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Impact of Atrial Fibrillation Ablation on Left Ventricular Filling Pressure and Left Atrial Remodeling

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Abstract

Background: **Left ventricular (LV) diastolic dysfunction is associated with new-onset atrial fibrillation (AF), and the estimation of elevated LV filling pressures by E/e′ ratio is related to worse outcomes in patients with AF. However, it is unknown if restoring sinus rhythm reverses this process.**

Objective: **To evaluate the impact of AF ablation on estimated LV filling pressure.**

Methods: **A total of 141 patients underwent radiofrequency (RF) ablation to treat drug-refractory AF. Transthoracic echocardiography was performed 30 days before and 12 months after ablation. LV functional parameters, left atrial volume index (LAVind), and transmitral pulsed and mitral annulus tissue Doppler (e′ and E/e′) were assessed. Paroxysmal AF was present in 18 patients, persistent AF was present in 102 patients, and long-standing persistent AF in 21 patients. Follow-up included electrocardiographic examination and 24-h Holter monitoring at 3, 6, and 12 months after ablation.**

Results: **One hundred seventeen patients (82.9%) were free of AF during the follow-up (average, 18 ± 5 months). LAVind reduced in the successful group (30.2 mL/m² ± 10.6 mL/m² to 22.6 mL/m² ± 1.1 mL/m², $p < 0.001$) compared to the non-successful group (37.7 mL/m² ± 14.3 mL/m² to 37.5 mL/m² ± 14.5 mL/m², p = ns). Improvement of LV filling pressure assessed by a reduction in the E/e′ ratio was observed only after successful ablation (11.5 ± 4.5 vs. 7.1 ± 3.7, $p < 0.001$) but not in patients with recurrent AF (12.7 ± 4.4 vs. 12 ± 3.3, p = ns). The success rate was lower in the long-standing persistent AF patient group (57% vs. 87%, p = 0.001).**

Conclusion: **Successful AF ablation is associated with LA reverse remodeling and an improvement in LV filling pressure. (Arq Bras Cardiol. 2014; 103(6):485-492)**

Keywords: **Atrial Fibrillation; Atrial Remodeling; Catheter Ablation; Echocardiography; Stroke Volume; Ventricular Function.**

Introduction

Grade I diastolic dysfunction is often considered a normal finding in the elderly, but it was found to be an independent predictor of atrial fibrillation (AF)[1]. Diastolic dysfunction contributes to the occurrence of AF because left ventricular (LV) relaxation abnormalities lead to a reduction in passive left atrial (LA) emptying, an increase in LA volume and pressure, and pulmonary vein (PV) distension, resulting in electrical remodeling[2-4]. It is not known if the occurrence of AF would worsen or even lead to increased LV filling pressure. The ratio of early diastolic mitral inflow velocity (E) and early diastolic mitral annular velocity (e´), E/e´, is useful in the estimation of LV filling pressure[5-9].

Catheter ablation has emerged as the most promising strategy for treating AF[10-13]. Because restoring sinus rhythm is associated with both LA reverse remodeling and the improvement in LA function[14-16], we hypothesize that successful AF ablation would improve LV filling pressure. This study sought to evaluate the impact of AF ablation on LV filling pressure, estimated by E/e´ ratio.

Methods

Patient population

In this study, we prospectively included consecutive patients with symptomatic, drug-refractory AF referred for ablation to the Instituto Brasília de Arritmia, between January 2007 and July 2010. Patients with paroxysmal, persistent, or long-standing persistent AF were classified according to AHA/ACC/ESC/HRS guidelines[17]. Exclusion criteria were as follows: significant cardiac valvular disease, first episode of AF, LA thrombus or tumors, hyperthyroidism, angina, or myocardial infarction occurring less than six months prior to ablation, or contraindications to warfarin use. Patients with previous percutaneous or surgical ablation procedures for AF were also excluded. Anticoagulation therapy with warfarin was used for at least one month pre-ablation and at least four months post-ablation in all patients, including those patients with paroxysmal AF. Seventy-three percent of patients were taking an angiotensin-I converting enzyme (ACE) inhibitor or angiotensin receptor blocker (ARB) during baseline and follow-up echocardiography. There was no important change in medication

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profile during the study period, except for the withdrawal of anticoagulation therapy for successful ablation patients with low risk (CHADS 2 ≤ 1) for embolic events.

