Clinical Significance of Peripheral Endothelial Function for Left Atrial Blood Stagnation in Nonvalvular Atrial Fibrillation Patients With Low-to-Intermediate Stroke Risk

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**Background:** In patients who have atrial fibrillation (AF) with CHADS2 score of 0–1 (categorized as low-to-intermediate risk), there is little information on stratifying the risk of stroke. This study aimed to determine whether impaired endothelial function assessed by reactive hyperemia-peripheral arterial tonometry (RH-PAT) predicted left atrial blood stagnation in these patients.

**Methods and Results:** We enrolled 81 consecutive patients with nonvalvular AF. The reactive hyperemia index (RHI) was measured using RH-PAT. Transesophageal echocardiography was performed to determine spontaneous echo contrast (SEC) before direct-current cardioversion or radiofrequency catheter ablation. SEC was found in 49 patients (60%). The RHI was significantly lower in patients with than without SEC. Multivariate analysis demonstrated that RHI was one of the independent determinants of SEC (OR per 0.1, 1.26; 95% CI, 1.11–1.49; \( P = 0.002 \)) in all patients. In addition, RHI was a significant determinant of SEC (AUC, 0.73; 95% CI, 0.63–0.89; \( P = 0.0017 \)) in patients with low-to-intermediate risk. At an RHI cut-off <1.62, the sensitivity and specificity for the identification of patients with SEC were 58% and 89%, respectively.

**Conclusions:** Impaired endothelial function assessed by RH-PAT might help to predict the presence of SEC in patients with low-to-intermediate risk of stroke.  

**Key Words:** Atrial fibrillation; CHADS2; Reactive hyperemia-peripheral arterial tonometry (RH-PAT); Spontaneous echo contrast
Each hand for continuous recording. The PAT signals are (Architect BNP-JP®, Abbott Japan Co, Ltd, Tokyo, Japan) using a specific immunoradiometric assay for human BNP (Arkray, Kyoto, Japan). Plasma BNP levels were measured by high-performance liquid chromatography intermediate risk.

Methods

Study Design and Patients

The study included 96 consecutive patients with nonvalvular AF who had been referred to Ehime University Hospital between January 2012 and June 2013. The exclusion criteria included patients with left ventricular ejection fraction (LVEF) <50%. As a control group, we included 10 patients without AF who were admitted to hospital during the same observational period. Electrocardiography, transthoracic echocardiography and coronary angiography were performed in all patients as screening examinations for angina pectoris, revealing no structural heart disease. The institutional medical ethics committee of Ehime University Hospital approved the study protocol, and all patients gave written informed consent.

Blood Sampling and Laboratory Measurements

Blood and urinary samples were taken after a 12-h overnight fast. We measured several indices including HbA1c, serum creatinine, B-type natriuretic peptide (BNP), high-sensitivity C-reactive protein (hs-CRP) and the urinary albumin-to-creatinine ratio. The estimated glomerular filtration rate (eGFR) was determined using the equation proposed by the Japanese Society of Nephrology and based on the equation described in the Modification of Diet in Renal Disease Study. HbA1c was elevated in the control group compared with the AF group. Systolic blood pressure was significantly lower in the control group compared with the AF group. The principle of PAT technology has been described previously. Briefly, the PAT probes are placed on one finger of each hand for continuous recording. The PAT signals are recorded electronically and analyzed online in an operator-independent manner (Endo-PAT 2000 software, version 3.0.4, Itamar Medical Ltd). The RH-PAT measurements are assessed using a computerized, automated algorithm (Itamar Medical Ltd). After a 5-min equilibration period, the blood pressure cuff on the study arm is inflated to 60 mmHg above the baseline systolic blood pressure or a maximum of 200 mmHg for 5 min. After a 5-min occlusion, the cuff is deflated, and the PAT measurement continues for another 5 min. The RH-PAT ratio reflects the extent of reactive hyperemia and is calculated as a ratio of the average amplitude of the PAT signal over 1 min starting 1.5 min after cuff deflation (control arm, A; occluded arm, C) divided by the average amplitude of the PAT signal recorded during the 2.5-min time period before cuff inflation (control arm, B; occluded arm, D). We used the RH-PAT index (RHI) for the present analysis. The RHI is derived from the following equation: $RHI = \ln\left(\frac{C/D}{A/B}\times0.226\times\ln(\text{baseline})-0.2\right)$. Previous studies have shown that the RH-PAT technology has excellent reproducibility.

