Apelin
A novel prognostic predictor for atrial fibrillation recurrence after pulmonary vein isolation
Ya Zhu Wang, MM, Jinqi Fan, MD, Bin Zhong, MM, Qiang Xu, MD

Abstract
Apelin, the ligand for the APJ receptor, is involved in the pathogenesis of atrial fibrillation (AF). However, whether serum apelin can predict the recurrence of AF after pulmonary vein isolation (PVI) has not been determined.

A prospective cohort study was performed in patients with AF (but without structural heart disease) who were undergoing first-time PVI. Serum apelin-12 was measured by enzyme-linked immunosorbent assay. Echocardiographic examination was performed at baseline, 3 months, and 6 months after PVI. Patients were followed up for 6 months after PVI, and the association between baseline apelin-12 and AF recurrence (early recurrence: within 3 months after ablation; late recurrence: 3–6 months after ablation) was analyzed.

A total of 61 patients were included in the study. Baseline serum level of apelin-12 was significant lower in patients with early (median [interquartile range]: 1844 [1607–2061] vs 2197 [1995–2455] ng/L, P = .01) and late (1639 [1524–1853] vs 1923 [1741–2303] ng/L, P = .02) AF recurrence compared with patients without these events. Results of Cox stepwise multivariate analysis demonstrated that lower baseline apelin-12 (<2265 ng/L) was independently associated with increased AF recurrence within 6 months after PVI (P < .05). The specificity and positive predictive value of apelin-12 for AF recurrence were significantly higher than those of baseline N-terminal brain proBNP (60.4% vs 28.6%, P < .001; 58.8% vs 34.4%, P = .01), although the sensitivity and negative predictive value were similar.

Reduced baseline serum apelin-12 may be an independent risk factor for the recurrence of AF after PVI in patients without structural heart disease.

Abbreviations: AF = atrial fibrillation, PVI = pulmonary vein isolation, PV = pulmonary veins.

Keywords: apelin, atrial fibrillation, pulmonary vein isolation, recurrence

1. Introduction
Atrial fibrillation (AF) has become one of the most common cardiac arrhythmias seen in clinics. Comorbidities of AF may increase the risks of thromboembolic events, such as stroke, and lead to deterioration of cardic function and incidence of heart failure, thereby contributing to morbidity and mortality. Electrophysiologically, many potential mechanisms are thought to be involved in the pathogenesis and progression of AF, including simultaneous re-entrant circuits and variability in wavelength initiated by rapidly firing foci. The latter originates from the pulmonary veins (PV) and targeted myocardial ablation by PV isolation (PVI) has become one of the most effective treatments for AF. However, previous evidence suggests considerable recurrence of AF after the PVI procedure, varying from 14% to 35% in paroxysmal AF and up to 70% in persistent AF.3-7 Therefore, developing biomarkers for predicting AF recurrence after ablation is clinically important in identifying AF patients who respond well to PVI treatment.

Many factors, mostly involved in the pathogenesis of AF, can potentially predict recurrence of AF after PVI, such as preexistent left atrial scarring, reduced cardiac systolic function, comorbidities of renal dysfunction, and increased serum markers, including high-sensitive C-reactive protein (CRP), endothelin-1, N-terminal pro-brain natriuretic peptide (NT-proBNP), and platelet-derived growth factor (PDGF).8-15

Apelin, an endogenous peptide ligand of the previously orphaned G-protein-coupled receptor APJ, is synthesized as a preproprotein, which is cleaved through N-terminal proteolysis to generate several mature biologically active forms including apelin-36, apelin-17, apelin-16, apelin-13, and apelin-12.16-18

Recent studies have suggested apelin plays an important role in the pathogenesis of cardiovascular diseases. For example, mice deficient in apelin exhibited a reduced response to cardiac pressure overload19 and the levels of serum apelin-12 were decreased in patients with essential hypertension.20 These findings suggest a potential role of apelin in the maintaining of cardiovascular homeostasis. Consistent with the above findings, a more recent prospective clinical study proposed a predictive value of apelin-12 in patients with ST elevation myocardial infarction.21

Previous studies also showed an implication of apelin in AF. For example, decreased circulating levels of apelin were observed in lone AF patients and in patients with persistent AF.22,23 The
latter study also showed that AF patients with low levels of serum apelin are associated with worse outcomes, suggesting a prognostic value of plasma apelin for patients with arrhythmia recurrence. However, to the best of our knowledge, few studies have evaluated the predictive effect of serum apelin on AF recurrence after PVI. In this prospective cohort study, we aimed to evaluate the levels of serum apelin-12 as a predictor for AF recurrence after PVI in AF patients without structural heart disease.

