Clinical and Applied Thrombosis/Hemostasis
Volume 25: 1-11

DOI: 10.1177/1076029619851570
journals.sagepub.com/home/cat
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# Dynamics of Soluble Thrombomodulin and Circulating miRNAs in Patients with Atrial Fibrillation Undergoing Radiofrequency Catheter Ablation

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**Abstract**

Atrial fibrillation (AF) is the most common cardiac arrhythmia in the world and has a high risk of thromboembolism. The most effective approach, catheter ablation, requires evaluation by electrocardiography. The aim of our study was to investigate novel clinical markers that predict restoration of sinus rhythm (SR) after catheter ablation. Seventy-eight consecutive patients with AF underwent catheter ablation and were separated into 2 groups: restored SR and recurrent AF. The levels of 4 blood proteins (serum or plasma) and 3 mature microRNAs (miRNAs) and their primary miRNAs (pri-miRNAs) in serum were measured before and after ablation, and the associations between each parameter were analyzed statistically. Soluble thrombomodulin (s-TM) and plasminogen activator inhibitor-1 (PAI-1) levels increased above baseline after ablation in both the restored SR (s-TM 11.55 [2.92] vs 13.75 [3.38], $P < .001$; PAI-1 25.74 [15.25] vs 37.79 [19.56], $P < .001$) and recurrent AF (s-TM 10.28 [2.78] vs 11.67 [3.37], $P < .001$; PAI-1 26.16 [15.70] vs 40.74 [22.55], $P < .001$) groups. Levels of C-reactive protein and asymmetric dimethylarginine were not significantly changed. Pri-miR-126 levels significantly decreased after ablation in the recurrent AF group, but the other miRNAs and pri-miRNAs did not. The measurement of s-TM and pri-miR-126 in blood was a useful tool to reflect the condition of AF patients with catheter ablation.

**Keywords**

atrial fibrillation, catheter ablation, soluble thrombomodulin, plasminogen activator inhibitor-1, miRNA, primary miRNA

Date received: 5 November 2018; revised: 17 April 2019; accepted: 18 April 2019.

## Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia and the prevalence of AF patients has increased as the population has grown older.[1] Patients with AF have an increased risk of stroke and thromboembolism.[2] Warfarin and direct oral anticoagulants (DOACs) effectively reduce the risk of ischemic stroke caused by AF,[3,4] however, anticoagulation increases the risk of major bleeding. Catheter ablation is an effective approach for the management of AF,[5-9] but evaluation of the effectiveness of AF ablation requires electrocardiography (ECG), and there are no specific blood-based biomarkers associated with the impact of AF ablation.

Vascular endothelial cells play a pivotal role in regulating the vascular inflammatory response as well as maintaining the

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homeostasis of blood vessels.[10] Atrial fibrillation leads to endothelial dysfunction,[11,12] and a reduced nitric oxide (NO) concentration in platelets has been demonstrated in patients with AF.[13] Measurements of flow-mediated dilatation (FMD) and reactive hyperemia peripheral arterial tonometry (RH-PAT) reflect peripheral endothelial function. Successful AF ablation has been shown by FMD and RH-PAT to improve endothelial dysfunction by restoring sinus rhythm (SR).[14-17] Several molecules, including plasminogen activator inhibitor-1(PAI-1), soluble thrombomodulin (s-TM), and asymmetric dimethylarginine (ADMA), which is a NO synthase inhibitor, have been identified as candidate biomarkers for endothelial function, but none have been directly proven.[14]

MicroRNAs (miRNAs) are small noncoding RNAs composed of about 22 ribonucleotides that regulate gene expression at the post-transcriptional level.[18] A variety of miRNAs have been shown to be linked to cardiovascular dysfunction, such as cardiac hypertrophy, ischemic heart disease, hypertension and arrhythmia.[19-22] There are a series of miRNAs involved in vascular function, of which miR-126 is selectively expressed in endothelial cells and plays the most important role in angiogenesis.[23,24] Another vascular miRNA, miR-22, acts as an oncogenic or tumor-suppressing miRNA in several human cancers, and also regulates vascular endothelial growth factor in colorectal cancer.[25] Circulating miR-22 was shown to be elevated in the serum of patients with heart failure (HF), suggesting that miR-22 is associated with cardiovascular function.[26]

The aim of the present study was to examine several blood proteins and miRNAs related to endothelial function in patients with AF, before and 6 months after catheter ablation. The associations between these blood proteins and miRNAs were also analyzed. In particular, we examined the primary miRNAs that were reported to reflect endothelial function.

## Methods

### Study Population

This study comprised 101 consecutive patients (20 women and 81 men; mean age, 61.8 $\pm$ 8.6 years), including 54 (53%) patients with paroxysmal AF, 36 (36%) patients with persistent AF, and 11 (11%) patients with long-standing persistent AF who were seen from November 2014 to August 2015. Forty-two patients had a history of AF ablation. Twenty-three patients were ultimately excluded from the study because the 6-month post-ablation follow-up was not carried out by the patient's local hospital.

All antiarrhythmic drugs were discontinued at least 5 half-lives before ablation, with the exception of amiodarone, which was discontinued at least 3 months before ablation. Warfarin or DOACs were not discontinued, and all patients provided written informed consent for all procedures. The protocol of this study was approved by the Institutional Ethics Committee of (Receipt number: 26-96). Atrial fibrillation was defined in accordance with the 2012 Heart Rhythm Society Expert Consensus Statement.[27] Paroxysmal AF was defined as recurrent AF ($\geq$ 2 episodes) that terminated spontaneously within 7 days. Persistent AF was defined as continuous AF that was sustained beyond 7 days, but necessitated pharmacologic or electrical cardioversion, and longstanding persistent AF was defined as continuous AF of a minimum 1-year duration.

