**Type III procollagen-N-peptide as a predictor of persistent atrial fibrillation recurrence after cardioversion**

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Received 19 March 2012; accepted after revision 15 April 2012; online publish-ahead-of-print 22 May 2012

**Aims**

Fibrosis and inflammation may play a significant role in the pathogenesis of atrial fibrillation (AF) recurrence. Type III procollagen-N-peptide (PIIINP) may be related to atrial fibrosis and play a role in predicting the recurrence of AF. We investigated whether PIIINP as a fibrosis marker predicts the recurrence of AF after cardioversion.

**Methods and results**

Serum PIIINP, interleukin-6, high-sensitivity C-reactive protein, brain natriuretic peptide, renin and aldosterone were measured at baseline and 24 months in 88 patients (62%) with sinus rhythm (SR) maintenance and 54 patients (38%) with AF recurrence. Furthermore, the root mean square voltage in the last 20 ms (RMS20) via P-wave signal-averaged electrocardiogram (P-SAECG) was measured and the relationship between fibrosis biomarkers and RMS20 was examined. Baseline PIIINP with AF recurrence was significantly higher than for those with SR maintenance (0.664 vs. 0.581 U/mL, \( P = 0.001 \)). However, there were no significant differences in other biomarkers. A logistic regression identified PIIINP (odds ratio 2.61, \( P = 0.008 \)) as an independent predictor of AF recurrence. The RMS20 as measured by P-SAECG with SR maintenance and PIIINP levels \( >0.72 \text{ U/mL (at baseline)} \) was significantly higher after 24 months than at baseline. Furthermore, the RMS20 with AF recurrence and PIIINP levels \( >0.72 \text{ U/mL (at baseline)} \) was significantly lower after 24 months than baseline.

**Conclusions**

Elevated baseline PIIINP concentration is an independent predictor for AF recurrence after cardioversion. Furthermore, there is a relationship between PIIINP and RMS20 and the fibrosis of AF.

**Keywords**

Type III procollagen-N-peptide • Atrial fibrillation • Antiarrhythmic therapy • Fibrosis marker • P-wave signal averaged electrocardiogram

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**Introduction**

Atrial fibrillation (AF) is the most prevalent sustained cardiac arrhythmia. The occurrence and development of AF are associated with changes in the electrical properties and structure of the atria, known as electrical and structural remodelling.¹⁻⁴

Atrial structural remodelling occurs due to inflammatory stressors and fibrosis. Furthermore, inflammatory changes and fibrosis might contribute to an increased propensity for persistent AF. C-reactive protein is the marker indicating inflammation of the atria.⁵⁻⁷ For example, C-reactive protein is elevated in patients with AF and is higher in patients with persistent AF compared with those with paroxysmal AF. In addition, lower levels of inflammatory markers at baseline predict maintenance of sinus rhythm (SR) after cardioversion and SR maintenance is associated with decreased levels of inflammatory markers. However, currently there is no marker of fibrosis to predict recurrence of AF after cardioversion. Type III procollagen-N-peptide (PIIINP) is released during collagen synthesis and can be used as a marker of rapidly increased collagen turnover and myocardial fibrosis scar formation after acute myocardial infarction.⁸⁻¹⁰ We previously reported that PIIINP might be related to the atrial fibrosis that occurs within AF.¹¹ It is not clear as to whether PIIINP is related to the fibrogenesis of AF or PIIINP is a fibrosis marker that can be used to predict

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the recurrence of AF after cardioversion. On the other hand, P-wave signal-averaged electrocardiogram (P-SAECG) measurement has been shown to have potential role-identifying patients at risk of developing paroxysmal AF and those likely to change from paroxysmal AF to permanent AF. There is demonstrable evidence that a prolonged P-SAECG indicates atrial conduction delay and cardiac electrical remodelling.\(^{12–14}\) The purpose of this study was to identify biomarkers, measured immediately after cardioversion, that predict the recurrence of AF and to investigate the relationship between fibrosis biomarkers and P-SAECG measurement.

**Methods**

**Study population**

This study conformed to the principles outlined in the declaration of Helsinki and was approved by the ethics committee at the University of Showa. All patients gave their written informed consent before they entered the trial. We assigned 142 patients (32–83 years of age) with persistent AF to undergo pharmacologic or electrical cardioversion. Exclusion criteria were myocardial infarction, cardiac surgery, congestive heart failure within the last 3 months, left ventricular dysfunction (ejection fraction <35%), and tissue fibrosis diseases, such as liver disease, renal dysfunction, pulmonary fibrosis, and malignancy. Patients who were over 85 years and below 20 years of age, had a pacemaker/implantable cardioverter-defibrillator, or were already treated with angiotensin receptor blockers (ARBs), angiotensin-converting enzyme inhibitors (ACE-Is) or beta-blockers were also excluded.