Transesophageal echocardiography (TEE) was performed within 24 h of ablation to exclude LA thrombus. Approval for this study was obtained from the local ethics committee, and written informed consent was obtained from all patients.

EP study and ablation technique

A multi-polar catheter was inserted into the coronary sinus from the jugular vein for recording and stimulation. Before the first transseptal puncture, 3,000 IU intravenous heparin was given, and additional heparin was administered to keep the activated coagulation time > 350 sec[18]. After transseptal access, a circular mapping catheter was used to guide the isolation of all four pulmonary veins (PVs). Ablation was performed using either 8-mm non-irrigated tip (35 W, 50 °C) or 3.5-mm irrigated tip catheter (25 - 30 W, 42 °C) to achieve PV antrum isolation. The ablation end-point was the elimination of all PV potentials. Additional linear lesions were performed in long-standing persistent AF as previously reported[18]. Ablation was performed in the antral region of all pulmonary veins (PV) guided by the presence of PV potentials on the circular mapping catheter with the aim of achieving pulmonary vein isolation. Additional LA substrate modification was performed at the discretion of the operator, mostly for patients with long-standing persistent AF, which included roof line, mitral isthmus line, coronary sinus ablation, and CFAE at the discretion of the operator. Briefly, CFAE was defined as continuous or multicomponent electrograms recorded during atrial fibrillation. Reinduction of AF was not tried after ablation.

If any left atrial tachycardia was induced, the entrainment mapping technique was used to identify critical areas for the reentrant circuit. In patients with documented typical atrial flutter, additional cavotricuspid isthmus ablation was performed.

Intracardiac echocardiography (ICE) was used to guide transseptal puncture and ablation using Sequoia™ or Cypress™ ultrasound systems (Siemens Medical Solutions USA Inc., Ultrasound Division, Mountain View, CA) and a deflectable, 5.5 - 10 MHz, phased-array AcuNav™ catheter (Siemens Medical Solutions USA, Inc.) with pulsed-wave Doppler. ICE images were also used to avoid ablation inside PVs or the LA appendage. Beginning in January 2007, ICEs were also used to guide visualization of the esophagus.

Transthoracic echocardiography (TTE)

TTE was performed at 30 days before and 12 months after AF ablation using a Sequoia™ ultrasound system (Siemens Medical Solutions USA, Inc.). The following data were measured: LV diameter and volume, LVEF (left ventricular ejection fraction) and LV mass, LA diameter and volume, transmitral pulsed Doppler E and A waves, mean of septal and lateral mitral valve annulus spectral tissue Doppler (TDI) e´ and a´ wave, and mitral regurgitation quantification. LA volume was measured using Simpson's method as recommended by the American Society of Echocardiography, and then LA volume was indexed (LAind) by dividing by body surface area (reference value < 29 mL/m^2)[19]. Because the mitral annulus TDI is less load-dependent and is more feasible to perform in AF patients, it was used to estimate LV filling pressure. Importantly, the LAVind and E/e´ ratio could be consistently recorded during sinus rhythm or AF. Ninety-three patients (66%) were in AF during baseline echocardiographic measurements. In these patients, five measurements were averaged to calculate all data.

Follow-up

After the ablation procedure, anticoagulation therapy was maintained for at least four months, and antiarrhythmic drugs (AAD) were used for three months in all patients. During the first year of follow-up, ECG was recorded monthly, exercise testing in the sixth month, TTE was done in the twelfth month, and Holter monitoring was done in the third, sixth, and twelfth months after ablation. Recurrence was defined as symptomatic or asymptomatic AF recorded by ECG, exercise testing, or Holter monitoring, after a blanking period of three months. All patients were followed for at least one year.

Statistic analysis

Continuous variables are reported as mean ± standard deviation (SD), and compared with Student's t-test or ANOVA. Categorical variables are reported as percentages and compared with chi-square test or Fisher's exact test, as appropriate. A probability value < 0.05 was considered significant. Interobserver and intraobserver variability were calculated and expressed as percent average value. All statistical analysis was performed with SPSS® 16 for Mac.