### Catheter Ablation Procedure

The AF ablation technique described by Nademanee et al[28,29] was utilized for this study. Briefly, the left atrium (LA) was accessed using a single trans-septal puncture with assisted intracardiac ultrasound catheter (AcuNav; Biosense Webster, Diamond Bar, CA, USA). After the coronary sinus (CS) was cannulated with a decapolar catheter (Dynamic XT Decapolar Steerable-Cath; C.R. Bard, Murray Hill, NJ, USA) for recording and induction, patients underwent non-fluoroscopic electroanatomical mapping with CARTO3 (Biosense Webster). A 3.5-mm NaviStar ThermoCool SmartTouch catheter (Biosense Webster) was used for ablation in all cases. Heparin (used to keep the activated clotting time > 300 seconds) was administered for anticoagulation during the procedure. All patients underwent complex fractionated atrial electrogram (CFAE)-targeted catheter ablation. Electroanatomical maps were displayed by shortest complex interval map on the CARTO3, and the areas of CFAE were also identified. The CFAE parameters on CARTO3 were modified settings: voltage range, 0.05 to 0.30 millivolts; interval range, 40 to 70 milliseconds.[30] Radiofrequency applications were used with a maximal power output of 35 watts with irrigation rates of 30 mL/minute (3.5-mm NaviStar ThermoCool SmartTouch catheter; Biosense Webster). The power was reduced to 15 watts in the posterior LA and CS because of their nearness to the esophagus.[31] The primary end points during catheter ablation were either complete elimination of areas of CFAE or conversion of AF to SR with occasional injection of nifekalant (0.3 mg/kg intravenously over 10 minutes, given twice maximum). If the atrial arrhythmias were not successfully terminated, internal cardioversion was performed.

### Ablation Follow-Up

All patients were followed up at 3 months and 6 months after ablation. Electrocardiography was also performed at each follow-up, with additional ECGs performed using a portable ECG event recorder or 24-hour Holter monitor as indicated by cardiac symptoms including palpitation continued for more than 30 seconds. Atrial fibrillation recurrence during this 6-month follow-up period was noted with a blanking period for the first 3 months. Arrhythmia recurrence was defined as any episode lasting more than 30 seconds and confirmed by either scheduled ECG.

### Blood Collection and Laboratory Analysis

Peripheral blood samples were collected from the antecubital vein before ablation and at 6 months post ablation during outpatient follow-up. Blood samples were subjected to

centrifugation at 1710*g* for 5 minutes to separate the serum, or at 920*g* for 15 minutes to separate the plasma, and stored at −80°C until use. The serum level of ADMA was measured using an in vitro enzyme-linked immunosorbent assay kit (Immunodiagnostik, Bensheim, Germany). The serum level of s-TM was measured using a chemiluminescent enzyme immunoassay kit. A latex photometric immunoassay kit was used to measure the serum levels of high-sensitivity C-reactive protein (hs-CRP) and the plasma levels of PAI-1, which were subjected to further analysis on the automated clinical laboratory system STACIA (LSI Medience, Tokyo, Japan).

### *RNA Purification and Measurement of Mature miRNAs and Primary miRNAs (pri-miRNAs)*

Total RNA including miRNAs was isolated using QIAzol lysis reagent and the miRNeasy Serum/Plasma kit (QIAGEN, Hilden, Germany). Mature miRNA and primary miRNA (pri-miRNAs) levels were measured as previously described.[32] Briefly, the cDNA of several miRNAs and their corresponding pri-miRNAs were synthesized using the High Capacity cDNA Reverse Transcription Kit and MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA), respectively. Quantitative real-time polymerase chain reaction (PCR) to measure the levels of miRNA and pri-miRNA was performed using TaqMan MicroRNA assays (Applied Biosystems) and FastStart Universal Probe Master (Roche, Basel, Switzerland), according to the manufacturer's protocol, with the 7300 Real-Time PCR System (Applied Biosystems). The threshold cycle (Ct) was defined as the fractional cycle number at which fluorescence cleared the prescribed threshold. Relative quantifications were calculated using the comparative Ct method ($2^{-\Delta\Delta Ct}$). Expression levels of miRNAs and pri-miRNAs were normalized to those of the RNA spike-in control cel-miR-39 and β-actin.

### *Statistical Analysis*

Continuous variables are expressed as the mean (standard deviation [SD]). Categorical data are expressed as count and percentage, except where indicated. Continuous variables were analyzed using *t*-tests for Gaussian-distributed data. The data were tested for Gaussian distribution using the Kolmogorov-Smirnov test. Comparisons of each baseline continuous variable between groups (restored SR vs recurrent AF) were analyzed using the unpaired *t*-test or Mann–Whitney *U* test. Comparisons of before- and after-ablation in each group were analyzed using the paired *t*-test or Wilcoxon rank-sum test. Categorical variables were analyzed using Fisher exact test. Spearman rank correlation coefficients were used to identify correlations between each value. The data were analyzed using the GraphPad Prism version 6.0 for Windows (GraphPad Software, San Diego, CA, USA). Statistical significance was defined as a $P$ value <.0025 for the comparison of 20 parameters and as a $P$ value <.0042 for the correlation of 12 parameters, according to Bonferroni correction.

