814 men and women aged 45–84 years without clinical cardiovascular disease and 4962 had complete baseline data on cardiac magnetic resonance imaging measures of LV dimension and ejection fraction and surface electrocardiogram. Linear regression models were constructed to determine the adjusted association between the PRi and measures of LV stroke volume, LV mass, LV end-systolic and end-diastolic volumes, and ejection fraction. Overall, mean age was 61.5 years, and 47.6% were male and race/ethnicity was white in 39.1%, Chinese in 13.1%, African-American in 25.7%, and Hispanic in 22.2%. The PRi ranged from 88 to 308 ms with a median value of 162 ms. As a continuous variable, every standard deviation unit (25 ms) increment in PRi was associated with a 2.00 mL (95% CI 1.52, 2.48) higher stroke volume, a 3.08 g (95% CI 2.30, 3.86) higher LV mass, a 1.36 g/m^2 (95% CI 0.96, 1.76) higher LV mass index, and 1.31 mL (95% CI 0.88, 1.73) higher end-systolic and 3.31 mL (95% CI 2.58, 4.03) higher end-diastolic volumes after adjustment for all covariates. No significant association was noted between the PRi and LV ejection fraction. *Conclusions.* A prolonged PRi is associated with LV measures and may in part explain the link between a prolonged PRi and cardiovascular outcomes.

1. Introduction

The slowing of conduction through the AV node may be assessed by the PR interval (PRi) on a surface electrocardiogram. The PRi reflects the time, measured in milliseconds (ms), for the electrical impulse to travel from the sinoatrial node to the atrioventricular node and to the Purkinje fibers [1] or time from onset of atrial depolarization to beginning of ventricular depolarization. Normally, the PRi ranges from 120 to 200 ms and intervals > 200 ms define a prolonged Pri [2]. Historically, a prolonged PRi by itself, in the absence of other conduction abnormalities, was believed to not progress to other forms of heart block [3]. Thus, presence of a prolonged PRi did not indicate a need for treatment other than correcting any electrolyte abnormalities or removing offending drugs [4, 5]. However, several recent studies have suggested that a shortened or prolonged PRi may indicate heightened risk for cardiovascular outcomes including congestive heart failure, atrial fibrillation, and mortality but most of these studies focused on adults with established cardiovascular disease [1, 6–11].

The PRi reflects the timing between atrial and ventricular systole and a longer period of ventricular filling will lead to higher stroke volumes and ventricular wall stress [11], heightening risk for future cardiovascular disease. The importance of the PRi is illustrated by right ventricular (RV) pacing, which increases risk of worsening LV function over time [12–14]. The objective of this study is to utilize data from the Multiethnic Study of Atherosclerosis, a well characterized cohort of adults without clinical cardiovascular disease or active implantable cardiac device at baseline, to examine the association between the PRi and LV dimensions and ejection

fraction. We hypothesize that a prolonged PRi is associated with higher LV stroke volume and a lower ejection fraction among adults without established cardiovascular disease.

2. Methods

2.1. Study Population. The Multiethnic Study of Atherosclerosis (MESA) is a population-based study of 6814 men and women aged 45–84 years, without clinical cardiovascular disease, recruited from six US communities (Baltimore, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; Northern Manhattan, NY; and St. Paul, MN). The main objective of the MESA Study is to determine the characteristics of subclinical cardiovascular disease and its progression. Sampling and recruitment procedures have been previously described in detail [12]. Subjects with symptoms or history of medical or surgical treatment for cardiovascular disease were excluded. During the recruitment process, potential participants were asked about their race/ethnicity. Questions on race/ethnicity were based on the US 2000 census questionnaire. Subjects who self-reported their race/ethnicity group as white or Caucasian, black or African-American, Chinese, or Spanish/Hispanic/Latino were asked to participate. Race/ethnicity was then categorized as white (non-Hispanic), black (non-Hispanic), Chinese, and Hispanic. Subjects were enrolled between 12/1/00 and 7/30/02. Adults weighing >300 pounds and participants with pacemakers and ECG-diagnosed atrial fibrillation/flutter were not eligible for participation. The institutional review boards at all participating centers approved the study, and all participants gave informed consent. A total of 57 participants with missing surface electrocardiogram were excluded along with 2 individuals with a PRi > 320 ms. An additional 1793 participants who did not undergo an MRI were excluded leaving a total of 4962 included in the analysis. Sensitivity analyses were completed after excluding MESA participants ($n = 967$) using medications that may impact the PRi (calcium channel blockers, beta blockers, digoxin, and any antiarrhythmic medications).

