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ORIGINAL RESEARCH

Improvement of Vascular Endothelial Function Reflects Nonrecurrence After Catheter Ablation for Atrial Fibrillation

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BACKGROUND: The clinical implication of vascular endothelial dysfunction in patients with atrial fibrillation (AF) remains unclear. This study aimed to elucidate the correlation between changes in vascular endothelial function assessed by reactive hyperemia-peripheral arterial tonometry and the effect of sinus rhythm restoration after catheter ablation (CA) for AF.

METHODS AND RESULTS: Consecutive 214 patients who underwent CA for AF were included in this single center, retrospective study. The natural logarithmic transformed reactive hyperemia-peripheral arterial tonometry index (LnRHI) of all patients was measured before CA as well as 3 and 6 months after CA. LnRHI in sinus rhythm was significantly higher than that in AF before CA. Multivariate logistic regression analysis revealed that the presence of AF was an independent risk factor for lowering of LnRHI (odds ratio, 4.092; P=0.002) before CA. The LnRHI was significantly improved 3 and 6 months after CA in patients without AF recurrence. Multivariate Cox hazard analysis revealed that changes in LnRHI from before to 3 months after CA independently correlated with recurrence of AF (hazard ratio, 0.106; P=0.001). Receiver operating characteristic analysis showed the decrease in LnRHI levels from before to 3 months after CA as a significant marker that suspects AF recurrence (area under the curve, 0.792; log-rank test, P<0.001).

CONCLUSIONS: The presence of AF was independently correlated with the impaired vascular endothelial function assessed by the reactive hyperemia-peripheral arterial tonometry. Long-term sinus rhythm restoration after CA for AF might contribute to the improvement of vascular endothelial function, which may reflect the nonrecurrence of AF.

Key Words: atrial fibrillation ■ catheter ablation ■ nonrecurrence predictor ■ reactive hyperemia-peripheral arterial tonometry ■ vascular endothelial function

The vascular endothelium plays an important role in the vasoregulation of coronary or peripheral vessels through the secretion of bioactive substances such as NO.[1] Vascular endothelial dysfunction is induced by the presence of coronary risk factors, and the presence of endothelial dysfunction has been shown to be an independent predictor of cardiovascular events.[2]

Meanwhile, recent studies have revealed that vascular endothelial dysfunction occurs even in patients with atrial fibrillation (AF).[3–8] Shin et al reported that patients with AF have significantly impaired endothelial function, which can be reversed through maintenance of sinus rhythm (SR) by successful catheter ablation (CA).[5] Furthermore, Kobayashi et al revealed that endothelial dysfunction

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CLINICAL PERSPECTIVE

What Is New?

- In the assessment of vascular endothelial function using the reactive hyperemia-peripheral arterial tonometry, the heart rhythm itself of atrial fibrillation (AF) impaired endothelial function, regardless of paroxysmal or persistent AF.
- Vascular endothelial function in patients with AF without recurrence after catheter ablation (CA) was improved to a level similar to those in normal control subject.
- Improvement of endothelial function 3 months after CA for AF compared with before CA was revealed as a significant predictor of nonrecurrence of AF after CA.

What Are the Clinical Implications?

- By assessing the change in vascular endothelial function in patients with AF over time, whether paroxysmal or persistent, or whether measured under sinus rhythm or AF rhythm, it might be possible to comprehensively clarify the successful treatment of AF, ie, nonrecurrence of AF after catheter ablation, including the control of risk factors of AF.

Nonstandard Abbreviations and Acronyms

CA	catheter ablation
LnRHI	natural logarithmic transformed reactive hyperemia-peripheral arterial tonometry index values
PAT	peripheral arterial tonometry
RHI	reactive hyperemia-peripheral arterial tonometry index
RH-PAT	reactive hyperemia-peripheral arterial tonometry
SR	sinus rhythm

was associated with AF recurrence after CA,[6] suggesting that endothelial function may be able to predict the recurrence of AF after CA. However, although Okawa et al reported that endothelial function in patients with persistent AF was significantly lower than that in patients with paroxysmal AF,[7] Matsue et al reported that there was no significant difference in endothelial function between paroxysmal and persistent AF.[8] Therefore, clinical implication of vascular endothelial dysfunction and the change in endothelial function in patients with AF have not been fully investigated, remaining inadequate.

METHODS

Study Population

The authors declare that all supporting data are available within the article. We conducted a retrospective observational study to investigate the clinical significance of vascular endothelial function in patients with AF. We recruited 308 consecutive patients who were referred to Kumamoto University Hospital and underwent CA for AF between January 2013 and December 2016. Patients with the following criteria were excluded: obstructive coronary artery disease, epicardial coronary artery spasm, microvascular coronary artery spasm, peripheral artery disease, cardiomyopathy, congestive heart failure, chronic kidney disease, and malignant tumor (Figure 1). We assessed their vascular endothelial function using reactive hyperemia-peripheral arterial tonometry (RH-PAT) before CA as well as 3 and 6 months after CA, whether under AF rhythm or SR. Furthermore, patients in whom we could not assess the vascular endothelial function accurately because of the loss of measurement or an unfavorable condition at the time of measurement were also excluded. Therefore, 214 patients with AF who underwent CA for paroxysmal AF (n=163) and persistent AF (n=51) were included in this study (Figure 1). In addition, we included 43 normal control patients without a history of AF and other exclusion criteria, to compare the vascular endothelial function between patients with AF and normal control subjects. Classification of paroxysmal and persistent AF was defined according to the 2017 expert consensus statement on catheter and surgical ablation of atrial fibrillation.[9] Thus, this study also included some patients with persistent AF who had been defibrillated before CA and returned to SR or spontaneously returned to SR after lasting beyond 7 days. The study protocol conformed to the principles of the Declaration of Helsinki. The study protocol was approved by the Institutional Review Board of Kumamoto University (approval number, Rinri 1406). Written informed consent was obtained from all participating patients. All antiarrhythmic medications were discontinued for 5 half-life periods before the procedure.

Measurement of Vascular Endothelial Function

All patients' vascular endothelial function was assessed by the RH-PAT using Endo-PAT2000 (Itamar Medical, Caesarea, Israel) before CA as well as 3 and 6 months after CA.[10,11] RH-PAT measurement was performed in the morning after the subjects had fasted, before taking medications, and absolutely without smoking and caffeine. A blood pressure cuff was placed on one upper arm (study arm), while the contralateral arm served as a control (control arm). Peripheral arterial tonometry

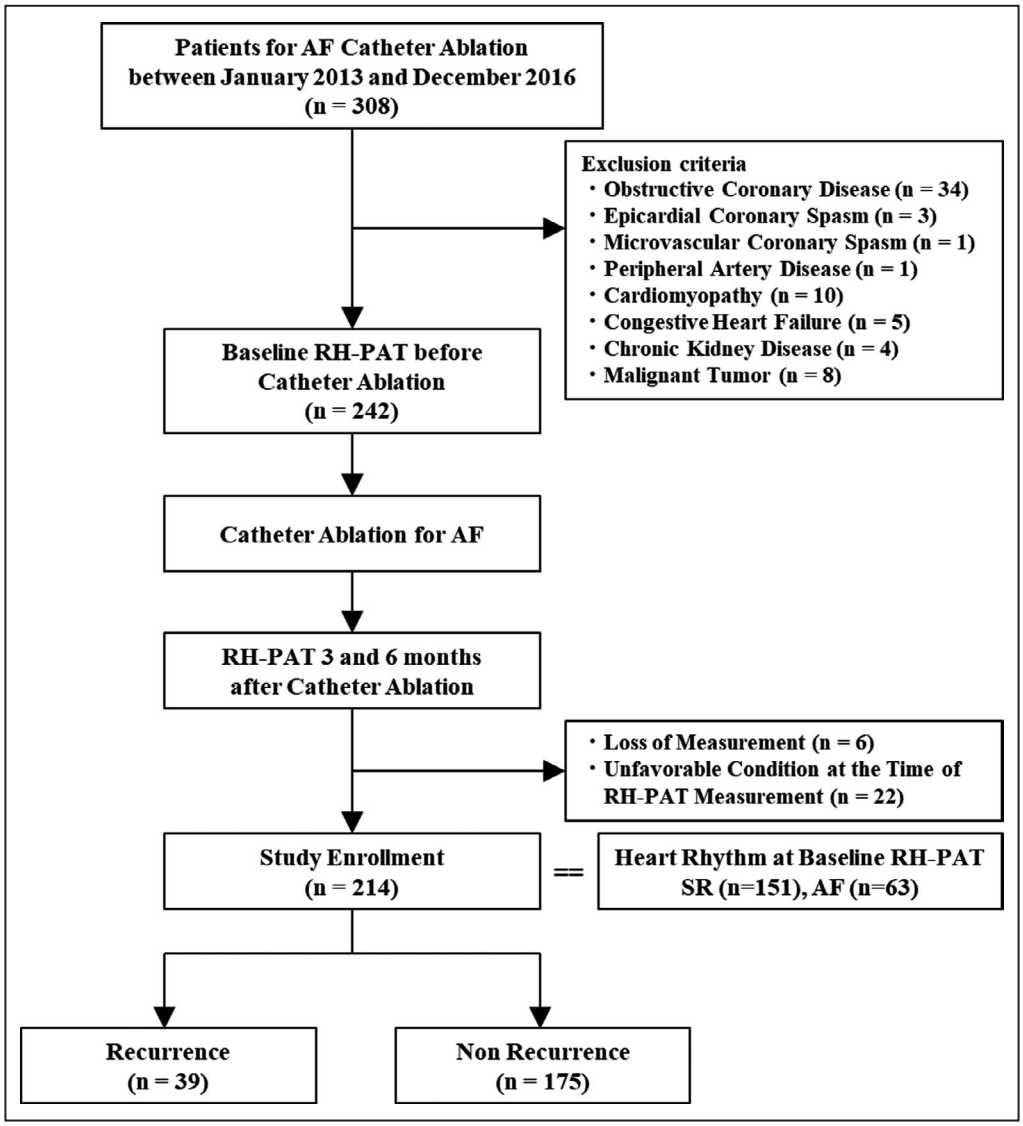
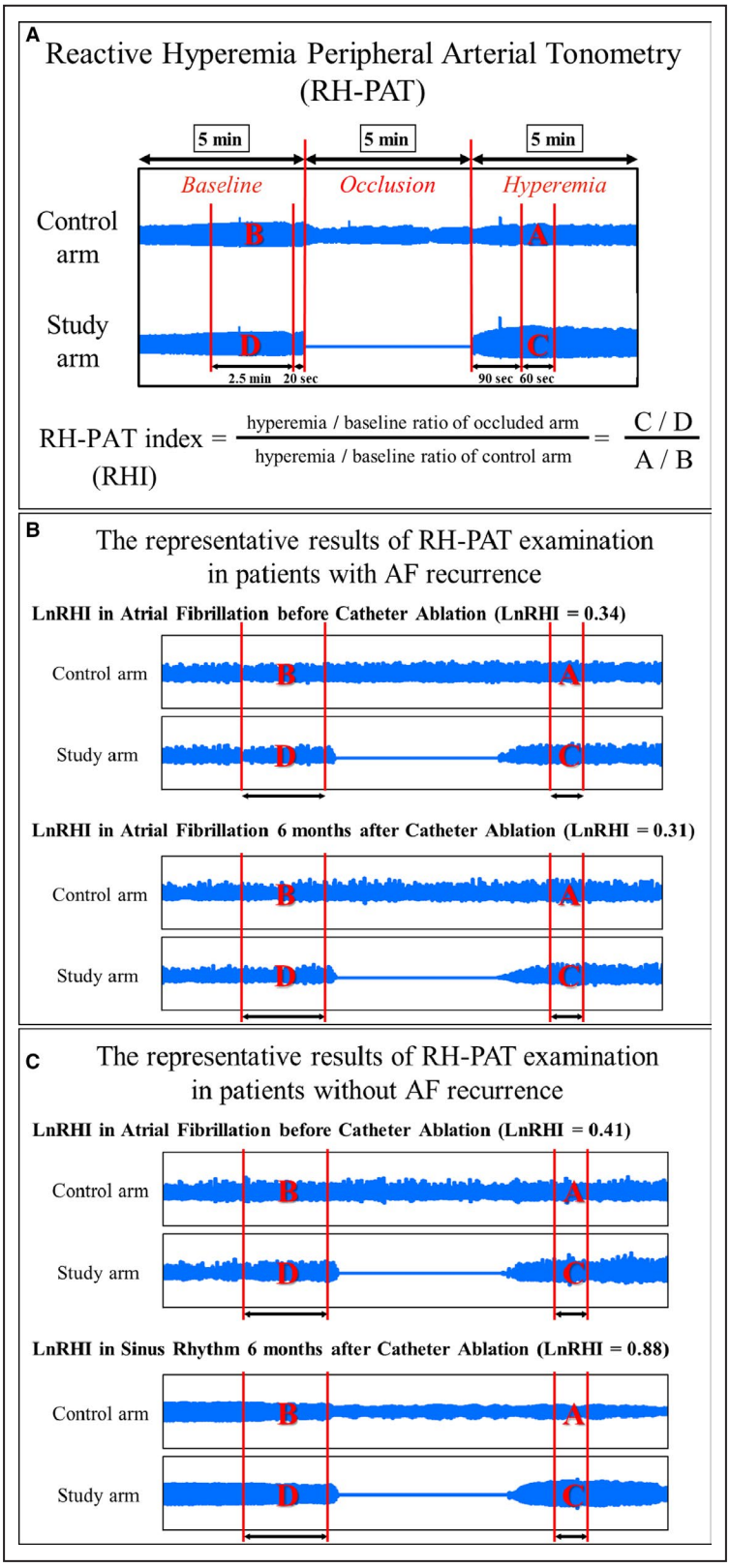


Figure 1. Flowchart of the patient enrollment in this study.
AF indicates atrial fibrillation; RH-PAT, reactive hyperemia-peripheral arterial tonometry; and SR, sinus rhythm.

probes were placed on one finger of each hand. After a 5-minute equilibration period, the cuff was inflated to 60 mm Hg above the systolic blood pressure or 200 mm Hg for 5 minutes, and then deflated to induce reactive hyperemia. The RH-PAT data were digitally analyzed online (Endo-PAT2000 software, version 3.0.4 and 3.4.4, Itamar Medical, Caesarea, Israel) (Figure 2A). The RH-PAT index (RHI) reflects the extent of reactive hyperemia and was calculated as the ratio of the average amplitude of the peripheral arterial tonometry (PAT) signal >1 minute, starting 1.5 minutes after cuff deflation (control arm, A; study arm, C), divided by the average amplitude of the PAT signal of a 2.5 minutes time period before cuff inflation (control arm, B; study arm, D) (Figure 2A). Thus, the RHI was calculated using the following equation: RHI=(C/D)/(A/B). However, because RHI values are not normally distributed, we calculated the natural logarithmically transformed RHI (LnRHI) values for use in analysis, as reported previously.[12,13] The clinical usefulness and reproducibility of RH-PAT technology in our faculty has been described in previous studies.[12–17]

CA and Follow-Up

The methods of CA and follow-up have been also described previously.[18–20] First, pulmonary vein isolation was performed during AF. If the AF was not terminated by pulmonary vein isolation alone, linear roof line lesion and complex fractionated atrial electrogram ablation were performed in all patients, subsequently.