Results

Three patients were excluded for having moderate mitral regurgitation. The study population comprised 141 patients [mean age, 67 ± 11 years, 71 (50.3%) male]. Eighteen (12.8%) patients had paroxysmal AF, 102 (72.3%) patients had persistent AF, and 21 (14.9%) patients had long-standing persistent AF. The mean failed AAD trials per patient were 2.1 ± 0.7. One hundred and twenty-six patients (89.3%) underwent one procedure, nine patients (6.4%) underwent two procedures, and six patients (4.3%) underwent three procedures.

All patients were followed for at least one year. Over a mean follow-up period of 18 ± 5 months, 117 (82.9%) patients were in sinus rhythm, 70.1% were not, and 12.8% were on previously failed AAD therapy. Twenty-four (17.1%) patients failed AF ablation and were defined as the non-successful group.

Baseline Characteristics

Baseline characteristics are presented in Table 1. There were more NYHA class II patients (56.4% vs. 75%, $p < 0.0001$) and long-standing persistent AF patients (10.2 vs. 37.5%, $p = 0.001$) in the non-successful group. At baseline, patients with successful ablation showed lower LAVind (30.2 ± 10.6 mL/m^2 vs. 37.7 ± 14.3 mL/m^2, $p = 0.023$), E/e' ratio (11.5 ± 4.5 vs. 12.7 ± 4, $p = 0.01$), and E/A ratio (0.9 ± 0.3 vs. 1.15 ± 0.5, $p < 0.0001$) and higher LVEF (68.6 ± 4.9% vs. 63.5 ± 8.6%, $p = 0.01$) and e'

Table 1 - Baseline characteristics in patients with successful and non-successful atrial fibrillation ablation

Variable	Successful Group (n=117)	Non-successful (n=24)	p
Gender, male, n (%)	61 (52.1%)	10 (41.7%)	ns
Age (years)	68 ± 12	63 ± 9	ns
NYHA FC	I 51 (43.5%) II 66 (56.4 %)	I 3 (12.5%) II 18 (75%) III 3 (12.5%)	< 0.0001
AF type	paroxysmal 18 (15.4%) persistent 87 (74.4%) Longstanding-Persistent 12 (10.2%)	paroxysmal 0 persistent 15 (62.4%) Longstanding-Persistent 9 (37.5%)	0.001
CAD	0	3 (2.6%)	ns
Alcohol abuse	18 (15.4%)	3 (12.5%)	ns
Chagas disease	9 (7.7%)	6 (25%)	ns
Smoking	3 (2.6%)	3 (12.5%)	ns
HBP	69 (59%)	18 (75%)	ns
DM	6 (5.1%)	3 (12.5%)	ns
CPOD	3 (2.6%)	0	ns
DD	61 (52.1%)	17 (52%)	< 0.01
LAV ind (mL/m²)	30.2 ± 10.6	37.7 ± 14.3	0.023
LVD (mm)	48.7 4.4	47.0 ± 1	ns
LVS (mm)	29.8 3.4	28.0 ± 2.5	ns
LV mass (g)	209.4 ± 49.8	186.2 ± 25.2	ns
LVEF (%)	68.6 4.9	63.5 ± 8.6	0.01
E/A ratio	0.9 0.3	1.15 ± 0.05	< 0.0001
e' (cm/s)	8.1 4.3	6.4 ± 1.8	0.009
a' (cm/s)	10.1 0.4	9.0 ± 1.3	ns
E/e' ratio	11.5 4.5	12.7 ± 4.4	0.01

FC: functional class; NYHA: New York Heart Association; AF: atrial fibrillation; CAD: coronary artery disease; HBP: high blood pressure; DM: diabetes mellitus; COPD: chronic obstructive pulmonary disease; DD: diastolic dysfunction; LAV ind: left atrial volume index; LVD: left ventricular diastole; LVS: left ventricular systole: LV: left ventricle; LVEF: left ventricular ejection fraction.

velocity (8.1 ± 4.3 cm/s vs. 6.4 ± 1.8 cm/s, p = 0.009) than non-successful patients. There was no remarkable variation in patient's body surface area during the study period.