2.2. PR Interval. Three sequential 10-second resting 12-lead ECGs were digitally acquired using a GE/Marquette MAC-PC electrocardiograph (Marquette Electronics, Milwaukee, Wisconsin) at 10 mm/mV calibration and speed of 25 mm/sec. All ECGs were centrally read and visually inspected for technical errors and inadequate quality at the Epidemiological Cardiology Research Center (EPICARE), Wake Forest School of Medicine (Winston-Salem, NC). A prolonged PRi was defined as a PRi > 200 ms. A shortened PRi was defined as a PRi < 120 ms.

2.3. Left Ventricular Mass Index, Dimensions, and Ejection Fraction. Participants underwent a cardiac MRI scan within a median of 16 days after the baseline evaluation and 95% were completed by 11 weeks after the baseline examination. The MESA cardiac MRI protocol, image analysis, and inter- and intrareader reproducibility have been previously reported [15]. Briefly, LV mass, volumes, and functional parameters were determined from short-axis fast gradient echo cine images covering the heart from base to apex throughout the cardiac cycle with temporal resolution ≤50 ms. LV mass was determined by the sum of the myocardial area (the difference between endocardial and epicardial contour) multiplied by the slice thickness plus image gap in the end-diastolic phase multiplied by the specific gravity of myocardium (1.05 g/mL). LV mass was examined with and without indexing for body surface area [16]. LV end-diastolic volume and LV end-systolic volume were calculated using Simpson's rule (the summation of areas on each separate slice multiplied by the sum of slice thickness and image gap). LV stroke volume was calculated as the difference between LV end-diastolic volume and LV end-systolic volume. LV ejection fraction was calculated as LV stroke volume divided by LV end-diastolic volume multiplied by 100 [15].

2.4. Covariates. All MESA participants completed self-administered questionnaires, provided fasting blood samples, and were interviewed and examined by trained research staff. Self-administered questionnaires were available in English, Spanish, and Chinese. Resting blood pressure and heart rate were measured 3 times with participants in the seated position with a Dinamap model Pro 100 automated oscillometric sphygmomanometer (Critikon, GE Healthcare, Waukesha, Wisconsin). The average of the last 2 measurements was used for the analysis. Presence of diabetes was defined as self-reported physician diagnosis, use of insulin or oral hypoglycemic agents, or fasting glucose ≥ 126 mg/dL. Current smoking status was based on self-report. Participants were instructed to bring in all existing medications, which were then recorded by research staff. Use of antihypertensive medication was defined as self-reported treatment for hypertension with one of six common classes of antihypertensive medications (thiazide diuretics, beta blockers, calcium channel blockers, angiotensin converting enzyme inhibitors (ACEi), angiotensin-2 receptor blockers (ARB), and other (alpha blockers or peripheral vasodilators)).

2.5. Statistical Analysis. Histograms were created to assess the shape of the distribution of the PRi and LV measures among the MESA participants included in the analyses. Scatterplots of PRi by LV end-diastolic volume, LV end-systolic volume, LV stroke volume, LV mass and LV mass index, and ejection fraction were examined. Spearman rank correlation coefficients between PRi and the LV measures and ejection fraction were calculated. Summary statistics for key baseline characteristics were compared by PRi categories. Continuous variables were compared using ANOVA and categorical variables were compared using the Fishers exact test. If these tests were statistically significant, then shortened and prolonged PRi groups were each compared to the normal PRi group. The level of statistical significance was set as $P < 0.01$ to account for multiple comparisons (normal versus prolonged PRi and normal versus shortened PRi).

Separate multivariable linear regression models were constructed to determine the associations with the PRi and LV measures and ejection fraction with the PRi fitted as a prolonged (>200 ms) or shortened (<120 ms) PRi compared

Table 1: Characteristics by presence of a prolonged PR interval (PRi).

