Association between Left Atrial Appendage Fibrosis and Thrombus Formation: A Histological Approach

Brief title: Left atrial appendage fibrosis and thrombosis

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Data availability statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Funding: Yukiko Nakano received a grant from The Japan Society for the Promotion of Science (Grant No. 17K09501), Tokyo, Japan.

Disclosures: All authors have nothing to disclose

Ethics approval/ Patient consent statement: The study was approved by the Ethics Committee of Hiroshima University Graduate School of Biomedical and Health Sciences and conducted in compliance with the ethical principles of the Declaration of Helsinki. All patients gave a written informed consent before participating.

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Abstract

Introduction:

Although recent echocardiographic studies have suggested that left atrial appendage (LAA) remodeling contributes to the development of LAA thrombus (LAAT), histological evidence is absent. The objective of this study was to examine clinical parameters and histological findings to clarify the factors involved in LAAT formation.

Methods:

A total of 64 patients (no atrial fibrillation [AF], N = 22; paroxysmal AF, N = 16; non-paroxysmal AF, N = 26) who underwent LAA excision during surgery were enrolled. Transthoracic and transesophageal echocardiography were performed before surgery. We evaluated the fibrosis burden (%) in the excised LAA sections with Azan–Mallory staining in patients with a LAAT compared with those without.

Results:

Patients with paroxysmal and non-paroxysmal AF had a higher LAA fibrosis burden than those without AF ($P = 0.005$ and $P < 0.0001$, respectively). Among the patients enrolled, 16 had a LAAT and 15 of them had non-paroxysmal AF. Among the non-paroxysmal AF patients, those with a LAAT had significantly higher LAA fibrosis burden than those without (23.8% [14.8%–40.3%] vs. 12.8% [7.4%–18.2%], $P = 0.004$) and echocardiographic parameters of the left atrial volume index ($R = 0.543$, $P = 0.01$), LAA depth ($R = 0.452$, $P = 0.02$), and LAA flow velocity ($R = -0.487$, $P = 0.01$) were correlated with the LAA fibrosis burden.

Conclusion:

This study provided histological evidence that LAA fibrosis is related to LAAT formation. Echocardiographic parameters of LAA remodeling and function were correlated with the LAA fibrosis burden.

Keywords: atrial fibrillation, fibrosis, left atrial appendage, thrombosis, transesophageal echocardiography

Introduction

Atrial fibrillation (AF) is associated with a 5-fold risk of stroke, and AF-related stroke patients have a 2.5-fold increased risk of mortality (1). Regarding nonvalvular AF patients, 90% of left atrial thrombus were formed in the left atrial appendage (LAA) and thrombotic embolisms were mostly derived from the LAA (2). Cardioversion, catheter ablation, and percutaneous LAA closure are contraindicated in cases with a LAA thrombus (LAAT) (3, 4). Therefore, elucidating the pathophysiological mechanism as well as improving the diagnostic accuracy of LAAT are clinically important for preventing thromboembolic complications. The AF burden is positively correlate with AF-related stroke events (5) and non-paroxysmal AF patients are more likely to develop a thromboembolism compared with those with paroxysmal AF (6). In a recent review article, Shen et al. suggested that atrial cardiomyopathy induced atrial remodeling and contributed to LAAT formation (7). Previous clinical studies suggested the involvement of LAA remodeling in LAAT (8). However, histological evidence is absent in this issue and the pathophysiological mechanisms of LAAT formation have not been fully elucidated. Recently, thoracoscopic stand-alone left atrial appendectomy has gained consensus as a novel surgical option for AF patients with a higher risk of thromboembolism (9). In this study, we aimed to compare the clinical and echocardiographic parameters with the histological evaluation of LAA tissue collected during cardiac surgery in patients with or without LAAT.

Methods

Enrollment

A total of 89 patients who underwent a thoracoscopic stand-alone left atrial appendectomy (N = 24) or a left atrial appendectomy concomitant with another cardiac surgery (N = 65) between January 2020 and February 2021 at Hiroshima University Hospital were enrolled in the study. Patients who underwent mitral valve surgery (N = 25) were excluded from the study and 64 patients were included in the analyses. Among the 64 patients, 16 had paroxysmal AF and 26 had non-paroxysmal AF. The other 22 were included in the non-AF group. Among the 64 patients, LAATs were documented in 16 with at least one TEE examination for each patient, while the other 48 patients did not have any evidence of a LAAT before surgery. The enrolled patients had retest transthoracic echocardiography (TTE) and transesophageal echocardiography (TEE) examinations within one month preceding the surgery. All the enrolled patients with paroxysmal and non-paroxysmal AF were appropriately anticoagulated by warfarin (prothrombin time-international normalized ratio 1.6 to 3.0) or direct oral anticoagulants. For patients with AF who underwent LAA excision due to intolerance of continuing anticoagulation, anticoagulation were continued until the successful LAA excision.

This study was approved by the Ethics Committee of Hiroshima University Graduate School of Biomedical and Health Sciences (E-1931) and conducted in compliance with the ethical principles of the Declaration of Helsinki. All patients provided written informed consent before participating.

AF definition

AF was diagnosed when observed in more than one test, including 12-lead electrocardiogram testing, 24-h Holter recording, long-term Holter recording, or mobile electrocardiogram monitoring. Paroxysmal AF was defined as recurrent AF that spontaneously terminated within seven days. Non-paroxysmal AF was defined as AF that was sustained

beyond seven days and included long-standing and permanent AF. Longstanding AF was defined as AF that was sustained more than a year without return to sinus rhythm. Patients who did not have a history of AF documentation before the operations were included as non-AF controls. Patients with AF that was first documented in the post-operative period were not included in the AF group but rather in the non-AF group.