Patients were followed-up in the outpatient clinic at 1, 3, 6, and 12 months after the CA with 12-lead ECGs, 24-hour Holter recordings, and an event recorder (Omron HeartScan801; OMRON, Kyoto, Japan). According to the 2017 expert consensus,[9] the recurrence of AF was defined as the occurrence of

Figure 2. Representative case of reactive hyperemia-peripheral arterial tonometry measurement.
A, The method of RH-PAT measurement and the formula to calculate the RH-PAT index (RHI). **B**, The representative result of RH-PAT examination in patient with atrial fibrillation recurrence. **C**, The representative result of RH-PAT examination in patients without atrial fibrillation recurrence. Since RH-PAT measures arterial dilatation from a 3-dimensional increase in the volumetric pulse wave of the fingertip arteriole vascular bed, the volume pulse wave change rate is large and the sensitivity of the data are also high. RH-PAT may be more suitable for measurement even in pathological conditions such as atrial fibrillation, where the pulse wave varies for each pulse such as upper and lower figure in (**B**) and upper figure in (**C**) which recorded during atrial fibrillation. AF indicates atrial fibrillation; and LnRHI, natural logarithmic transformed reactive hyperemia-peripheral arterial tonometry index.

AF >3 months following AF ablation. The episodes of atrial tachycardia or atrial flutter was also included as a recurrence.

Statistical Analysis

To evaluate the relationship between AF and vascular endothelial function, we compared the clinical variables including LnRHI in each heart rhythm at the time of RHI measurement before CA. The data were expressed as mean±SD, median value with interquartile range, or frequencies (%). The Shapiro-Wilk test was used to evaluate the distribution of continuous data, and the continuous parameters were compared using the Student *t*-test for normally distributed data and the Mann-Whitney *U*-test for parameters with skewed data distribution. Categorical parameters were compared using the Chi-squared test. To examine whether any of the baseline parameters were independently associated with the LnRHI before CA, all patients were split by median and divided into the high-LnRHI and low-LnRHI groups. The correlation between LnRHI and clinical variables containing the presence of AF at the time of RHI measurement were assessed by univariate and multivariate logistic regression analysis before CA.

Furthermore, to clarify the extent to which restoration of SR by CA can improve vascular endothelial function in patients with AF, we examined the serial change in LnRHI for a 6-month period following CA in patients with and without recurrence of AF. Furthermore, to reveal the vascular endothelial dysfunction under the SR in patients with AF, eliminating the influence of heart rhythm, the LnRHI in patients without AF recurrence obtained before CA as well as 3 and 6 months after CA, all recorded during sinus rhythm, were compared with those in the normal control subjects. The differences in the LnRHI were analyzed using a one-way analysis of variance, followed by Tukey multiple comparison.

Finally, to investigate the factors correlated with AF recurrence, we compared clinical variables between patients with and without recurrence of AF. Univariate and multivariate Cox proportional hazard analyses were conducted to determine whether any of the baseline parameters were independently associated with AF recurrence. Then, the receiver operating characteristic curve was constructed for the significant parameter, and the area under the curve, sensitivity, and specificity were calculated to predict the recurrence of AF, with an area under the curve value of 0.50 indicating no accuracy and a value of 1.00 indicating maximal accuracy. The Kaplan–Meier method was used to estimate the AF recurrence and nonrecurrence rate, and the differences between survival curves were evaluated using the log-rank test.

The subanalysis of the differences in the LnRHI, blood pressure, and heart rate were also performed using a one-way analysis of variance, followed by Tukey multiple comparison, or using the Student *t*-test because the data were normally distributed. In addition, the association among LnRHI and blood pressure, pulse pressure, and heart rate were investigated by the linear regression analysis.

Statistical tests were performed using SPSS software, version 25 (IBM, NY) and BellCurve for Excel (SSRI, Tokyo). A 2-tailed *P* value of <0.05 was considered statistically significant.

RESULTS

Relationship Between AF and Vascular Endothelial Function

The baseline characteristics in groups that had SR or AF rhythm at the time of the RHI measurement before CA are shown in Table 1. Before CA, 151 patients had SR and 63 patients had AF rhythm at the time of the RHI measurement. LnRHI was significantly lower in AF rhythm than in SR (0.70±0.24 versus 0.50±0.23, *P*<0.001) (Figure 3A).

The baseline characteristics in the high-LnRHI and low-LnRHI groups are shown in Table 2. Univariate logistic regression analysis revealed that men, height, weight, the presence of AF at the time of RHI measurement, persistent form of AF, systolic blood pressure, diastolic blood pressure, pulse pressure, heart rate, smoking, and left ventricular ejection fraction significantly correlated with the lowering of LnRHI (Table 3). After adjustment for men, height, weight, persistent form of AF, heart rate, smoking, and left ventricular ejection fraction, multivariate logistic regression

Table 1. Baseline Variables in the Groups That Had SR and AF at the Time of RHI Measurement Before CA

Variable	SR (n=151)	AF (n=63)	*P* Value
Age, y	61.7±10.0	61.3±10.0	0.815
Men, n (%)	98 (64.9)	56 (88.9)	<0.001
Height, cm	164.7±10.0	167.5±7.2	0.072
Weight, kg	64.3±11.7	66.7±10.8	0.073
Body mass index, kg/m^2	23.62±3.42	23.71±3.09	0.647
Waist, cm	87.7±9.2	87.0±8.4	0.876
Duration of AF, y	3.0 [1.0–7.0]	3.0 [1.0–6.0]	0.583
Persistent AF, n (%)	13 (8.6)	38 (60.3)	<0.001
Diabetes mellitus, n (%)	17 (11.3)	5 (7.9)	0.466
Hypertension, n (%)	85 (56.3)	37 (58.7)	0.743
Systolic blood pressure, mm Hg	123.87±14.08	117.60±14.09	0.003
Diastolic blood pressure, mm Hg	74.97±10.60	80.41±10.57	0.001
Pulse pressure, mm Hg	48.91±11.35	37.19±11.24	<0.001
Heart rate, beats/min	59 [54–66]	77 [71–86]	<0.001
Dyslipidemia, n (%)	45 (29.8)	23 (36.5)	0.337
Smoking history, n (%)	69 (45.7)	42 (66.7)	0.005
Left atrial diameter, mm	36.3±5.1	38.1±5.3	0.025
LVEF, %	64.6±4.2	59.9±6.3	<0.001
hsCRP, mg/dL	0.05 [0.02–0.10]	0.05 [0.03–0.11]	0.240
eGFR, mL/min per 1.73 m^2	75.0±14.2	70.6±13.3	0.036
BNP, pg/mL	19.8 [11.3–44.1]	68.3 [30.5–96.8]	<0.001
LnRHI	0.70±0.24	0.50±0.23	<0.001
Baseline medication			
β-blockers, n (%)	46 (30.5)	29 (46.0)	0.024
ACEIs or ARBs, n (%)	56 (37.1)	26 (41.3)	0.566
Calcium channel blockers, n (%)	48 (31.8)	25 (39.7)	0.267
AADs, n (%)	139 (92.1)	57 (90.5)	0.705

Data are presented as mean±SD, median value with interquartile range, or frequencies and percentages (%). AAD indicates anti-arrhythmic drugs; ACEI, angiotensin-converting enzyme inhibitors; AF, atrial fibrillation; ARB, angiotensin receptor blockers; BNP, brain natriuretic peptide; CA, catheter ablation; eGFR, estimated glomerular filtration ratio; hsCRP, high-sensitivity C-reactive protein; LnRHI, natural logarithmic transformed reactive hyperemia-peripheral arterial tonometry index values; LVEF, left ventricular ejection fraction; RHI, reactive hyperemia-peripheral arterial tonometry index; and SR, sinus rhythm.

analysis identified the presence of AF at the time of RHI measurement as a most significant correlated factor with the lowering of LnRHI (odds ratio, OR; 4.092; *P*=0.002) (Table 3).

Restoration of the Vascular Endothelial Function by CA

AF recurrence was observed in 39 patients (18.2%; recurrence group), but was not observed in the remaining 175 patients (81.8%; nonrecurrence group) (Figure 1). The LnRHI did not improve 6 months after CA (0.78±0.25 versus 0.66±0.19, *P*=0.055) in patients with AF recurrence (Figure 3B), however the LnRHI was significantly improved 6 months after CA (0.61±0.25 versus 0.74±0.22, *P*<0.001) in patients without AF recurrence (Figure 3C). In particular, the difference in LnRHI between before and 3 or 6 months after CA (ΔLnRHI) was shown in Figure 3D in patients with and without recurrence of AF. The value of LnRHI did not increase 3 months after CA with AF recurrence, whereas the value of LnRHI was already significantly increased 3 months after CA without AF recurrence. Representative results of the RH-PAT examination in patients with and without AF recurrence are shown in Figure 2B and 2C. The LnRHI in AF rhythm before CA did not improve in the AF rhythm 6 months after CA (Figure 2B), however the LnRHI in AF rhythm before CA was improved in the SR 6 months after CA (Figure 2C).

Furthermore, 122 consecutive patients with AF in whom the SR was present at the time of the RHI measurement and in whom AF recurrence was not observed (AF SR group) were compared with 43 normal control subjects (control group) (Figure 3E). The LnRHI in the AF SR group before CA was significantly lower than that in the control group (0.78±0.19 versus 0.66±0.24, *P*=0.031). However, the LnRHI in the AF SR group 3 and 6 months after CA was significantly improved compared with that