2. Methods

2.1. Patient characteristics

This study included 61 consecutive patients who underwent circumferential PVI for symptomatic and drug-refractory paroxysmal AF or persistent AF between May 2010 and April 2011 in our center. Patients having one of the following conditions were excluded from this study: mitral valvular heart disease, pulmonary disease, a recent infection, surgery, acute coronary syndrome during the past 2 months prior to enrollment, and/or previous catheter ablation for AF. All selected patients signed informed consent and this study was approved by the ethics committee of Chongqing Medical University.

2.2. Study protocol

All included patients had transthoracic echocardiograms (Philips iE33 ultrasound PHILPS (USA) Investment Co., Ltd. Bothell. www.medical.philips.com) before as well as at 3 and 6 months after PVI. Left atrium diameter, valvular abnormality, left ventricular (LV) wall motion, and LV ejection fraction (LVEF) were assessed. The blood samples were obtained from the antecubital vein of each participant, and stored in ethyl-diaminetetra-acetic acid-containing and aprotinin (2 antiocoagulant). Blood samples were obtained at 12 hours before PVI, and at 1, 3, and 6 months after PVI. Blood was centrifuged at 4°C until the biochemical analyses were performed. The plasma levels of apelin-12, NT-proBNP, and PDGF-AA were measured by the enzyme-linked immunosorbent assay with commercially available kits according to the manufacturer’s instructions (RayBio Human ELISA Kit, Norcross, GA).

2.3. PVI procedure

PVI was performed according to the procedure described by O’Carroll et al.[17] Briefly, a multipolar mapping catheter was placed into the coronary sinus via the left subclavian vein to record coronary sinus electrograms. The ablation catheter and circular mapping catheter were placed via the right femoral vein to the left atrium using a double trans-septal puncture technique. PVI was performed by applying radiofrequency energy at ostial sites which showed the earliest bipolar pulmonary vein (PV) potentials during sinus rhythm or paced rhythm. Radiofrequency energy was delivered with a target temperature of 45°C and a maximum power output of 30 to 40 W for 30 seconds (Stockert, Biosense-Webster Inc., South Diamond Bar, CA) using a 4-mm irrigated tip with saline cooling of the ablation electrode (Navistar, Biosense-Webster) under the guidance of the CARTO system and fluoroscopy. Successful PVI was defined as having either the abolition or dissociation of the distal PV potentials from the left atrial electrograms.

2.4. Follow-up

All patients were examined at 1, 3, and 6 months after PVI. A 12-lead ECG and 12-lead 24-hour Holter or 1-week long-term Holter were performed at every visit, or whenever patients reported symptoms of palpitations. During the follow-up period, antiarrhythmic agents were discontinued for all patients, unless continuous medication was considered necessary for patients with persistent arrhythmia.

2.5. Definition of AF recurrence

The existence of AF was defined as atrial tachyarrhythmia (including AF, AFL, AT) continuing for ≥30 seconds as determined by 12-lead ECG and 12-lead 24-hour Holter or 1-week long-term Holter during follow-up. We classified the patients into 2 different groups according to clinical outcomes as follows: early recurrence of AF (ERAF), that is, recurrence within 3 months post-ablation; and late recurrence of AF (LRAF), that is, recurrence between 3 and 6 months, that is, the end of the follow-up period.

