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Original Article

Enlargement of the left atrium is associated with increased infiltration of immune cells in patients with atrial fibrillation who had undergone surgery

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ABSTRACT

Background: Enlargement of the left atrium (LA) is a risk factor of atrial fibrillation (AF) recurrence after pharmacological and nonpharmacological interventions for AF. However, structural changes associated with LA enlargement have not been fully elucidated.
Methods: To examine inflammation in the structural changes associated with LA enlargement, human left appendages obtained from 27 patients who underwent cardiac surgery by using the maze procedure were subjected to immunohistochemical analysis.
Results: The extent of interstitial fibrosis increased according to the increase in LA dimension (LAD) as assessed by using ultrasound echocardiography. The extent of the infiltration of CD68-positive macrophages and CD3-positive T cells increased simultaneously according to the increments in LAD. The areas infiltrated by immune cells were positively and significantly correlated with LAD ($r^2=0.58$, $p<0.01$ for CD68; $r^2=0.49$, $p<0.01$ for CD3).
Conclusions: In the patients with AF, LA enlargement was associated not only with the increase in the extent of interstitial fibrosis but also with the changes in the LA component cells, including an increase in number of immune cells resident in tissues.

1. Introduction

Atrial fibrillation (AF), the most common type of sustained tachyarrhythmia, affecting approximately 0.9% of the population, is known to be associated with significant morbidity and mortality [1–4]. Recently, circumferential pulmonary vein isolation (CPVI) via catheter ablation has been established as a curative strategy for this type of arrhythmia, suggesting an important role of the thoracic veins in the mechanisms underlying AF. However, the success rate of CPVI ablation has been reported to decrease with increases in left atrial dimension (LAD) [5–7], similar to that of pharmacotherapy. This suggests potential pathological processes that invade the atrium from the pulmonary veins in a progressive manner in concert with the enlargement of the left atrium (LA).

A substantial body of evidence linking inflammation to the pathophysiology of a broad spectrum of cardiovascular diseases, including coronary artery disease, diabetes mellitus, and hypertension, has been developed [8–11]. Similarly, the hypothesis that inflammatory processes are involved in the pathogenesis of AF has attracted renewed attention. In previous studies, other researchers and we demonstrated the recruitment of immune cells across the atrial endocardium in patients with AF [12,13]; however, the degree of infiltration of immune cells widely varied between patients.

In the present study, we hypothesized that inflammatory processes occur and progress according to LA enlargement, which may contribute in part to the refractoriness to interventions for AF with an enlarged LA. Atrial appendage specimens from patients with AF who had undergone cardiac surgery were used for immunohistochemical analysis to assess potential relationships between LAD and the infiltration of immune cells.

2. Materials and methods

2.1. Patients

Left atrial appendage specimens were obtained from patients who had undergone cardiac surgery involving the maze

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procedure. LAD was evaluated based on transthoracic echocardiograms obtained within 1 week before the operation. All the patients had AF (persistent AF, 21; paroxysmal AF, 6). None of the patients had a previous myocardial infarction, febrile disorders, systemic inflammatory diseases, malignancy, or chronic renal failure. This investigation conformed to the principles outlined in the Declaration of Helsinki and was approved by an institutional review board (2005/7/13), and written informed consent was obtained from all the patients.

Table 1
Patient characteristics.

	n (%)
Male/female	17/10
Age (years), mean ± SD	59.9 ± 10.1
EF (%), mean ± SD	63.4 ± 9.2
LAD (mm), mean ± SD	55.2 ± 11.0
Paroxysmal (%)	6 (22.2)
AF duration (months), mean ± SD	82.4 ± 79.9
Concomitant heart diseases	
MR	16 (59)
MR+TR	3 (11)
MR+AR/AS	3 (11)
AR/AS	3 (11)
ASD	2 (7)
Medications	
VKA	27 (100)
Diuretics	26 (96)
RAS inhibitor	17 (63)
Beta-blocker	16 (59)

EF, ejection fraction; LAD, left atrial dimension; VKA, vitamin K antagonist; RAS, renin-angiotensin system; MR, mitral regurgitation; TR, tricuspid regurgitation; AR, aortic regurgitation; AS, aortic stenosis; and ASD, atrial septal defect.

2.2. Histological examination and immunohistochemistry

Blocks of tissues were embedded in optimal cutting temperature compound and immediately frozen in liquid nitrogen after resection. Frozen cryostat sections (8 μm) were cut, air-dried, fixed in acetone, and thereafter evaluated with standard protocols for staining with hematoxylin–eosin and Masson trichrome stain. Immunostaining was performed in sequential sections by using Dako EnVision+Systems (Dako) with primary antibodies for CD68 (KT117, TransGenic, Inc.) and CD3 (Nichirei Bioscience 413591).