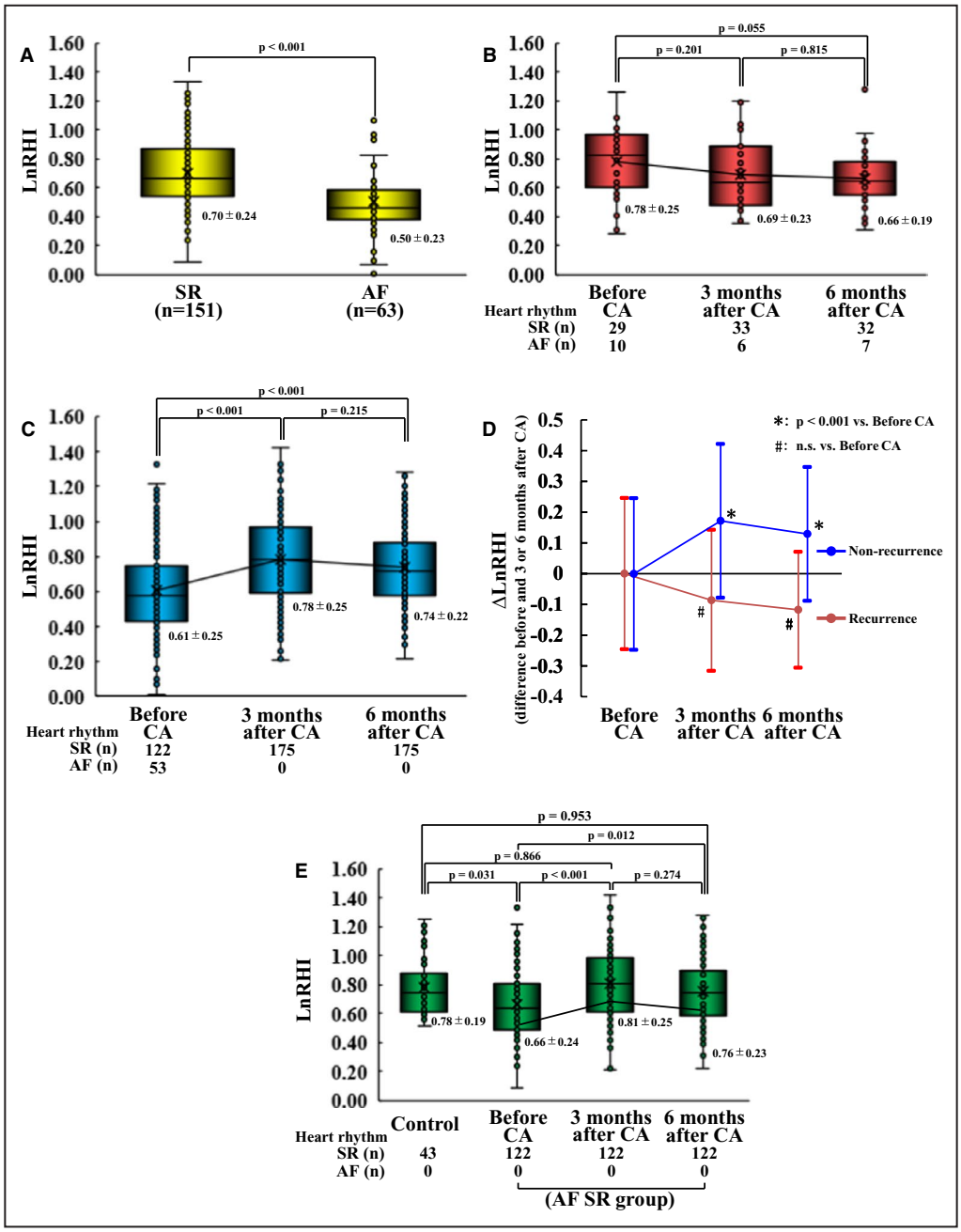


Figure 3. Comparison of the reactive hyperemia-peripheral arterial tonometry index.
A, The comparison of the LnRHI between in SR or in AF at the time of reactive hyperemia-peripheral arterial tonometry measurement before CA. **B** and **C**, The comparison of LnRHI between before CA, 3 months after CA, and 6 months after CA in patients with (**B**) and without recurrence of AF (**C**). **D**, The change in LnRHI (ΔLnRHI) from before to 3 or 6 months after CA in patients with and without recurrence of AF. **E**, The comparison of LnRHI between in patients without AF recurrence, all recorded under SR (AF SR group), and in normal control subjects. The summary data of each parameter in (**A**, **B**, **C**, and **E**) are described by the box-and-whisker plot. In these plots, the lines within the boxes represent the median values. The upper and lower lines of the boxes represent the 25th and 75th percentiles, respectively. The upper and lower bars outside the boxes represent the 10th and 90th percentiles, respectively. The mean±SD value was described, and the mean values are connected by a line. Furthermore, the data in (**D**) are described by line chart. The central point represents the mean value and upper and lower point represent the SD.

Table 2. Comparison of Patients Characteristics Between High-LnRHI Group and Low-LnRHI Group Before Catheter Ablation

Variable	High-LnRHI Group (n=l07)	Low-LnRHI Group (n=107)	*P* Value
Age, y	61.8±9.8	61.5±10.2	0.995
Men, n (%)	66 (61.7)	88 (82.2)	0.001
Height, cm	164.1±10.0	170.0±8.4	0.035
Weight, kg	63.2±11.2	66.9±11.5	0.017
Body mass index, kg/m^2	23.41±3.35	23.89±3.29	0.165
Waist, cm	86.9±8.8	88.1±9.41	0.347
Duration of AF, y	3.0 [0.5–6.5]	3.0 [1.0–7.0]	0.729
Baseline cardiac rhythm (AF), n (%)	13 (12.1)	50 (46.7)	<0.001
Persistent AF, n (%)	16 (15.0)	35 (32.7)	0.002
Diabetes mellitus, n (%)	10 (9.3)	12 (11.2)	0.653
Hypertension, n (%)	58 (54.2)	64 (59.8)	0.407
Systolic blood pressure, mm Hg	124.11±14.32	119.94±14.12	0.033
Diastolic blood pressure, mm Hg	74.89±11.30	78.25±10.16	0.023
Pulse pressure, mm Hg	49.22±11.31	41.69±12.54	<0.001
Heart rate, beats/min	58 [54–69]	68 [60–77]	<0.001
Dyslipidemia, n (%)	30 (28.0)	38 (35.5)	0.337
Smoking history, n (%)	48 (44.9)	63 (58.9)	0.040
Left atrial diameter, mm	36.4±5.3	37.3±5.1	0.213
LVEF, %	64.6±4.8	61.9±5.6	<0.001
hsCRP, mg/dL	0.04 [0.03–0.11]	0.05 [0.04–0.12]	0.240
eGFR, mL/min per 1.73 m^2	75.1±14.4	72.3±13.7	0.232
BNP, pg/mL	25.4 [14.9–68.1]	29.5 [17.2–53.8]	<0.001
Baseline medication			
β-blockers, n (%)	37 (34.6)	38 (35.5)	0.846
ACEIs or ARBs, n (%)	39 (36.4)	43 (40.2)	0.543
Calcium channel blockers, n (%)	31 (30.0)	42 (39.3)	0.113
AADs, n (%)	97 (90.7)	99 (92.5)	0.622

Data are presented as mean±SD, median value with interquartile range, or frequencies and percentages. AAD indicates anti-arrhythmic drugs; ACEI, angiotensin-converting enzyme inhibitors; AF, atrial fibrillation; ARB, angiotensin receptor blockers; BNP, brain natriuretic peptide; eGFR, estimated glomerular filtration ratio; hsCRP, high sensitivity C-reactive protein; LnRHI, natural logarithmic transformed reactive hyperemia-peripheral arterial tonometry index values; and LVEF, left ventricular ejection fraction.

before CA (3 months: 0.66±0.24 versus 0.81±0.25, *P*<0.001; 6 months: 0.66±0.24 versus 0.76±0.23, *P*=0.012). Furthermore, the LnRHI at 3 and 6 months after CA in AF SR group were not different from that of the control group (3 months: 0.78±0.19 versus 0.81±0.25, *P*=0.866; 6 months: 0.78±0.19 versus 0.76±0.23, *P*=0.953) (Figure 3E). The LnRHI in patients without AF recurrence after CA was significantly improved to a level similar to those in normal control subjects.

Prediction of the Recurrence of AF After CA by Evaluating the Vascular Endothelial Function

The comparison of characteristics between the recurrence and the nonrecurrence after CA for AF are shown in Table 4. The LnRHI 3 and 6 months after CA in the recurrence group was significantly lower than that in the nonrecurrence group (3 months: 0.69±0.23 versus 0.78±0.25, *P*=0.045; 6 months: 0.66±0.19 versus 0.74±0.22, *P*=0.044). Furthermore, the change in LnRHI from before to 3 months after CA in the recurrence group was significantly lower than that in the nonrecurrence group (–0.09±0.26 versus 0.17±0.27, *P*<0.001) (Table 4).

Univariate Cox proportional hazard analysis revealed that the AF disease duration, prevalence of dyslipidemia, usage of β-blockers, LnRHI before CA, and the change in LnRHI from before to 3 months after CA were correlated with the recurrence of AF (Table 5). After adjustment for the usage of β-blockers and LnRHI before CA, multivariate Cox proportional hazard analysis revealed that the change in LnRHI from before to 3 months after CA were most independently correlated with the recurrence of AF (hazard ratio, [HR]; 0.106, *P*=0.001) (Table 5).

Table 3. Univariate and Multivariate Logistic Regression Analysis for the Lowering of LnRHI Before CA

Variable	Univariate Logistic Regression			Multivariate Logistic Regression With Significant Factors in Univariate Analysis		
	OR	95% CI	*P* Value	OR	95% CI	*P* Value
Age, per y	0.997	0.971–1.024	0.826			
Men (yes)	2.877	1.531–5.406	0.001	1.781	0.604–5.250	0.295
Height, per cm	1.035	1.005–1.066	0.023	0.979	0.927–1.033	0.434
Weight, per kg	1.030	1.005–1.055	0.019	1.021	0.987–1.055	0.226
Body mass index, per kg/m^2	1.045	0.963–1.135	0.292			
Waist, per cm	1.015	0.984–1.046	0.346			
Duration of AF, per y	1.014	0.957–1.075	0.636			
Baseline cardiac rhythm (AF) (yes)	6.343	3.171–12.687	<0.001	4.092	1.654–10.125	0.002
Persistent AF (yes)	2.765	1.418–5.389	0.003	0.760	0.304–1.902	0.558
Diabetes mellitus (yes)	1.225	0.505–2.971	0.653			
Hypertension (yes)	1.257	0.731–2.163	0.408			
Systolic blood pressure, per mm Hg	0.979	0.961–0.999	0.035			
Diastolic blood pressure, per mm Hg	1.030	1.004–1.057	0.025			
Pulse pressure, per mm Hg	0.949	0.926–0.972	<0.001	0.972	0.945–1.000	0.047
Heart rate, per beats/min	1.045	1.022–1.069	<0.001	1.010	0.981–1.040	0.491
Dyslipidemia (yes)	1.414	0.793–2.521	0.241			
Smoking history (yes)	1.760	1.024–3.025	0.041	0.992	0.496–1.984	0.981
Left atrial diameter, per mm	1.034	0.981–1.089	0.213			
LVEF, per %	0.904	0.855–0.956	<0.001	0.952	0.891–1.017	0.147
hsCRP, per mg/dL	0.697	0.224–2.165	0.532			
eGFR, per mL/min per 1.73 m^2	0.986	0.967–1.005	0.151			
BNP, per pg/mL	1.005	0.999–1.010	0.095			
Baseline medication						
β-blockers (yes)	1.057	0.602–1.855	0.846			
ACEIs or ARBs (yes)	1.171	0.675–2.034	0.574			
Calcium channel blockers (yes)	1.584	0.896–2.801	0.114			
AADs (yes)	1.276	0.483–3.369	0.623			

AAD indicates anti-arrhythmic drugs; ACEI, angiotensin-converting enzyme inhibitors; AF, atrial fibrillation; ARB, angiotensin receptor blockers; BNP, brain natriuretic peptide; CA, catheter ablation; eGFR, estimated glomerular filtration ratio; hsCRP, high-sensitivity C-reactive protein; LnRHI, natural logarithmic transformed reactive hyperemia-peripheral arterial tonometry index values; LVEF, left ventricular ejection fraction; and OR, odds ratio.

Furthermore, receiver operating characteristic curve analysis revealed that the value of LnRHI 3 months after CA, which decreased to ≥0.01 compared with that before CA, was an independent marker that suspects AF recurrence (sensitivity, 0.806; specificity, 0.821; area under the curve, 0.792; *P*<0.001) (Figure 4A). Kaplan–Meier analysis demonstrated a significantly higher probability of AF recurrence when the LnRHI value 3 months after CA is lower than that before CA (log-rank test, *P*<0.001) (Figure 4B).

Subanalysis of the RHI Before Catheter Ablation

Figure 5A and 5B show the subanalysis of the comparison of LnRHI between SR and AF rhythm at the time of RH-PAT measurement before CA in patients with paroxysmal AF (Figure 5A) and persistent AF (Figure 5B). Figure 5C and 5D show the comparison of LnRHI, measured under SR (Figure 5C) or AF rhythm (Figure 5D) at the time of RH-PAT measurement before CA, between patients with paroxysmal AF and patients with persistent AF. In the SR group, there were 13 patients with persistent AF (Table 1). In other words, in patients with persistent AF, 13 patients performed RHI measurement under SR because AF was defibrillated before CA or spontaneously terminated after lasting beyond 7 days (Table 1). On the other hand, remaining 38 patients performed RHI measurement under AF rhythm. Furthermore, in patients with paroxysmal AF, 138 patients performed RHI measurement under SR and 25 patients performed under AF rhythm.

Whether in paroxysmal AF or in persistent AF, LnRHI measured under AF rhythm was lower than that

Table 4. Comparison of Characteristics Between the Recurrence Group and the Nonrecurrence Group After CA of AF

Variable	Recurrence (n=39)	Nonrecurrence (n=175)	*P* Value
Age, y	62.l±9.8	61.5±10.1	0.787
Men, n (%)	28 (71.8)	126 (72.0)	0.979
Height, cm	164.8±8.5	165.7±9.5	0.419
Weight, kg	65.4±12.4	64.9±11.3	0.900
Body mass index, kg/m^2	24.13±4.75	23.54±2.92	0.930
Waist, cm	88.3±9.7	87.4±8.8	0.569
Duration of AF, y	5.0 [2.0–10.0]	2.0 [1.0–6.0]	0.006
Persistent AF, n (%)	7 (17.9)	44 (25.1)	0.340
Diabetes mellitus, n (%)	3 (7.7)	19 (10.9)	0.402
Hypertension, n (%)	22 (56.4)	100 (57.1)	0.933
Systolic blood pressure before CA, mm Hg	123.21±13.66	121.77±14.51	0.572
Diastolic blood pressure before CA, mm Hg	76.72±10.64	76.54±10.93	0.925
Pulse pressure before CA, mm Hg	46.49±13.42	45.23±12.31	0.571
Heart rate before CA, beats/min	66 [55–74]	63 [56–75]	0.676
Dyslipidemia, n (%)	19 (48.7)	49 (28.0)	0.012
Smoking history, n (%)	23 (59.0)	88 (50.3)	0.326
Left atrial diameter, mm	37.0±4.8	36.8±5.3	0.826
LVEF, %	63.2±6.0	63.2±5.2	0.686
hsCRP, mg/dL	0.04 [0.02–0.07]	0.05 [0.02–0.11]	0.210
eGFR, mL/min per 1.73 m^2	71.7±12.4	74.2±14.4	0.355
BNP, pg/mL	33.4 [17.4–65.5]	26.1 [13.4–55.3]	0.600
Baseline medication			
β-blockers, n (%)	20 (51.3)	55 (31.4)	0.020
ACEIs or ARBs, n (%)	15 (38.5)	67 (38.3)	0.984
Calcium channel blockers, n (%)	10 (25.6)	63 (36.0)	0.217
AADs, n (%)	35 (89.7)	161 (92.0)	0.422
Postoperative β-blockers, n (%)	18 (46.2)	54 (30.9)	0.068
LnRHI (before CA)	0.78±0.25	0.61±0.25	<0.001
LnRHI (3 mo after CA)	0.69±0.23	0.78±0.25	0.045
LnRHI (6 mo after CA)	0.66±0.19	0.74±0.22	0.044
ΔLnRHI (difference before and 3 mo after CA)	−0.09±0.26	0.17±0.27	<0.001

Data are presented as mean±SD median value with interquartile range, or frequencies and percentages (%). AAD indicates anti-arrhythmic drugs; ACEI, angiotensin-converting enzyme inhibitors; AF, atrial fibrillation; ARB, angiotensin receptor blockers; BNP, brain natriuretic peptide; CA, catheter ablation; eGFR, estimated glomerular filtration ratio; hsCRP, high sensitivity C-reactive protein; LnRHI, natural logarithmic transformed reactive hyperemia-peripheral arterial tonometry index values; and LVEF, left ventricular ejection fraction.

measured under SR (paroxysmal AF: 0.70±0.25 versus 0.44±0.22, *P*<0.001; persistent AF: 0.65±0.21 versus 0.55±0.23, *P*=0.160) (Figure 5A and 5B). However, in patients with persistent AF, there was no significant difference between LnRHI measured under AF rhythm and SR (Figure 5B). In addition, LnRHI measured under AF rhythm in patients with paroxysmal AF was tended to lower than that in patients with persistent AF (0.44±0.22 versus 0.55±0.23, *P*=0.059) (Figure 5D).