Transthoracic Echocardiography

Transthoracic echocardiography was performed in all patients before electrical cardioversion or catheter ablation. Conventional echocardiographic parameters were measured according to the recommendations of the American Society of Echocardiography. LVEF and LA volume index (LAVI) were calculated using the biplane Simpson’s method from the apical 4- and 2-chamber views.

Assessment for SEC With TEE

TEE was performed immediately after measurement of the RHI. An ultrasound system (Philips iE33; Philips Medical Systems, Andover, MA, USA) was used for TEE. During the TEE examination, special attention was paid to assessing the presence or absence of LA thrombi and SEC. SEC was defined as dynamic smoke-like echoes within the LA cavity with a characteristic swirling motion that could not be eliminated by changes in the gain settings. The Doppler sample volume was placed in proximity to the LA appendage (LAA) orifice for the measurement of the highest emptying blood flow velocity (LAAV). The outflow profiles were recorded over 5–10 cycles, and the values of 3 consecutive cycles were averaged.

Statistical Analysis

A chi-square test, Fisher’s exact probability test, or Wilcoxon signed-rank test was used for the comparison of categorical variables; continuous variables were compared using a one-way ANOVA followed by a Bonferroni post-hoc test. Univariate and multivariate logistic regression models were used to evaluate the influence of the presence of SEC. Multivariate analysis was performed with variables that were statistically significant in the univariate analysis. All statistical analyses were performed using JMP 5.0.1 (SAS Institute Inc, Cary, NC, USA).

Results

Patients Characteristics

We enrolled 96 patients with AF before cardioversion or catheter ablation; 15 patients with LVEF <50% were excluded (Figure 1). Ultimately, 81 patients with nonvalvular AF were evaluated. SEC was observed in 49 patients (60%). The clinical characteristics of the AF patients as well as the control subjects are shown in Table 1. Systolic blood pressure was significantly elevated in the control group compared with the AF group. Diastolic blood pressure was lower in AF patients without structural heart disease. The institutional medical ethics committee of Ehime University Hospital approved the study protocol, and all patients gave written informed consent.
Table 1. Baseline Characteristics of AF Patients With and Without SEC

<table>
<thead>
<tr>
<th></th>
<th>Control without AF</th>
<th>AF total cohort</th>
<th>AF without SEC</th>
<th>AF with SEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>81</td>
<td>32</td>
<td>49</td>
</tr>
<tr>
<td>Age, years</td>
<td>67 (64–71)</td>
<td>65 (56–70)</td>
<td>61 (54–70)</td>
<td>67 (61–70)</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>5/5</td>
<td>59/22</td>
<td>26/6</td>
<td>33/16</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.6 (22.4–25.8)</td>
<td>23.6 (21.6–26.0)</td>
<td>23.3 (21.4–25.9)</td>
<td>24.8 (21.7–27.3)</td>
</tr>
<tr>
<td>CHADS2 score ≥2, %</td>
<td>2 (20)</td>
<td>28 (35)</td>
<td>10 (31)</td>
<td>18 (37)</td>
</tr>
<tr>
<td>Current smoking</td>
<td>3 (30)</td>
<td>30 (12)</td>
<td>4 (13)</td>
<td>8 (15)</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>139 (132–146)</td>
<td>124 (116–138)</td>
<td>126 (113–138)*</td>
<td>123 (117–138)*</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>83 (77–87)</td>
<td>74 (68–83)</td>
<td>71 (64–80)*</td>
<td>76 (70–85)</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>68 (60–76)</td>
<td>69 (59–79)</td>
<td>64 (57–74)</td>
<td>73 (61–82)</td>
</tr>
<tr>
<td>HbA1c (NGSP), %</td>
<td>6.0 (5.7–6.2)</td>
<td>5.9 (5.6–6.1)</td>
<td>5.8 (5.3–6.0)</td>
<td>5.9 (5.7–6.2)</td>
</tr>
<tr>
<td>eGFR, ml/min/1.73m²</td>
<td>74 (69–84)</td>
<td>65 (57–77)</td>
<td>71 (63–82)</td>
<td>64 (54–74)*</td>
</tr>
<tr>
<td>BNP, pg/ml</td>
<td>19 (9–29)</td>
<td>100 (31–200)</td>
<td>43 (17–146)</td>
<td>136 (52–349)*</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>41.3 (39.5–43.8)</td>
<td>42.3 (40.4–45.5)</td>
<td>42 (40.2–45)</td>
<td>42.6 (40.1–45.6)</td>
</tr>
<tr>
<td>hs-CRP, mg/dl</td>
<td>0.06 (0.02–0.005)</td>
<td>0.06 (0.03–0.10)</td>
<td>0.06 (0.02–0.07)</td>
<td>0.07 (0.04–0.12)</td>
</tr>
<tr>
<td>UACR, mg/g</td>
<td>12 (9–16)</td>
<td>6 (4–10)</td>
<td>6 (4–10)</td>
<td>6 (4–13)</td>
</tr>
</tbody>
</table>