**Blood measurements**

Table 1

<table>
<thead>
<tr>
<th>Echocardiographic parameters</th>
<th>Maintenance of SR</th>
<th>Recurrence of AF</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEF (%)</td>
<td>66 ± 10</td>
<td>63 ± 9</td>
<td>0.17</td>
</tr>
<tr>
<td>LAD (mm)</td>
<td>41 ± 6</td>
<td>43 ± 5</td>
<td>0.03</td>
</tr>
<tr>
<td>P-wave signal averaged ECG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FPD (ms)</td>
<td>134 ± 17</td>
<td>135 ± 12</td>
<td>0.71</td>
</tr>
<tr>
<td>RMS20 (μV)</td>
<td>2.7 ± 1.0</td>
<td>2.2 ± 4.0</td>
<td>0.05</td>
</tr>
<tr>
<td>Therapies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bepridil</td>
<td>88 (100%)</td>
<td>54 (100%)</td>
<td></td>
</tr>
<tr>
<td>ARB or ACE-I</td>
<td>31 (36%)</td>
<td>11 (20%)</td>
<td>0.06</td>
</tr>
<tr>
<td>Beta blockers</td>
<td>28 (32%)</td>
<td>19 (35%)</td>
<td>0.77</td>
</tr>
<tr>
<td>Diuretics</td>
<td>17 (20%)</td>
<td>8 (15%)</td>
<td>0.46</td>
</tr>
<tr>
<td>Statins</td>
<td>15 (17%)</td>
<td>12 (22%)</td>
<td>0.54</td>
</tr>
<tr>
<td>Oral anticoagulants</td>
<td>85 (97%)</td>
<td>52 (97%)</td>
<td>0.89</td>
</tr>
</tbody>
</table>

AF, atrial fibrillation.

**Results**

**Patients**

Patient characteristics are presented in Table 1. The mean age was 66 ± 12 years and there were no statistically significant differences
in age, gender, or duration of AF between the two groups. Maintenance of SR was achieved in 88 patients (62%), while 54 patients (38%) experienced recurrence of AF. Left atrial dimension was increased in patients with AF recurrence compared with those with SR maintenance (43 ± 5 vs. 41 ± 6 mm, P = 0.03). The RMS20 was decreased in patients with AF recurrence compared with those with SR maintenance (2.2 ± 4 vs. 2.7 ± 6 μV, P = 0.05). There were no statistically significant differences in the use of medication at baseline between the two groups.

Concentrations of biomarkers
Patients with AF recurrence after cardioversion had significantly higher concentrations of PIIINP at baseline compared with patients with SR maintenance (0.66 ± 0.09 vs. 0.58 ± 0.07, P = 0.03). At baseline, the median concentrations of IL-6, BNP, Hs-C-reactive protein, renin, and aldosterone were not significantly different between the two groups (Figure 1). The time course of the biomarkers was examined at baseline, 12, and 24 months (Table 2). In patients with SR maintenance, levels of PIIINP and BNP at 24 months were significantly lower than those at baseline. There was also a tendency towards lower aldosterone levels at 24 months in these patients (P = 0.07). However, there were no significant differences in Hs-C-reactive protein, IL-6, or renin levels between baseline and 24 months. In patients with AF recurrence, levels of PIIINP at 24 months were significantly higher than those at baseline. There were no statistically significant differences on other biomarker levels between baseline and 24 months in patients with AF recurrence.

Predictors of recurrent atrial fibrillation
Kaplan–Meier survival analysis of PIIINP levels showed a significantly higher relapse probability in patients with PIIINP levels in the highest quartile (PIIINP > 0.72 U/mL) compared with the three lowest quartiles (P = 0.03; Figure 2). The logistic regression model (Table 3) shows that PIIINP was the most significant predictor of AF recurrence (odds ratio 2.63, 95% CI 1.32–3.56, P = 0.001). Left atrial dimension was independently associated with AF recurrence. Hs-C-reactive protein, IL-6, BNP, and age were not independently associated with AF recurrence.