2.6. Statistical analysis

Statistical analysis was performed with SPSS v17.0 statistical software (SPSS Inc., Chicago, IL). The Kolmogorov–Smirnov test was used to verify the normality of the distribution of continuous variables. The normally distributed variables were expressed as mean±standard deviation (SD) and compared with Student t test, while the non-normally distributed variables were reported as median (first and third quartiles) and compared with the Mann–Whitney U test. A Chi-squared test was used for statistical analysis of categorical variables. Receiver-operator characteristic (ROC) curves were established to calculate areas under the curve (AUC) to evaluate the predictive values of apelin-12 and other factors for the recurrence of AF. The best cut-off values of the above mentioned markers for the prediction of AF recurrence were also derived from the ROC curves. Univariate Cox regression analysis was performed to determine the association between the serum markers and the recurrent AF at follow-up. Adjusted odds ratios and 95% confidence interval (CI) were applied for estimation of the associations. All statistical tests were two-sided probability tests, and a P-value <.05 was considered statistically significant.

3. Results

3.1. Baseline characteristics and outcomes of participants

Baseline characteristics of these 61 patients are presented in Table 1. The mean age of the patients was 55.8 years and 59.0% were men. During follow-up, ERAF occurred in 36.1% (22/61) of the patients, and the incidence of overall LRAF was 18.0% (11/61).

3.2. Clinical characteristics of patients with ERAF

In the present study, a total of 22 ERAF patients were enrolled. Among these 22 ERAF patients, 12 were treated with amiodarone for arrhythmia (but 6 did not resume sinus rhythm after 1 month treatment and then changed to beta-blocker to control ventricular rate), 2 underwent secondary ablation (1 successfully restored sinus rhythm, and the other one was treated with beta-blocker to control ventricular rate), 5 received DC cardioversion (3 successfully recovered sinus rhythm, and 2 received beta-blocker controlled ventricular rate), and 3 were
directly treated with beta-blocker to control ventricular rate. By the end of the study follow-up period, a total of 12 patients in ERAF had not recovered sinus rhythm, and these 12 ERAF patients were considered clinical failure.

For patients with or without ERAF, the baseline characteristics were generally matched in demographic features, comorbidities of coronary artery disease (CAD) and diabetes mellitus (DM), baseline thyroid functions, and concurrent medications (Table 1). However, the baseline level of serum apelin-12 was significantly higher level of NT-proBNP was also noticed in patients with ERAF compared with that of those without ERAF (P = .03, Table 2). Moreover, the levels of serum apelin-12 and NT-proBNP were not significantly different at 1, 3, and 6 months after PVI in patients with and without ERAF. The echocardiographic study showed that patients with ERAF had significantly larger left atrial diameter and lower LVEF at baseline compared with those without ERAF (Table 3), although the echocardiographic parameters were not significantly different between these 2 groups at 3 and 6 months post-PVI.