**Table 1.** Patient Characteristics.[a]

| | Restored SR, n = 32 | Recurred AF, n = 46 | *P* Value |
|---|---|---|---|
| Age (years) | 61 ± 11 | 63 ± 7 | .721 |
| Male | 28 (87.5) | 36 (78.3) | .376 |
| Body mass index (kg/m$^2$) | 23.5 ± 2.9 | 24.3 ± 3.0 | .214 |
| AF type | | | |
| Paroxysmal AF | 21 (65.6) | 22 (47.8) | .166 |
| Persistent AF | 9 (28.1) | 17 (37.0) | .471 |
| Long-standing persistent AF | 2 (6.3) | 7 (15.2) | .295 |
| Histories of AF ablation | 14 (43.8) | 19 (41.3) | 1.000 |
| Casual factors | | | |
| Congestive heart failure | 3 (9.4) | 5 (10.9) | 1.000 |
| Hypertension | 12 (37.5) | 26 (56.5) | .133 |
| Diabetes mellitus | 4 (12.5) | 5 (10.9) | 1.000 |
| Previous stroke/TIA | 2 (6.3) | 5 (10.9) | .694 |
| Cardiovascular disease | 2 (6.3) | 4 (8.7) | 1.000 |
| Medications | | | |
| ARBs or ACE inhibitors | 10 (31.2) | 14 (30.4) | 1.000 |
| β-blockers | 17 (53.1) | 25 (54.3) | 1.000 |
| Calcium channel antagonists | 12 (37.5) | 14 (30.4) | .627 |
| Statins | 5 (15.6) | 14 (30.4) | .182 |
| Class I AADs | 16 (50.0) | 21 (45.7) | .819 |
| Echocardiographic parameters | | | |
| LAD (mm) | 38.51 ± 5.69 | 42.88 ± 5.71 | .003 |
| LAV (mL) | 57.36 ± 20.11 | 76.49 ± 23.84 | <.001 |
| LVEF (%) | 62.23 ± 10.99 | 59.13 ± 11.47 | .177 |
| Anticoagulants | | | |
| Warfarin | 7 (21.9) | 18 (39.1) | .141 |
| Rivaroxaban | 16 (50.0) | 17 (37.0) | .352 |
| Apixaban | 3 (9.4) | 5 (10.9) | 1.000 |
| Edoxaban | 6 (18.8) | 5 (10.9) | .344 |
| Dabigatran | 0 (0) | 1 (2.2) | 1.000 |

Abbreviations: AAD, antiarrhythmic drug; ACE, angiotensin-converting enzyme; ARB, angiotensinII receptor blocker; DOACs, direct oral anticoagulants; LAD, Left atrial diameter; LAV, Left- atrial volume; LVEF, left ventricular ejection fraction; TIA, transient ischemic attack.
[a]Values are mean ± SD, or n (in percent).

## Results

### *Baseline Patient Characteristics*

Catheter ablation was performed and followed up on all patients, who were then assigned to one of 2 groups: restored SR after catheter ablation (restored SR) or recurrent AF after catheter ablation (recurrent AF). The clinical characteristics of the 2 patient groups are shown in Table 1. There were no significant differences between the groups in terms of age, gender, body mass index, type of AF, or causal factors and medications, including anticoagulants. While the left atrial diameter was relatively wider ($P = .003$) and the left atrial volume was greater ($P < .001$) at baseline in the recurrent AF group compared with the restored SR group (Table 1), the left ventricular ejection fraction did not show a significant difference. Complete blood count data were not changed between the groups (Table 2).

**Table 2.** Change of Parameter.

| | Restored SR | | | Recurred AF | | |
|---|---|---|---|---|---|---|
| | Baseline | 6-Month f/u | *P* Value | Baseline | 6-Month f/u | *P* Value |
| Bloodmarkers | | | | | | |
| ADMA (μM/L) | 0.625 (0.163) | 0.589 (0.101) | .241 | 0.637 (0.143) | 0.616 (0.102) | .500 |
| s-TM (U/mL) | 11.55 (2.92) | 13.75 (3.38) | <.001 | 10.28 (2.78) | 11.67 (3.38) | <.001 |
| PAI-1 (ng/mL) | 25.74 (15.25) | 37.79 (19.56) | <.001 | 26.16 (15.70) | 40.74 (22.55) | <.001 |
| hs-CRP (μg/dL) | 120.5 (245.5) | 70.9 (68.0) | .599 | 187.7 (371.4) | 107.9 (134.0) | .905 |
| Mature microRNAs | | | | | | |
| miR-22 | 1.134 (1.654) | 0.627 (0.600) | .278 | 1.355 (1.928) | 0.707 (0.576) | .185 |
| miR-126 | 1.973 (2.003) | 1.430 (1.294) | .421 | 1.549 (1.563) | 1.647 (2.087) | .737 |
| miR-142 | 2.618 (2.487) | 2.390 (2.269) | .524 | 2.500 (2.169) | 2.236 (1.786) | .294 |
| Primary microRNAs | | | | | | |
| Pri-miR-22 | 2.429 (2.692) | 1.997 (2.387) | .443 | 2.915 (3.036) | 2.388 (2.219) | .380 |
| Pri-miR-126 | 1.878 (2.378) | 1.150 (0.846) | .369 | 3.394 (4.831) | 0.941 (0.438) | <.001 |
| Pri-miR-142 | 100.2 (232.1) | 69.9 (149.9) | .134 | 38.5 (101.9) | 48.1 (181.6) | .066 |
| Echocardiographic data | | | | | | |
| LAD (mm) | 38.51 (5.69) | 38.58 (6.23) | .909 | 42.88 (5.71) | 42.67 (5.91) | .682 |
| LAV (mL) | 57.36 (20.11) | 59.99 (19.82) | .248 | 76.49 (23.84) | 73.15 (26.99) | .244 |
| LVEF (%) | 62.23 (10.99) | 65.16 (9.09) | .101 | 59.13 (11.47) | 59.16 (10.72) | .829 |
| Complete blood count data | | | | | | |
| WBC ($\times 10^3/\mu L$) | 5.43 (1.17) | 5.77 (1.61) | .183 | 5.65 (1.32) | 5.87 (1.55) | .399 |
| RBC ($\times 10^6/\mu L$) | 4.73 (0.53) | 4.65 (0.47) | .128 | 4.68 (0.37) | 4.64 (0.38) | .294 |
| PLT ($\times 10^3/\mu L$) | 211.6 (44.5) | 201.2 (48.1) | .044 | 207.8 (47.0) | 209.8 (48.9) | .899 |
| Biochemical data | | | | | | |
| AST (U/L) | 25.44 (9.73) | 30.34 (15.61) | .028 | 27.02 (8.53) | 27.59 (11.45) | .836 |
| ALT (U/L) | 23.22 (9.81) | 27.91 (13.50) | .013 | 23.35 (9.82) | 23.43 (11.24) | .899 |
| BUN (mg/dL) | 15.36 (3.86) | 15.57 (3.97) | .872 | 15.50 (4.62) | 16.18 (3.67) | .220 |
| CRE (mg/dL) | 0.883 (0.237) | 0.878 (0.243) | .595 | 0.895 (0.180) | 0.889 (0.164) | .696 |

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; BUN, blood urea nitrogen; CRE, creatinine; LAD, Left atrial diameter; LAV, Left- atrial volume; LVEF, left ventricular ejection fraction; PLT, platelet; RBC, red blood cell; WBC, white blood cell.

