Endothelial Dysfunction in Paroxysmal Atrial Fibrillation as a Prothrombotic State
— Comparison with Permanent/Persistent Atrial Fibrillation

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Aim: Atrial fibrillation (AF), regardless of subtype, is associated with a prothrombotic state, which is related to endothelial dysfunction (ED). We hypothesized that paroxysmal atrial fibrillation (PAF) patients have endothelial dysfunction, and this may partially explain the high thromboembolic risk and poorer outcome in this category of patients.

Methods: The study population consisted of 100 consecutive outpatients with AF (mean age 65.9 ± 7.9 years; 68 (68%) male) and 21 characteristics and comorbidity matched control subjects (mean age 64.8 ± 7.0 years; 13 (61.9%) male). AF patients were divided into the PAF group (n = 50) and permanent/persistent AF (PeAF) group (n = 50). Reactive hyperemia pulse amplitude tonometry index (RHI) was measured to evaluate endothelial function.

Results: RHI was significantly lower in the PAF (1.67 ± 0.30) and PeAF (1.63 ± 0.28) groups in comparison with control subjects (2.12 ± 0.40, both P < 0.001). There was no significant difference in RHI between the PAF and PeAF groups (P = 0.88). On linear regression analysis, both PeAF and PAF are significant independent predictors of RHI.

Conclusions: In conclusion, ED in PAF patients was comparable to PeAF patients, and the presence of PAF itself is a contributing factor for ED independent of other coexisting comorbidities. This may provide a mechanism explaining why the risk of thromboembolism in PAF is comparable with PeAF patients.


Key words; Endothelial dysfunction, Atrial fibrillation, Thrombosis

Introduction

Atrial fibrillation (AF), which is the most common type of arrhythmia, represents the most frequent cardiac cause of cerebral embolism. The risk of stroke is increased by about 5-fold in AF patients.1

There have been a number of recent studies to determine the pathogenesis of hypercoagulability. Atrial fibrillation is associated with a prothrombocytic and hypercoagulative state, as demonstrated by high levels of plasma fibrinogen, von Willebrand factor (vWF), and high fibrin D-dimer concentration.2-5 In general, the so-called Virchow’s triad of factors, i.e., flow disturbance, endothelial dysfunction (ED), and hypercoagulability, is necessary for pathological thrombosis.

ED is now recognized as the first step in atherosclerosis, and several studies have been performed to evaluate the correlation between AF and ED. Although ED is the result of many factors that impair endothelial function, there is a great deal of evidence indicating that AF is a risk factor of ED. In 1997, Minamino et al. first documented the impairment of endothelial function in patients with AF compared.
with sinus rhythm. The reasons for this observation were investigated, and it was reported that beat-tobeat variations in cardiac cycle length, stroke volume, and flow velocity produce turbulent shear stress and may cause ED.

AF is divided into paroxysmal, persistent, and permanent subtypes. Paroxysmal AF (PAF) is defined as episodes that resolve spontaneously within less than 7 days, usually less than 24 hours. In persistent AF, arrhythmia fails to resolve spontaneously within 7 days. Episodes may eventually resolve spontaneously or can be terminated by cardioversion. Permanent AF is considered to be present if the arrhythmia lasts for more than 1 year and cardioversion has either not been attempted or has failed.

The risk of embolization is well established in permanent AF, and several large trials indicated no difference in the risk of stroke between subjects with PAF and those with permanent AF.

There was no difference in the risk of thromboembolic events between paroxysmal and permanent atrial fibrillation, and the reason for this remains unclear. We hypothesized that not only permanent AF but also PAF patients have endothelial dysfunction even though the time of exposure to turbulent flow is shorter in the latter than in the former, and this may partially explain the high thromboembolic risk and poorer outcome in this category of patients.

**Subjects and Methods**

**Study populations**

Over a period of 3 months, 50 patients with paroxysmal AF (PAF), 50 patients with permanent/persistent AF (PeAF), and 21 control subjects without AF were enrolled in this study. All patients were being treated on an outpatient basis.

Clinical history, including symptoms of palpitation, was obtained for all patients. At the time of enrollment, all past electrocardiography records were reviewed.

PAF was defined as the documentation of no more than 2 consecutive electrocardiographs of AF and confirmed intercession by documented sinus rhythm electrocardiography.

PeAF was defined as 2 consecutive episodes of AF documented electrocardiographically with an interval of longer than 2 months.