Results are median values (interquartile range in parentheses). *P<0.05 vs. control. †P<0.05 vs. AF without SEC. ACEI/ARB, angiotensin-converting enzyme inhibitors/angiotensin-receptor blockers; AF, atrial fibrillation; BMI, body mass index; BNP, brain natriuretic peptide; BP, blood pressure; eGFR, estimated glomerular filtration rate; hs-CRP, high-sensitivity C-reactive protein; LAAV, left atrial appendage velocity; LAVI, left atrial volume index; LVEF, left ventricular ejection fraction; RHI, reactive hyperemia index; SEC, spontaneous echo contrast; TEE, transesophageal echocardiography; UACR, urinary albumin-to-creatinine ratio.

SEC than in control subjects. There were no significant differences in heart rate among the 3 groups. AF patients with SEC had lower eGFR and higher levels of BNP compared with AF patients without SEC and control subjects. We evaluated whether hs-CRP and albuminuria were associated with the presence of SEC, but there was no significant difference in either factor. Statin use was significantly higher in control subjects compared with the AF groups. LAVI was significantly larger and LAAV was significantly reduced in AF patients with than without SEC. The prevalence of AF during TEE was more frequent in AF patients with SEC.

Relationship Between Endothelial Function and SEC

Figure 2 shows the RH-PAT signals of representative patients with and without SEC. The recorded data from a patient with SEC showed a blunted finger PAT response during the reactive hyperemia phase (Figure 2A). The mean RHI was significantly lower in patients with than without SEC (P=0.002, Table 1). Single logistic regression analysis demonstrated that sex, CHADS2 score ≥2, eGFR, BNP, AF rhythm during TEE, heart rate, LAVI and LAAV, and lower RHI were significantly correlated with the presence of SEC. In the multivariate analysis that included these factors, RHI as well as LAAV and LAVI were independent determinants of SEC (Table 2).

RH-PAT Index in Low-to-Intermediate Risk AF Patients

According to the CHADS2 score, patients with a score of 0 or 1 and patients with a score ≥2 were defined as low-to-intermediate risk and high risk, respectively. The RHI was significantly higher in low-to-intermediate risk patients with than without SEC. Conversely, there was no significant difference between high risk patients with and without SEC (Figures 3A,B). Receiver-operating characteristic curve analysis demonstrated that the RHI was a significant predictor of SEC in patients with ow-to-intermediate risk (area under the curve, 0.73; 95% confidence interval, 0.63–0.89; P=0.0017). At a cut-off value of RHI ≤1.62, the sensitivity and specificity for the presence of SEC were 58% and 89%, respectively, and the positive and negative predictive values were 82% and 58%, respectively (Figure 3C).

Discussion

Major Findings

Impaired endothelial function assessed by RH-PAT was significantly associated with the presence of LASEC in patients with nonvalvular AF. According to multivariate analysis, RHI was the most powerful determinant of the presence of LASEC. In addition, the assessment of endothelial function using RH-PAT showed promise in determining the presence of LASEC even
According to another report, AF led to not only local but also systemic endothelial dysfunction as assessed by levels of asymmetric dimethylarginine. Additionally, Cai et al. demonstrated that AF caused a marked decrease in endocardial NOS expression and NO bioavailability, and an increase in PAI-1 expression in the porcine LA. These reports support the hypothesis that impaired atrial contraction induced by AF decreases the NO concentration and contributes to the thromboembolic phenomena in vivo.