Relations between P-wave signal-averaged electrocardiogram measurements and type III procollagen-N-peptide
Figure 3 shows the P-SAECG measurements at baseline in all patients and in those being in SR at 12 and 24 months. Filtered P-wave duration measurements were available at baseline and at 24 months for 88 patients in SR maintenance. Filtered P-wave duration measurements were available at baseline for 54 patients, at 12 months for 45 patients, and at 24 months for 38 patients in AF recurrence. In those with SR maintenance and baseline PIIINP levels >0.72 U/mL, FPD was not significantly different between baseline (132 ± 16 ms) and 24 months (136 ± 16 ms, P = 0.06). Filtered P-wave duration in patients with baseline PIIINP levels <0.72 U/mL was also not significantly different between baseline (132 ± 14 ms) and 24 months (134 ± 12 ms, P = 0.14). In patients with AF recurrence and baseline PIIINP levels >0.59 U/mL, there was no significant difference between baseline (137 ± 13 ms) and 24 months (140 ± 17 ms, P = 0.11). In contrast, for patients with baseline PIIINP levels <0.72 U/mL, FPD after 12 months (141 ± 11 ms) was significantly longer than that at baseline (135 ± 12 ms, P = 0.01). Figure 4 shows the P-SAECG measurements at baseline in all patients and in those being in SR at 12 and

![Figure 1](https://academic.oup.com/europace/article-abstract/14/12/1719/526746/14121719587146) Dot plot of biomarkers concentration at baseline.
Table 2 | Time course of biomarker concentration

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>SR maintenance (n = 88)</th>
<th>AF recurrence (n = 54)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Base line</td>
<td>12 months</td>
</tr>
<tr>
<td>PIIINP (U/mL)</td>
<td>0.58 ± 0.22</td>
<td>0.55 ± 0.24</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>2.41 ± 1.22</td>
<td>2.10 ± 1.45</td>
</tr>
<tr>
<td>Hs-C-reactive protein (mg/L)</td>
<td>0.16 ± 0.04</td>
<td>0.15 ± 0.03</td>
</tr>
<tr>
<td>BNP (pg/mL)</td>
<td>140 ± 98</td>
<td>111 ± 87</td>
</tr>
<tr>
<td>Renin (ng/mL/h)</td>
<td>3.81 ± 1.34</td>
<td>3.84 ± 1.95</td>
</tr>
<tr>
<td>Aldosterone (pg/mL)</td>
<td>12.1 ± 3.2</td>
<td>11.1 ± 4.2</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD.
Hs-C-reactive protein, high-sensitivity C-reactive protein; IL-6, interleukin 6; BNP, brain natriuretic peptide; PIIINP, type III procollagen-N-peptide.

Figure 2 | Kaplan–Meier estimates of the percentage of patients remaining free from recurrence of persistent atrial fibrillation. X-axis shows days of follow-up (day) timed after bepridil administration. Highest quartile of type III procollagen-N-peptide vs. the three lowest quartiles.

Table 3 | Independent predictors of atrial fibrillation according to stepwise logistic regression analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIIINP</td>
<td>2.63</td>
<td>1.32–3.56</td>
<td>0.001</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.78</td>
<td>0.48–1.21</td>
<td>0.792</td>
</tr>
<tr>
<td>Hs-CR</td>
<td>1.18</td>
<td>0.89–2.02</td>
<td>0.121</td>
</tr>
<tr>
<td>BNP</td>
<td>0.74</td>
<td>0.46–1.19</td>
<td>0.812</td>
</tr>
<tr>
<td>LAD</td>
<td>1.34</td>
<td>1.12–1.78</td>
<td>0.032</td>
</tr>
</tbody>
</table>

CI, confidence interval.

Discussion

This is the first study to determine that higher PIIINP levels at baseline were associated with recurrence of AF and that PIIINP was the only biomarker that independently predicted recurrence of AF in a multivariate analysis. In addition, PIIINP might be related to the formation of atrial fibrosis in patients with persistent AF and PIIINP baseline levels were related to the RMS20 as measured by P-SAECG.