Outcome

During follow-up, ablation was successful in a greater percentage of patients with paroxysmal and persistent AF than patients with long-standing persistent AF (87% vs. 57%, respectively, p = 0.001). Acute PV isolation was achieved in all patients in this cohort. Additional LA substrate modification was frequently performed in patients with non-paroxysmal AF (roof line: paroxysmal = 3 patients vs. non-paroxysmal = 35 patients, p = 0.001; mitral isthmus line: paroxysmal = 0 patients vs. non-paroxysmal = 54 patients, p < 0.001; coronary sinus ablation: paroxysmal = 0 patients vs. non-paroxysmal = 27 patients, p < 0.001; CFAE: paroxysmal = 1 patient, non-paroxysmal = 15 patients, p < 0.01). The average procedure time for the entire cohort was 265 ± 55 min (204 ± 51 min for paroxysmal AF, 277 ± 79 min for non-paroxysmal AF, p = 0.009).

In patients with successful ablation, there was a significant improvement in NYHA functional class compared to recurrent AF patients (p < 0.001) (Table 2). Furthermore, there was a significant reduction in parameters of LA reverse remodeling in successful patients (LAVind: 30.2 mL/m² ± 10.6 mL/m² vs. 22.6 mL/m² ± 1.1 mL/m², p < 0.001) (Figure 1). Also, in successful patients, LV filling pressure improved, as E/e´ ratio was reduced significantly after ablation (11.5 ± 4.5 vs. 7.1 ± 3.7, p < 0.001, respectively). These observations were not seen in patients with recurrent AF. Analyzing only patients with paroxysmal AF, we also observed a reduction in LAVind (21.8 mL/m² ± 11.9 mL/m² to 14.1 mL/m² ± 5.5 mL/m², p < 0.001) and in the E/e´ ratio (10.3 ± 3 vs. 5.0 ± 0.5, p < 0.001).

Reproducibility

Intraobserver variability for E wave velocity, e´ wave velocity, and LA volume were 2.0%, 3.3%, and 2.3%, respectively. Interobserver variability for E wave velocity, e´ wave velocity, and LA volume were 3.7%, 3.5% and 4.3%, respectively.

Table 2 - Follow-up characteristics in patients with successful and non-successful atrial fibrillation ablation

Variable	Successful Group (n = 117)		p	Non-Successful (n = 24)		p
	Pre-ablation	Post-ablation		Pre-ablation	Post-ablation	
NYHA FC	1.57 ± 0.5	1.1 ± 0.3	< 0.001	2.0 ± 0.5	2.0 ± 0.4	ns
LAV ind (ml/m²)	30.2 ± 10.6	22.6 ± 1.1	< 0.001	37.7 ± 14.3	37.5 ± 14.5	ns
LVD (mm)	49.0 ± 4.7	49.0 ± 5.1	ns	46.0 ± 0.1	49.0 ± 1.0	0.001
LVS (mm)	29.9 ± 3.6	29.4 ± 3.4	ns	27.0 ± 3.2	27.0 ± 2.1	ns
LV mass (g)	195.3 ± 44.5	192.4 ± 51.8	ns	173.5 ± 27.9	178.5 ± 21.3	ns
LVEF (%)	68.6 ± 4.9	70.0 ± 4.7	ns	63.5 ± 8.6	66.0 ± 3.2	ns
E/A ratio	0.9 ± 0.3	1.0 ± 0.3	ns	1.1 ± 0.05	1.5 ± 0.02	ns
e' (cm/s)	8.1 ± 4.3	9.5 ± 2.5	ns	6.4 ± 1.8	7.9 ± 0.5	ns
a' (cm/s)	10.1 ± 0.4	10.0 ± 0.2	ns	9.0 ± 1.3	8.7 ± 0.9	ns
E/e' ratio	11.5 ± 4.5	7.1 ± 3.7	< 0.001	12.7 ± 4	12.0 ± 3.3	ns
LAV ind (ml/m²)	-7.9 ± 5.5			-1.0 ± 0.9		< 0.0001
Δ E/e' ratio	-3.9 ± 3.4			-1.1 ± 0.9		< 0.0001

FC: functional class; NYHA: New York Heart Association; LAV ind: left atrial volume index; LVD: left ventricular diastole; LVS: left ventricular systole; LV: left ventricle; LVEF: left ventricular ejection fraction.