Table 2. Multivariate Analysis of SEC Using Cox Regression Analysis

<table>
<thead>
<tr>
<th></th>
<th>HR (95% CI)</th>
<th>P value</th>
<th>HR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHADS2 score ≥2, %</td>
<td>2.03 (0.57–7.85)</td>
<td>0.278</td>
<td>1.44 (0.40–5.35)</td>
<td>0.574</td>
</tr>
<tr>
<td>AF during TEE, %</td>
<td>2.12 (0.56–8.30)</td>
<td>0.272</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>1.00 (0.95–1.06)</td>
<td>0.574</td>
<td>1.44 (0.40–5.35)</td>
<td>0.574</td>
</tr>
<tr>
<td>eGFR, ml/min/1.73m²</td>
<td>1.01 (0.96–1.05)</td>
<td>0.768</td>
<td>0.99 (0.94–1.03)</td>
<td>0.620</td>
</tr>
<tr>
<td>BNP, pg/ml</td>
<td>1.00 (0.99–1.00)</td>
<td>0.768</td>
<td>0.99 (0.94–1.03)</td>
<td>0.620</td>
</tr>
<tr>
<td>LAVI, ml/m²</td>
<td>0.97 (0.93–1.01)</td>
<td>0.095</td>
<td>0.96 (0.92–0.99)</td>
<td>0.037</td>
</tr>
<tr>
<td>E/e’</td>
<td>0.98 (0.84–1.14)</td>
<td>0.828</td>
<td>1.02 (0.87–1.17)</td>
<td>0.801</td>
</tr>
<tr>
<td>LAAV, cm/s</td>
<td>1.03 (0.99–1.06)</td>
<td>0.117</td>
<td>1.04 (1.01–1.07)</td>
<td>0.012</td>
</tr>
<tr>
<td>RHI, per 0.1</td>
<td>1.31 (1.12–1.58)</td>
<td>0.011</td>
<td>1.26 (1.11–1.49)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

CI, confidence interval; HR, hazard ratio. Other abbreviations as in Table 1.

in patients with low-to-intermediate risk (ie, CHADS2 score of 0 or 1).

**Endothelial Function and AF**

Recent studies suggest that AF adversely affects endothelial function. Several mechanisms might be responsible for this correlation. In the endothelium, it has been shown that nitric oxide synthase (NOS) expression is regulated by flow-mediated shear stress and is downregulated at sites with low flow. In an experimental study, rapid atrial pacing reduced the mRNA expression of ventricular and aortic endothelial NOS. According to another report, AF led to not only local but also systemic endothelial dysfunction as assessed by levels of asymmetric dimethylarginine. Additionally, Cai et al. demonstrated that AF caused a marked decrease in endocardial NOS expression and NO bioavailability, and an increase in PAI-1 expression in the porcine LA. These reports support the hypothesis that impaired atrial contraction induced by AF decreases the NO concentration and contributes to the thromboembolic phenomena in vivo.

**Figure 2.** Representative cases of RH-PAT examination and TEE in patients with LASEC (A, B) and without LASEC (C, D). In patients with LASEC, the RHI was 1.58. In patients without LASEC, the RHI was 2.34. LASEC, left atrial spontaneous echo contrast; RHI, reactive hyperemia index; RH-PAT, reactive hyperemia-peripheral arterial tonometry.
Endothelial Function as Clinical Risk Stratification
The endothelium has a protective role in vascular function because it regulates vascular tone, cellular adhesion, smooth muscle cell proliferation and vessel wall inflammation, and helps to maintain blood flow and regulate the blood coagulation system. Impairment of endothelial function triggers the platelet adhesion and aggregation, and fibrin formation that play a critical role in systematic hypercoagulability. Several recent reports suggested that endothelial NOS downregulation in the LA was associated with the development of LA thrombi formation even in the presence of sinus rhythm. In addition, Suzuki et al reported that impairment of endothelial function assessed by RH-PAT was related to systemic thrombosis in patients after lower limb arthroplasty. That study also demonstrated an overlap of many risk factors, which are included in clinical risk stratification using the CHA2DS2-VASc score as recommended by the European Society of Cardiology. and these factors were associated with the development of spontaneous deep vein thrombosis. Our study results suggested that a noninvasive test of peripheral endothelial function using RH-PAT could assess the possible thromboembolic source or severity of atherosclerosis, and might explain the improvement in clinical risk stratification in patients with AF.

Measurement of Peripheral Endothelial Function
Flow-mediated dilatation (FMD) and RH-PAT are 2 major methods for assessing peripheral endothelial function in clinical practice. FMD can vary because of operator experience and technical complexity during the measurement. In this study, we evaluated the use of RH-PAT, which is a FDA-approved device that provides a noninvasive, automatic and quantitative assessment of endothelial function. A previous study reported that the risk factors associated with endothelial function assessed by RH-PAT (higher body mass index, higher cholesterol, and the presence of diabetes as well as smoking and female sex), were not identical with those associated with FMD. These risk factors were also reported to highlight the hemorheological imbalance that is implicated in the pathogenesis of SEC. 