### Table 1
Baseline characteristics of patients with and without early recurrence of atrial fibrillation (ERAF).

| Parameters                          | Baseline (n=39) | No ERAF (n=39) | ERAF (n=22) | P-value |
|-------------------------------------|----------------|---------------|-------------|---------|
| Age, y                              | 55.80±13.42    | 53.81±15.84   | 58.50±7.56  | .137    |
| Male sex, n (%)                     | 36 (69.02%)    | 23 (58.97%)   | 13 (59.09%) | .993    |
| Weight, kg                          | 62.36±9.78     | 63.67±10.07   | 60.43±8.07  | .318    |
| Height, m                           | 1.64±0.07      | 1.65±0.08     | 1.62±0.06   | .127    |
| Smoking, n (%)                      | 15 (24.59%)    | 10 (25.64%)   | 5 (22.73%)  | .800    |
| Body mass index, kg/m²              | 23.11±2.92     | 23.23±2.88    | 23.10±2.77  | .887    |
| Duration of AF history, y           | 4.41±3.83      | 4.94±4.36     | 3.72±3.08   | .248    |
| Non-paroxysmal AF, n (%)            | 17 (27.87%)    | 8 (20.51%)    | 9 (40.91%)  | .088    |
| Hypertension, n (%)                 | 22 (36.07%)    | 15 (38.46%)   | 7 (31.82%)  | .604    |
| Diabetes mellitus, n (%)            | 6 (10.07%)     | 4 (10.26%)    | 2 (9.09%)   | .883    |
| Coronary artery disease, n (%)      | 10 (16.39%)    | 6 (15.38%)    | 4 (18.18%)  | .777    |
| Systolic blood pressure, mmHg       | 125.77±20.74   | 126.97±23.29  | 123.10±16.08| .511    |
| Diastolic blood pressure, mmHg      | 77.18±11.85    | 78.46±12.98   | 74.90±9.91  | .290    |
| Heart rate, beats/min               | 76.20±15.31    | 74.08±14.53   | 80.79±16.59 | .127    |
| T3, pmol/L                          | 4.81±0.77      | 4.68±0.91     | 4.42±1.21   | .444    |
| T4, pmol/L                          | 39.67±25.47    | 20.58±14.93   | 21.31±12.93 | .876    |
| TSH, μU/mL                          | 5.07±2.74      | 3.79±2.69     | 3.13±1.71   | .410    |
| Creatinine, μmol/L                  | 77.54±14.97    | 77.63±17.41   | 78.74±12.36 | .872    |
| High-sensitivity C-reactive protein, mg/L | 3.18±6.98      | 3.63±7.87     | 3.53±6.91   | .979    |
| Beta-blocker, n (%)                 | 30 (49.18%)    | 18 (46.15%)   | 12 (54.55%) | .529    |
| Calcium-channel blocker, n (%)      | 7 (11.48%)     | 4 (10.26%)    | 3 (13.64%)  | .691    |
| Angiotensin-converting enzyme inhibitor, n (%) | 25 (40.98%)  | 15 (38.46%)   | 10 (45.45%) | .584    |
| Angiotensin receptor blocker, n (%) | 13 (21.31%)    | 8 (20.51%)    | 5 (22.73%)  | .839    |
| Statin, n (%)                       | 7 (11.48%)     | 5 (12.82%)    | 2 (9.09%)   | .661    |

AF = atrial fibrillation, T = thyroxine, TSH = thyroid-stimulating hormone.

### Table 2
Biomarker levels following pulmonary vein isolation (PVI) in patients with and without early recurrence of atrial fibrillation (ERAF).

| Parameters                          | No ERAF (n=39) | ERAF (n=22) | P-value |
|-------------------------------------|----------------|-------------|---------|
| NT-proBNP, pg/mL                    | 2197 (1895–2453) | 2251 (1525–2516) | .023    |
| PDGF-AA, pg/mL                      | 2043 (1411–2141) | 2074 (1999–2109) | .967    |
| Apelin-12, ng/L                     | 280 (1848–3800)  | 2279 (2082–3130) | .868    |
| NT-proBNP, pg/mL                    | 280 (1584–2172)  | 3130 (2009–3840) | .778    |
| PDGF-AA, pg/mL                      | 200 (1848–2141)  | 1943 (1325–1430) | .394    |
| Apelin-12, ng/L                     | 2810 (1848–3800) | 2279 (2082–3130) | .868    |

Median and 25th and 75th percentiles are presented.

AF = atrial fibrillation, ERAF = early recurrence of AF, NT-proBNP = N-terminal pro-brain natriuretic peptide, PDGF = platelet-derived growth factor, PVI = pulmonary vein isolation.
3.3. Clinical characteristics of patients with LRAF

Patients with LRAF were older and had higher diastolic blood pressure (DBP), but were matched for other baseline characteristics such as demographic features, comorbidities of CAD and DM, baseline thyroid functions, as well as the concurrent medications with those without LRAF (Table 4). Compared with non-ERAF patients, LRAF patients had higher NT-proBNP at baseline and 3 and 6 months after PVI (Table 5). Echocardiographic parameters in both groups were generally similar except that LRAF patients had significantly enlarged LVSD at 6 months after PVI compared with non-ERAF patients (Table 3).