Baseline data acquisition

Baseline clinical characteristics, including age, gender, body mass index, risk factors (hypertension, diabetes mellitus, and hyperlipidemia), history of heart failure and cerebral infarction, anticoagulation therapy, and laboratory data were recorded within three days before the operation. The CHA2DS2-VASc score was calculated in the fixed manner (10) using baseline data.

TTE and TEE

TTE examinations were performed using commercially-available systems (Artida2, Toshiba, Tokyo, Japan; Vivid E9, GE Medical Systems, Milwaukee, WI, USA). Experienced echocardiographers conducted all TTE examinations and analyses of parameters. All images were obtained in parasternal short- and long-axis views or apical two- and four-chamber views according to the American Society of Echocardiography guidelines (11). The left atrial volume was measured in apical two- and four-chamber views at end-diastole. The Left atrial volume index was calculated as left atrial volume (mL)/body surface area (m^2). The left ventricular ejection fraction was measured in apical two- and four-chamber views with a modified Simpson's method. The left ventricular dimension was measured in a parasternal long-axis view at end-diastole. Transmitral early diastolic inflow (E) wave data were obtained in the three-chamber view. Pulsed-wave tissue Doppler early diastolic mitral annular velocity (e')

was measured at the septal annular site, and the ratio of E to e' (E/e') was calculated.

TEE examinations were performed using an EPIQ7 ultrasound imaging system (Philips, Andover, MA, USA) and a X8-2t 3D TEE transducer (Philips, Andover, MA, USA). LAA images were obtained from the base of the heart with rotation of the probe (0° to 180°). The LAA flow velocity was measured in the basal short-axis view using the pulsed Doppler method as the maximum value of five continuous cardiac cycles. In the longitudinal view, the maximal LAA area was measured with the planimetry method (***Figure 1-A***). The LAA orifice diameter and LAA depth in end-diastole were measured at four views (0°, 45°, 90°, and 135°; ***Figure 1-B***). The maximum values were adopted as the LAA orifice diameter and LAA depth. Spontaneous echo contrast (SEC) was visually classified into five grades (none, 0; mild, 1+; moderate, 2+; moderate to severe, 3+; severe, 4+) according to previous reported criteria (12). We defined grades 0 to 2+ as the non-dense SEC group and grades 3+ and 4+ as the dense SEC group. LAA sludge was a markedly pronounced SEC without becoming a LAAT (***Figure 1-C***). A LAAT was a mass with a different high echogenic density from the LAA wall that was attached to the LAA wall and moved independently of it. The evidence for a LAAT was obtained in more than one TEE view. A representative image of a LAAT is presented in ***Figure 1-D***. All the TEE examinations were performed and interpreted on and off-line by experienced echocardiographers who were not always blinded to the patient baseline data but were completely blinded to the histological results of LAA. All the patients in the non-paroxysmal AF group had an AF rhythm and all in the paroxysmal AF group had a sinus rhythm during the TTE and TEE measurements.

LAA excision

Patients with a LAAT or LAA sludge despite adequate anticoagulation therapy and AF patients with higher CHA_2DS_2-VASc scores who were intolerant of continuing anticoagulation

therapy received a thoracoscopic stand-alone left atrial appendectomy. Thoracoscopic stand-alone left atrial appendectomies were performed as previously reported by Ohtsuka et al. (9). For the other patients, LAA excisions were performed concomitant with other surgical procedures. The primary cardiac disease and operative methods for each patient are listed in ***Supplemental Table 1***.

Histological assessment of LAA fibrosis

All the excised 64 LAAs were immediately formalin fixed and evaluated histologically. Two 10 × 10 mm sections were randomly resected from the distal side of each LAA sample (***Figure 2-A***). The paraffin-embedded sections of LAA (4.5 μm) were prepared for morphological analysis. The fibrosis burdens were evaluated with Azan–Mallory staining in addition to routine hematoxylin–eosin staining (***Figure 2-B and C***). For measurement of the LAA fibrosis burden, one macroscopic field was randomly selected in each LAA section with Azan–Mallory staining and were imaged under 12.5× magnification. In each photograph, red-colored cardiac muscle areas and blue-colored fibrosis areas were measured using the image processing software ImageJ and were analyzed as follows: fibrosis burden = fibrosis area [blue] / (fibrosis area [blue] + cardiac muscle area [red]) × 100 (%). Then, the average of the fibrosis burden of the two fields was calculated. The epicardium, endocardium, fatty tissue, and perivascular tissue were excluded from the analysis (***Figure 2-C and D***). ***Supplemental Figure*** shows representative LAA staining images. All the histological evaluations were performed by two certified pathologists who were blinded to the clinical data.

Statistical analysis

Normally distributed continuous data were compared with a one-way analysis of variance (ANOVA) as appropriate and presented as the mean ± SD. If the data were not

normally distributed, they were presented as the median and interquartile range and analyzed with nonparametric methods. In the nonparametric analyses, comparisons among the two groups were performed using the Wilcoxon rank sum test and comparisons among the three groups were performed using the Kruskal–Wallis test. Multiple comparison tests among the three groups were performed using a one-way ANOVA followed by Tukey's post-hoc test when normally distributed, and by Steel–Dwass test when not normally distributed. Categorical variables were compared with a chi-squared test or Fisher's exact test, as appropriate, and presented as percentages of the group total. Correlations between two continuous variables were analyzed with Pearson's correlation coefficient test. The correlation coefficient (R) was analyzed with the restricted maximum likelihood method. All statistical analyses were performed using JMP software version 12.0 (SAS Institute, Cary, NC, USA), with a P-value < 0.05 considered to indicate statistical significance.

Results

Baseline characteristics, echocardiographic parameters, and AF types

The baseline characteristics and echocardiographic parameters among the three groups (non-AF, paroxysmal AF, and non-paroxysmal AF) are shown in ***Supplemental Table 2***.