Subanalysis of Serial Changes in the RHI Before CA to 6 Months After CA

Figure 6 shows the subanalysis of the serial changes in LnRHI before CA to 6 months after CA depending on the heart rhythm at the time of RH-PAT measurement before CA or the type of AF. In particular, the serial changes of the LnRHI in patients with recurrence of AF whose rhythm was SR or AF rhythm when RHI was measured before CA were shown in Figure 6A and 6B. The serial change of the LnRHI in patients with paroxysmal or persistent AF with recurrence were shown in Figure 6C and 6D. Furthermore, the serial changes of the LnRHI in patients without recurrence of AF whose rhythm was SR or AF rhythm when RHI was measured before CA were shown in Figure 6E and 6F. The serial changes of the LnRHI in patients with paroxysmal or persistent AF without recurrence were shown in Figure 6G and 6H. LnRHI in patients without recurrence of AF was significantly improved in all subanalysis whether measured

Table 5. Univariate and Multivariate Cox Proportional Hazards Analysis for the Recurrence of AF

Variable	Univariate Cox Hazard			Multivariate Cox Hazards With Significant Factors in Univariate Analysis		
	HR	95% CI	*P* Value	HR	95% CI	*P* Value
Age, per y	1.006	0.974–1.038	0.722			
Men (yes)	0.978	0.487–1.964	0.949			
Height, per cm	0.992	0.960–1.025	0.620			
Weight, per kg	1.003	0.976–1.031	0.839			
Body mass index, per kg/m^2	1.050	0.957–1.151	0.303			
Waist, per cm	l.010	0.975–1.046	0.571			
Duration of AF, per y	l.074	1.016–1.134	0.011	1.081	1.024–1.142	0.005
Persistent AF (yes)	0.682	0.301–1.546	0.359			
Diabetes mellitus (yes)	0.708	0.218–2.299	0.566			
Hypertension (yes)	0.972	0.516–1.830	0.930			
Systolic blood pressure before CA, per mm Hg	1.007	0.985–1.029	0.561			
Diastolic blood pressure before CA, per mm Hg	1.002	0.973–1.032	0.895			
Pulse pressure before CA, per mm Hg	1.007	0.982–1.033	0.577			
Heart rate before CA, per beats/min	1.009	0.985–1.033	0.482			
Dyslipidemia (yes)	2.123	1.133–3.979	0.019	1.948	1.009–3.759	0.047
Smoking history (yes)	1.336	0.706–2.528	0.374			
Left atrial diameter, per mm	1.008	0.949–1.070	0.807			
LVEF (per %)	1.000	0.943–1.061	0.990			
hsCRP, per mg/dL	0.336	0.028–4.002	0.388			
eGFR, per mL/min per 1.73 m^2	0.989	0.966–1.011	0.325			
BNP, per pg/mL	0.998	0.991–1.005	0.565			
Baseline medication						
β-blockers (yes)	2.071	1.105–3.881	0.023	1.785	0.916–3.478	0.088
ACEIs or ARBs (yes)	1.016	0.533–1.936	0.962			
Calcium channel blockers (yes)	0.635	0.309–1.303	0.216			
AADs (yes)	0.813	0.289–2.287	0.695			
Postoperative β-blockers (yes)	1.811	0.964–3.399	0.065			
LnRHI (before CA)	9.844	2.992–32.383	<0.001	2.947	0.657–13.216	0.158
LnRHI (3 mo after CA)	0.283	0.078–1.031	0.056			
LnRHI (6 mo after CA)	0.218	0.046–1.033	0.055			
ΔLnRHI (difference between before and 3 mo after CA)	0.058	0.020–0.165	<0.001	0.106	0.028–0.405	0.001

AAD indicates anti-arrhythmic drugs; ACEI, angiotensin-converting enzyme inhibitors; AF, atrial fibrillation; ARB, angiotensin receptor blockers; BNP, brain natriuretic peptide; CA, catheter ablation; eGFR, estimated glomerular filtration ratio; HR, hazard ratio; hsCRP, high sensitivity C-reactive protein; LnRHI, natural logarithmic transformed reactive hyperemia-peripheral arterial tonometry index values; and LVEF, left ventricular ejection fraction.

in SR (Figure 6E) or AF rhythm (Figure 6F) before CA, or whether in patients with paroxysmal AF (Figure 6G) or persistent AF (Figure 6H). However, in patients with recurrence of AF, LnRHI was tended to be unimproved (Figure 6D) or rather declining (Figure 6A and 6C), since some patients measured RHI under AF rhythm 3 and 6 months after CA. On the other hand, LnRHI measured under AF rhythm before CA in patients with recurrence (Figure 6B) was only tended to improve, since some patients measured RHI under SR 3 and 6 months after CA. Furthermore, whether with or without recurrence of AF, LnRHI before CA in patients with persistent AF was lower than that in patients with paroxysmal AF (with recurrence: 0.81±0.24 versus 0.66±0.26, *P*=0.172, Figure 6C and 6D; without recurrence: 0.63±0.25 versus 0.56±0.22, *P*=0.109, Figure 6G and 6H).

Subanalysis of Comparison of RHI Between Patients Without Recurrence of AF and Normal Control Subjects

Figure 7 shows the subanalysis of comparison of LnRHI between patients without recurrence of AF and

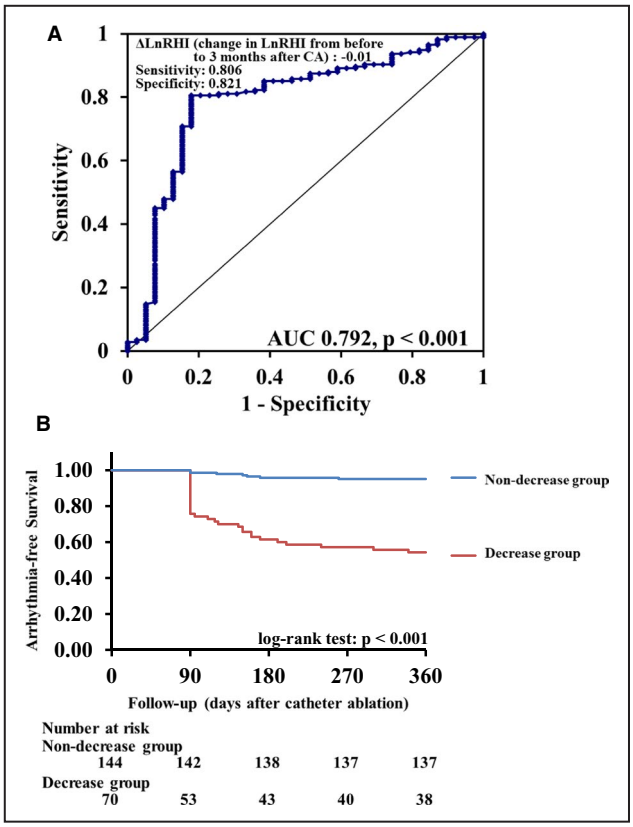


Figure 4. Receiver operating characteristic curve and Kaplan–Meier analysis to identify the recurrence of atrial fibrillation.

A, Receiver operating characteristic curve analysis. The value of LnRHI 3 months after catheter ablation, which decreased to ≥0.01 compared with that before catheter ablation, was an independent factor of atrial fibrillation recurrence (sensitivity, 0.806; specificity, 0.821; area under the curve, 0.792; *P*<0.001). **B**, Kaplan–Meier analysis. The decrease group in LnRHI 3 months after catheter ablation compared with before catheter ablation (red) had a significantly higher probability of atrial fibrillation recurrence compared with the non-decrease group (blue) (log-rank test, *P*<0.001). AUC indicates area under the curve; CA, catheter ablation; and LnRHI, natural logarithmic transformed reactive hyperemia-peripheral arterial tonometry index.

normal control subjects depending on the heart rhythm at the time of RH-PAT measurement before CA or the type of AF. In particular, the comparisons of the LnRHI between patients without AF recurrence in whom the AF rhythm was present at the time of the RHI measurement before CA and normal control subjects were shown in Figure 7A. The comparisons of LnRHI between patients with paroxysmal AF without recurrence in whom the SR or AF rhythm was present at the time of the RHI measurement before CA and normal control subjects were shown in Figure 7B and 7C. The comparisons of LnRHI between patients with persistent AF without recurrence in whom the SR or AF rhythm was present at the time of the RHI measurement before CA and normal control subjects were shown in Figure 7D and 7E.

In all subanalysis, the LnRHI was significantly improved to a level similar to those in normal control subjects if there was no recurrence of AF after CA.

Association Between RHI and Blood Pressure or Heart Rate

The association between the RHI and blood pressure were shown in Figure 8. The comparison of systolic blood pressure, diastolic blood pressure (Figure 8A), and pulse pressure (Figure 8B) between in SR or in AF rhythm at the time of RH-PAT measurement before CA in this study, and the correlation between systolic blood pressure, diastolic blood pressure, or pulse pressure and the LnRHI were shown in Figure 8. Systolic blood pressure and pulse pressure in SR at the time of RH-PAT measurement before CA were significantly higher than those in AF rhythm (123.87±14.08 versus 117.60±14.09, *P*=0.003; 48.91±11.35 versus 37.19±11.24, *P*<0.001), and diastolic blood pressure in SR was significantly lower than that in AF rhythm (74.97±10.60 versus 80.41±10.57, *P*=0.001) (Figure 8A and 8B). Systolic blood pressure and pulse pressure were significantly correlated with LnRHI, however, both coefficients of determination were low (*P*=0.004, R^2=0.038; *P*<0.001, R^2=0.094) (Figure 8C through 8E). Furthermore, the comparison of blood pressure and pulse pressure between before CA, 3 months after CA, and 6 months after CA in patients with and without recurrence of AF, and the comparison of blood pressure and pulse pressure between patients without AF recurrence, all recorded in SR (AF SR group), and normal control subjects were shown in Figure 9. There were no significant differences between all blood pressure and pulse pressure.

On the other hand, the association between the RHI and heart rate were shown in Figure 10. Heart rate under SR at the time of RH-PAT measurement before CA was significantly lower than that under AF rhythm (60.69±9.60 versus 78.52±14.75, *P*<0.001) (Figure 10A), and heart rate under AF rhythm at the time of RH-PAT measurement before CA in patients with paroxysmal AF tended to be higher than that in patients with persistent AF (83.16±19.52 versus 75.47±9.64, *P*=0.077) (Figure 10B). Heart rate was significantly inverse correlated with LnRHI. However, the coefficients of determination were low (*P*<0.001, R^2=0.070) (Figure 10C), and there were no significant differences in heart rate before CA, 3 months after CA, and 6 months after CA in patients with and without recurrence of AF (Figure 10D and 10E). Furthermore, changes in heart rate in AF SR group did not inversely correlate with changes in LnRHI (Figure 10F).