### *Increase in s-TM, but not ADMA, after Catheter Ablation*

To evaluate the function of endothelial cells, the levels of 4 important molecules were measured: s-TM, ADMA, PAI-1, and hs-CRP. There were no significant differences in these molecules in patients with AF who had or had not received ablation before entering into this study. There was also no significant difference in these molecules in a comparison between patients with paroxysmal or persistent AF at baseline (data not shown).

The level of s-TM at baseline was higher in the restored SR group than the recurrent AF group ($P = .030$). After catheter ablation, the s-TM levels had increased at the 6-month follow-up compared with baseline in both the restored SR and recurrent AF groups (11.55 [2.92] vs 13.75 [3.38], $P < .001$; 10.28 [2.78] vs 11.67 [3.37], $P < .001$, respectively; Figure 1A). There were no significant differences in ADMA levels between the 2 groups at baseline ($P = .403$), nor were there any significant differences in ADMA levels at the 6-month follow-up compared with the baseline for either the restored SR group (0.625 [0.163] vs 0.589 [0.101], $P = .241$) or the recurrent AF group (0.637 [0.143] vs 0.616 [0.102], $P = .500$; Figure 1B). Contrary to the results for s-TM, the hs-CRP level in the restored SR group was lower than that in the recurrent AF group at baseline ($P = .038$), but the hs-CRP levels at the 6-month follow-up were unchanged in both groups (120.5 [245.5] vs 70.9 [68.0], $P = .599$; 187.7 [371.4] vs 107.9 [134.0], $P = .905$), respectively (Figure 1C). There were no significant differences in PAI-1 levels between the 2 groups at the baseline ($P = .907$). After catheter ablation, similar to the result of s-TM, PAI-1 levels increased at the 6-month follow-up compared with baseline in both the restored SR group (25.74 [15.25] vs 37.79 [19.56], $P < .001$) and the recurrent AF group (26.16 [15.70] vs 40.74 [22.55], $P < .001$), respectively (Figure 1D). A Receiver Operating Characteristic (ROC) curve was created and the area under the curve for AF recurrence in s-TM at baseline was 0.645 (Supplementary Figure 1). Sensitivity and specificity for AF recurrence were determined using the Youden Index, which was 68.8% and 58.7% at 10.07 U/mL, respectively.

### *Pri-miR-126 Decreased after Ablation in the Recurrent AF Group*

To evaluate mature and primary miRNA in AF before and after catheter ablation, the levels of miR-22, miR-126, and miR-142 were measured. There were no significant differences in the levels of these miRNAs between the restored SR group and the recurrent AF group at baseline (miR-22, $P = .640$; miR-126, $P = .476$; miR-142, $P = 0.924$), or at the 6-month follow-up (miR-22, $P = .278$ and $P = .185$; miR-126, $P = .421$ and $P = .737$; miR-142, $P = .524$ and $P = .294$; Figure 2A).

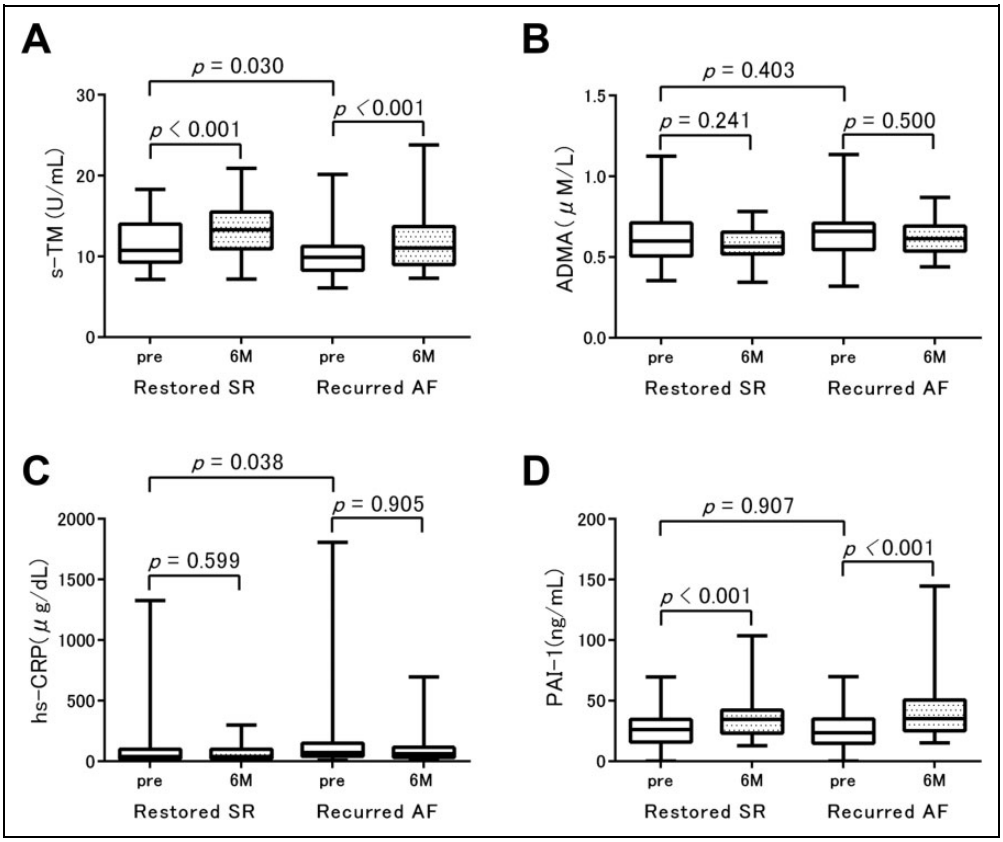


**Figure 1.** Serum levels of soluble thrombomodulin (s-TM) (A), asymmetric dimethylarginine (ADMA) (B), and high-sensitivity C-reactive protein (hs-CRP) (C), and plasma levels of plasminogen activator inhibitor-1(PAI-1) (D) before and 6 months after catheter ablation in patients in the restored sinus rhythm (SR) and recurrent atrial fibrillation (AF) groups. Lines in the middle of the boxes represent the median values. The lower and upper edges of the boxes represent the first and third quartiles, respectively.