LAA fibrosis burden and AF types

The LAA fibrosis burden was significantly higher in both the paroxysmal and non-paroxysmal AF groups compared with the non-AF group (***Figure 3***). A significant correlation was found between the fibrosis burden and AF types (non-AF, 2.9% [2.0%–6.1%]; paroxysmal AF, 10.5% [5.5%–17.9%]; non-paroxysmal AF, 18.6% [12.1%–36.5%]; paroxysmal AF vs.

non-AF, $P = 0.005$; non-paroxysmal AF vs. non-AF, $P < 0.0001$; non-paroxysmal AF vs. paroxysmal AF, $P = 0.057$). The associations of the LAA fibrosis burden with the other clinical parameters (age, body mass index, estimated glomerular filtration rate, and CHADS2-VASc score) are shown in ***Figure 4***. The LAA fibrosis burden was negatively correlated with the estimated glomerular filtration rate ($R = –0.28$, $P = 0.04$).

Baseline characteristics, echocardiographic parameters, and LAAT

In the study cohort, 16 patients developed LAATs and 15 of them were in the non-paroxysmal AF group. ***Table 1*** shows the baseline characteristics and echocardiographic parameters according to the presence or absence of LAAT. All patients with a LAAT had AF. Patients with a LAAT had a significantly higher prevalence of non-paroxysmal AF and higher NT-proBNP levels than those without a LAAT. Among the TTE parameters, the left atrial volume index was significantly higher in the patients with a LAAT than in those without. TEE showed that the LAA orifice diameter and the LAA area were significantly larger, the LAA depth was significantly deeper, the prevalence of dense SEC and LAA sludge were significantly higher, and the LAA flow velocity was significantly lower in the patients with a LAAT than in those without. In ***Table 2***, non-paroxysmal AF patients were analysed separately. Among the non-paroxysmal AF patients, the LAA depth was significantly deeper and the LAA flow velocity was significantly lower in the patients with a LAAT than in those without.

LAA fibrosis burden and LAAT

Figure 5 shows the comparison between patients with and without a LAAT regarding LAA fibrosis burden. Patients with a LAAT had a significantly higher LAA fibrosis burden than those without (23.8% [14.8%–40.3%] vs. 6.5% [2.8%–13.2%] , $P < 0.0001$; ***Figure 5-A***). The association was also significant when the non-paroxysmal AF group was

analyzed separately (24.8% [15.2%–40.3%] vs. 12.8% [7.4%–18.2%], P = 0.002; ***Figure 5-B***).

LAA fibrosis burden and echocardiographic parameters

The LAA fibrosis burden was positively correlated with the left atrial volume index ($R = 0.533$, $P < 0.0001$), the LAA orifice diameter ($R = 0.309$, $P = 0.02$), and the LAA depth ($R = 0.451$, $P = 0.0002$), while it was inversely correlated with the LAA flow velocity ($R = -0.576$, $P < 0.0001$; ***Figure 6***). When the non-paroxysmal AF group was analyzed separately, the LAA fibrosis burden was positively correlated with the left atrial volume index ($R = 0.543$, $P = 0.01$) and the LAA depth ($R = 0.452$, $P = 0.02$), while it was inversely correlated with the LAA flow velocity ($R = -0.486$, $P = 0.01$; ***Figure 7***).

Discussion

In this histological study, we reveal the following findings: 1) patients with AF had an increased fibrosis burden in the LAA tissue compared with those without; 2) patients who developed a LAAT had a higher LAA fibrosis burden compared with those without a LAAT; 3) LAA fibrosis burden was positively correlated with the echocardiographic parameters related to LAA remodeling and function. To the best of our knowledge, this is the first report to histologically reveal a positive association between LAA fibrosis and LAAT.

Clinical and histological factors associated with LAAT

Non-paroxysmal AF, NT-proBNP levels, and echocardiographic parameters related to left atrial/LAA enlargement, and decreased LAA function were found to be associated with LAAT. Typically, AF burden is an important factor that contributes to the development of

thromboembolic events (5, 6). NT-proBNP level has also been reported as an independent predictor of LAAT in nonvalvular AF patients (13). Elevated NT-proBNP levels are linked to long-term increases in left atrial/LAA pressure, which leads to left atrium/LAA structural remodeling. Although the NT-proBNP level did not relate to LAAT among non-paroxysmal AF patients in our study, this may because patients without a LAAT must have an underlying cardiac disease that can increase the NT-proBNP level. Previous echocardiographic studies compared morphologic parameters of LAA and LAAT, and suggested that left atrium/LAA enlargements contributed to LAAT formation (14-16). Consistent with previous reports, we found that left atrial volume, LAA orifice dimension, LAA depth, and LAA flow velocity were associated with LAAT. However, histological evidence has been absent regarding this issue.

LAA fibrosis and LAAT

The progression of atrial fibrosis is related to different factors such as aging, atrial stretch, inflammation, and oxidative stress (7). In this small cohort, LAA fibrosis burden was negatively correlated with the estimated glomerular filtration rate, although not correlated with the age and CHADS2-VASc score. On the other hand, the relationship between LAA fibrosis and LAAT has not been confirmed. Delayed-enhanced magnetic resonance imaging can visualize atrial fibrosis with slow washout kinetics of gadolinium (17). Daccarett et al. estimated the atrial fibrosis burden with delayed-enhanced magnetic resonance imaging (MRI) and found a positive correlation between atrial fibrosis burden and previous stroke among patients with AF (18). The left atrial low-voltage zone assessed with a multielectrode catheter has been reported to be a hallmark of left atrial fibrosis and is related to thromboembolic risk in AF patients (19). On the other hand, evaluation of local fibrosis in the LAA by clinical modalities is still challenging because of the thin LAA wall and morphological complexity (20). In this study, we elucidated the relationship between LAA

fibrosis and LAAT using histological evaluation. Patients who developed a LAAT were shown to have a higher LAA fibrosis burden. This finding may provide important evidence for issues related to clinical parameters.