The pathophysiology of AF is complex, with increasing evidence suggesting that fibrosis and inflammation contribute to the pathogenesis of AF.\(^{15,16}\) Although the exact pathophysiological mechanisms are poorly understood, persistent AF is thought to result from atrial remodelling, as electrical remodelling alone cannot explain the development of sustained AF.\(^{1,17,18}\) Atrial fibrosis, which has a slower time course, could be involved. In this study, we examined biomarker levels at baseline and at 12 and 24 months. Type III procollagen-N-peptide levels at 24 months were significantly lower than at baseline in patients with SR maintenance. In addition, a Kaplan–Meier survival analysis of PIIINP levels showed a...
significantly higher relapse probability in patients whose PIIINP levels were in the highest quartile compared with the other quartiles. Type III procollagen-N-peptide is a peptide liberated during collagen III biosynthesis. It is up-regulated during normal tissue repair and is used as a marker of fibrogenesis and scar formation.\(^{19,20}\) For example, after acute myocardial infarction, PIIINP levels increased for several months, and in patients with dilated cardiomyopathy the presence of AF is associated with a higher PIIINP/PINP ratio.\(^{21}\) Furthermore, PIIINP is an important prognostic marker in disorders characterized by abnormal and excessive collagen accumulation such as sclerosis, lung and liver fibrosis.\(^{22–24}\) Richter et al.\(^{24}\) reported that PIIINP levels over the first 6 months after ablation might be predictive of recurrent AF. According to these reports, PIIINP is affected by factors other than atrial fibrosis. Patients with a history of myocardial infarction, cardiac surgery, congestive heart failure, or tissue fibrosis diseases, such as liver disease, renal dysfunction, pulmonary fibrosis, and malignancy, were excluded from our study. It was therefore assumed that the results of our study were not influenced by other fibrosis factors.

Chung et al.\(^{5}\) reported that C-reactive protein was elevated in patients with AF and was higher in patients with persistent AF compared with those with paroxysmal AF. Lower levels of inflammatory markers at baseline predict maintenance of SR after cardioversion and the maintenance of SR is associated with a decrease in the levels of inflammatory markers.\(^{6,7}\) In the present study, the inflammation markers studied, Hs-C-reactive protein and IL-6, were not significantly different at baseline between patients with SR maintenance and those with AF recurrence. There were also no significant changes in Hs-C-reactive protein and IL-6 levels at 24 months. It is unclear at present as to whether Hs-C-reactive protein and IL-6 levels have a beneficial effect on the clinical incidence of persistent AF.

The lack of association between inflammation markers and persistent AF in our study may be explained by the timing of blood sampling. Several studies reported that plasma C-reactive protein level peaks acutely after restoration of SR and normalize slowly thereafter.\(^{25–27}\) These results suggest that episodes of AF may result in an increase of inflammatory markers rather than inflammation acting only as a background substrate for AF. In this study, blood samples were drawn immediately after restoration of SR by electrical cardioversion. However, we were unable to obtain blood samples immediately after restoration of SR by pharmacological cardioversion, and timing of blood sampling was sometimes done a few days after the pharmacological...
cardioversion and restoration of SR. Therefore, inflammatory marker values were different between electrical and pharmacological cardioversion. Consequently, there was no relationship between inflammation markers and recurrence of AF in our study. Several studies reported significant correlation between intra-atrial conduction time and the duration of the P-wave as measured by P-SAECG. Moreover, persistence of abnormal atrial conduction detected by P-SAECG is able to identify patients who are at high risk of AF recurrence after cardioversion. A short atrial refractory period or a prolonged intra-atrial conduction time predicts recurrence of AF in patients after electrical cardioversion. In our study, RMS20 measurements at 24 months were significantly higher than those at baseline in patients with SR main-

conflict of interest: none declared.

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conflict of interest: none declared.

Conclusions
Our study demonstrated that elevated baseline PIIINP concentration is an independent predictor for AF recurrence after cardioversion. Furthermore, PIIINP baseline levels were related to the RMS20 as measured by P-SAECG. However, further studies are needed to clarify the relationship between PIIINP and persistent AF.

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Pulmonary vein isolation in a patient with dextrocardia

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A 61-year-old lady with known dextrocardia and paroxysmal atrial fibrillation (AF) was referred for ablation. Computed tomography (CT) of the thorax (frontal plan as shown in Figure 1A) and CT geometry reconstruction (Figure 1B) were performed prior to ablation. Intracardiac echocardiography (Figure 1C) showed a secondum atrial septal defect (ASD). Atrial trans-septal access was achieved through the ASD. Three-dimensional mapping of the atria (Figure 1D) using Ensite NavX system (St Jude Medical) showed complete reversal of the left and right atrium and pulmonary veins. Pulmonary vein isolation was performed successfully in the reverse manner (Figure 1E). The procedural time was 140 min and fluoroscopy time was 42.5 min. The patient tolerated the procedure well and there were no complications. This case demonstrated the complete reversal of cardiac anatomy during AF ablation.

Conflict of interest: none declared.

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