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Original Article

Histopathologic analysis of atrial tissue in patients with atrial fibrillation: comparison between patients with atrial septal defect and patients with mitral valvular heart disease☆

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ABSTRACT

Background: Atrial fibrillation (AF) in adult patients with atrial septal defect (ASD) accompanies an enlarged right atrium (RA) with a less enlarged left atrium (LA), which is the opposite situation in patients with AF and mitral valvular disease. This study was to compare the histopathological change in the atrium of patients with AF of two different etiologies: ASD and mitral disease.
Methods: Twenty-four patients were enrolled. Group 1 included patients with ASD (8), Group 2 included patients with ASD with AF (6), and Group 3 included patients with mitral disease with AF (10). Preoperative atrial volumes were measured. Atrial tissues were obtained during surgical procedures and stained with periodic acid-Schiff, smooth muscle actin, Sirius red, and Masson's trichrome to detect histopathologic changes compatible with AF. The severity of histopathological changes was represented with "positivity" and "strong positivity" after analyzing digitalized images of the staining. We investigated the relationship between the degree of atrial dilatation and severity of histopathological changes according to the groups and tissues.
Results: Group 2 and Group 3 patients showed a tendency toward an enlarged RA volume and enlarged LA volume, respectively, compared with each others. However, in the histopathologic analysis, "positivity" and "strong positivity" showed no significant positive correlations with the degree of atrial volume in special staining.
Conclusions: A similar degree of histopathologic changes was observed in both atria in patients with AF (Group 2 and 3) regardless of the degree of dilatation of atrial volume and disease entities.

1. Introduction

For the past couple of decades, the Cox maze III procedure, for which lesion sets are made in both the right atrium (RA) and left atrium (LA), has been chosen as the standard surgical option for treating atrial fibrillation (AF). A major cause of AF in adults is associated with left-sided valvular heart disease, especially in mitral valve stenosis or regurgitation. In a recent paper, Cox noted the effectiveness of the Cox maze III procedure for correcting various types of atrial arrhythmias, such as AF or atrial flutter, due to an enlarged LA with or without an enlarged RA [1]. However, the paper contained no comments about AF occurring with an enlarged RA in the presence of a normal or insignificantly enlarged LA. We frequently find significantly enlarged RA with a normal-sized LA in many cases of congenital heart disease, including atrial septal defect (ASD) or Ebstein's anomaly, in adult patients. Some surgeons have treated atrial tachyarrhythmias, including AF, associated with congenital cardiac anomalies that present with an enlarged RA and nearly normal-sized LA using the RA maze procedure, and they reported tolerable surgical outcomes with this modified technique [2–4]. From the results of above previous studies, we hypothesized that patients

Abbreviations: AF, Atrial fibrillation; ASD, Atrial septal defect; GA, Glutaraldehyde; H&E, Hematoxylin and eosin; LA, Left atrium; MT, Masson's Trichrome; Np, Positive pixel count; Nsp, Strongly stained positive pixel count; PAS, Periodic acid-Schiff; RA, Right atrium; SMA, Smooth muscle actin.

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with AF and accompanying valvular heart disease and adult patients with AF and accompanying ASD would have different atrial histopathologic findings. In this study, we tried to verify the above hypothesis via histopathologic analyses of patients' atrial tissue. We also investigated the associations between the severity of histopathologic changes and the degree of preoperative atrial volume dilatation, which may be used as an indicator of whether to perform a RA maze in patients with ASD and AF. We expected the results of this study to provide information on whether the RA maze procedure in patients with ASD and AF can show similar effectiveness in eradicating AF and preventing its recurrence compared with an original maze procedure in the same patients.

2. Materials and methods

2.1. Patients' grouping

Between January 2010 and July 2011, 24 consecutive adult patients (>20 years of age) were enrolled in this prospective study. We divided these patients into three groups. Patients in Group 1 had simple ASD (n=8), patients in Group 2 had ASD with AF (n=6), and patients in Group 3 had mitral valvular disease with AF (n=10).

2.2. Preoperative evaluation

We performed catheterization on the patients with ASD (Group 1 and 2) to evaluate the pulmonary artery resistance, pressure of right ventricle and pulmonary artery, and the reversibility of pulmonary hypertension to determine whether to close the ASD completely or partially and also to check for coronary arterial status. For patients with mitral valvular disease (Group 3), an angiographic procedure was performed by interventionists to check for coronary arterial disease. However, the pressure profiles of cardiac chambers and pulmonary artery were not measured in Group 3.