Echocardiographic parameters and LAA fibrosis

Atrial structural remodeling is characterized by changes in the atrial size and function and has been macroscopically explained by increased interstitial fibrosis with cardiac structural alterations (21, 22). Although the echocardiographic measurements of left atrium and LAA enlargements are often considered to be parameters of structural remodeling, there has been no comparison of these parameters with LAA fibrosis. In this study, the left atrial volume index, LAA orifice diameter, and LAA depth were positively correlated with the LAA fibrosis burden. These findings support the validity of using echocardiographic measurements as an indicator of structural remodeling. On the other hand, the evaluation of LAA function is challenging due to the complexity of its shape, especially during AF rhythms. The LAA flow velocity measured with the pulsed-wave Doppler method is considered the standard parameter of LAA function (23) and it has been reported to be associated with thromboembolic risk in AF patients (24, 25). Our findings demonstrated that the progression of LAA fibrosis was negatively correlated with impaired LAA function. The LAA flow velocity was revealed to be a clinical parameter of LAA fibrosis.

Possible mechanism linking LAA fibrosis and LAAT

Atrial fibrosis is considered to be the substrate for AF perpetuation (26), and atrial fibrosis burden contributes to the development of an electrophysiological reentrant region and electrical drivers of AF (27). The LAA is responsible for about 25% of AF recurrences after pulmonary vein isolation (28), and some studies have demonstrated that electrical isolation of

LAA improves the catheter ablation outcome in non-paroxysmal AF patients (29, 30). A recent histological study reported that LAA fibrosis is associated with AF recurrence after endoscopic ablation (31). Considering this evidence, LAA fibrosis seems to contribute to AF perpetuation through the reentrant region and electrical drivers, which leads to LAAT formation. On the other hand, AF promotes atrial fibrosis (32). Mayyas et al. reported that cardiac endothelin-1, which reflects atrial wall stress, related to AF persistence and atrial fibrosis (33). In addition, we found a negative correlation between LAA fibrosis and LAA function. LAA fibrosis impairs LAA contractility and causes local blood stasis in the LAA cavity. In a previous report, Thambidorai et al. found that LAAT was less common in patients with mitral regurgitation, which may be due to the "washing" of the LAA by the regurgitant blood flow (34). This finding can support the association of local blood stasis with LAAT formation. Inflammation can also link to AF development, atrial fibrosis, endothelial dysfunction, and LAAT (34–36). In this study, serum high sensitivity C-reactive protein level did not relate to LAAT. This may because patients without a LAAT had another underlying cardiac disease that was subject to the operation. Now we are conducting further histological study to elucidate underlying mechanism relates to LAAT formation.

Study limitations

First, patients without a LAAT and non-AF control subjects had another underlying cardiac disease that was an indication for cardiac surgery. However, obtaining LAA samples from healthy subjects would have been ethically impossible. In this study, we excluded mitral valve disease, which is considered to have a great impact on left atrial/LAA hemodynamics and remodeling. Second, this was an observational study with a small sample size. Third, although we calculated the fibrosis burden using an average of two different points of the LAA, maldistribution of fibrosis may remain as a possible bias. Fourth, we did not perform cardiac

MRI which can clinically estimate the overall amount of left atrial fibrosis. Fifth, the cardiac rhythm during the echocardiographic examinations would affect the echocardiographic parameters. In addition, a referral bias may exist especially in patients referred for a thoracoscopic stand-alone left atrial appendectomy.

Conclusion

Using histological evaluation of LAA tissue, we demonstrated an association between LAA fibrosis and LAAT. This finding is valuable because it brings new evidence to consider together with previous clinical studies.

Acknowledgments: We thank the members of the clerical and medical staff at Hiroshima University Hospital for their assistance. We thank the ENAGO Group (English editing system) for editing a draft of this manuscript.

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Figure legends

Figure 1. Transesophageal echocardiography parameters

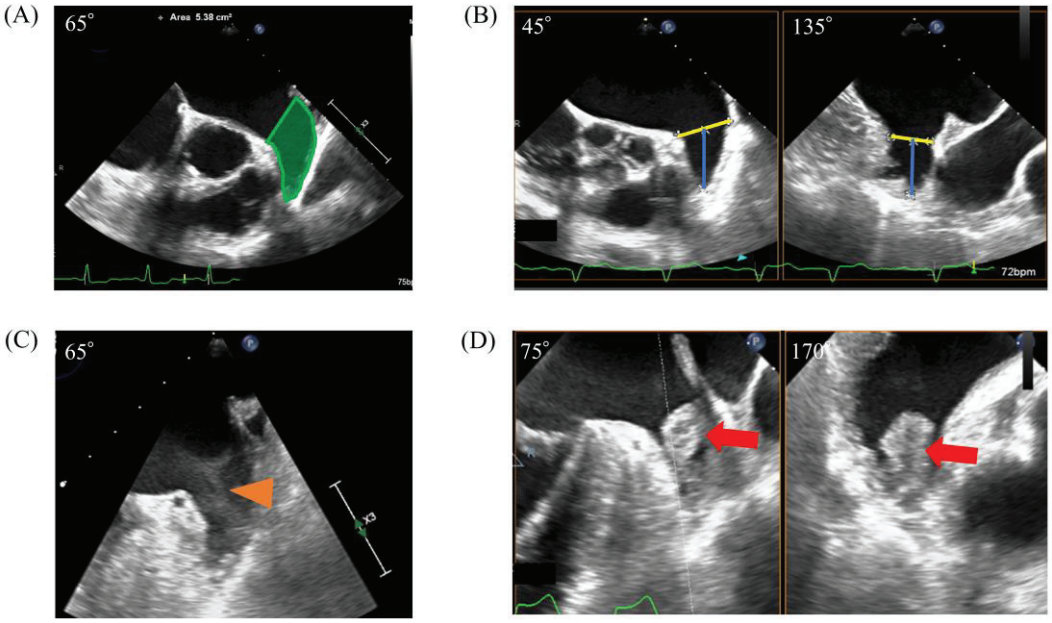


Figure 1-A: The maximal LAA area was traced in the longitudinal view (green).