Discussion

The main findings of this study were related to LV filling pressure and LA reverse remodeling. Using Doppler echocardiography, we showed that successful AF ablation reduces LA volume and improves LV filling pressure estimated by E/e´ ratio.

Reverse remodeling of LA post-AF ablation

Volume and pressure overload are recognized as the main factors leading to the adaptive responses that occur in LA remodeling. This overload promotes distension of the LA and PVs, which generates rapid and disorganized electrical activity favoring AF occurrence[3,4]. The adaptive responses leading to this phenomenon are myocyte growth, hypertrophy, necrosis, alterations of fibrocollagenous connective tissue, and changes in the expression of cellular ion channels and atrial hormones, including atrial natriuretic peptide[20,21]. Furthermore, in the setting of LA volume overload and/or LA pressure overload, LA myocytes are more likely to depolarize, increasing vulnerability to AF[22]. After AF occurrence, the arrhythmia may be maintained by a remodeled substrate, but AF itself also promotes atrial remodeling ("AF begets AF")[23]. Thus, as previously hypothesized, the restoration of sinus rhythm leads to LA reverse remodeling after successful AF ablation, electrical cardioversion or cardiac surgery[24-26]. Beukema et al[16] found that LA diameter decreased from the baseline significantly in patients with persistent sinus rhythm after ablation, and they also found that recurrent AF was associated with larger diameters. Verma et al[27] reported similar results in LA diameter reduction in a series of patients who underwent extensive PV antrum ablation[27]. Recently, Machino-Ohtsuka et al[28] reported a significant LA volume reverse remodeling after 12 months of successful AF ablation. Similar findings were demonstrated using cardiac magnetic resonance[29]. Our results are in agreement with these studies because a significant improvement in LA reverse remodeling was found in our successful ablation population, in which LAVind decreased from 30.2 ± 10.6 mL/m² to 22.6 ± 1.1 mL/m² ($p < 0.001$). We measured LA volume, which is more accurate than LA diameter, to evaluate reverse LA remodeling after AF treatment. LA diameter is measured in M-mode, using a single LA dimension and may, thus, underestimate LA size. On the other hand, the LA index volume is measured using biplane Simpson's rule (4- and 2-chamber views), which is more reliable. This method has been validated with computed tomography[30] and magnetic resonance measurement[31].

It is important to emphasize that LA reverse remodeling was also observed in patients with paroxysmal AF (21.8 ± 11.9 mL/m² to 14.1 ± 5.5 mL/m², $p < 0.001$). Although our series had only 18 patients with paroxysmal AF, this finding was consistent. Similarly, 49% of patients from the study by Beukema et al[16] had paroxysmal AF, and these patients showed a reduction in LA diameter from 40.5 ± 4.4 to 37.5 ± 3.5 mm ($p < 0.01$). In the study by Tops et al[32], 61% of patients had paroxysmal AF, but they did not compare this subgroup with persistent and long-standing persistent AF patients. These findings were also observed in the study by Reant et al[33], which showed a reduction in longitudinal diameter from 59.7 ± 7.3 mm to 53.19 ± 7.7 mm six months after ablation in the subgroup of patients with paroxysmal AF.

Remodeling of the LA substrate appears to play an important role in the success rate of AF ablation, but the mechanism responsible for LA reverse remodeling after successful ablation is not clear yet. Our results support the findings reported by Reant et al[33], who suggested that diastolic dysfunction may cause isolated AF by the combination of LA and PV stretch and enlargement, which results from LV end-diastolic pressure. One could argue that extensive ablation shrinks the left atrium, and that reverse remodeling would not be related to sinus