Endothelial Dysfunction and SEC Occurrence in AF Patients With Low-to-Intermediate CHADS2 Risk Score
Several schemes have been proposed for stroke risk stratification of patients with AF. Among these schemes, the CHADS2 score and the CHA2DS2-VASc score are commonly acknowledged in clinical practice, and have been validated to provide significant risk stratification. The CHADS2 score assigns 1 point each for heart failure, hypertension, age ≥75 years, and diabetes mellitus and 2 points for prior stroke or transient ischemic attack. In an epidemiological study of 1,733 patients with nonvalvular AF without anticoagulation therapy, higher CHADS2 score was associated with increased incidence of stroke. The Japanese Circulation Society guidelines recommend that patients with a CHADS2 score ≥2, categorized as high risk, should be treated with anticoagulation therapy because the risk of ischemic stroke outweighs the increased risk of bleeding induced by anticoagulation therapy.

Conversely, 38.9–50% of patients with nonvalvular AF are found to have a low-to-intermediate risk CHADS2 score of 0–1. However, the annual expected stroke rate in these patients is still considered to be elevated. The decision to treat with antithrombotic therapy in these patients remains controversial and the use of antithrombotic therapy is limited in clinical practice. The CHA2DS2-VASc score consists of 2 additional risk factors, “Vascular diseases” and “Sex category”, in addition to the CHADS2 score. For patients with CHADS2 scores of 0–1, categorized as low-to-intermediate risk, the CHA2DS2-VASc score was introduced to improve predictive accuracy; however, there is a lack of evidence concerning the usefulness of this score in the Japanese population. A previous study demonstrated that TEE was useful to stratify the risk of cardiovascular events in Japanese patients with CHADS2 scores of 0–1. Another study suggested that the stroke or other embolic event rate reached 12%/year in patients with SEC, compared with 3%/year in those without SEC. TEE findings in AF patients with low-to-intermediate CHADS2 scores could improve prognostic accuracy for ischemic stroke, cardiovascular events and death.

We included a control group of 10 patients without AF who
did not have organic heart disease or coronary stenosis. RH-PAT in the control group was similar to that in AF patients without SEC, but significantly higher than that in AF patients with SEC. These results support our hypothesis that AF patients with SEC have advanced endothelial dysfunction. A previous report also suggested that SEC was strongly associated with LAA thrombus and embolic events, and the occurrence of SEC was independently associated with LAAV and LA area in a multivariate analysis. Another study demonstrated that in patients with LA dilation and reduced ventricular function despite a low CHADS2 score of 0–1, 3% of patients had LA thrombus and 8% patients had dense SEC. Virchow's triad traditionally explains a pathophysiologic mechanism of thrombus formation, and the triad includes abnormalities in blood composition, vessel wall components and blood flow. In the present study, the occurrence of SEC was independently associated with LAVI, LAAV and RHI, suggesting that endothelial dysfunction was an important determinant of SEC in addition to LA remodeling and low LAA flow. However, TEE is a semi-invasive procedure and is not free from clinical complications. Because of these concerns, TEE is not widely performed as a routine examination in clinical practice. In this investigation, we verified that the impairment of endothelial function assessed by RH-PAT was a powerful determinant for LA blood stagnation, TEE risk predicting for cardiovascular events, in patients with nonvalvular AF with low-to-intermediate risk. RH-PAT is the only FDA-approved noninvasive device for evaluating endothelial function in clinical practice. The assessment of endothelial function using RH-PAT might be a promising and a feasible procedure/strategy for risk stratification in these patients.

Study Limitations

The major limitations are that the sample size was relatively small, and that the study was performed at a single center. Furthermore, it was a cross-sectional study, which limits any determination of causality. Second, endothelium-independent vasodilatation was not measured, because RH-PAT cannot reliably assess nitroglycerin-induced vasodilatation. In addition, we did not evaluate FMD simultaneously in this study. Third, no data were obtained that showed equivalent endothelial function in the atria and the peripheral arteries. Finally, regardless of the absence of significant coronary stenosis evaluated by coronary angiography, the control subjects might have subclinical endothelial dysfunction because of their relatively high age, elevated blood pressure and more frequent use of statins than in the group of AF patients without SEC. In addition, high age, elevated blood pressure and more frequent use of coronary angiography, the control subjects might have subclinical endothelial dysfunction despite a low CHADS2 score of 0–1, 3% of patients had LA thrombus and 8% patients had dense SEC. Virchow's triad traditionally explains a pathophysiologic mechanism of thrombus formation, and the triad includes abnormalities in blood composition, vessel wall components and blood flow. In the present study, the occurrence of SEC was independently associated with LAVI, LAAV and RHI, suggesting that endothelial dysfunction was an important determinant of SEC in addition to LA remodeling and low LAA flow.

Conclusions

We propose that impaired endothelial function assessed by RH-PAT is related to the presence of SEC in patients with low-to-intermediate risk of stroke according to the CHADS2 score.

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Conflicts of Interest


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