Figure 1-B: LAA orifice diameter (yellow line) and LAA depth (blue line) in end-diastole were measured in four views (0°, 45°, 90°, and 135°). LAA depth was measured as the maximum distance from the orifice line to the apex of the LAA.

Figure 1-C: LAA sludge was a markedly pronounced spontaneous echo contrast without becoming a LAAT (allow head orange).

Figure 1-D: LAAT was a mass with a different echogenic density from the LAA wall that attached to the LAA wall and moved independent of it. The evidence for a LAAT was detected in more than one TEE view (allow head red).

LAA, left atrial appendage; LAAT, left atrial appendage thrombus

Figure 2: LAA sample and assessment of LAA fibrosis

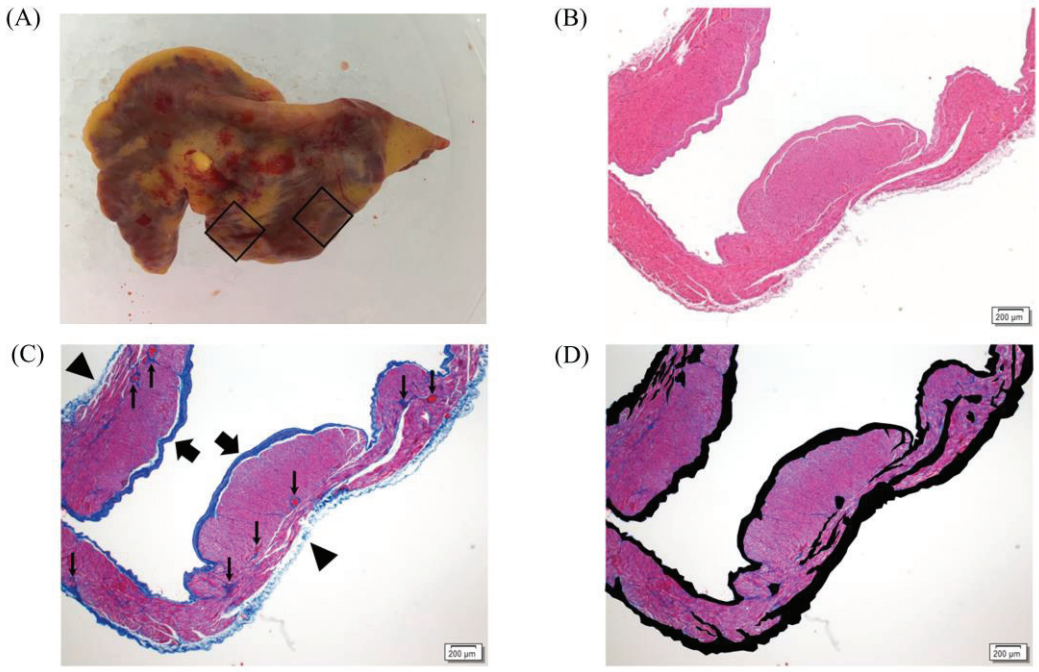


Figure 2-A: 10 × 10 mm tissue sections are obtained from the end side of the LAA.

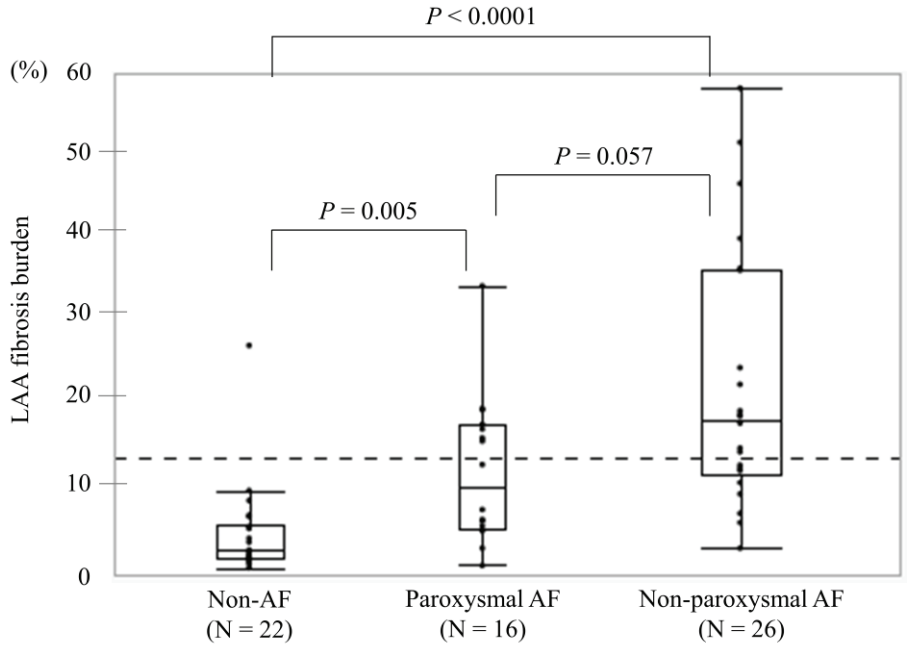
Figure 2-B: Hematoxylin–eosin staining of the LAA tissue (×12.5).