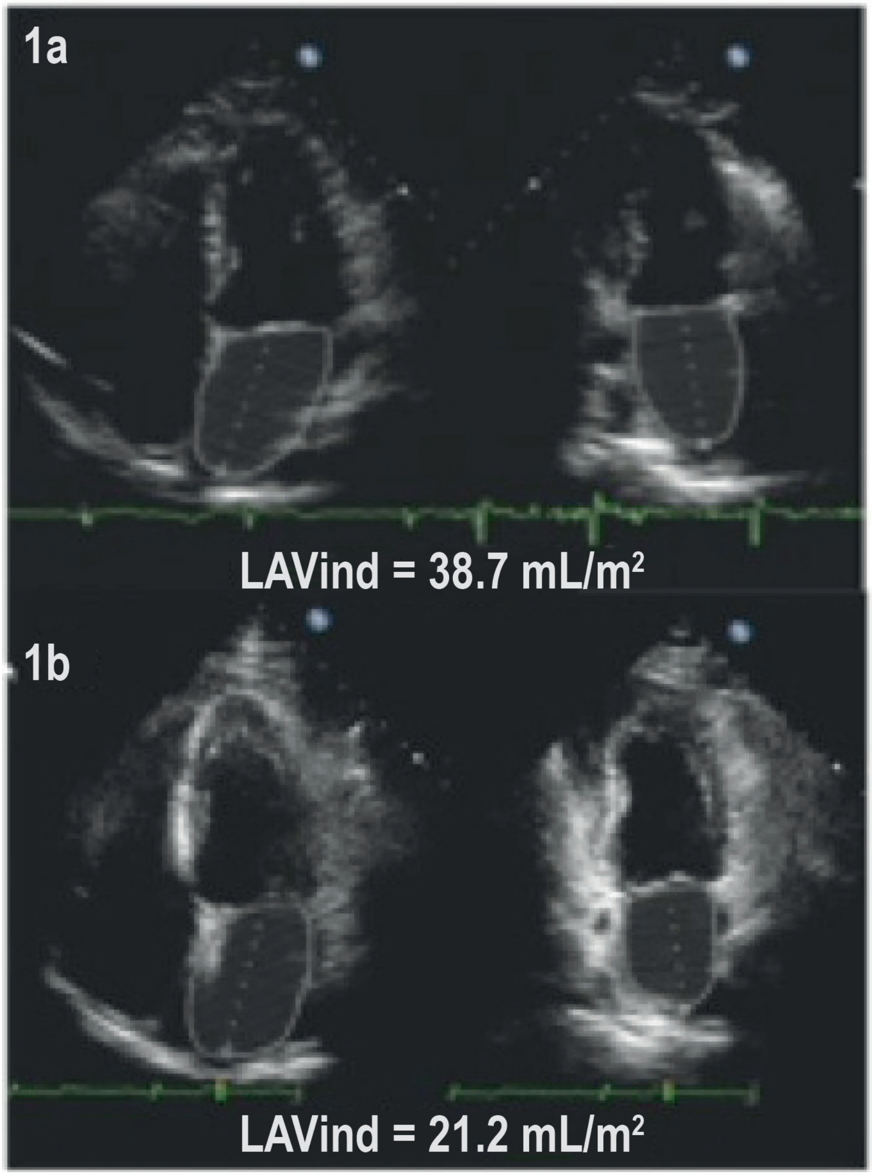

Figure 1 - *Example of left atrium (LA) reverse remodeling after successful atrial fibrillation ablation. 1a) LA volume index before ablation; 1b) LA volume index 12 months after ablation.*

rhythm restoration. However, in our series, reverse remodeling was not observed in patients with recurrent AF.

Improvement of left ventricle diastolic function

Diastolic dysfunction is a common cause of volume and/or pressure overload, which leads to LA remodeling and may further result in structural and electrophysiological heterogeneity, promoting a substrate for AF occurrence. Thus, it is not surprising that the restoration of sinus rhythm could also improve diastolic function. Sinus rhythm restoration after ablation results in LA reverse remodeling, but these studies have not noted a clear benefit in LV diastolic function. The lack of a clear benefit may be explained by the fact that although the LA volume index is a reliable method to evaluate abnormalities in LV diastolic loading[34,35], this index is a measure of LA function. However, Hurrell et al[36] showed that there is a very good correlation between the LA volume index and invasive LV diastolic loading[36]. The LA volume index has been used in different settings as a measure of diastolic function[4,35,37-39]. By incorporating other methods to estimate LV filling pressure, we were able to show its improvement after restoration and maintenance of sinus rhythm following AF ablation.