Variable	PRi < 120 ms (n = 49)	PRi 120–200 ms (n = 4503)	PRi > 200 ms (n = 410)	P value
Age (years)	58.0 (0.1)	61.2 (10.0)	65.7 (10.1)*	<0.001
Male (%)	28.6*	46.4	62.7+	<0.001
Race/ethnicity				<0.001
White (%)	57.1*	38.6	39.3	
Black (%)	14.3*	24.9	36.0	
Hispanic (%)	16.3*	23.1	14.3+	
Chinese (%)	12.2	13.4	10.4	
Waist circumference (cm)	92.7 (13.5)	96.3 (13.3)	99.3 (12.5)+	<0.001
Height (cm)	164.4 (8.1)	166.6 (9.9)	170.1 (9.8)	<0.001
Weight (kg)	70.3 (14.2)*	76.6 (16.1)	82.5 (16.1)+	<0.001
Current smoker (%)	16.3	13.0	10.3	NS
Systolic blood pressure (mmHg)	122.6 (22.1)	125.1 (21.2)	130.0 (21.0)	<0.001
Diastolic blood pressure (mmHg)	72.0 (10.8)	71.8 (10.3)	72.8 (10.0)	0.2
Diabetes (%)	8.2	10.8	12.9	0.5
Heart rate (beats/minute)	65.2 (9.2)	63.1 (9.4)	59.9 (9.2)+	<0.001
QRS duration (ms)	93.3 (13.5)	92.9 (13.4)	97.7 (16.0)+	<0.001
PR interval (ms)	111.6 (6.6)	160.8 (17.7)	218.2 (19.7)+	<0.001

+$P < 0.001$ compared to PRi interval 120–200 ms. *$P < 0.01$ compared to PRi interval 120–200 ms.

to a normal PRi (120–200 ms). Linear regression models were then created with the dependent PRi examined as a continuous variable (per standard deviation unit or 25 ms). The Kolmogorov-Smirnov and the Shapiro-Wilk expanded tests were used to examine the assumption of a normal distribution for the LV measures. For each dependent variable, three regression models were examined. Model 1 adjusted for age, sex, race, height, and weight. Model 2 added site and heart rate to Model 1. Model 3 then added use of antihypertensive medications (ace inhibitor, angiotensin II antagonist, beta blocker, calcium channel blocker, and diuretics), systolic blood pressure, current smoking status, and presence of diabetes to Model 2. Potential covariates including total cholesterol and use of antiarrhythmic medications, digitalis preparations, cholesterol lowering mediations, or glucose lowering medications were not included in the final model because they were not associated with any change in the parameter estimates for PRi after adjustment for all variables in Model 3. The multivariate linear regression models were then repeated after excluding 967 participants using medications that may slow AV nodal conduction (beta-blockers, calcium channel blockers, digoxin, and/or antiarrhythmic medications).

To explore whether the associations between PRi and LV mass, LV mass index, LV dimensions, and ejection fraction were modified by race/ethnicity, interaction terms for race × PRi were fitted in the models with all covariates and with all participants. If the interaction term reached a statistical significance level of $P < 0.1$, then interaction terms for each nonwhite race × PRi were included in a model with all covariates. Race-specific associations for the association between the PRi and LV measures were obtained via linear combinations of the model's main PRi coefficient and the race × PRi interaction coefficient.

3. Results

Overall, mean age was 61.5 years (10.1) and 47.6% were male. Race/ethnicity was white in 39.1%, Chinese in 13.1%, African-American in 25.7%, and Hispanic in 22.2%. The PRi ranged from 88 to 308 ms with a median value of 162 ms and mean value of 165 ms (standard deviation 25). Of the 4962 subjects in the analysis cohort, 49 (1.0%) had a PRi < 120 ms, 4503 (90.7%) had a PRi from 120–200 ms, and 410 subjects (8.3%) had a prolonged PRi (>200 ms). Individuals with a PRi > 200 ms were older, taller, heavier, and more likely to be male (Table 1). Both heart rate and systolic blood pressure were significantly higher and QRS duration was significantly longer among participants with a PRi > 200 ms compared to those with a PRi 120–200 ms. Participants with a prolonged PRi were more likely to be using calcium channel blockers, beta-blockers, and diuretics compared to participants with a PRi 120–200 ms (Table 2).