Figure 2-C: Azan–Mallory staining of the LAA tissue (×12.5). Arrow head (black), epicardium; thick arrow (black), endocardium; thin arrow (black), small vascular and peri-vascular connective tissue.

Figure 2-D: The epicardium, endocardium, vascular, connective tissue, and fatty tissue were excluded (colored in black). The fibrosis burden were calculated as 10.5% in this image.

LAA, left atrial appendage

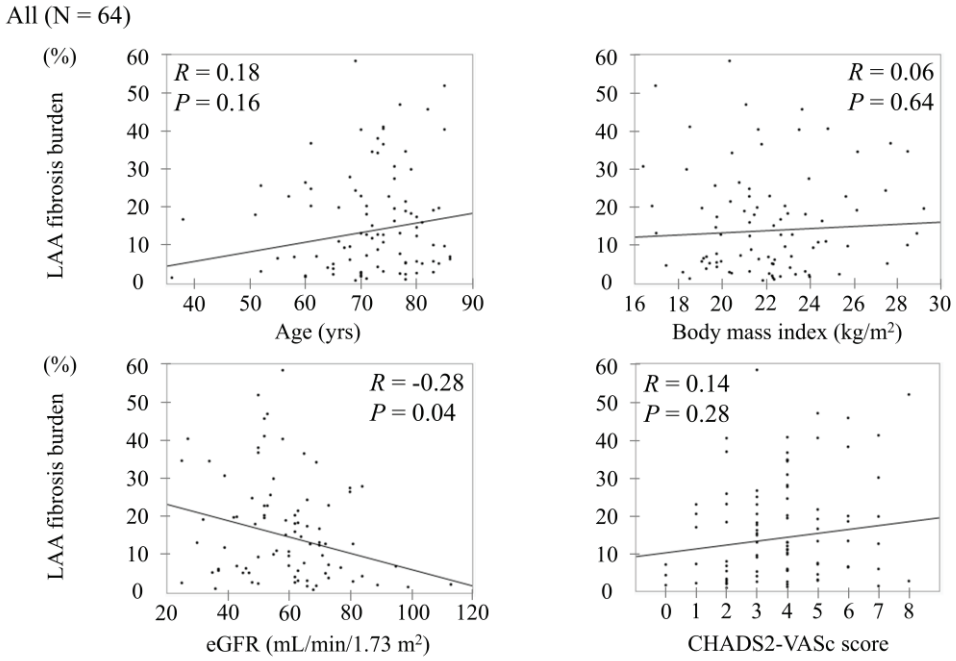
Figure 3. Association of the LAA fibrosis burden with AF types



Statistical differences among the three groups (non-AF, paroxysmal AF, and non-paroxysmal AF) were evaluated with the Steel–Dwass test. The paroxysmal and non-paroxysmal AF patients had a significantly higher LAA fibrosis burden than non-AF patients ($P = 0.005$ and $P < 0.0001$, respectively). The LAA fibrosis burden in the non-paroxysmal AF patients tended to be higher than that in the paroxysmal AF. The difference approached statistical significance ($P = 0.057$).

AF, atrial fibrillation; LAA, left atrial appendage

Figure 4. Correlation between the LAA fibrosis burden and clinical parameters



The LAA fibrosis burden was negatively correlated with the eGFR (R = –0.28, P = 0.04), although not correlated with the age (R = 0.18, P = 0.16), body mass index (R = 0.06, P = 0.64), and CHADS2-VASc score (R = 0.14, P = 0.64).

LAA, left atrial appendage; eGFR, estimated glomerular filtration rate

Figure 5. Association of the LAA fibrosis burden with a LAAT

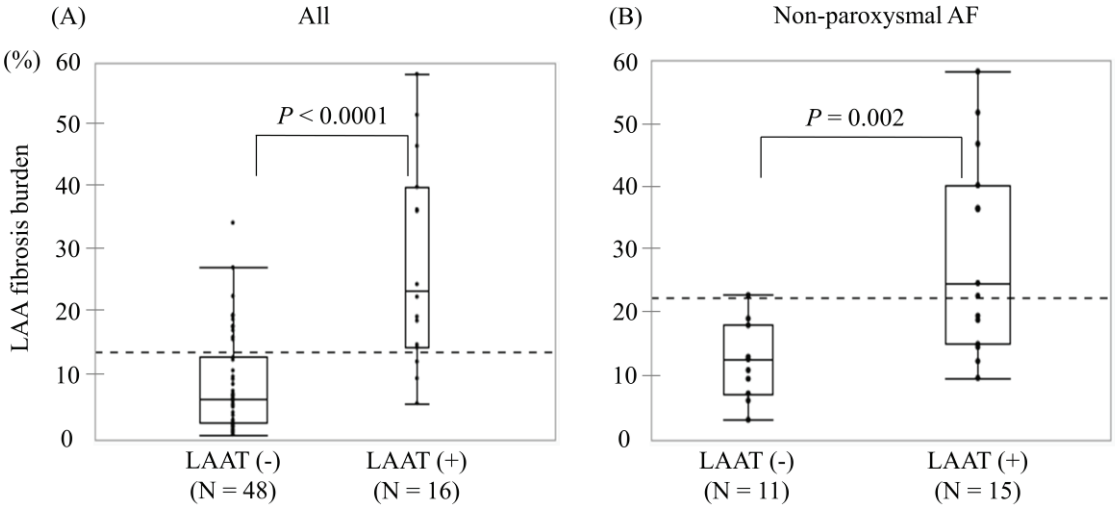
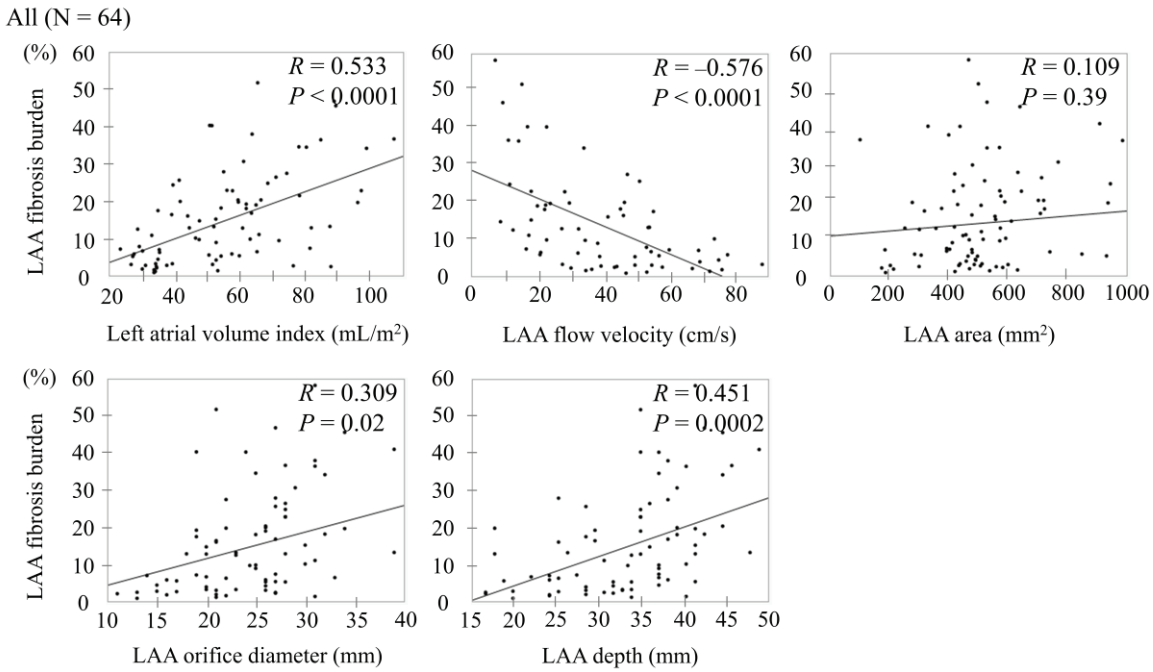