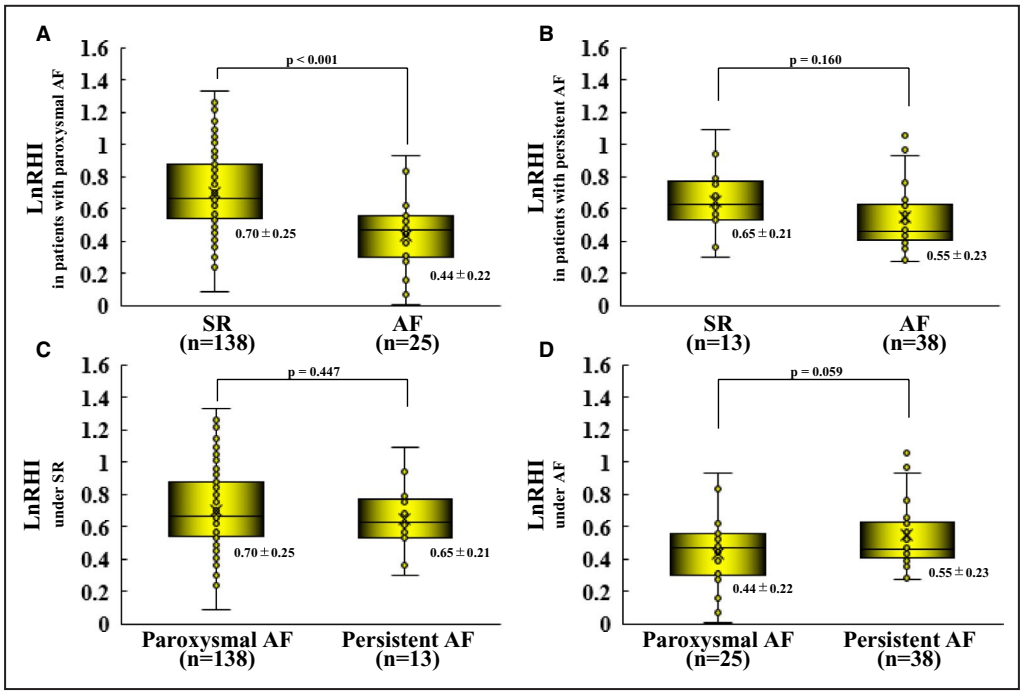
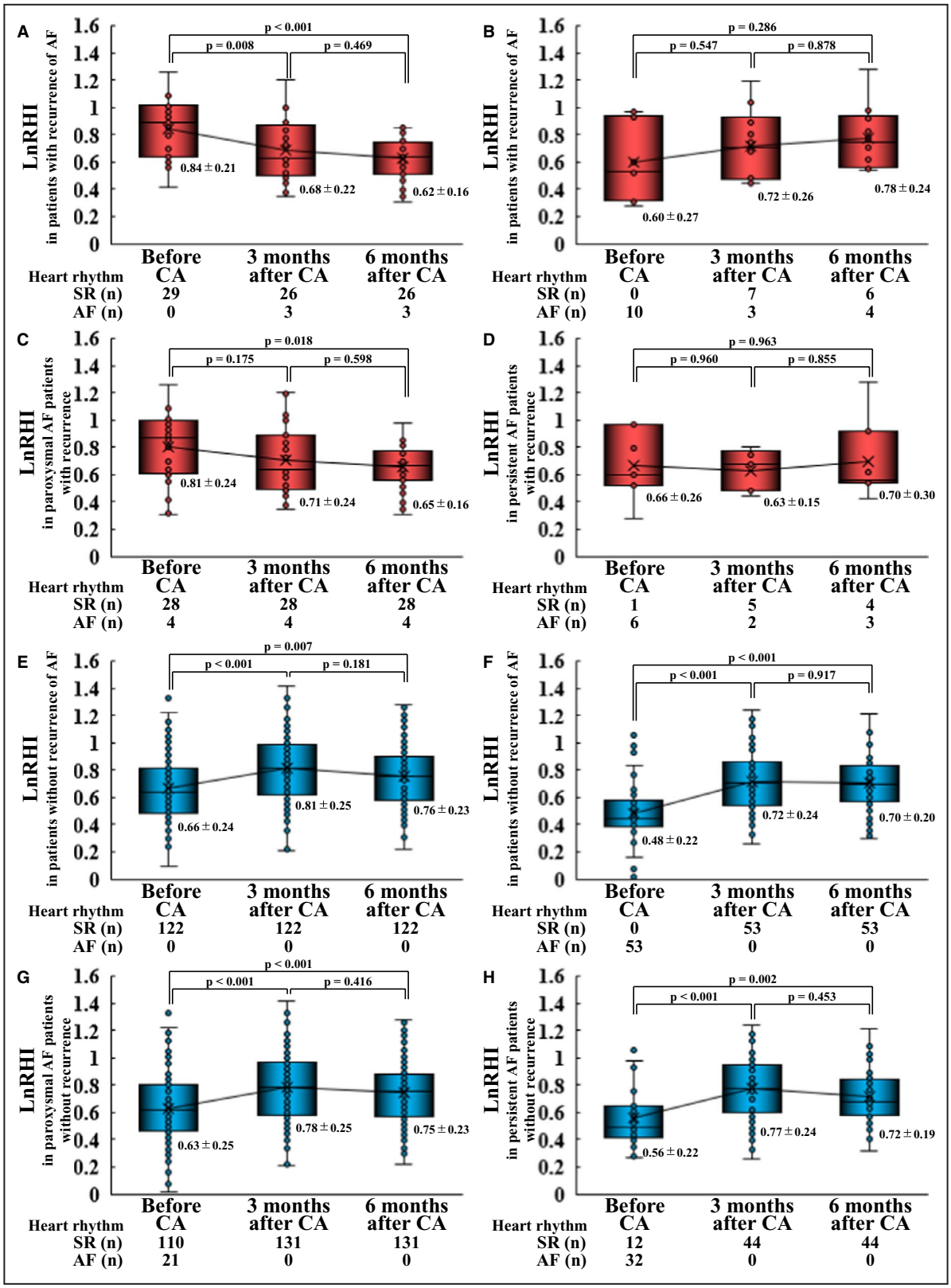


Figure 5. Subanalysis of comparison of the reactive hyperemia-peripheral arterial tonometry index before catheter ablation.
A and **B**, The comparison of the LnRHI between in SR and in atrial fibrillation (AF) at the time of reactive hyperemia-peripheral arterial tonometry (RH-PAT) measurement before catheter ablation (CA) in patients with paroxysmal AF (**A**) and persistent AF (**B**). **C**, The comparison of LnRHI measured under SR at the time of RH-PAT measurement before CA between in patients with paroxysmal AF and in patients with persistent AF. **D**, The comparison of LnRHI measured under AF rhythm at the time of RH-PAT measurement before CA between in patients with paroxysmal AF and in patients with persistent AF. See Figure 3 for the box-and-whisker plot, and the mean±SD. AF indicates atrial fibrillation; LnRHI, natural logarithmic transformed reactive hyperemia-peripheral arterial tonometry index; and SR, sinus rhythm.

Subanalysis of LnRHI Focused on Patients With Paroxysmal AF Under SR and Patients With Persistent AF Under AF Rhythm

As previously reported,[7] subanalysis focused on patients with paroxysmal AF whose LnRHI were measured under only SR at the time of RH-PAT measurement before CA (n=138) and patients with persistent AF whose LnRHI measured under only AF rhythm (n=38) were shown in Figure 11. LnRHI in patients with paroxysmal AF under SR was significantly higher than that in patients with persistent AF under AF rhythm at the time of RH-PAT measurement before CA (0.70±0.25 versus 0.55±0.23, *P*<0.001) (Figure 11A). Furthermore, in the comparison of LnRHI among control patients, patients with paroxysmal AF under SR, and patients with persistent AF under AF rhythm, LnRHI was highest in control patients (0.78±0.19), and was lowest in patients with persistent AF (0.55±0.23) (Figure 11B). Although LnRHI in patients with paroxysmal AF remained relatively high from before to 6 months after CA, LnRHI in patients with persistent AF was significantly increased at the 3 months after CA, and remained consistent during the 6 months follow-up period (Figure 11C through 11E). The same results were also obtained only in patients without recurrence of AF (Figure 11F through 11H), and these findings were similar to the previous report.[7]

Furthermore, the results of Figures 3 and 4 were also reanalyzed focused on patients with paroxysmal AF under SR (n=138) and patients with persistent AF under AF rhythm (n=38) at the time of RH-PAT measurement before CA. LnRHI also did not improve 6 months after CA (0.81±0.23 versus 0.65±0.19, *P*=0.007) in patients with AF recurrence (Figure 12A), however, the LnRHI was significantly improved 6 months after CA (0.63±0.24 versus 0.73±0.22, *P*=0.002) in patients without AF recurrence (Figure 12B). Univariate and multivariate Cox proportional hazard analysis revealed that the change in LnRHI from before to 3 months after CA were also most independently correlated with the recurrence of AF (HR, 0.043, *P*<0.001) (Table 6). Receiver operating characteristic curve analysis revealed that the value of LnRHI 3 months after CA, which decreased to ≥0.01 compared with that before CA, was also an



independent marker that suspects AF recurrence (sensitivity, 0.882; specificity, 0.745; area under the curve, 0.844; *P*<0.001) (Figure 12C). Kaplan–Meier analysis demonstrated a significantly higher probability of AF recurrence when the LnRHI value 3 months after CA is lower than that before CA (log-rank test: *P*<0.001)

Figure 6. Subanalysis of serial changes in the reactive hyperemia-peripheral arterial tonometry index.
A and **B**, The serial changes of the natural logarithmic transformed reactive hyperemia-peripheral arterial tonometry index in patients with recurrence of atrial fibrillation (AF) measured under sinus rhythm (SR) before catheter ablation (CA) (**A**) and measured under AF rhythm (**B**). **C** and **D**, The serial changes of the LnRHI in patients with paroxysmal AF with recurrence (**C**) and patients with persistent AF with recurrence (**D**). **E** and **F**, The serial changes of the LnRHI in patients without recurrence of AF measured under SR before CA (**E**) and measured under AF rhythm (**F**). **G** and **H**, The serial changes of the LnRHI in patients with paroxysmal AF without recurrence (**G**) and patients with persistent AF without recurrence (**H**). See Figure 3 for the box-and-whisker plot, the mean±SD, and the line connecting the mean values. AF indicates atrial fibrillation; and LnRHI, natural logarithmic transformed reactive hyperemia-peripheral arterial tonometry index. [Corrections added on September 07, 2021, figure caption is updated to read correctly.]

(Figure 12D). These results of subanalysis focused on patients with paroxysmal AF under SR and patients with persistent AF under AF rhythm were same to the major findings in the present study.

Long-Term Outcome of the Recurrence After CA in Patients With AF

Regarding the recurrence within 1 year, Figure 4 showed the decrease in LnRHI levels from before to 3 months after CA as a significant marker that suspects AF recurrence. On the other hand, in a follow-up after over 1 year, as many ECGs of arrhythmia as possible were recorded based on physical findings, symptoms, and regular electrocardiography. Kaplan–Meier analysis demonstrated a significantly higher probability of AF recurrence even in an average of 40.7±2.5 years of follow-up when the LnRHI value 3 months after CA is lower than that before CA (log-rank test, $P<0.001$) (Figure 13). However, since a very late recurrence >1 year later was seen even if the LnRHI value 3 months after CA is higher than that before CA, the difference between non-decrease group and decrease group was tended to be smaller year by year.

DISCUSSION

The present study demonstrated that the presence of AF, regardless of paroxysmal AF or persistent AF, was independently correlated with impaired vascular endothelial function. Furthermore, impaired vascular endothelial function was present in patients with AF even under SR. However, the impaired vascular endothelial function in patients with AF improved to a level similar to those in normal control subjects when the SR was maintained after CA. In contrast, impaired vascular endothelial function did not improve in patients with AF recurrence after CA. Therefore, the change in vascular endothelial function following CA sensitively reflects the recurrence or nonrecurrence of AF. The decrease of LnRHI 3 months after CA compared with that before CA was an independent predictor that suspects AF recurrence.

AF is one of the most frequent arrhythmias. Once AF has occurred, a change in turbulent blood flow from laminar blood flow because of AF can reduce shear stress, resulting in vascular endothelial dysfunction. The blood vessel wall is loaded with shear stress caused by blood flow and stretch because of blood pressure, and normal shear stress releases NO from the vascular endothelium, maintaining vascular function. However, the synthesis of NO decreases under low shear stress because of the decrease in the activation of intracellular signals, such as the transcription factor Krüppel-like factor 2, which activates endothelial NO synthase.[21] Therefore, the flow conditions during AF reproduce turbulent shear stress, which itself do not upregulate NO synthase or increase NO release.[22] In the present study, vascular endothelial function assessed by the RH-PAT was reduced just because of the rhythm of AF (Figures 3A and 5A, 5B). In addition, the vascular endothelial function was significantly improved by CA for AF if there is no recurrence (Figures 3C and 6E through 6H), similar to the report by Shin et al.[5] These findings have suggested that the decrease in shear stress because of AF rhythm itself is a major cause of vascular endothelial dysfunction in patients with AF.