We excluded patients with acute cardiovascular or cerebrovascular events within 1 month, infection, malignancy, connective tissue inflammatory disease, diabetes mellitus, and hepatic or renal impairment. We also excluded patients with hyperthyroidism and valvular disease. Patients with systolic blood pressure over 140 mmHg and/or diastolic pressure over 90 mmHg, and/or taking antihypertensive agents were considered to have hypertension. Dyslipidemia was defined as total cholesterol over 220 mg/dL or current treatment with lipid-lowering agents. Diabetes mellitus was diagnosed according to the World Health Organization criteria.

Venous blood samples were collected from all patients, and echocardiography and electrocardiography were performed within 1 month before or after measuring endothelial function (EndoPAT 2000; Itamar Medical Ltd., Caesarea, Israel).

Control subjects with no clinical history of atrial fibrillation were recruited at our clinic, and careful history taking, physical examination, blood sampling, electrocardiography, echocardiography, and blood pressure measurement were performed.

Venous blood was obtained from all subjects in the fasted state, and examined to determine the plasma levels of total cholesterol, HDL cholesterol, triglyceride, LDL cholesterol, and brain natriuretic peptide (BNP). Each lipid factor was measured with an automated analyzer using commercially available antibodies to total cholesterol (Sekisui Medical, Tokyo, Japan), HDL cholesterol and LDL cholesterol (Kyowa Medex, Tokyo, Japan), triglyceride (Serotec, Sapporo, Japan), and BNP (Tosoh, Tokyo, Japan).

All patients gave their written informed consent, and the study was approved by the local ethics committee.

**Endothelial function measurement**

We used EndoPAT for digital reactive hyperemia pulse amplitude tonometry (RH-PAT) to evaluate endothelial function. All patients were instructed to avoid eating or drinking, except water, for 8 h before RH-PAT. The same dim room maintained at a temperature of 26.5°C was used for RH-PAT in all patients. Before commencing RH-PAT, patients remained in bed for 15 minutes. A PAT probe was positioned on 1 finger of each hand and set by computer to inflate to 70 mmHg, and baseline pulse amplitude was recorded from both fingers. After this procedure, the blood pressure cuff was inflated on 1 arm to 200 mmHg for 5 min and released. Throughout the period of inflation and release, recordings were taken simultaneously from both fingers. The increase in pulse amplitude in the hyperemic finger was recorded digitally and analyzed using an automated operator-independent proprietary algorithm as the RH-PAT index (RHI).
Statistics

Continuous variables are reported as the means ± standard deviation. The data among patient groups and control subjects were analyzed by the $\chi^2$ test, one-way ANOVA and post-hoc Tukey-Kramer analysis or Kruskal-Wallis test, as appropriate.

As the RHI had a nonnormal distribution, the Kruskal-Wallis test and Mann-Whitney U test were used to analyze the data among 3 groups.

Linear multiple regression analyses were performed to determine independent predictors of RHI.

Data analysis was performed with SPSS 10.0 statistical software. All tests were 2-sided. A value of $p<0.05$ was considered significant in all statistical analyses.

Patient characteristics

Clinical characteristics, including comorbidities and concurrent drug use, for AF patients and control subjects are shown in Table 1. Echocardiography parameters for AF patients and control subjects are shown in Table 2. No patient had symptomatic peripheral artery disease in all groups. There were no significant differences between PAF and PeAF groups except for the rates of warfarin and antiarrhythmic drug administration, which were lower and higher, respectively, in the PAF group than in the PeAF group. No patients in the control group were administered beta-blockers, warfarin, aspirin, or antiarrhythmic drugs.

Hemostatic markers and left atrial dimension

The serum level of BNP was also significantly elevated in the PeAF group compared to control ($p<0.001$).
The left atrial dimension was significantly larger in patients in the PeAF group than in both control and PAF groups (p < 0.001 for both) (Table 2). There were no significant differences in left atrial dimension (p = 0.300) or BNP (p = 0.069) between the PAF and control groups. No significant differences were observed between the AF groups with regard to other hemostatic markers.

**RH-PAT index**

Fig. 1 shows the RHI, which was significantly lower in both PAF (1.67 ± 0.30) and PeAF (1.63 ± 0.28) groups than in the controls (2.12 ± 0.40, p < 0.001 for both). There was no significant difference in RHI between the PAF and PeAF groups (p = 0.88).

The stepwise regression method was used to select factors significantly associated with RHI. The result of the final model is summarized in Table 3, and significant and independent associations were shown between RHI and the administration of statin positively, and LAD, age, smoking, and the presence of PAF and PeAF negatively.