3.4. Predictive value of baseline serum apelin-12 for AF recurrence

We next used multivariate Cox regression analysis to determine the correlation of factors with the AF recurrence. As shown in

| Table 3 |
|----------------------------------|
| Transthoracic echocardiographic parameters following pulmonary vein isolation (PVI) in patients with and without atrial fibrillation recurrence. |
| Parameters | No ERAF (n = 39) | ERAF (n = 22) | P-value | No LRAF (n = 50) | LRAF (n = 11) | P-value |
|----------------------------------|
| Before PVI |
| LAD, mm | 36.51 ± 5.01 | 40.08 ± 4.76 | <.001 | 37.02 ± 5.30 | 38.29 ± 4.39 | .550 |
| LVDD, mm | 46.62 ± 4.06 | 49.42 ± 7.84 | .058 | 47.45 ± 6.04 | 47.14 ± 3.30 | .857 |
| LVSD, mm | 29.47 ± 4.67 | 33.32 ± 7.65 | .012 | 30.73 ± 6.38 | 29.71 ± 3.58 | .570 |
| IVST, mm | 10.76 ± 1.50 | 10.37 ± 1.65 | .292 | 10.67 ± 1.49 | 10.29 ± 1.82 | .417 |
| LVEF (%) | 67.30 ± 8.47 | 62.34 ± 8.08 | <.001 | 65.71 ± 8.86 | 66.43 ± 7.25 | .586 |
| 3 months after PVI |
| LAD, mm | 35.75 ± 5.44 | 37.83 ± 5.76 | .227 | 36.11 ± 5.76 | 38.80 ± 4.02 | .194 |
| LVDD, mm | 45.84 ± 3.25 | 47.91 ± 4.06 | .083 | 47.30 ± 6.04 | 47.40 ± 2.72 | .964 |
| LVSD, mm | 28.95 ± 3.93 | 31.75 ± 11.80 | .284 | 30.26 ± 8.54 | 28.60 ± 3.24 | .556 |
| IVST, mm | 10.25 ± 1.52 | 9.83 ± 1.55 | .375 | 10.15 ± 1.61 | 9.80 ± 1.03 | .530 |
| LVEF (%) | 66.90 ± 6.32 | 65.00 ± 15.40 | .585 | 65.52 ± 11.23 | 69.80 ± 5.87 | .261 |
| 6 months after PVI |
| LAD, mm | 35.40 ± 4.35 | 36.25 ± 3.15 | .650 | 35.30 ± 4.24 | 36.50 ± 3.42 | .516 |
| LVDD, mm | 45.20 ± 4.37 | 43.25 ± 5.9 | .433 | 44.50 ± 4.70 | 45.00 ± 5.50 | .838 |
| LVSD, mm | 26.30 ± 3.30 | 26.25 ± 3.49 | .976 | 20.69 ± 3.86 | 25.00 ± 4.07 | .032 |
| IVST, mm | 9.50 ± 1.51 | 9.83 ± 1.85 | .653 | 9.40 ± 1.35 | 10.33 ± 2.06 | .234 |
| LVEF (%) | 69.80 ± 5.09 | 69.00 ± 3.30 | .706 | 70.20 ± 5.14 | 68.00 ± 2.51 | .255 |

ERA = early recurrence of AF, IVST = left ventricular septum thickness, LRAF = late recurrence of AF, LVDD = left ventricular diastolic dimension, LVEF = left ventricular ejection fraction.