Table 3 shows the mean values of LV measures and ejection fraction by presence of a PRi < 120 ms, 120–200 ms, or > 200 ms. Compared to participants with a PRi 120–200 ms, participants with a PRi > 200 ms had higher mean LV stroke volume indexed for body surface area (48.6 (10.4) versus 46.6 (8.8); $P < 0.001$), higher LV end-systolic (22.9 mL/m^2 (9.4) versus 21.2 mL (7.9); $P < 0.001$), and higher LV end-diastolic volumes indexed for body surface area (71.6 mL/m^2 (16.1) versus 67.8 mL (13.2); $P < 0.001$). A prolonged PRi was also associated with higher LV mass (163.3 g (46.3) versus 143.7 g

Table 2: Baseline medication use by presence of a prolonged PR interval (PRi).

Medication type	PRi < 120 ms (n = 49)	PRi 120–200 ms (n = 4503)	PRi > 200 ms (n = 410)	Overall P value
ACE inhibitor (%)	6.1	11.6	16.8*	0.003
Angiotensin 2 antagonist (%)	2.0	4.7	6.5	0.2
Beta-blocker (%)	4.1	8.5	15.1+	<0.001
Calcium channel blocker (%)	2.0	11.3	18.5+	<0.001
Antiarrhythmic medication (%)	0	0.04	1.7*	0.001
Digitalis preparation (%)	0	0.3	1.0	0.07
Diuretic medication (%)	0*	11.6	19.2+	<0.001
Cholesterol medication (%)	10.2	15.4	18.2	0.2
Any hypertension medication (%)	12.2*	34.0	51.6+	<0.001

+ $P < 0.001$ compared to PRi 120–200 ms; * $P < 0.01$ compared to PRi 120–200 ms.

Table 3: Left ventricle (LV) ejection fraction and measures by PR interval (PRi).

Variable	PRi < 120 ms (n = 49)	PRi 120–200 ms (n = 4552)	PRi > 200 ms (n = 410)	Overall P value
LV mass (g)	130.6 (38.5)	143.7 (38.4)	163.5 (46.3)+	<0.001
†LV mass index (g/m^2)	73.5 (16.5)	71.4 (15.9)	83.9 (19.5)+	<0.001
†LV end-systolic volume (mL)	20.6 (10.4)	21.2 (7.9)	22.9 (9.4)+	<0.001
†LV end-diastolic volume (mL)	64.2 (15.2)*	67.8 (13.2)	71.6 (16.1)+	<0.001
†LV stroke volume (mL)	43.6 (9.0)*	46.6 (8.8)	48.6 (10.4)+	<0.001
LV ejection fraction (%)	68.6 (8.3)	69.1 (7.4)	68.5 (7.8)	0.3

Data shown as mean (standard deviation).
+ $P < 0.001$ compared to PRi 120–200 ms; * $P < 0.01$ compared to PRi 120–200 ms.
† Indexed for body surface area [16].

Table 4: Multivariable adjusted differences in LV measures and ejection fraction by presence of a prolonged PR interval (>200 ms) versus PR interval 120–200 ms.

LV measures	Model 1 β (95% CI)	Model 2 β (95% CI)	Model 3 β (95% CI)
LV mass (g)	7.17 (4.41, 9.94)	6.13 (3.38, 8.89)	5.51 (2.89, 8.13)
†LV mass index (g/m^2)	3.57 (2.11, 5.03)	2.91 (1.45, 4.36)	2.56 (1.18, 3.94)
LV end-systolic volume (mL)	2.55 (1.09, 4.01)	2.56 (1.09, 4.04)	2.72 (1.25, 4.20)
LV end-diastolic volume (mL)	8.21 (5.69, 10.73)	6.93 (4.46, 9.41)	6.80 (4.32, 9.28)
LV stroke volume (mL)	5.67 (3.94, 7.39)	4.37 (2.71, 6.03)	4.07 (2.41, 5.72)
LV ejection fraction (%)	0.14 (−0.57, 0.85)	−0.12 (−0.83, 0.59)	−0.26 (−0.97, 0.45)

Model 1 adjusted for age, sex, race, height, and weight. Model 2 adds heart rate and site to Model 1. Model 3 adds systolic blood pressure, use of antihypertensive medications, current smoking status, and diabetes to Model 2.
† LV mass indexed for body surface area [16].