**Table 2. Echocardiography parameters in the control group, paroxysmal atrial fibrillation group, and persistent/permanent atrial fibrillation group**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (mm)</th>
<th>PAF (mm)</th>
<th>PeAF (mm)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVSTh</td>
<td>9.0 ± 0.9</td>
<td>9.5 ± 1.0</td>
<td>9.2 ± 1.3</td>
<td>0.187</td>
</tr>
<tr>
<td>PWTh</td>
<td>9.5 ± 1.0</td>
<td>9.4 ± 1.2</td>
<td>9.3 ± 0.9</td>
<td>0.962</td>
</tr>
<tr>
<td>LVDD</td>
<td>50.5 ± 1.9</td>
<td>48.5 ± 3.0</td>
<td>48.7 ± 3.7</td>
<td>0.111</td>
</tr>
<tr>
<td>LVDS</td>
<td>31.1 ± 2.1</td>
<td>30.3 ± 5.0</td>
<td>30.6 ± 4.0</td>
<td>0.840</td>
</tr>
<tr>
<td>FS (%)</td>
<td>38.4 ± 2.7</td>
<td>35.3 ± 6.9</td>
<td>37.1 ± 8.2</td>
<td>0.120</td>
</tr>
<tr>
<td>LAD (mm)</td>
<td>36.1 ± 5.0</td>
<td>39.2 ± 5.3</td>
<td>46.7 ± 6.0</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are the mean ± SD.

IVSTh: intraventricular septal thickness; PWTh: posterior wall thickness; LVDD: left ventricular dimension in diastole; LVDS: left ventricular dimension in systole; FS: fractional shortening; LAD: left atrial dimension.

Statistical differences between controls and patients are shown by p values; *p < 0.05, **p < 0.001. Statistical differences between patients with PAF group and PeAF group are shown by p values; †p < 0.05, ‡p < 0.001.

**Table 3. Stepwise multiple regression analysis of variables associated with RHI**

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Selected factors</th>
<th>Standardized regression coefficient</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>RHI</td>
<td>LAD</td>
<td>-0.280</td>
<td>-2.99</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Smoking</td>
<td>-0.218</td>
<td>-2.92</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Statin</td>
<td>0.161</td>
<td>2.12</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>PAF</td>
<td>-0.529</td>
<td>-4.95</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>PeAF</td>
<td>-0.447</td>
<td>-3.56</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Multiple regression coefficient, R = 0.60, F = 13.1, p < 0.001

For analysis, the following variables were included: age, sex, body mass index, history of hypertension, smoking, dyslipidemia, use of statins, ACE-I/ARB, beta blockers, calcium channel blocker, aspirin, LDL-cholesterol, LAD, and presence of PAF and PeAF.
Endothelial dysfunction in paroxysmal atrial fibrillation patients

The present study demonstrated that even in patients with PAF, RHI is lower than in background-matched control subjects, and the presence of PAF was also shown to be an independent predictor of RHI. Several studies demonstrated that ED occurred in patients with PAF. Li-Saw-Hi et al. reported that the levels of vWF, which is considered a reliable marker of endothelial damage or dysfunction, were high in PAF patients while in sinus rhythm. Sohara et al. also demonstrated that prothrombotic factors are present in PAF patients; however, these studies evaluated the plasma level of vWF as an index of endothelial dysfunction, which is affected by many known cardiovascular risk factors. It is frequently argued that abnormalities in markers of endothelial function in AF patients could be due to some known cardiovascular risks that often coexist with AF. For example, elevated plasma levels of vWF are associated with established cardiovascular risk factors, such as age, smoking, diabetes mellitus, cholesterol, and hypertension. Although serum levels of vWF in AF patients were compared to controls in these studies, control subjects had few or no cardiovascular risk factors, and this may have influenced the serum levels of vWF.

In stepwise multivariate regression analysis, age, LAD, and taking statins are other independent predictors of RHI. Several lines of evidence suggest that statin therapy can improve endothelial function, and this also suggests the validity of using RHI to measure endothelial function.

In the present study, the RHI was lower in PAF patients and PeAF patients than in control subjects even outside the AF period. These observations suggest that endothelial dysfunction occurs in AF patients independent of the duration of the AF period, and contributes to the high prevalence of thromboembolic events even in PAF patients. This hypothesis is also supported by the observation that ischemic stroke occurs in patients with PAF even during the non-AF period. In addition, Motoki et al. have reported that coagulation activity in the LA was increased in PAF patients, even when they were in the non-AF period. This may also contribute to the high prevalence of thromboembolic events in PAF patients as a one of three factors of Virchow’s triad.