| Table 4 |
|----------------------------------|
| Baseline characteristics of patients with and without late recurrence of atrial fibrillation (LRAF). |
| Parameters | No LRAF (n = 50) | LRAF (n = 11) | P-value |
|----------------------------------|
| Age, y | 58.35 ± 13.98 | 65.69 ± 12.11 | <.001 |
| Male sex, n (%) | 23 (46.00%) | 6 (54.55%) | .607 |
| Weight, kg | 63.08 ± 6.98 | 63.05 ± 4.53 | .928 |
| Height, m | 1.66 ± 0.08 | 1.66 ± 0.05 | .253 |
| Body mass index, kg/m² | 23.19 ± 2.36 | 23.30 ± 1.24 | .461 |
| Duration of AF history, y | 4.26 ± 3.00 | 4.76 ± 1.58 | .472 |
| Nonparoxysmal AF, n (%) | 11 (22.00%) | 6 (54.55%) | .029 |
| Hypertension, n (%) | 18 (36.00%) | 4 (36.36%) | .982 |
| Diabetes mellitus, n (%) | 5 (10.00%) | 1 (0.99%) | .927 |
| Coronary artery disease, n (%) | 8 (16.00%) | 2 (18.18%) | .860 |
| Systolic blood pressure, mmHg | 131.12 ± 24.51 | 131.04 ± 11.35 | .265 |
| Diastolic blood pressure, mmHg | 80.55 ± 13.17 | 84.78 ± 12.40 | <.001 |
| Heart rate, beats/min | 72.32 ± 8.23 | 72.39 ± 5.90 | .805 |
| T3, pmol/L | 4.85 ± 0.70 | 4.86 ± 1.05 | .946 |
| T4, pmol/L | 18.00 ± 2.12 | 17.73 ± 1.90 | .129 |
| TSH, µIU/mL | 5.62 ± 3.07 | 6.06 ± 2.09 | .314 |
| Creatinine, µmol/L | 74.58 ± 11.07 | 74.08 ± 15.17 | .399 |
| High-sensitivity C-reactive protein, mg/L | 3.13 ± 6.36 | 3.57 ± 6.86 | .865 |
| Beta-blocker, n (%) | 25 (50.00%) | 5 (45.45%) | .785 |
| Calcium-channel blocker, n (%) | 5 (10.00%) | 2 (18.18%) | .441 |
| Angiotensin-converting enzyme inhibitor, n (%) | 21 (42.00%) | 4 (36.36%) | .731 |
| Angiotensin receptor blocker, n (%) | 11 (22.00%) | 2 (18.18%) | .780 |
| Statin, n (%) | 6 (12.00%) | 1 (0.99%) | .784 |

AF = atrial fibrillation, T = thyroxine, TSH = thyroid-stimulating hormone.
### Table 5

Biomarker levels following pulmonary vein isolation (PVI) in patients with and without late recurrence of atrial fibrillation (LRAF).

| Parameters        | No LRAF (n=50) | LRAF (n=11) | P-value |
|-------------------|----------------|-------------|---------|
| Before PVI        |                |             |         |
| NT-proBNP, pg/mL  | 477 (401–488)  | 339 (278–404) | .001    |
| PDGF-AA, pg/mL    | 1974 (1298–2232) | 2015 (1220–2117) | .171    |
| Apelin-12, ng/L   | 1639 (1524–1853) | 1922 (1741–2303) | .023    |
| 1 month after PVI |                |             |         |
| NT-proBNP, pg/mL  | 262 (206–347)  | 291 (253–369)  | .501    |
| PDGF-AA, pg/mL    | 1732 (1474–1906) | 1869 (1693–1984) | .179    |
| Apelin-12, ng/L   | 2058 (2004–3644) | 2746 (1967–3782) | .974    |
| 3 months after PVI|                |             |         |
| NT-proBNP, pg/mL  | 2105 (2009–2194) | 217 (197–393)  | .292    |
| PDGF-AA, pg/mL    | 2170 (1771–2325) | 2043 (1974–2127) | .322    |
| Apelin-12, ng/L   | 1600 (1435–2273) | 2966 (2251–3488) | .027    |
| 6 months after PVI|                |             |         |
| NT-proBNP, pg/mL  | 268 (246–288)  | 218 (164–292)  | .111    |
| PDGF-AA, pg/mL    | 1853 (1299–2085) | 1432 (887–1998) | .385    |
| Apelin-12, ng/L   | 1600 (1435–2273) | 2288 (2242–3589) | .015    |

AF = atrial fibrillation, Median and 25th and 75th percentiles are presented, NT-proBNP = N-terminal pro-brain natriuretic peptide, PDGF = platelet-derived growth factor.