(38.4); $P < 0.001$) and LV mass indexed for body surface area (83.9 g/m^2 (19.5) versus 71.4 (15.9) g/m^2) compared to a PRi 120–200 ms (Table 3). A shortened PRi was associated with significantly lower levels of LV stroke volume indexed for body surface area and LV end-diastolic volume indexed for body surface area compared to a PRi 120–200 ms (Table 3). No significant difference was noted in ejection fraction across the PRi groups.

Figure 1 shows the scatterplots and Spearman's rank correlation coefficients for PRi and the LV measures and LV ejection fraction. The correlation between PRi and the LV measures ranged from as low as −0.05 ($P < 0.001$) for LV ejection fraction to as high as 0.22 ($P < 0.001$) for LV mass. In the regression analyses, presence of a PRi > 200 ms was associated with significantly higher LV stroke volume (4.07 mL; 95% CI 2.41, 5.72), higher LV mass (5.51 g 95% CI 2.89, 8.13) and LV mass index (2.56 g/m^2; 95% CI 1.18, 3.94), and higher LV end-systolic (2.72 mL; 95% CI 1.25, 4.20) and end-diastolic volumes (6.80 mL; 95% CI 4.32, 9.28) compared to presence of a PRi 120–200 ms after adjustment for all covariates (Table 4). Presence of a PRi < 120 ms was associated with significantly lower LV stroke volume (−4.78 mL; 95% CI