Similarly, there were no significant differences in any of the pri-miRNA levels between the 2 groups at baseline (pri-miR-22, $P = .329$; pri-miR-126, $P = .099$; pri-miR-142, $P = .160$; Figure 2B). After catheter ablation, the pri-miR-22 and pri-miR-142 levels were unchanged in both the restored SR group and the recurrent AF group, respectively, at the 6-month follow-up (pri-miR-22, $P = .443$ and $P = .380$; pri-miR-142, $P = .134$ and $P = .066$). However, the pri-miR-126 level decreased at the 6-month follow-up compared with the baseline in the recurrent AF group (pri-miR-126, 1.878 [2.378] vs 1.150 [0.846], $P = .369$; 3.394 [4.831] vs 0.941 [0.438], $P < .001$; Figure 2B). Interestingly, the pri-miR-126 levels in patients with diabetes mellitus (DM) and/or hypertension (HT) relatively decreased after ablation ($P = .009$), but not in patients without DM and/or HT ($P = .813$).

## *Correlation between pri-miRNAs and Mature miRNAs*

Since miR-142 is not a major endothelial miRNA, the remaining analyses were conducted on miR-22 and miR-126 data. The serum levels of miR-126 were highly and positively correlated with miR-22 at the change before and after catheter ablation ($r = 0.873$, $P < .001$; Figure 3A). Similarly, the serum levels of pri-miR-126 were positively correlated with pri-miR-22 at the change before and after catheter ablation ($r = 0.298$, $P = .008$; Figure 3A). Unexpectedly, there were no positive correlations for changes in miR-22 versus pri-miR-22, or miR-126 versus pri-miR-126 ($r = -0.259$, $P = .022$; $r = -0.108$, $P = .348$, respectively; Figure 3B).

## *Association between Primary miRNAs and Other Molecules*

The changes in s-TM levels before and after catheter ablation were inversely correlated with the changes in miR-22 and miR-126 levels ($r = -0.341$, $P = .002$; $r = -0.255$, $P = .024$, respectively; Figure 4A). In contrast, the changes in ADMA levels were highly positively correlated with the changes in miR-22 and miR-126 levels ($r = 0.533$, $P < .001$; $r = 0.510$, $P < .001$, respectively; Figure 4B). With regard to the pri-miRNAs, the changes in pri-miR-126 levels were positively correlated with those of hs-CRP ($r = 0.299$, $P = .008$) and inversely correlated with those of s-TM ($r = -0.320$, $P = .004$; Figure 5A). In turn, the changes in pri-miR-22 were positively correlated with those of hs-CRP ($r = 0.229$, $P = .044$) and inversely correlated with those of ADMA ($r = -0.245$, $P = .032$; Figure 5B).

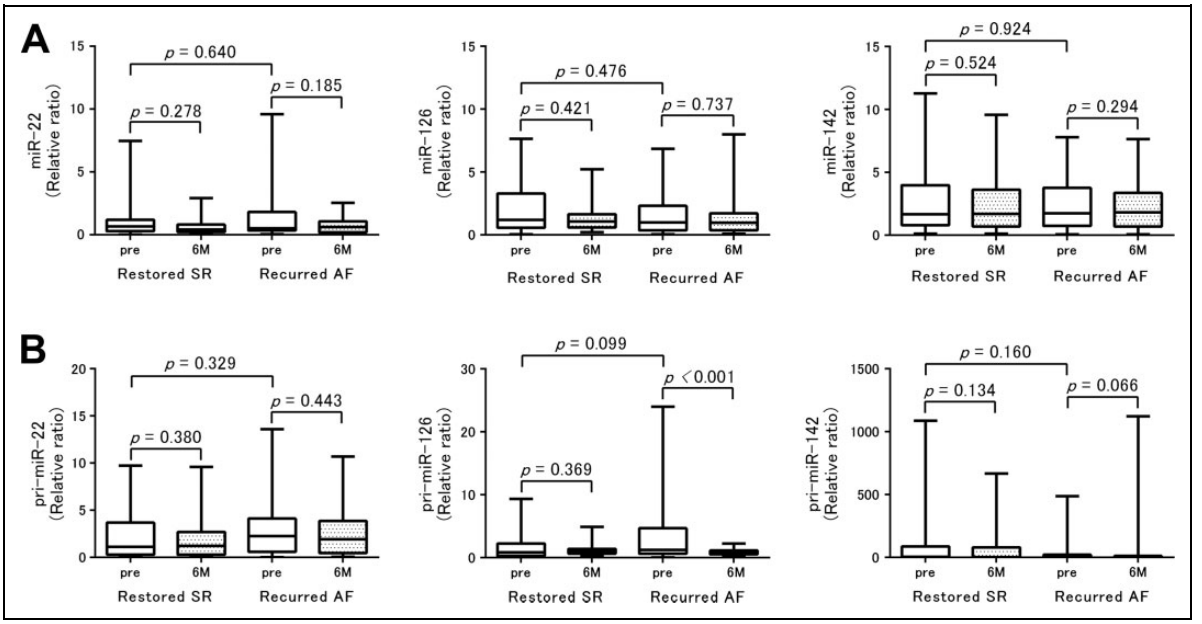


**Figure 2.** Serum levels of mature microRNAs (miRNAs) (A) and primary miRNAs (B) before and 6 months after catheter ablation in patients in the restored sinus rhythm (SR) and recurrent atrial fibrillation (AF) groups. Lines in the middle of the boxes represent the median values. The lower and upper edges of the boxes represent the first and third quartiles, respectively.