On the other hand, Okawa et al reported that LnRHI in patients with persistent AF was significantly lower than that in patients with paroxysmal AF.[7] However, in that study, the RHI in patients with paroxysmal AF was all measured under SR, and the RHI in patients with persistent AF was all measured under AF rhythm. In fact, focusing on the patients with paroxysmal AF under SR and patients with persistent AF under AF rhythm, LnRHI in patients with persistent AF under AF rhythm was also significantly lower than that in patients with paroxysmal AF under SR at the time of RH-PAT measurement before CA in the present study (Figure 11A). However, Matsue et al have reported that there was no significant difference in RHI between patients with paroxysmal AF and patients with persistent AF.[8] Furthermore, Freestone et al have revealed that there were no significant differences in patients with paroxysmal, persistent, and permanent AF in von Willebrand factor, soluble E-selectin, or soluble thrombomodulin levels used as indexes of endothelial activation, damage/dysfunction, and endothelial damage, respectively.[23] In the present study, LnRHI before CA in patients with persistent AF was lower than that in patients with paroxysmal AF (0.81±0.24 versus 0.66±0.26, $P=0.172$, Figure 6C and

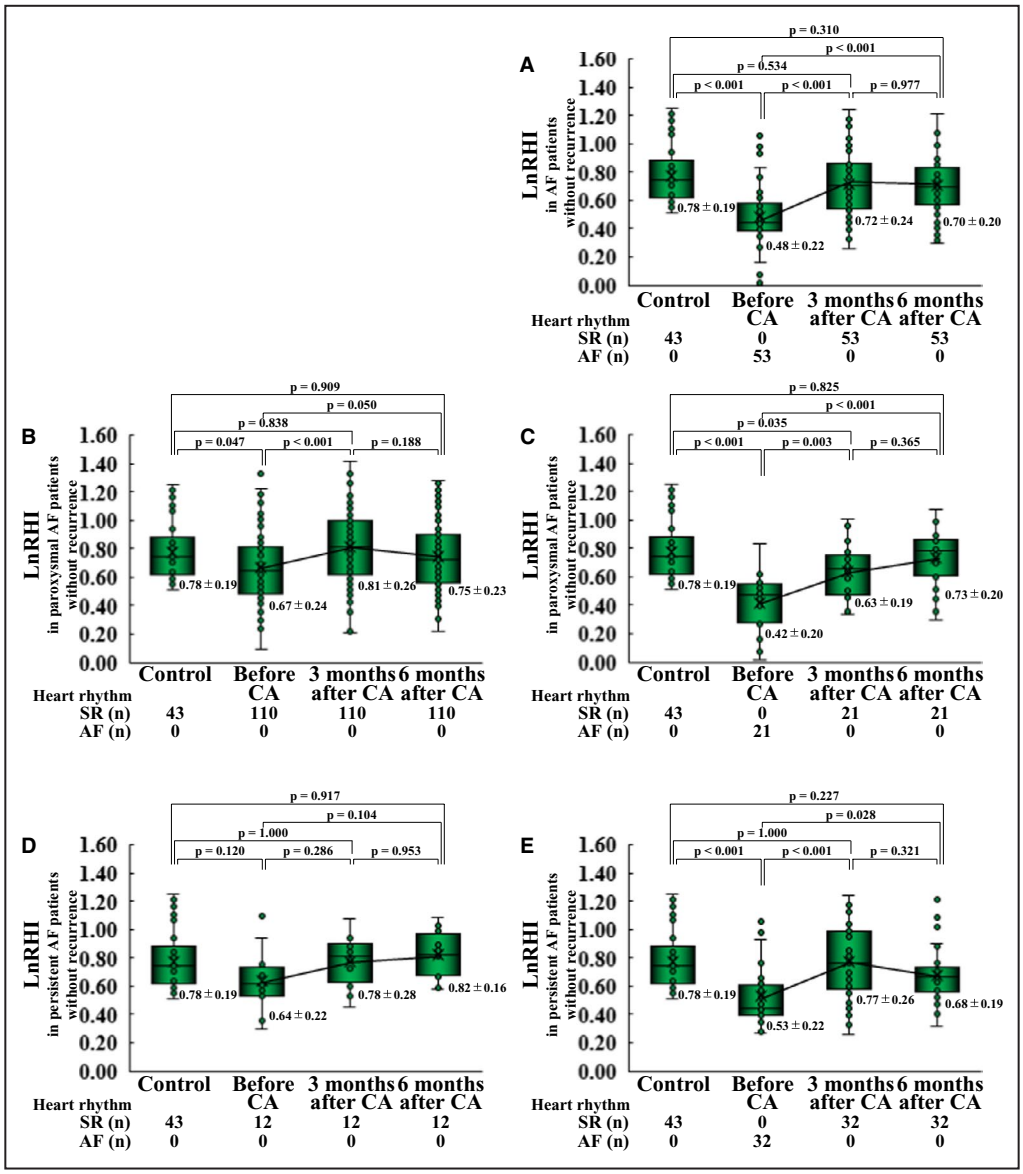


Figure 7. Subanalysis of comparison of reactive hyperemia-peripheral arterial tonometry index between patients without recurrence of atrial fibrillation and normal control subjects.
A, The comparison of the natural logarithmic transformed reactive hyperemia-peripheral arterial tonometry (RH-PAT) index (LnRHI) between patients without atrial fibrillation (AF) recurrence in whom the AF rhythm was present at the time of the RH-PAT measurement before catheter ablation (CA), and normal control subjects. **B** and **C**, The comparison of LnRHI between patients with paroxysmal AF without recurrence in whom the sinus rhythm (SR) (**B**) or AF rhythm (**C**) was present at the time of the RH-PAT measurement before CA, and normal control subjects. **D** and **E**, the comparison of LnRHI between patients with persistent AF without recurrence in whom the sinus rhythm (**D**) or AF rhythm (**E**) was present at the time of the RH-PAT measurement before CA, and normal control subjects. See Figure 3 for the box-and-whisker plot, the mean±SD, and the line connecting the mean values.

6D; 0.63±0.25 versus 0.56±0.22, *P*=0.109, Figure 6G and 6H), whether with or without recurrence of AF, as reported previously.[7] However, this might be also because of the fact that the ratio of AF rhythm at the time of RH-PAT measurement in patients with persistent AF was significantly higher than that in patients with paroxysmal AF, regardless of the recurrence of AF (with recurrence: 4 of 32 (11%) versus 6 of 7 (86%), *P*<0.001, Figure 6C and 6D; without recurrence: 21 of 131 (16%) versus 32 of 44 (73%), *P*<0.001, Figure 6G and 6H). In fact, LnRHI measured under AF rhythm in patients with paroxysmal AF was not only no significant difference from, but also tended to be lower than that measured under AF rhythm in patients with

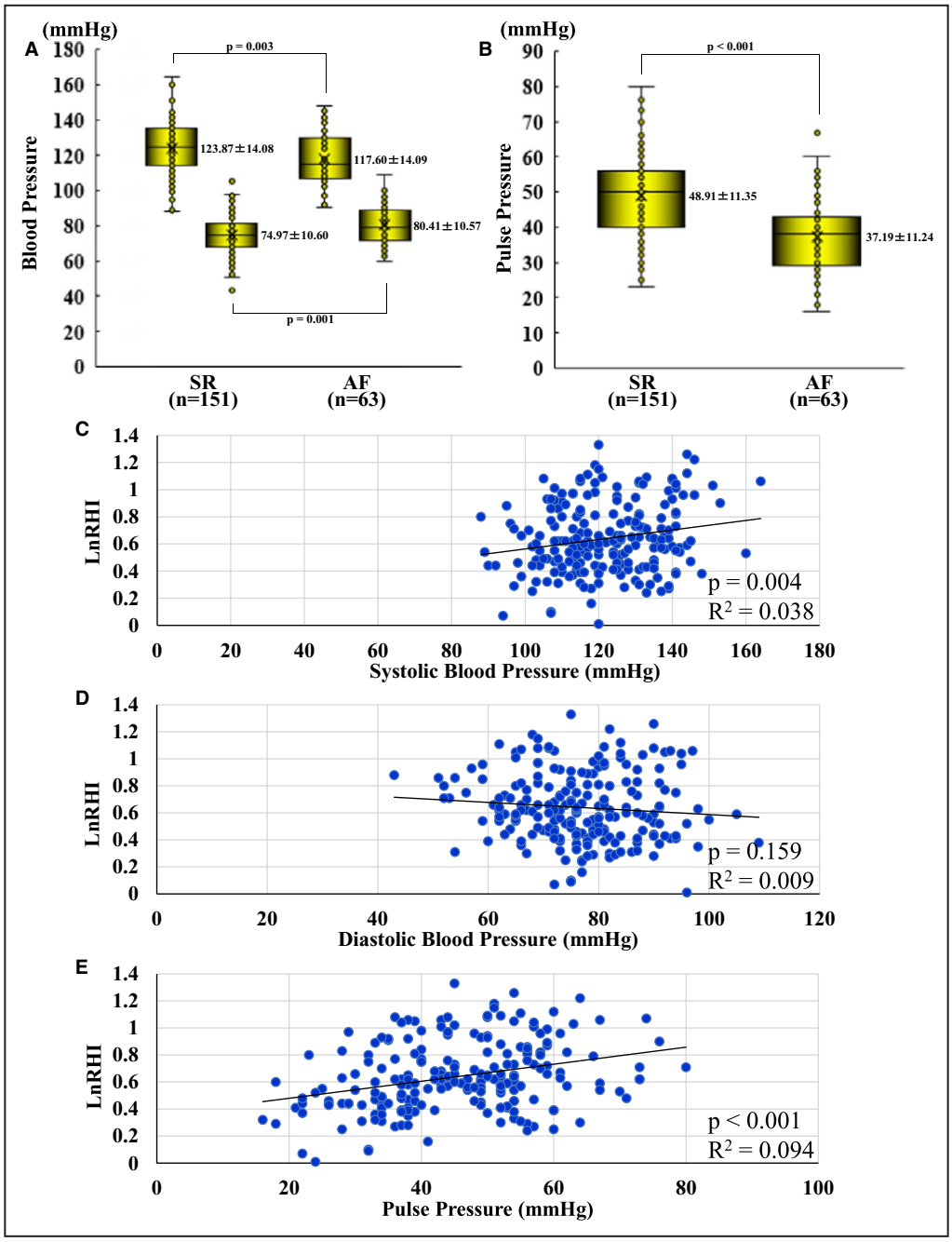


Figure 8. Comparison of the blood pressure according to the heart rhythm before catheter ablation and correlation between blood pressure and reactive hyperemia-peripheral arterial tonometry index.

A, The comparison of systolic blood pressure and diastolic blood pressure between in sinus rhythm or in atrial fibrillation at the time of reactive hyperemia-peripheral arterial tonometry measurement before catheter ablation. **B**, The comparison of pulse pressure between in sinus rhythm or in atrial fibrillation at the time of reactive hyperemia-peripheral arterial tonometry measurement before catheter ablation. **C** through **E**, The correlation between systolic blood pressure, diastolic blood pressure, or pulse pressure and the natural logarithmic transformed reactive hyperemia-peripheral arterial tonometry index. See Figure 3 for the box-and-whisker plot, and the mean±SD. AF indicates atrial fibrillation; LnRHI, natural logarithmic transformed reactive hyperemia-peripheral arterial tonometry index; and SR, sinus rhythm.

persistent AF (Figure 5D). The higher heart rate under AF rhythm in patients with paroxysmal AF compared with that in patients with persistent AF in this study (Figure 10B) might be associated with lower LnRHI, as reported previously.[24] These findings have suggested that LnRHI even in patients with persistent AF

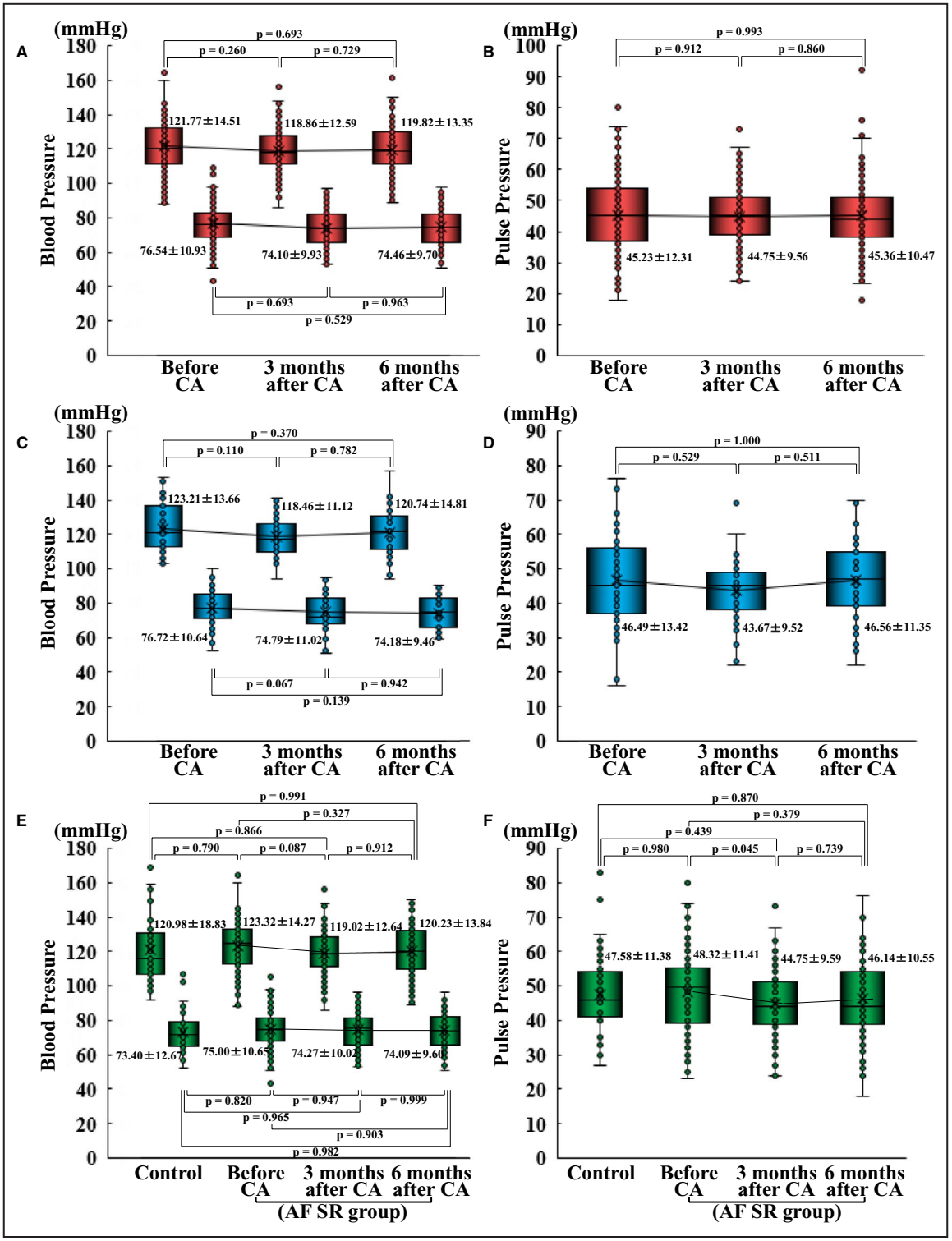


Figure 9. The serial changes in blood pressure and pulse pressure.
A and **B**, The comparison of systolic blood pressure and diastolic blood pressure (**A**), and pulse pressure (**B**) between before CA, 3 months after CA, and 6 months after CA in patients with recurrence of AF. **C** and **D**, The comparison of systolic blood pressure and diastolic blood pressure (**C**), and pulse pressure (**D**) between before CA, 3 months after CA, and 6 months after CA in patients without recurrence of AF. **E** and **F**, The comparison of systolic blood pressure and diastolic blood pressure (**E**), and pulse pressure (**F**) in patients without AF recurrence, all recorded in sinus rhythm (SR) (AF SR group), and normal control subjects. See Figure 3 for the box-and-whisker plot, the mean±SD, and the line connecting the mean values. AF indicates atrial fibrillation; CA, catheter ablation; and SR, sinus rhythm.