Figure 5-A: Patients with a LAAT had a significantly higher LAA fibrosis burden than those without (23.8% [14.8%–40.3%] vs. 6.5% [2.8%–13.2%], $P < 0.0001$).

Figure 5-B: Among non-paroxysmal AF patients, patients with a LAAT had a significantly higher LAA fibrosis burden than those without (24.8% [15.2%–40.3%] vs. 12.8% [7.4%–18.2%], $P = 0.002$).

LAAT, left atrial appendage thrombus; LAA, left atrial appendage; AF, atrial fibrillation

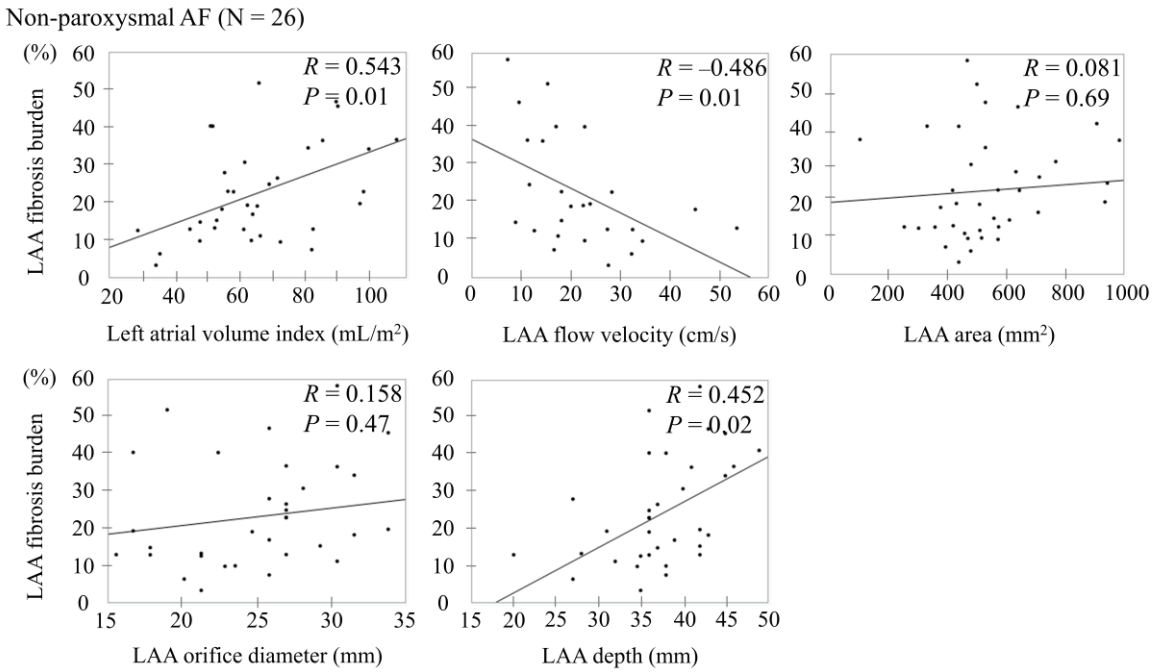
Figure 6. Correlation between the LAA fibrosis burden and echocardiographic parameters



The LAA fibrosis burden was positively correlated with the left atrial volume index (R = 0.533, P < 0.0001), the LAA orifice diameter (R = 0.309, P = 0.02), and the LAA depth (R = 0.451, P = 0.0002), but negatively correlated with the LAA flow velocity (R = –0.576, P < 0.0001).