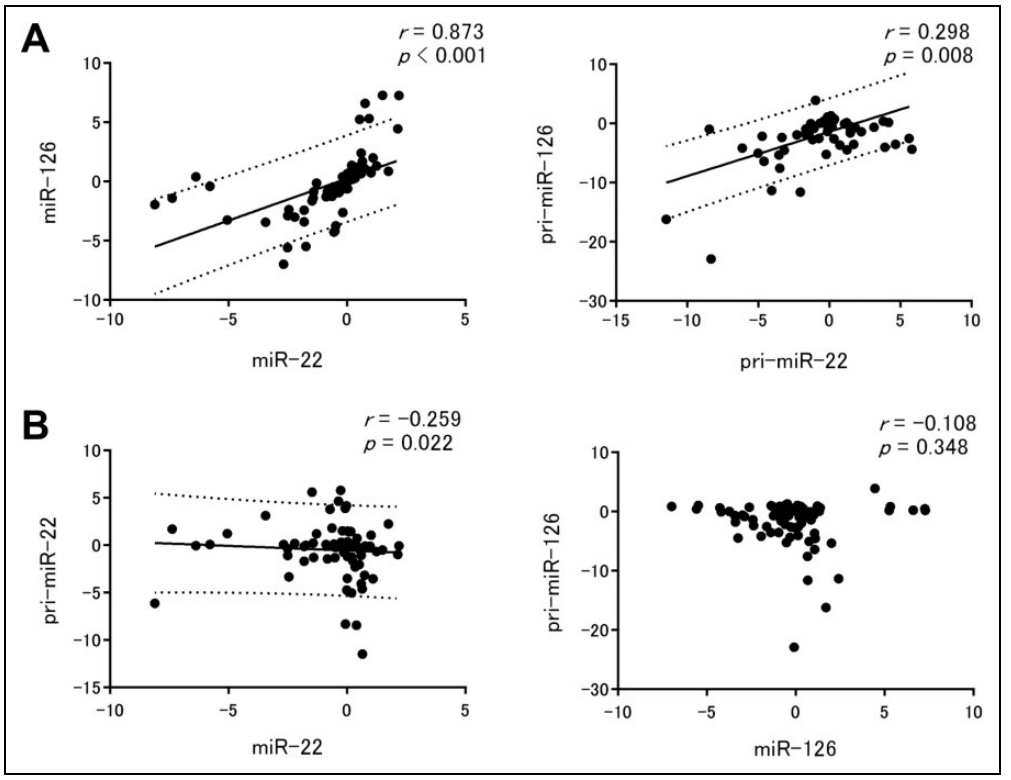


**Figure 3.** (A) Correlations between the serum levels of mature miR-22 and miR-126 (left), and primary (pri)-miR-22 and pri-miR-126 (right), in 78 patients with atrial fibrillation (AF; r = 0.873, $P < .001$; r = 0.298, $P = .008$, respectively). (B) Correlations between the serum levels of mature miR-22 and pri-miR-22 (left), and mature miR126 and pri-miR-126 (right), in 78 patients with AF (r = 0.873, $P < .001$; r = 0.298, $P = .008$, respectively).

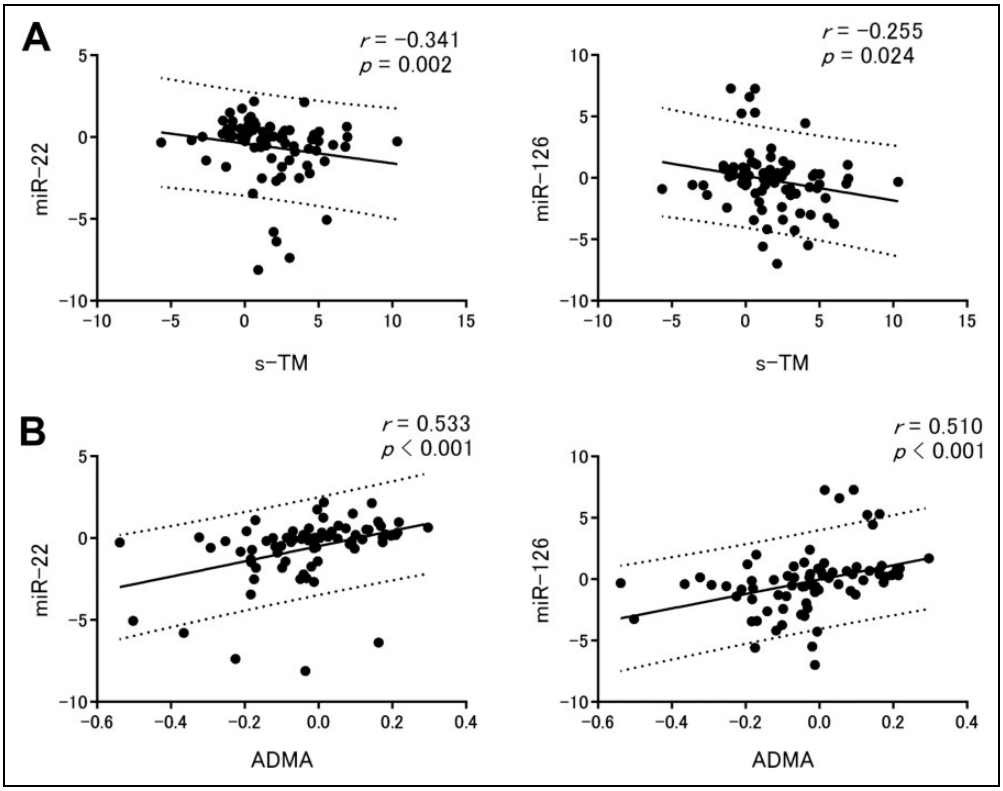


**Figure 4.** (A) Correlations between the serum levels of miR-22 and soluble thrombomodulin (s-TM; left), and miR-126 and s-TM (right), in 78 patients with atrial fibrillation (AF; r = −0.341, *P* = .002; r = −0.255, *P* = .024, respectively). (B) Correlations between the serum levels of miR-22 and asymmetric dimethylarginine (ADMA; left), and miR-126 and ADMA (right), in 78 patients with AF (r = 0.533; *P* < .001; r = 0.510, *P* < .001, respectively).