Although pulsed-wave Doppler analysis of the mitral valve inflow and PV flow are commonly used, these measurements may be influenced by preload and afterload[9]. This limitation is even more pronounced in patients with AF due to changes in LA filling and loss of LA contraction. In this situation, TDI imaging is the most accurate method to estimate LV filling pressure. In our study population, patients with non-successful AF ablation had a lower e´ and higher E/e´ ratio than patients with successful AF ablation, suggesting a relationship between elevated LV filling pressure and poor AF ablation outcome. Furthermore, there was a significant reduction in the E/e´ ratio during follow-up in successful patients, suggesting that sinus rhythm restoration has a positive impact on LV filling pressure. The same findings were observed in patients with paroxysmal AF. Only a few studies have tried to assess the relationship between AF and ventricular diastolic filling patterns. Melek[40] et al. reported the cardioversion effect on TDI parameters in persistent AF patients. They were not able to find any significant improvement on diastolic function after restoration of sinus rhythm with the exception of atrial function, but the follow-up period was very short. In contrast, some studies found that impaired diastolic filling was an independent predictor of late recurrence following cardioversion[41] and ablation[42].

Patients with pronounced ventricular filling impairment are at greater risk for AF. What is not clear is if AF can worsen LV diastolic function. During acute rapid AF, there are some mechanisms explaining the worsening of LV diastolic function, for example, loss on LA contraction, irregular LV filling, and rate and worse AV synchrony; long-standing AF data are still scarce. Some mechanistic insights came from the studies of Goette et al[43,44], who showed in animal and human models that AF lead to down-regulation of the endothelial nitric oxide synthase and elevated asymmetric dimethylarginine, which are associated with vascular dysfunction and heart failure. They also showed a reduction in aldosterone levels after restoration of sinus rhythm.

There is lack of data showing improvement of diastolic function after successful AF ablation. Reant et al[33] found that diastolic function improved significantly 12 months since the procedure, with an increase in lateral early diastolic peak velocity of 29% ($p < 0.001$) in paroxysmal AF and 46% ($p < 0.05$) in chronic AF patients. Cha et al[45] reported a diastolic function improvement following successful AF ablation. Diastolic function was graded from 1 to 4 according to ASE guidelines[46], however all patients included were in sinus rhythm at the time of their transthoracic echocardiograms. Our study included a large number of patients with persistent and permanent AF, so that 66% were not in sinus rhythm during echocardiographic measurements. Nevertheless, we suggest an association between successful AF ablation and estimated LV filling improvement.

Study limitations

Although we have shown an association between LA volume index reduction and improvements in LV filling pressure in patients with successful AF ablation, we acknowledge that this association may not be related just to ablation. LA reverse remodeling after successful AF ablation may result not only from sinus rhythm restoration but may also be a consequence of better left atrial-ventricular synchronism. Further prospective studies are necessary to confirm this hypothesis.

We did not perform seven-day ambulatory monitoring, and asymptomatic AF recurrences may have been missed. However, we investigated patients for asymptomatic AF recurrences during the first year of follow-up: ECG was recorded monthly, and Holter monitoring was done in the third, sixth, and twelfth month after ablation.

Finally, this was a non-randomized, single-arm study, and some echocardiography measurements that are thought to be a consequence of the ablation procedure may have been influenced by other factors. For example, after invasive procedures, some patients become more compliant with pharmacological treatment and exercise, and this may have altered the echocardiography measurements regardless of the ablation procedure.

Because this is the initial experience of a single private practice service, most patients referred for ablation were classified into persistent or long standing persistent AF, according to HRS 2007 consensus, making this study difficult to compare with other AF ablation studies, which have mostly paroxysmal AF patients.

Conclusion

AF ablation showed better results in patients with paroxysmal and persistent AF than long-standing persistent AF. When sinus rhythm was maintained consistently, there was an improvement in estimated LV filling pressure and LA reverse remodeling.

Author contributions

Conception and design of the research: Santos, SN; Henz, BD; Zanatta, AR; Barreto, JR; Leite, LR. Acquisition of data: Santos, SN; Henz, BD; Zanatta, AR; Barreto, JR; Loureiro, KB; Novakoski, C; Santos, MVN; Giuseppin, FF; Oliveira, EM; Leite, LR. Statistical analysis: Santos, SN; Leite, LR. Analysis and interpretation of the data: Santos, SN; Leite, LR. Writing of the manuscript: Santos, SN; Leite, LR. Critical revision of the manuscript for intellectual content: Santos, SN; Henz, BD; Leite, LR.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

This study is not associated with any thesis or dissertation work.

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