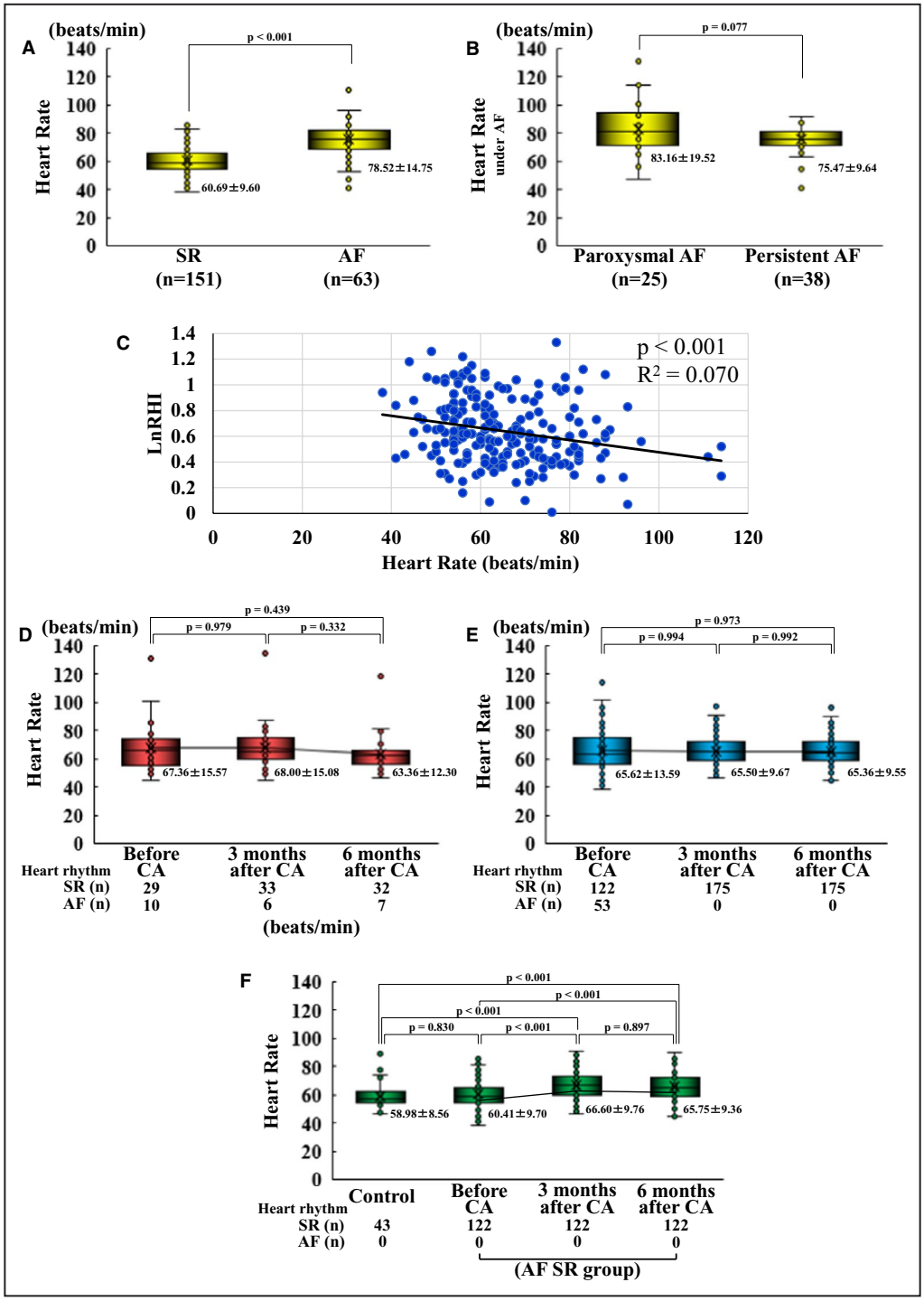
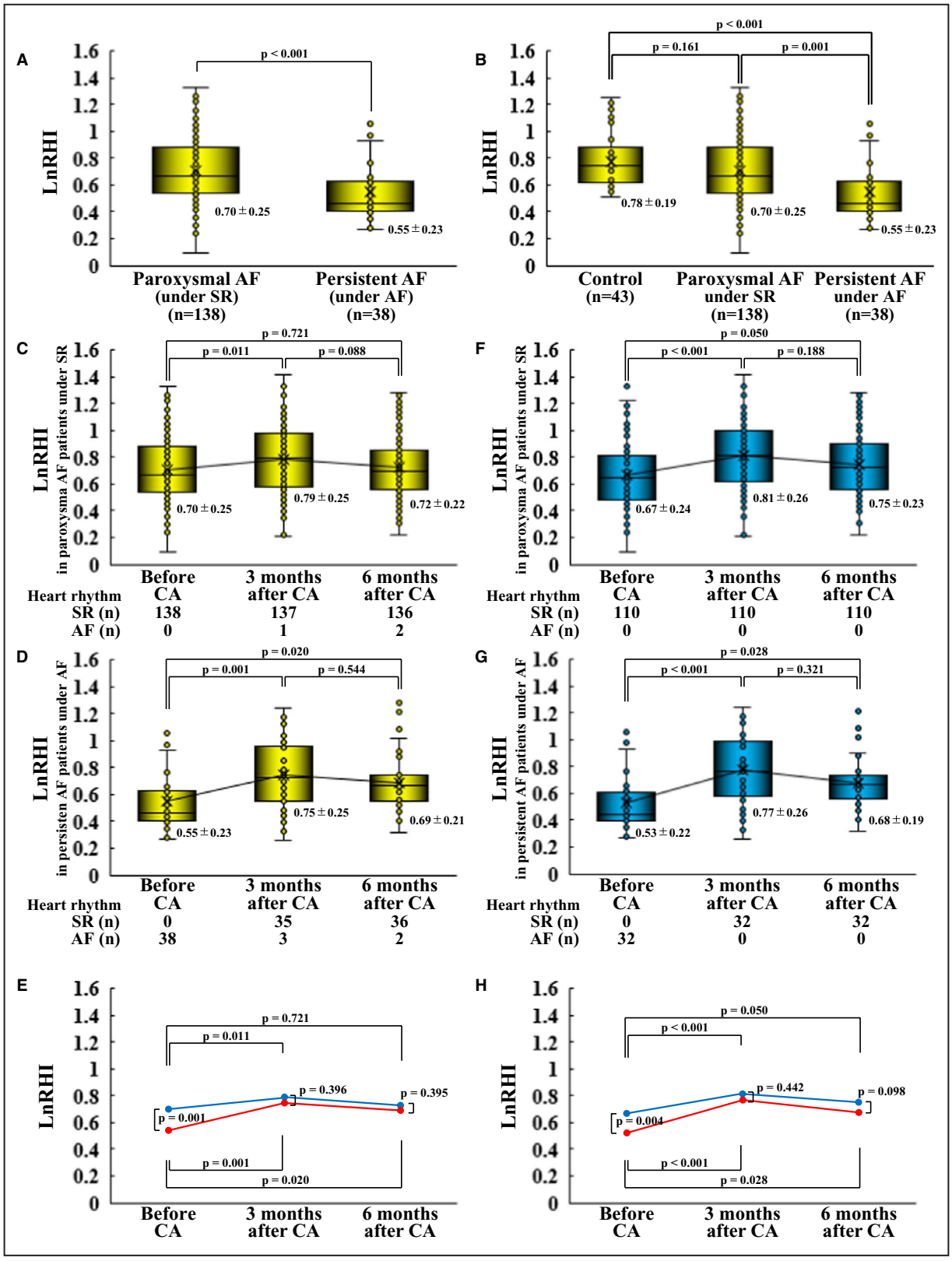


Figure 10. Investigation of the heart rate.

A, The comparison of heart rate between in SR or in atrial fibrillation (AF) rhythm at the time of reactive hyperemia-peripheral arterial tonometry measurement before catheter ablation (CA). **B**, The comparison of heart rate under AF rhythm at the time of reactive hyperemia-peripheral arterial tonometry measurement before CA between patients with paroxysmal AF and patients with persistent AF. **C**, The correlation between heart rate and the natural logarithmic transformed reactive hyperemia-peripheral arterial tonometry index. **D** and **E**, The comparison of heart rate between before CA, 3 months after CA, and 6 months after CA in patients with recurrence of AF (**D**) and without recurrence of AF (**E**). **F**, The comparison of heart rate between patients without AF recurrence, all recorded in SR (AF SR group), and normal control subjects. See Figure 3 for the box-and-whisker plot, the mean±SD, and the line connecting the mean values. AF indicates atrial fibrillation; CA, catheter ablation; LnRHI, natural logarithmic transformed reactive hyperemia-peripheral arterial tonometry index; and SR, sinus rhythm.



may not decrease depending on their heart rate compared with that in patients with paroxysmal AF.

Moreover, it is thought that chronic inflammation also contributes to impairment of vascular endothelial functions in patients with AF. Inflammation involved in the pathophysiology of AF, which provoked by diabetes mellitus, hypertension, obesity, and so on, reduces NO synthesis through mechanisms such as reduced

Figure 11. Subanalysis of natural logarithmic transformed reactive hyperemia-peripheral arterial tonometry index focused on patients with paroxysmal AF under sinus rhythm and patients with persistent AF under AF rhythm.
A, The comparison of the natural logarithmic transformed reactive hyperemia-peripheral arterial tonometry index (LnRHI) between patients with paroxysmal atrial fibrillation (AF) whose LnRHI were measured under only sinus rhythm (SR) at the time of reactive hyperemia-peripheral arterial tonometry measurement before catheter ablation (CA) (n=138) and patients with persistent AF whose LnRHI measured under only AF rhythm (n=38). **B**, The comparison of LnRHI between control patients (n=43), patients with paroxysmal AF under SR(n=138) and patients with persistent AF under AF rhythm (n=38). **C** through **E**, The serial changes of the LnRHI in patients with paroxysmal AF under SR (**C**), patients with persistent AF under AF rhythm (**D**), and both patients with AF (**E**). **F** through **H**, The serial changes of the LnRHI focused on patients without recurrence in patients with paroxysmal AF under SR (**F**), patients with persistent AF under AF rhythm (**G**), and both patients with AF (**H**). See Figure 3 for the box-and-whisker plot, the mean±SD, and the line connecting the mean values.

endothelial NO synthase expression associated with calcium signaling and phosphorylation[25] and accelerated degradation of endothelial NO synthase mRNA and secretion of excessive inflammatory cytokines, causing vascular endothelial dysfunction.[26] In fact, the Framingham Heart Study reported that advancing age, female sex, lower systolic blood pressure, higher heart rate, higher body mass index, increasing total/high-density lipoprotein cholesterol ratio, diabetes mellitus, smoking, and lipid-lowering medication were associated with abnormal RHI.[24] In addition, lower pulse pressure was also correlated with a decrease in LnRHI before CA in this study (OR, 0.972; P=0.047) (Table 3), though the coefficient of determination between pulse pressure and LnRHI was weak (R^2=0.094, P<0.001), and blood pressure, pulse pressure, and heart rate were not different between before CA, 3 months after CA, and 6 months after CA (Figures 8 through 10). Therefore, vascular endothelial function in patients with AF might be reduced even under SR depending on their individual background, like this study (Figures 3E and 7B, 7D). Not only CA but also treatment for its risk factors is considered to be important for the treatment of AF. Since vascular endothelial function assessment reflects the extent to which endothelial dysfunction occurs as a comprehensive biological response of each individual, vascular endothelial function test is expected to be an individual indicator of AF risk control when the person is exposed to various risk factors of AF.

Thus, vascular endothelial function test is affected by both AF rhythm itself and risk factors of AF. The changes in vascular endothelial function after CA may reflect recurrence or nonrecurrence of AF. In the present study, if the value of LnRHI 3 months after CA did not decrease compared with before CA, it became clear that it would be an independent marker that suspects nonrecurrence of AF (Figure 4). Vascular endothelial function test might be an indicator for assessing whether AF treatment is successful or not. Especially, regarding anticoagulation after CA, anticoagulants must be used to prevent intracardiac thrombosis for at least 3 months after CA.[9] At the time of discontinuation of anticoagulants, the risk of thromboembolism is also considered; however, it is also necessary to determine whether AF has been cured. Therefore, using a vascular endothelial function test, it might be possible to comprehensively evaluate whether AF had not recurred, including the control of risk factors of AF; that is the clinical implication of the present study.