## Discussion

This study was performed to investigate the pattern of changes in multiple parameters for endothelial function, and to examine the associations between these data in patients with AF. Previous studies in patients with AF only evaluated endothelial markers and circulating mature miRNAs on an individual basis, with the main findings as follows: (1) the level of s-TM in the restored SR group was higher than that in the recurrent AF group at baseline; (2) s-TM and PAI-1 were increased at 6 months after catheter ablation; and (3) pri-miR-126 was decreased after catheter ablation in the recurrent AF group.

Thrombomodulin plays a pivotal role in regulating thrombosis on the surface of endothelial cells. Thrombin binding to TM causes protein C activation, which results in anticoagulant and anti-inflammatory events in vessels.[33] Recently s-TM has been recognized as a biochemical marker for endothelial damage. The serum level of s-TM is higher in patients with DM, while a decreased s-TM level has been observed in patients with pulmonary hypertension.[34,35] There is some controversy regarding the relationship between AF and s-TM. In one study, the baseline serum level of s-TM in patients with newly diagnosed non-valvular AF was higher than in controls,[36] while others showed that the s-TM level and expression of TM in tissue was lower in AF patients compared with healthy control subjects.[37,38] Our data showed that the s-TM level in patients with AF increased after catheter ablation (Figure 1A). This is consistent with the association between increased levels of endothelial-derived molecules, including s-TM, with adverse outcomes in patients with AF.[39] Moreover, the level of s-TM was higher in the restored SR group than the recurrent AF group (Figure 1A). The cut-off value of s-TM for AF recurrence was 10.07 U/mL, although specificity and sensitivity were relatively low. In an experimental rat model, rapid atrial pacing downregulated the gene expression of TM in atrial endocardium,[40] suggesting that the AF condition downregulated TM expression in the heart or endothelial cells and decreased serum s-TM.

While the levels of PAI-1 and s-TM were high in patients at 6 months after catheter ablation, no significant change in hs-CRP with catheter ablation was observed (Figure 1B and 1C). In previous studies of PAI-1, this fibrinolysis marker was significantly increased in AF cases compared with controls.[41,42] The Hs-CRP is a more precise biomarker than CRP, and the hs-CRP test is performed to assess the condition of vascular inflammation; an increased level of hs-CRP is also correlated with the prognosis of cardiovascular disease.[43] Moreover, hs-CRP is recognized as a useful parameter of endothelial cellular function.[44] We found a significantly higher level of hs-CRP at the baseline in patients in the AF recurrence group

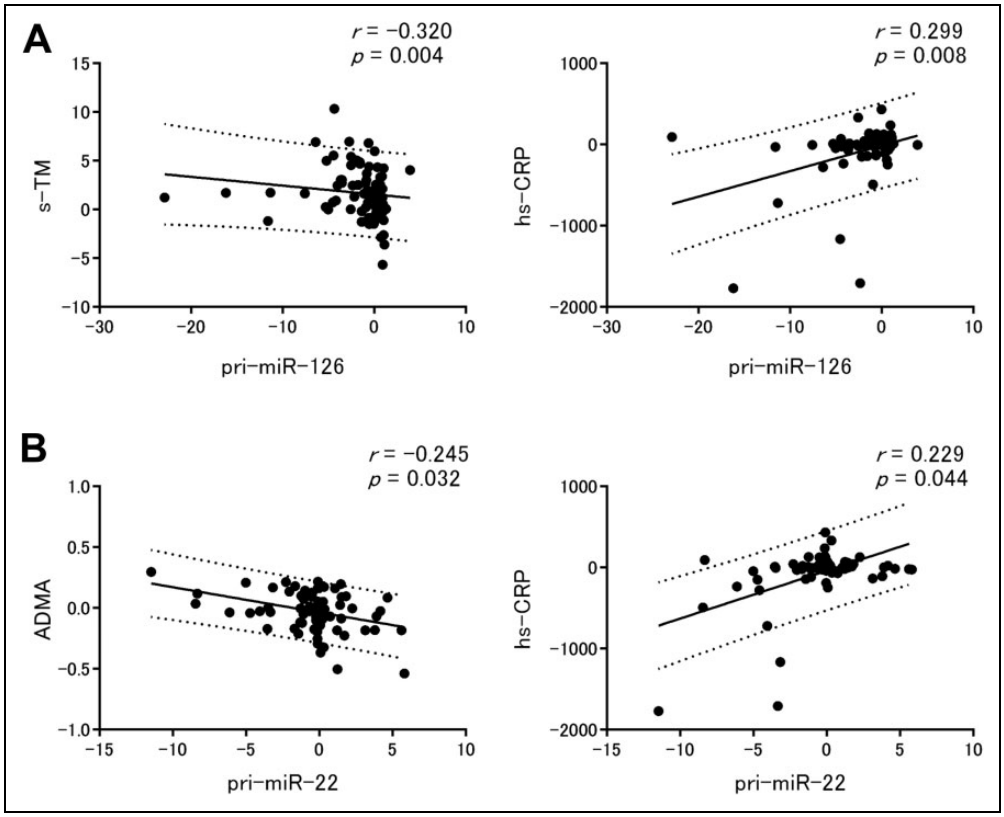


**Figure 5.** (A) Correlations between the serum levels of primary (pri)-miR-126 and soluble thrombomodulin (s-TM; left), and pri-miR-126 and serum high-sensitivity C-reactive protein (hs-CRP; right), in 78 patients with atrial fibrillation (AF; r = −0.320, *P* = .004; r = 0.299; *P* = .008, respectively). (B) Correlations between the serum levels of pri-miR-22 and asymmetric dimethylarginine (ADMA; left), and miR-22 and serum hs-CRP (right), in 78 patients with AF (r = −0.245; *P* = .032; r = 0.229, *P* = .044, respectively).

compared with those in the restored SR group. Preoperative higher hs-CRP was shown to be a predictor for the incidence of postoperative AF in patients receiving off-pump coronary artery bypass grafting (CABG).[45] There are several reports showing that hs-CRP is positively related to the recurrence of AF after cardioversion or ablation.[46] These results together with our data suggest that AF recurrence might bring chronic cardiovascular inflammation, following a high reading of hs-CRP.