### Table 6

Univariate regression analyses for the predictors of late recurrence of atrial fibrillation (LRAF).

| Parameters                        | Relative risk | 95%CI     | P-value |
|-----------------------------------|---------------|-----------|---------|
| Age, y                            | 0.983         | 0.957–1.010 | .226    |
| BMI, kg/m²                        | 1.064         | 0.914–1.239 | .421    |
| Hypertension                      | 0.802         | 0.336–1.913 | .619    |
| DM                                | 0.278         | 0.037–2.072 | .212    |
| CAD                               | 0.839         | 0.284–2.480 | .751    |
| Diastolic blood pressure baseline, mmHg | 1.055    | 1.011–1.101 | .015    |
| LAP at baseline, mm               | 1.019         | 0.942–1.102 | .638    |
| LVEF at baseline (%)              | 1.037         | 0.991–1.085 | .121    |
| NT-proBNP at baseline, pg/mL      | 1.011         | 1.007–1.014 | <.001   |
| PDGF-AA at baseline, pg/mL        | 1.000         | 0.999–1.000 | .343    |
| Apelin-12 at baseline, ng/L       | 0.997         | 0.995–0.998 | <.001   |

BMI = body mass index, CAD = coronary artery disease, CI = confidence interval, DM = diabetes mellitus, LAP = left atrial dimension, LVEF = left ventricular ejection fraction, NT-proBNP = N-terminal pro-brain natriuretic peptide, PDGF = platelet-derived growth factor.

### Figure 1

Determination of the predictive value of baseline serum apelin-12 within 6 months after PVI. Receiver-operator characteristic curves were established as detailed in statistical analysis section. AUC = area under the curve, CI = confidence interval, PVI = pulmonary vein isolation.

AUC=0.634
95%CI=0.557–0.712
P=0.001

AF = atrial fibrillation, Median and 25th and 75th percentiles are presented, NT-proBNP = N-terminal pro-brain natriuretic peptide, PDGF = platelet-derived growth factor.
Table 6, among all factors tested, the higher mean diastolic BP, higher plasma NT-proBNP, and lower plasma apelin-12 at baseline were independent predictors of AF recurrence at 6 months after PVI ($P = .015$, $P < .001$, and $P < .001$, respectively). We also generated ROC curves to determine the 95% CI and the optimal cutoff value of serum apelin-12 and NT-proBNP, respectively, for the prediction of AF recurrence within 6 months after PVI. As shown in Figs. 1 and 2, respectively, serum apelin-12 had an AUC of 0.63 (95% CI: 0.56–0.71) with a cutoff value of 2265.14 ng/L, while serum NT-proBNP had an AUC of 0.60 (95% CI: 0.53–0.78) with a cutoff value of 392.41 pg/mL.

Kaplan–Meier curves (for probability of AF recurrence by median levels of serum apelin-12) were generated for the 180 days of follow-up (Fig. 3; log-rank test, $P = .001$). Similar trends were also found for NT-proBNP (Fig. 4; log-rank test, $P < .001$), whereas no differences were observed for PDGF-AA. The specificity and positive predictive value of serum apelin-12 for AF recurrence were significantly higher than those of baseline N-terminal brain proBNP (60.4% vs 28.6%, $P < .001$; 58.8% vs 34.4%, $P = .01$), although the sensitivity and negative predictive value were similar (90.91% vs 84.00%, $P = .424$; 91.43% vs 80.00%, $P = .221$).

4. Discussion
Our study of patients with recurring AF (but without structural heart disease) revealed that reduced baseline serum apelin-12 may be an independent risk factor for the recurrence of AF after PVI in these patients. Moreover, our results expanded previous observations showing that changes in plasma apelin level occurred in lone AF subjects and that apelin had a predictive value for AF recurrence after electric cardioversion. Our findings also indicated that measuring baseline serum apelin-12 may be important for identifying AF patients with a higher risk of AF recurrence after PVI, and that the predictive value of baseline serum apelin-12 for AF recurrence may be similar to NT-proBNP.

Apelin is highly expressed in cardiomyocytes, endothelial cells, and vascular smooth muscle cells (VSMC), suggesting that apelin may play an important role in physiology and pathophysiology of cardiovascular system. Meanwhile, the heart atrium also has high expression of apelin. A previous study by Szokodi et al indicated that administration with apelin-16, an active form of apelin, increased mean arterial filling pressure and cardiac contractility in the isolated perfused rat heart through stimulation of Ca$^{2+}$ influx and activation of phospholipase C systems. In addition, it has been suggested that apelin also reduced preload and afterload in the heart, leading to a significant
reduction of left atrial pressure. This is particularly important since reduction of intra-atrial pressure may improve the physical characteristics of atria and decrease pulmonary vein ectopic electrical activity, thereby exerting potent vasodilator and positive inotropic activities leading to an increase in the conduction velocity and a decrease in the field potential duration in in vitro neonatal cardiomyocytes—all of which have been proved to be important mechanisms underlying the pathogenesis and progression of AF. These protective effects of apelin may be the mechanisms involved in increased serum apelin at baseline, which may be used to predict favorable outcomes after PVI procedures.