To our knowledge, this is the first study to demonstrate the significant impairment of endothelial function in patients with PAF in comparison with sex-, age-, and risk factor-matched control subjects, and this impairment of endothelial function is independent of other comorbidity factors. By comparing with background-matched control subjects and using statistical methods, we evaluated ED with exclusion of the effects of risk factors that could affect endothelial function.

Measurement of endothelial function using a novel device

We used a novel device, EndoPAT, which is the only FDA-approved noninvasive device for evaluating endothelial function. Assessment of vascular function with PAT involves measuring pulse amplitude in the fingertip at rest and under conditions of increased shear stress that result in the release of NO. Dhindsa et al. examined the relation of PAT hyperemia to established measures of endothelial function, and PAT showed good correlations with these measures. Reproducibility of the digital vascular responses over time was examined in 2 small studies, and the results confirmed the reproducibility of PAT.

Although FMD is a well-established measure of endothelial function, problems with reproducibility and the influence of sympathetic nervous activity persist. Additionally, its results are highly operator-dependent and can be confounded by changes in the baseline diameter of the brachial artery. In contrast, 2 methods are used to minimize the impact of confounding factors on RH-PAT results. First, the reactive hyperemia response was referenced to a baseline derived from the same finger to eliminate local finger-related effects. Second, the effects of systemic factors were minimized by normalizing the RH-PAT value of the study arm to the corresponding PAT signal of the control arm. Furthermore, RH-PAT, when compared to FMD, requires less specialized training. These characteristics support the validity of using RH-PAT for precise measurement of ED.

Based on these results, we propose that AF itself, regardless of subtype, may also be the result of systemic ED; that is, ED leads to AF. This hypothesis was supported by a recent study indicating no significant association between AF duration and the frequency of attacks of stroke based not only on the AF subtype.

Future prospects

Recently, Schnabel et al. presented a scoring system to predict an individual’s absolute risk of developing AF within the next 10 years. If our hypothesis is correct, it may be possible to recognize patients who are at high risk of developing AF, and prevent or postpone its development by improving endothelial function.
In fact, 2 meta-analyses supported the protective effects of statins and angiotensin-converting enzyme inhibitors, which improve endothelial function, against the onset or recurrence of AF. It has been suggested that statins may be effective because of their anti-inflammatory effects, but in a study of patients undergoing thoracic surgical, the protective effect was independent of the high sense C-reactive protein levels. These observations may be due to improved endothelial function in AF patients.

In the present study, there was dispersion in the RHI of PAF and PeAF patients. Further studies are needed to determine whether the degree of ED is correlated with the rate of thromboembolism or prognosis, and whether RH-PAT-guided drug therapy could improve the prognosis of AF patients.

Study limitations

The present study had some limitations. First, as this was a cross-sectional study, we cannot imply causality. Second, due to the community-based nature of our cohort, we did not administer nitroglycerin to measure endothelium-independent vasodilatation. Although this means that the PAT response to ischemia may not be fully endothelial dependent, RHI has been validated against acetylcholine-mediated vasodilatation of coronary arteries, the gold standard in endothelial function testing. Third, we determined the clinical subtype of AF based solely on electrocardiography and the attending physician's interpretation, and may therefore have underestimated the frequency of AF episodes in the PAF group. This means that some PeAF patients may have been identified as PAF patients, and this could be a reason why equal endothelial dysfunction was shown in a recent study. Fourth, we did not divide persistent and permanent AF patients; however, the patients in this group were frequently asymptomatic, making it difficult to divide these patients into two groups only from the symptoms. Fifth, no data have shown that atrial and peripheral arteries have equivalent endothelial function. Further limitations include significant differences between PAF and PeAF groups in the rate of beta-blocker and antiarrhythmic drug administration. It is possible that these differences may have influenced the findings of RHI.

Although this study included relatively large numbers of patients compared with those reported previously, the size of the study cohort may still have been insufficient to detect differences in the degree of endothelial dysfunction between the PAF and PeAF groups.

Conclusions

The findings of the present study suggested that patients with PAF, even outside the AF period, are comparable in the degree of ED to PeAF patients. This impairment of endothelial function was caused by the presence of PAF itself, independent of other coexisting comorbidities. This may provide a mechanism explaining why the risk of thromboembolic risk in PAF is comparable with that in PeAF patients.

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