The ADMA is an analogue of L-arginine, the precursor of NO, which is an endogenous inhibitor of NO synthase that can cause endothelial dysfunction, inflammation and oxidative stress in cardiovascular diseases.[47] In our study, ADMA was not significantly associated with AF recurrence after ablation. However, there are reports that describe alterations in serum ADMA in patients with AF and the role of ADMA in the pathophysiology of AF. Lim et al. showed that blood levels of ADMA were decreased after successful AF ablation,[48] while Sasaki et al. demonstrated that blood levels of ADMA did not change after AF ablation, either in the treatment success group or the recurrence group.[49] The discrepancy between the results of our study and those of others is probably due to differences in patient background (eg, diabetes or hypertension), or the use of different methods for sample preparation (plasma or serum) or commercially available assay kits.

The research area of circulating miRNAs has expanded dramatically and data on many useful biomarkers have been collected. Although the mechanism of miRNA regulation in the occurrence and development of AF is not completely clear, there are several revealing reports. Compared with controls, the levels of miR-21 and miR-150 were lower in patients with AF, especially those with paroxysmal AF, and the levels of these miRNAs increased after AF ablation.[50] The levels of miR-409-3p and miR-432 in the plasma of patients with AF were lower than those in healthy control subjects, but were restored after catheter ablation.[51] McManus et al. performed a large study to find circulating miRNAs associated with new-onset AF in whole blood among 2445 Framingham Heart Study Offspring participants and identified several miRNAs, including miR-328, that were associated with preexisting AF, but not with AF onset.[52] Harling et al. identified potential miRNAs that were dysregulated in patients with postoperative AF undergoing CABG and one of these miRNAs, miR-483-5p, was significantly higher in the preoperative serum of patients with postoperative AF.[53] There are more studies to identify AF-related miRNAs and to explore the role of these miRNAs in the regulation of heart remodeling.

We investigated the dynamics of 3 miRNAs: miR-126 (endothelial miRNA), miR-22 (ubiquitously expressed miRNA), and miR-142-3p (peripheral blood mononuclear

cell-derived miRNA). Since miR-126 is mainly expressed in endothelial cells and platelets, circulating miR-126 is considered an endothelial marker. The loss of endothelial miR-126 in the plasma of patients with type 2 DM suggests that the circulating miR-126 level represents endothelial damage.[54] Previous reports have shown that miR-126 levels decreased with participation in the onset of acute myocardial infarction,[55] and decreases have been shown in patients with AF and HF compared with healthy control subjects.[56] However, the biological mechanism by which miR-126 is downregulated is not fully understood.[57] In this study, there was no significant difference between the levels of 3 miRNAs (miR-126, miR-22, and miR-142-3p), either before or after catheter ablation, in the 2 groups of patients with AF (Figure 2A). In contrast, the level of pri-miR-126 in serum tended to be higher in the recurrent AF group and decreased significantly after catheter ablation; pri-miR-22 and pri-miR-142-3p did not show these differences (Figure 2B). According to miRNA biogenesis, since mature miRNAs are generated from pri-miRNAs by 2 endonucleases (Drosha and Dicer), the ratio between the expression of pri-miRNAs and their mature miRNAs is not 1:1. Therefore, the changes in pri-miR-126 and mature miR-126 could not be accurately correlated with those for pri-miR-22 and mature miR-22 (Figure 5B), suggesting that pri-miR-126, not miR-126, might be an independent useful parameter for AF recurrence. This is noteworthy as there have been no previous reports on circulating pri-miRNAs related to any human diseases. The mechanism by which the serum levels of pri-miR-126 were downregulated after catheter ablation should be investigated further. We observed significant negative associations between the changes in s-TM and those of miR-126, miR-22 and pri-miR-126 (Figures 4A and 5A), and positive associations between the changes in ADMA and those of miR-22, miR-126 and pri-miR-22 (Figures 4B and 5B). In vitro experiments to evaluate the role of these miRNAs in regulating the expression of TM or ADMA should be explored in the future.

### *Limitation*

Previously left atrial enlargement is one the major predictor of catheter ablation efficacy and left atrial volume (LAV) has been used for a risk factor of AF recurrence after catheter ablation, however, only several blood markers have been recognized as the predictive parameters for the conditions of patients with AF with catheter ablation. We investigated to find the new parameters by multiple comparison, not by multivariate analysis. When we checked associations of the changes in s-TM, PAI-1, pri-miR-126, LAD, and LAV by multivariate logistic regression analysis, the change in pri-miR-126 was relatively an important, but not significant, candidate of predictive parameters to know the AF recurrence ($P = .070$; Supplementary Table 1). The number of patients needs to be increased to integrate these parameters, proteins, and miRNAs in future studies.

## Author's Note

All procedures involving human participants were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Ethical approval to report this case series was obtained from Ethics Committee on Life Sciences and Genetic Analysis, Kagoshima University Graduate School of Medical and Dental Sciences (Approval number: 26-96). Written informed consent was obtained from the patients for their anonymized information to be published in this article.

## Acknowledgments

We thank Nobue Uto and Ryoko Narimatsu (Department of Laboratory and Vascular Medicine) for their technical support. We also thank Michelle Kahmeyer-Gabbe, PhD, from Edanz Group (www.edanz.com/ac) for editing a draft of this manuscript.

## Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This research was supported by the following; Grant-in-Aid for Scientific Research (18H02734) (M.Y.); Grant-in-Aids for Exploratory Research (18K19523) (M.Y.), (15K15197) (T.H.), and (17K09593) (M.M.); Fukuda Foundation Technology of Japan for Medical (F.N.); and the Japan Society for the Promotion of Science (26461077) (N.O.).

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## Supplemental Material

Supplemental material for this article is available online.

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