Infiltration of immune cells in the atrium was examined via light microscopy by using immunostained images at a magnification of 100× captured with a digital camera (Nikon). Using the Image-Pro Plus software (Media Cybernetics, Carlsbad, CA, USA), the extent (% area) of interstitial fibrosis and positive staining representing immunoreactivity was recorded and corrected to the total section areas. The mean value was obtained from 10 different fields selected blindly for each patient.

2.3. Statistical analysis

Data were expressed as mean ± SD values. Correlations of LAD to interstitial fibrosis and immunoreactive areas were evaluated by using linear regression analysis. Statistical significance was set at a $p < 0.05$.

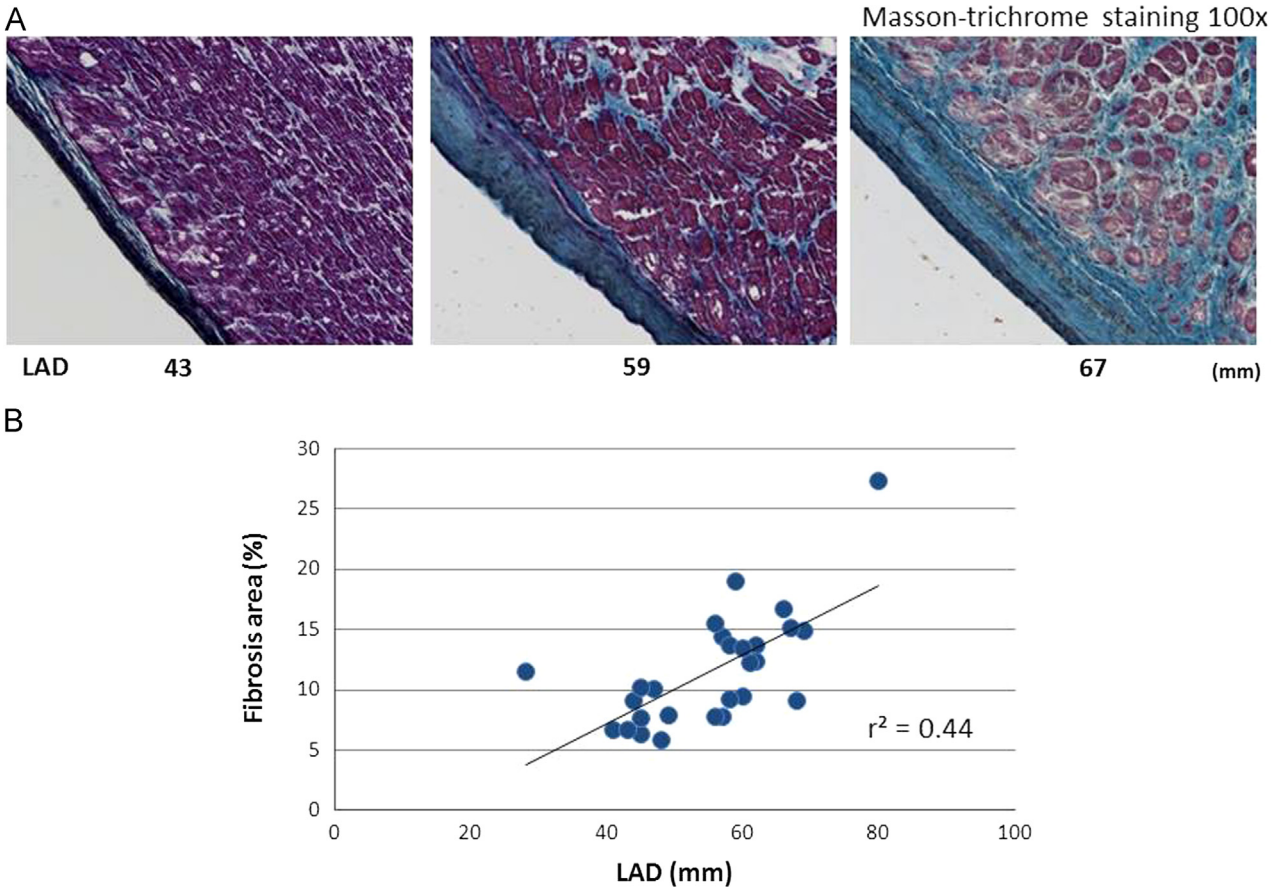

Fig. 1. (A) Masson-trichrome staining of left atrial appendage specimens from patients with left atrial dimensions (LADs) of 43 mm (left), 59 mm (middle), and 67 mm (right), respectively. The extent of interstitial fibrosis (blue area) increased as the LAD increased. (B) Correlation between LAD (mm) and fibrosis area (% of the total section area). A statistically significant positive correlation was observed.