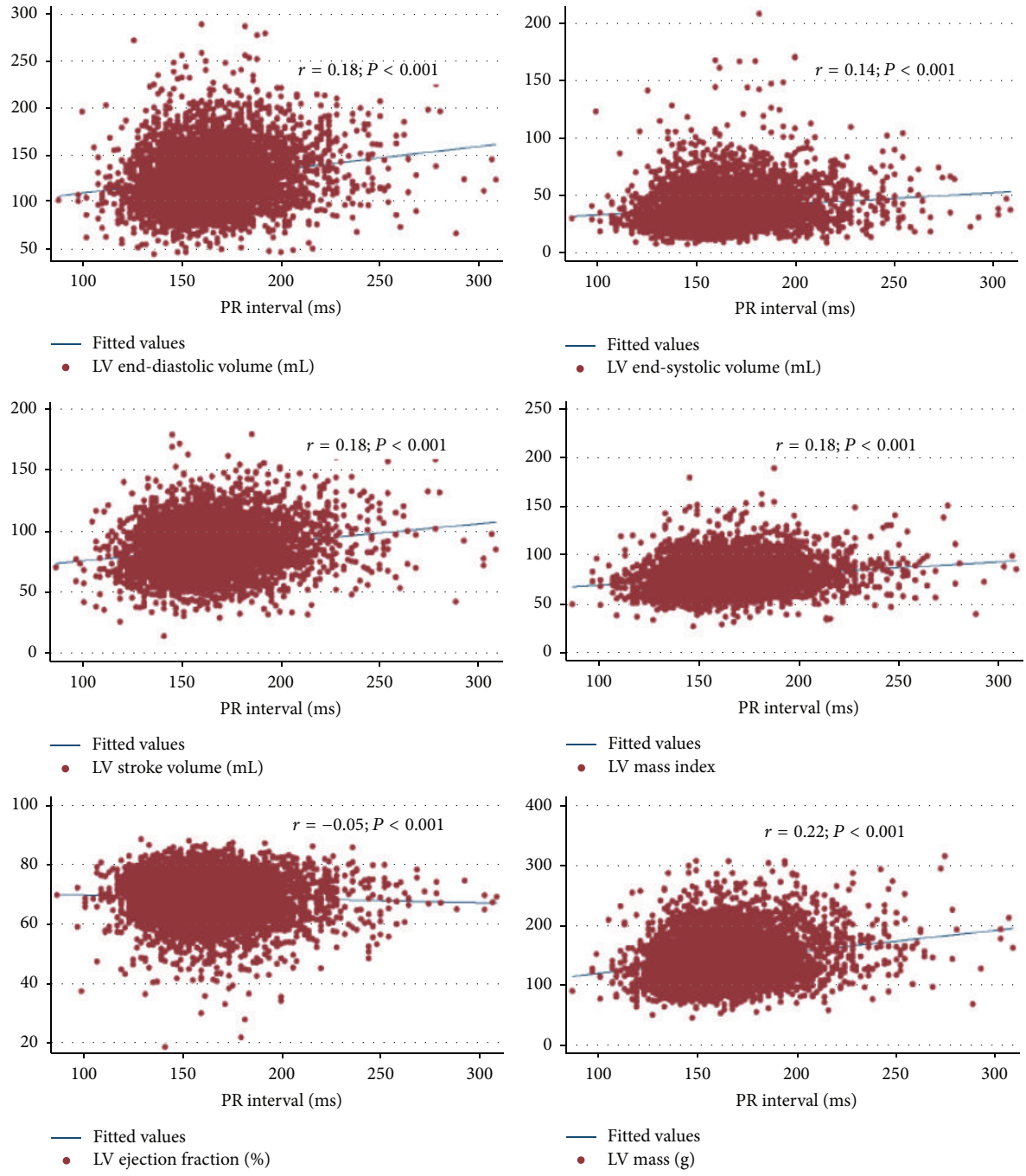

Figure 1: Scatterplots of PR interval by left ventricle dimensions, left ventricle mass index, and ejection fraction.

−9.17, −0.39) compared to a PRi 120–200 ms after adjustment for all covariates. However, compared to a PRi 120–200 ms, no significant association was noted between a PRi < 120 ms and LV mass, LV mass index, LV end-systolic or end-diastolic volume, or ejection fraction in any of the models (data not shown).

As a continuous variable, every standard deviation unit (25 ms) increment in PRi was associated with higher LV stroke volume (2.00 mL 95% CI 1.52, 2.48), higher LV mass (3.08 g; 95% CI 2.30, 3.86) and LV mass index (1.36 g/m^2; 95% CI 0.96, 1.76), and higher LV end-systolic (1.31 mL; 95% CI 0.88, 1.73) and end-diastolic (3.31 mL 95% CI 2.58, 4.03) volumes after adjustment for all covariates. In the sensitivity analyses which excluded participants using medications that may slow AV nodal conduction, every incremental standard deviation unit increase in PRi remained associated with significantly higher LV stroke volume (1.19 mL; 95% CI 0.72, 1.67), LV mass (2.87 g; 95% CI 2.06, 3.68) and LV mass index (1.00 g/m^2; 95% CI 0.56, 1.44), and higher LV end-systolic (1.19 mL; 95% CI 0.72, 1.67) and end-diastolic

(2.87 mL; 95% CI 2.06, 3.67) volumes after adjustment for all covariates. No significant association was noted between the PRi and ejection fraction (−0.20; 95% CI −0.43, −0.03) after adjustment for all covariates in the sensitivity analyses.

The interaction term for race × PRi fitted in the model with all participants and adjusting for all covariates was not significant in models with LV stoke volume, LV end-systolic volume, or LV end-diastolic volume as the dependent variable. However, the interaction term for race × PRi did meet statistical significance in the model with LV mass and LV mass index as the dependent variable ($P < 0.001$). Among whites, every standard deviation unit increment in PRi was associated with a 1.33 g (95% CI 0.26, 2.41) higher LV mass after adjustment for all covariates. Compared to whites, the association between every standard deviation unit increment in PRi and LV mass was 2.29 g higher in African Americans (95% CI 1.06, 3.51) and 2.96 g higher in Hispanics (95% CI 1.33, 2.96). No significant difference in the association between PRi and LV mass was noted between Asians and whites after adjustment for all covariates (−0.05; 95% CI −0.33, 0.43). Similar results were noted for LV mass index (data not shown).