AF, atrial fibrillation; LAA, left atrial appendage

Figure 7. Correlation between the LAA fibrosis burden and echocardiographic parameters for non-paroxysmal AF



Among non-paroxysmal AF patients, the LAA fibrosis burden was positively correlated with the left atrial volume index ($R = 0.543$, $P = 0.01$) and the LAA depth ($R = 0.452$, $P = 0.02$), but negatively correlated with the LAA flow velocity ($R = –0.486$, $P = 0.01$).

AF, atrial fibrillation; LAA, left atrial appendage

Table 1. Comparison of baseline characteristics and echocardiographic parameters according to the presence or absence of a LAAT.

	LAAT (+) (N = 16)	LAAT (-) (N = 48)	*P*-value
AF, N (%)	16 (100)	25 (52)	< 0.0001
Non-paroxysmal AF, N (%)	15 (94)	12 (25)	< 0.0001
Longstanding AF, N (%)	8 (50)	6 (12.5)	0.003
Age, yrs	76.7 ± 7.1	71.2 ± 9.9	0.10
Male, N (%)	11 (69)	33 (69)	0.65
Body mass index, kg/m^2	22.5 ± 3.2	22.3 ± 2.7	0.81
Hypertension, N (%)	12 (71)	37 (77)	0.51
Diabetes mellitus, N (%)	4 (25)	16 (33)	0.53
Hyperlipidemia, N (%)	6 (38)	18 (38)	1.00
CHA_2DS_2-VASc score	4.3 ± 1.8	3.6 ± 1.8	0.23
eGFR, mL/min/1.73 m^2	54.6 ± 11.4	61.8 ± 20.0	0.18
Hs-CRP, mg/L	0.08 (0.04–0.18)	0.11 (0.04–0.44)	0.26
NT-pro BNP, pg/mL	1467 (689–2466)	527 (282–1616)	0.02
Transthoracic echocardiography			
Left ventricular ejection fraction, %	56.6 ± 13.0	57.0 ± 10.8	0.90

Left ventricular end-diastolic dimension, mm	47.1 ± 6.8	50.4 ± 8.1	0.15
Left atrial volume index, mL/m^2	65.7 ± 20.7	47.8 ± 18.2	0.002
E/e’	16.3 ± 7.3	17.9 ± 10.4	0.59
Transesophageal echocardiography			
LAA flow velocity, cm/s	16.6 ± 6.2	46.7 ± 17.8	< 0.0001
LAA orifice diameter, mm	26.3 ± 4.2	22.2 ± 5.9	0.01
LAA depth, mm	38.9 ± 3.3	31.6 ± 7.2	0.0002
LAA area, mm^2	576 ± 248	465 ± 193	0.07
LAA dense SEC, N (%)	15 (94)	10 (21)	< 0.0001
LAA sludge, N (%)	14 (88)	10 (21)	< 0.0001

LAAT, left atrial appendage thrombus; AF, atrial fibrillation; eGFR, estimated glomerular filtration rate; hs-CRP, high sensitivity C-reactive protein; NT-proBNP, N-terminal pro-brain natriuretic peptide; LAA, left atrial appendage; SEC, spontaneous echo contrast

Table 2. Comparison of baseline characteristics and echocardiographic parameters according to the presence or absence of a LAAT among non-paroxysmal AF patients.

	LAAT (+) (N = 15)	LAAT (-) (N = 11)	*P*-value
Age, ys	75.2 ± 7.7	71.5 ± 4.5	0.16
Male, N (%)	10 (67)	9 (82)	0.38
Longstanding AF, N (%)	8 (53)	6 (55)	0.95
AF duration, month	15 (6-124)	15 (6-43)	0.66
OAC, DOAC, N (%)	12 (80)	10 (91)	0.43
OAC, warfarin, N (%)	3 (20)	1 (9)	0.43
Body mas index, kg/m2	22.6 ± 3.2	23.6 ± 2.6	0.42
Hypertension, N (%)	11 (73)	8 (73)	0.97
Diabetes mellitus, N (%)	4 (27)	2 (18)	0.61
Hyperlipidemia, N (%)	5 (33)	4 (36)	0.87
CHA2DS2-VASc score	4.3 ± 1.9	3.7 ± 1.3	0.42
eGFR, mL/min/1.73m2	55.7 ± 10.7	57.6 ± 17.3	0.73
Hs CRP, mg/L	0.08 (0.04–0.19)	0.08 (0.05–0.54)	0.33
NT-pro BNP, pg/mL	1318 (676–2552)	1268 (718–2607)	0.70
Transthracic echocardiography			
Left ventricular ejection fraction, %	55.7 ± 13.0	55.7 ± 9.0	1.00
Left ventricular end-diastolic dimension, mm	47.5 ± 6.7	46.6 ± 4.1	0.70
Left atrial volume index, mL/m2	66.3 ± 21.3	54.1 ± 13.7	0.11
E/e'	15.5 ± 7.3	21.0 ± 13.6	0.22

Transesopheageal echocardiography			
LAA emptying flow velocity, cm/s	16.3 ± 2.3	29.9 ± 2.7	0.0008
LAA orfice diameter, mm	26.3 ± 4.4	23.9 ± 4.8	0.21
LAA depth, mm	38.9 ± 3.4	32.5 ± 6.4	0.003
LAA area, mm2	558.2 ± 245.7	422.1 ± 81.0	0.09
LAA dense SEC, N (%)	14 (93)	7 (64)	0.06
LAA sludge, N (%)	13 (87)	7 (64)	0.17

LAAT, left atrial appendage thrombus; AF, atrial fibrillation; OAC, oral anticoagulant; DOAC, direct oral anticoagulant; eGFR, estimated glomerular filtration rate; hs-CRP, high sensitivity C-reactive protein; NT-proBNP, N-terminal pro-brain natriuretic peptide; LAA, left atrial appendage; SEC, spontaneous echo contrast