The volume of the preoperative atrial chamber was measured by a cardiologist based on echocardiographic findings using a method developed by Sanfilippo et al. [5]. The anteroposterior dimension (D1) of the RA and LA was measured on the parasternal view. The mediolateral (D2) and superior-inferior (D3) dimensions of the RA and LA were measured on the apical four-chamber view. All measurements were taken along the line of the maximum diameter between the chamber walls on the respective planes. The volume of both atriums was calculated with the formula indicated for an atrium shaped as a prolate ellipsoid $\left(\text{Volume of the RA or LA (mL)} = \frac{4\pi}{3} \times \frac{D1}{2} \times \frac{D2}{2} \times \frac{D3}{2}\right)$. We corrected these values for the patients' body surface area (mL/m^2).

2.3. Operations

For the patients in Group 1, we performed ASD patch closure with glutaraldehyde (GA)-fixed autologous pericardium and atrial reduction plasty with or without tricuspid valve annuloplasty according to the severity of tricuspid annulus dilatation. Atrial reduction plasty was performed on patients whose atrial chambers were significantly dilated. A LA appendectomy was performed as prophylaxis against possible thromboembolic events resulting from AF occurrence despite ASD closure in this group, as described in previous studies [6]. After resection of LA appendage, only base of LA appendage was remained. The RA appendage was resected with a triangle shape, about 1.5–2 cm of base and 1cm of height when a venous cannula was inserted into the RA appendage. Patients in Group 2 underwent ASD closure with GA-fixed autologous pericardium and a modified Cox maze III procedure. RA and LA appendage resection was performed during the Cox maze III procedure. The free wall of both atria was resected for atrial reduction plasty in patients whose atria were dilated more than normal. For the patients in Group 3, mitral valve repair or replacement and a Cox maze III procedure were concomitantly performed. Two patients in Group 3 underwent a concomitant coronary artery bypass graft surgery. Similar to Groups 1 and 2, atrial reduction plasty was performed on the patients whose atrial tissue was significantly dilated (Table 1).

The resected areas of the atrial tissue were the same for all groups. For the RA reduction, we extended a usual right atriotomy (1–1.5 by 2–3 cm) for the main procedures such as ASD closure or valve repair. LA reduction plasty was performed on the area between the inferior pulmonary veins and posterior annulus of mitral valve, about 1 by 2–3 cm. This resected LA area can also be a part of the LA maze lesion sets (the mechanical barriers for arrhythmogenic circuits on the atrial tissue induced by the maze procedure) [7].

2.4. Pathologic analysis

All atrial tissues were immediately immersed in formalin solution after resection and embedded in a paraffin block. First, we stained the tissues with hematoxylin and eosin and selected one or two of the most severely diseased areas to create a microarray kit. One microarray kit included a 4×5 matrix, which meant that one slide contained four types of tissues (RA free wall and appendage, LA free wall and appendage) obtained from five patients. This kit could facilitate more effective staining and comparison of tissues. In patients from whom we did not obtain tissues from all four atrial areas, the spot for the missing tissue in the microarray slide was left empty (Fig. 1).

Special stains used to define pathologic changes in the atrial tissue included periodic acid-Schiff (PAS, Fig. 2A), Masson's trichrome (MT, Fig. 2C), Sirius red (Fig. 2D), and smooth muscle actin (SMA, Fig. 2B). After completing the staining procedures, the microarray slide images were digitalized for quantitative measurement of positive findings with an image analysis program (ApergioImageScope version 10.2.1.2315, Apergio Technologies, Inc, Vista, CA, USA). From the digital image analysis, we obtained the "positive pixel count (Np)", "strongly stained positive pixel count (Nsp)", "positivity", which was defined as the ratio of positive pixels to the total pixel number within a single spot, and "strong positivity", which was defined as the ratio of strong positive pixels to the total pixel number within a single spot. We compared these parameters among the three groups.

2.5. Statistical analysis and ethics

Quantitative data are presented as the mean±standard deviation, and P <.05 was considered statistically significant. The statistical analysis and comparison of continuous variables among the groups or subgroups were performed with an independent samples t test or a Mann–Whitney U test. Pearson's or Spearman's correlation analysis was used to verify the correlation between the degree of RA or LA dilatation and "positivity" or "strong positivity" on the histopathologic analysis from each special staining. Statistical analysis was performed

Table 1
Operative procedures ("Cox-maze III" procedure, which was concomitantly performed in all patients in Groups 2 and 3, was not described in this table)

Group 1 (n=8)		Group 2 (n=6)		Group 3 (n=10)	
ASD closure+TVP	4	ASD fenestrated closure+TVP	4	MVR+TVP	4
ASD closure only	4	ASD fenestrated closure	1	MVP+TVP	2
		ASD closure+TVP	1	MVP+CABG	1
				MVR+AVR+TVP	1
				MVP+TVP+CABG	1
				MVR+AVR+TVP +CABG	1

AVR, aortic valve replacement; CABG, coronary artery bypass graft; MVP, mitral valve repair; MVR, mitral valve replacement; TVP, tricuspid valve repair (including annuloplasty).