4. Discussion

This study demonstrates that a prolonged PRi is associated with significantly higher LV stroke volume and LV mass and LV mass index but not ejection fraction. This study also shows that that the associations between the PRi and LV mass and LV mass index differ by race/ethnicity with stronger associations noted among African American and Hispanic adults. In the CARE-HF trial [14], a prolonged PRi was one of three independent predictors of cardiovascular hospitalization and mortality in patients with severe heart failure. In the Health, Aging, and Body Composition study, a cohort of 2722 white and black adults with a mean age of 74 years at baseline, every 29 ms higher PRi was associated with a 13% increase in the 10 year risk of developing heart failure (95% CI 1.02, 1.25). A prolonged PRi was also associated with a heightened risk for the combined endpoint of heart failure or cardiovascular mortality (HR 1.61; 95% CI 1.02, 2.54) in the Heart and Soul Study, a cohort of adults with stable coronary artery disease [8]. It is possible that associations between a prolonged PRi and future risk of heart failure are mediated, at least in part, by a prolonged PRi reflecting higher LV mass index [7, 17, 18]. Although a few studies that examined associations between the PRi and cardiovascular outcomes adjusted for left ventricular hypertrophy, residual confounding may have existed due to lack of direct measures of LV mass [1, 10, 19, 20].

A prolonged PRi has also been linked with increased risk for atrial fibrillation [1, 21]. Using data from the Framingham Heart Study, Schnabel et al. found that the PRi adds discriminatory value to a 5-year risk prediction model for atrial fibrillation, which also included demographic data, systolic blood pressure, use of blood pressure lowering medications, and presence of heart failure [20]. Although this risk prediction model was validated in both whites and African Americans [19], other studies have not consistently demonstrated a significant association between the PRi and risk for atrial fibrillation [21]. Inconsistent associations have also been noted between PRi and mortality [6, 7, 22, 23]. These inconsistent associations have been attributed to differences in the level of contribution of *P* duration to the length of the PRi within and across populations [22].

While the MESA study was limited by lack of information on left atrial dimensions, higher LV mass could potentially link a prolonged PRi with increased future risk for atrial fibrillation [24–27]. The hypothesized mechanistic link between elevated LV mass and atrial fibrillation is supported by studies demonstrating strong associations between long standing hypertension and increased risk for atrial fibrillation [28–30]. In the MESA study, individuals with a PRi > 200 ms had higher systolic blood pressure and were more likely to be using antihypertensive medications compared to those with a PRI ≤ 200 ms. However, the role of elevated LV mass for risk of atrial fibrillation likely depends on its interaction with other factors such as ventricular wall stress, ischemia, scar tissue, and electrolyte abnormalities [24].

Our study noted that the association between PRi and LV mass index differs by race. Few studies have explored racial differences in the association between the PRi and cardiovascular risk factors and outcomes. The Atherosclerosis Risk in Communities (ARIC) study included 14, 433 adults (25% African American and 75% white) and in this cohort both obesity and hypertension, strong risk factors for both heart failure and atrial fibrillation, were associated with a prolonged PRi and associations were stronger among African Americans compared to whites [31]. In contrast, the Health, Aging, and Body Composition study did not find differences in risk of heart failure or atrial fibrillation between African American and white adults [7]. Shulman et al. examined the PRi among 50,870 adults followed for a mean of 3.7 years and 5,1999 developed atrial fibrillation. While atrial fibrillation risk by presence of a prolonged PRI was significantly higher among whites compared to Hispanics and African Americans, a significant increase in the risk for atrial fibrillation was noted at lower PRi levels for both Hispanic and African Americans as compared to whites. Thus, it is likely that the association between the PRi and atrial fibrillation, and perhaps other cardiovascular outcomes, differs by race [1].

The strengths of this study include the inclusion of adults from four different racial/ethnic groups and standardized measures of multiple measures of LV dimensions and ejection fraction by MRI. Because all MESA participants were free of clinical cardiovascular disease at baseline; the findings of this study may not be applicable to individuals with established clinical cardiovascular disease such as heart failure. Information on atrial dimensions and electrolyte abnormalities was not available. The associations between PRi and LV dimensions were not strong and could be due to residual confounding. The cross-sectional design of this study precludes determination of temporal associations.

In conclusion, the PRi is associated with measures of LV stroke volume and LV mass but not ejection fraction. The association between a prolonged PRi and cardiovascular outcomes including heart failure and atrial fibrillation noted

in previous studies may be due in part to a prolonged PRi indicating higher LV mass.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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