The pathophysiological mechanisms underlying the pathogenesis of AF are complicated. For instance, inflammation and oxidative stress contribute significantly to the pathogenesis of AF. An increased activity of apelin may also exert beneficial effects on cardiac function, which contributes to the reduced risk of AF recurrence post-PVI in patients with relatively higher serum apelin. Indeed, a previous study of ischemic cardiomyopathy using a rat model indicated that one of the active forms, apelin-13, improved cardiac function, which was further confirmed by subsequent observations showing that expression of apelin was upregulated in an early compensated phase of heart failure and was reversely correlated with the disease progression and severity. Moreover, Siddiquee et al. reported that apelin protected against angiotensin II-induced cardiovascular fibrosis. Since an upstream therapy including ACEI or ARB likely prevents AF recurrence via regulation of atrial remodeling, we hypothesized that the increased apelin levels may also benefit patients with AF with the same mechanisms. Indeed, our findings together with those from other groups suggested that the increased activity of apelin may have a beneficial effect on AF patients, and that higher serum apelin predicts a lower risk of AF recurrence after PVI.

NT-proBNP is a natriuretic hormone which, in the absence of heart failure, is secreted from the atrium in response to atrial distension and overload. Consistent with the previous findings, we showed that an increased plasma NT-proBNP level predicted a higher risk for AF recurrence post-PVI. Previous studies have demonstrated that BNP inhibited the resting sympathetic activity of the heart, thereby potentiating vagal activity through activating the cGMP pathway and inciting atrial overload and atrial remodeling, which results in sustaining atrial fibrillatory rotors and potentially initiating and sustaining AF. Therefore, NT-proBNP was suggested to predict AF recurrence early in AF patients who underwent PVI. By comparing AUC under ROC curves of apelin-12 and NT-proBNP, our study showed that the predictive value of baseline serum apelin-12 for AF recurrence seemed to be similar to that of NT-proBNP. These results should be confirmed in further studies.

The strengths of our study include our study was a prospective study and we examined the potential for the baseline serum apelin-12 as a predictor for the occurrence of AF recurrence after ablation. However, we also note that our study had some limitations. First,
the sample size (61 patients) was relatively small. Therefore, the findings of the present study should be validated in the future studies with a large cohort with more statistical power. Also, our observational study could not indicate a causative relationship between increased serum apelin-12 and reduced risk of AF recurrence. Hence, the therapeutic value of the treatment targeting the apelin-APJ system deserves further investigation. Finally, our study only had a follow-up duration of 6 months, and the predictive significance of serum apelin-12 in patients with AF warrants further evaluation.

In summary, reduced baseline serum apelin-12 may be an independent risk factor for recurrence of AF after PVI. Therefore, measuring the baseline serum apelin-12 may be useful for identifying AF patients at a higher risk of AF recurrence after PVI. Moreover, the predictive value of baseline apelin-12 for AF recurrence may be similar to the commonly used biomarker, NT-proBNP.

**Author contributions**

Conceptualization: Bin Zhong, Qiang Xu.

Data curation: Ya Zhu Wang, Jinqi Fan, Qiang Xu.

Investigation: Ya Zhu Wang, Jinqi Fan.

Methodology: Ya Zhu Wang.

Project administration: Bin Zhong.

Resources: Qiang Xu.

Software: Jinqi Fan.

Supervision: Bin Zhong, Qiang Xu.

Validation: Bin Zhong.

Visualization: Qiang Xu.

Writing – original draft: Ya Zhu Wang.

Writing – review & editing: Ya Zhu Wang, Jinqi Fan, Qiang Xu.

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