However, vascular endothelial function test before CA for AF could not predict a recurrence of AF in the present study. The LnRHI before CA in the recurrence group was conversely higher than that in the nonrecurrence group (0.78±0.25 versus 0.61±0.25, P<0.001) (Table 4, and Figure 3B and 3C). There are several reasons for this. First, a larger number of patients with AF recurrence had measured their endothelial function before CA under SR coincidentally compared with patients without AF recurrence (29 of 39, 74% versus 122 of 175, 70%, P=0.565) (Figure 3B and 3C). In fact, in patients with recurrence of AF (Figure 6A and 6B), LnRHI measured in SR before CA was significantly higher than that measured in AF rhythm before CA (0.84±0.21 versus 0.60±0.27, P=0.005). In addition, in patients without recurrence of AF (Figure 6E and 6F), LnRHI that measured in SR before CA was also significantly higher than that measured in AF rhythm before CA (0.66±0.24 versus 0.48±0.22, P<0.001). Second, the proportion of patients with persistent AF tended to be lower in the recurrence group than in the nonrecurrence (17.9% versus 25.1%, P=0.340) (Table 4). In this study, whether in recurrence group or non-recurrence group, LnRHI in patients with persistent AF before CA was lower than that in patients with paroxysmal AF (Figure 6C and 6D, and Figure 6G and 6H). Therefore, LnRHI in the recurrence group, which had a lower incidence of persistent AF, might be higher than that in the nonrecurrence group. Third, Since LnRHI measured under AF rhythm in patients with paroxysmal AF was tended to be lower than that measured under AF rhythm in patients with persistent AF (Figure 5D), LnRHI before CA in patients without recurrence of AF who measured RHI under AF rhythm (Figure 6F), which included a large number of paroxysmal AF (n=53; paroxysmal=21, persistent=32), was lower than that in patients with recurrence of AF who measured RHI under AF rhythm (Figure 6B), which included a small number of paroxysmal AF (n=10; paroxysmal=4, persistent=6) (0.48±0.22 versus 0.60±0.27, P=0.152). Fourth, on baseline medications, β-blocker

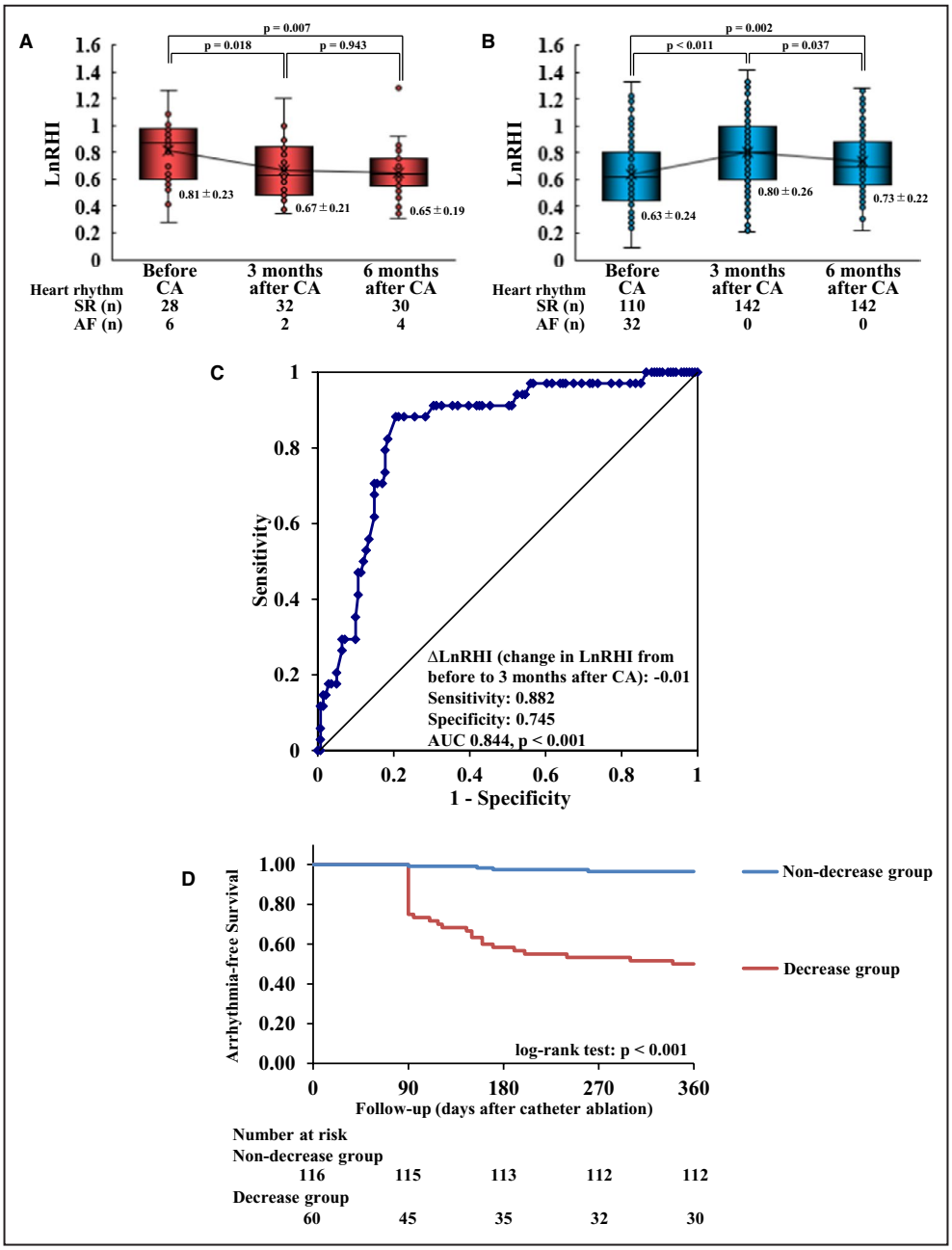


Figure 12. Re-analysis of the major findings focused on patients with paroxysmal AF under sinus rhythm and patients with persistent AF under AF rhythm.

A and **B**, The comparison of the LnRHI in patients with (**A**) and without recurrence of AF (**B**) focused on patients with paroxysmal AF under SR and patients with persistent AF under AF rhythm. **C**, Receiver operating characteristic curve analysis focused on patients with paroxysmal AF under SR and patients with persistent AF under AF rhythm. The value of LnRHI 3 months after catheter ablation, which decreased to ≥0.01 compared with that before catheter ablation, was an independent factor of AF recurrence (sensitivity, 0.882; specificity, 0.745; area under the curve, 0.844; *P*<0.001). **D**, Kaplan−Meier analysis focused on patients with paroxysmal AF under SR and patients with persistent AF under AF rhythm. The decrease group in LnRHI 3 months after catheter ablation compared with before catheter ablation (red) had a significantly higher probability of AF recurrence compared with the non-decrease group (blue) (log-rank test: *P*<0.001). See Figure 3 for the box-and-whisker plot, the mean±SD, and the line connecting the mean values. AF indicates atrial fibrillation; AUC, area under the curve; CA, catheter ablation; LnRHI, natural logarithmic transformed reactive hyperemia-peripheral arterial tonometry index; and SR, sinus rhythm.

Table 6. Univariate and Multivariate Cox Proportional Hazards Analysis for the Recurrence of AF Focused on Patients With Paroxysmal AF Under SR and Patients With Persistent AF Under AF Rhythm

Variable	Univariate Cox Hazard			Multivariate Cox Hazards With Significant Factors in Univariate Analysis		
	HR	95% CI	*P* Value	HR	95% CI	*P* Value
Age, per y	1.000	0.965–1.036	0.985			
Male (yes)	1.050	0.502–2.196	0.897			
Height, per cm	0.994	0.960–1.029	0.733			
Weight, per kg	1.005	0.977–1.034	0.711			
Body mass index, per kg/m^2	1.057	0.962–1.161	0.251			
Waist, per cm	l.013	0.976–1.052	0.491			
Duration of AF, per y	l.070	1.009–1.135	0.023	1.079	1.019–1.142	0.010
Persistent AF (yes)	0.761	0.315–1.837	0.543			
Diabetes mellitus (yes)	0.600	0.144–2.502	0.483			
Hypertension (yes)	0.884	0.451–1.733	0.719			
Systolic blood pressure before CA, per mm Hg	1.004	0.980–1.029	0.750			
Diastolic blood pressure before CA, per mm Hg	0.994	0.964–1.025	0.717			
Pulse pressure before CA, per mm Hg	1.010	0.982–1.039	0.479			
Heart rate before CA, per beats/min	0.995	0.996–1.026	0.763			
Dyslipidemia (yes)	2.249	1.148–4.406	0.018	1.953	0.945–4.035	0.071
Smoking history (yes)	1.843	0.912–3.725	0.088			
Left atrial diameter, per mm	1.001	0.940–1.066	0.978			
LVEF, per %	1.005	0.941–1.072	0.891			
hsCRP, per mg/dL	0.384	0.028–5.272	0.474			
eGFR, per mL/min per 1.73 m^2	0.992	0.968–1.016	0.491			
BNP, per pg/mL	0.995	0.986–1.004	0.239			
Baseline medication						
β-blockers (yes)	2.316	1.180–4.544	0.015	1.881	0.896–3.948	0.095
ACEIs or ARBs (yes)	1.051	0.526–2.100	0.887			
Calcium channel blockers (yes)	0.587	0.266–1.297	0.188			
AADs (yes)	0.921	0.282–3.013	0.892			
Postoperative β-blockers (yes)	1.490	0.135–16.430	0.745			
LnRHI (before CA)	11.382	3.112–41.621	<0.001	2.540	0.483–13.365	0.271
LnRHI (3 mo after CA)	0.135	0.033–0.550	0.005	…	…	…
LnRHI (6 mo after CA)	0.209	0.039–1.117	0.067			
ΔLnRHI (difference between before and 3 mo after CA)	0.021	0.006–0.072	<0.001	0.043	0.010–0.180	<0.001

AAD indicates anti-arrhythmic drugs; ACEI, angiotensin-converting enzyme inhibitors; AF, atrial fibrillation; ARB, angiotensin receptor blockers; BNP, brain natriuretic peptide; CA, catheter ablation; eGFR, estimated glomerular filtration ratio; HR, hazard ratio; hsCRP, high sensitivity C-reactive protein; LnRHI, natural logarithmic transformed reactive hyperemia-peripheral arterial tonometry index values; LVEF, left ventricular ejection fraction; and SR, sinus rhythm.

usage in the recurrence group was significantly higher than that in the nonrecurrence group (51.3% versus 31.4%, *P*=0.020) (Table 4). The use of β-blocker has been shown to improve RHI independent of blood pressure improvement.[27] These findings may have influenced the inverse phenomenon of LnRHI levels between patients with and without recurrence of AF before CA. Also, since vascular endothelial function is influenced not only by the rhythm of AF itself or chronic inflammation associated with the risk factor of AF but also by any other factors that affect to the individual, it might be difficult to predict the recurrence of AF by comparing 1-point numerical values of vascular endothelial function between individuals. In patients without recurrence of AF, whether paroxysmal or persistent, or whether measured under SR or AF rhythm before CA, LnRHI improved to the similar level as normal control subjects (Figures 3E and 7). Intraindividual variation and improvement, rather than interindividual differences, are considered to be important in vascular endothelial function test in the treatment of AF.

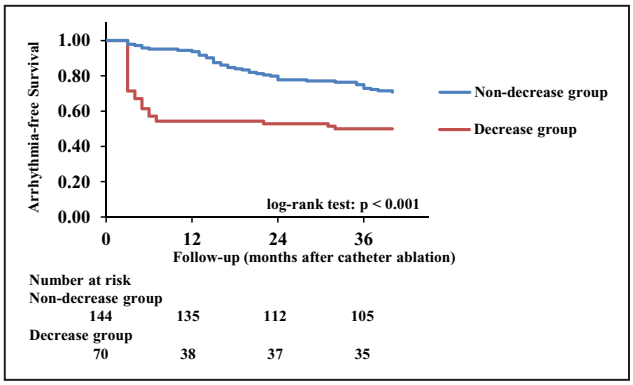


Figure 13. Long-term outcome of the recurrence after catheter ablation in patients with atrial fibrillation.
Kaplan–Meier analysis showed the decrease group in the natural logarithmic transformed reactive hyperemia-peripheral arterial tonometry index 3 months after catheter ablation compared with before catheter ablation (red) had a significantly higher probability of long-term outcome of atrial fibrillation recurrence compared with the non-decrease group (blue) (log-rank test: P<0.001).

Study Limitations

This study included several limitations. First, this was a single-center observational study with a small number of patients. Second, although we complied with the indication for CA for AF, selection bias cannot be denied. Third, vascular endothelial function includes not only NO production but also smooth muscle cell proliferation inhibition, coagulation, vascular permeability, and regulation of inflammation. The present study only looks at vascular endothelial function assessed by the RH-PAT. Fourth, RHI might have improved a little in patients with recurrence because of atrial tachycardia or atrial flutter compared with recurrence attributable to AF because of the regularity of the pulse wave. In case of recurrence with atrial tachycardia or atrial flutter, a different RHI value may be needed as a predictive value of recurrence. Fifth, since RHI is greatly affected by the rhythm of AF itself, RHI may not decrease in the condition that the AF burden is low. Similarly, if the recurrence of AF occurs for the first time in the late phase such as over 1 year after CA, RHI just within 6 months after CA may not be an indicator of recurrence of AF as shown in Figure 13. Very late AF recurrences have been reported to occur up to 10 years after ablation.[9] Conversely, in the phase immediately just after CA such as within 3 months, acute inflammatory changes owing to energy delivery is involved, therefore RHI may not be reliable. Sixth, the follow-up period for this study was only 1 year, therefore there was no significant difference in recurrence rates between paroxysmal AF and persistent AF (paroxysmal AF: 32 of 163, 20% versus persistent AF: 7 of 51, 14%; P=0.340). Because the judgment of recurrence after >1 year was performed based on the occasional ECG, evaluation of long-term outcome might not be sufficient.

CONCLUSIONS

The rhythm of AF was independently correlated with impaired vascular endothelial function assessed by the RH-PAT. However, the vascular endothelial dysfunction was improved by the long-term SR restoration after CA for AF. The change in vascular endothelial function following CA might sensitively reflect the recurrence or nonrecurrence of AF. In the treatment of AF, the assessment of vascular endothelial function has been found to be one of an important examination for evaluating the freedom from AF after CA and the control of risk factors associated with the development of AF.

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