Impaired endothelial function in lone atrial fibrillation


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Abstract

Background/Aim. Impaired endothelial function has been previously documented in patients with atrial fibrillation (AF) and underlying comorbidities or older patients with idiopathic AF. The aim of this study was to evaluate systemic endothelial function in younger AF patients (less than 60 years old) with lone AF (that is, without associated cardiopulmonary comorbidities, including arterial hypertension), by comparing brachial artery flow-mediated dilation (FMD) in lone AF patients with FMD of healthy subjects in sinus rhythm. Methods. Two groups of participants were prospectively enrolled. The first group comprised of 38 AF patients (the mean age 45 ± 11 years, 68% male) matched by age, gender and atherosclerotic risk factors. All the participants underwent physical examination, laboratory analysis [including determination of C-reactive protein (CRP)], standard echocardiography and exercise-stress testing. Brachial artery FMD and endothelium independent dilation (NMD) were assessed with a high-resolution ultrasound probe and arterial diameters taken from 5 consecutive cardiac cycles were averaged for each measurement to accommodate to beat-to-beat flow variations in AF. Results. There were no differences between the 2 groups regarding age, gender and most clinical, laboratory and echocardiographic characteristics (all p > 0.05), apart from the increased heart rate (p = 0.018), body mass index (p = 0.027), CRP levels (p = 0.007) and left atrial anteroposterior dimension (p < 0.001) in AF patients. FMD of AF patients [median value 5.0%, interquartile range (IQR) 2.87%–7.50%] was significantly lower (p < 0.001) than FMD of healthy controls (median value 8.85%, IQR 5.80%–12.50%), whereas there were no differences in median NMD values (p > 0.05). In the multivariate analysis, the independent FMD determinants in our study population were the presence of AF, smoking and total cholesterol levels (all p < 0.001). In patients with AF, the strongest independent FMD determinant was arrhythmia duration (p < 0.001), followed by smoking (p = 0.013) and total cholesterol levels (p = 0.045). Conclusions. Our findings confirm that sustained AF is associated with systemic endothelial dysfunction even in relatively young patients with no cardiovascular disorders or risk factors. AF is an independent contributor to lower FMD and a prolonged arrhythmia duration may confer the risk for more profound endothelial damage.

Key words: atrial fibrillation; endothelium, vascular; brachial artery; echocardiography; risk assessment; heart rate; body mass index; c-reactive protein.

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Introduction

Atrial fibrillation (AF) is the most prevalent sustained cardiac arrhythmia in adult population. It is usually associated with underlying comorbidities (e.g. arterial hypertension, coronary artery disease, valvular heart disease, diabetes mellitus, thyroid and pulmonary disorders) and a variety of risk factors (e.g. obesity, metabolic syndrome, sleep apnea, excessive alcohol consumption and competitive sports). AF is considered a benign condition with favorable long-term prognosis. However, even in patients with lone AF, an evidence of damage/dysfunction of atrial endocardium, platelet activation and increased inflammatory and oxidative stress has been found.

Over the past decade, systemic arterial endothelial dysfunction has been functioned both experimentally and clinically in various subsets of AF patients. In clinical research, brachial artery flow-mediated dilation (FMD) is the most used method to investigate systemic endothelial function. This technique relies on brachial artery dilation produced by endothelial release of endogenous vasodilators [principally nitric-oxide (NO)] in response to increased blood flow and shear stress. Although there has been some concern about FMD application in the settings of oscillatory blood flow, recent studies have demonstrated good reproducibility and correlation with other determinants of endothelial damage in AF.

It has been recognized that circulating indices of endothelial damage are related to increased risk of stroke in AF and endothelial dysfunction in peripheral vessels has been associated with adverse vascular events in patients in sinus rhythm. However, the prognostic implications of systemic endothelial dysfunction, determined by FMD in AF patients are still unknown. Nevertheless, impaired endothelial function is considered to be an important facilitator of thrombus formation.

To determine the association of AF and endothelial dysfunction, it would be the most appropriate to investigate apparently healthy individuals with AF, such as patients with lone AF. However, most previous research on endothelial function in lone AF included patients with hypertension or subjects older than 60 years, clearly breaching the definition of lone AF.

Therefore, the aim of this study was to evaluate the association of AF with endothelial dysfunction by comparing brachial artery FMD of younger patients with persistent lone AF with FMD of healthy control subjects in sinus rhythm.

Methods

This single-center, cross-sectional study was conducted between November 2009 and April 2011. Patients with lone AF and healthy volunteers in sinus rhythm, matched by age, gender and atherosclerotic risk factors, were prospectively enrolled.

Before recruitment, all the participants underwent physical examination, routine biochemistry analyses, thyroid function assessment, determination of C-reactive protein (CRP) levels (by a commercially available immunoassay for high-sensitivity detection – detection limit 0.1 mg/L), 12-lead electrocardiogram (ECG), exercise stress testing and standard transthoracic echocardiographic examination.

The patients were eligible if persistent, lone AF was confirmed by 12-lead ECG. Persistent AF was defined as a sustained arrhythmia lasting for more than 7 days with repeated ECG demonstration of AF without intervening periods of sinus rhythm. AF duration was determined as accurately as possible according to patient-reported symptom onset and available medical documentation. AF was considered lone in patients younger than 60 years of age if there were no known associated cardiovascular disorders, or precipitating factors for AF. Therefore, none of the AF patients had a history of hypertension or other cardiovascular disorders prior to AF onset and all the patients were normotensive on the initial clinical evaluation before the initiation of medical therapy. All the patients had normal baseline laboratory tests, thyroid function, ECG and echocardiographic findings (mild left atrial dilatation < 4.5 cm was allowed). Ischemic heart disease or positive exercise stress test, valvular dysfunction (including mitral valve prolapse), cardiomyopathies, heart failure, preexcitation syndrome, diabetes mellitus, chronic pulmonary diseases, acute or chronic inflammatory disorders, malignancy, recent body trauma or surgery were exclu-
sion criteria. AF patients received a beta-blocker or verapamil for heart rate control and digoxin was added when rate control (<80 beats/min at rest) was not achieved with the highest tolerated dose of either agent alone. Warfarin was administered to all AF patients, targeting international normalized ratio (INR) of 2.0 to 3.0. No other medications were allowed.

The control subjects were considered eligible if they had no history of cardiovascular or other disorders and their physical examination, biochemistry, ECG, exercise stress test and echocardiogram were normal. Control subjects received no medications.

Written informed consent was obtained from all the participants.

Endothelial function was assessed using a high resolution (7.5 MHz, Agilent Image Point HX) vascular ultrasound probe. Vascular studies were performed by the 2 experienced investigators in a temperature-controlled room between 11 am and 1 pm. All the subjects were instructed not to eat, drink caffeinated beverages or take vitamin C supplements at least 12 hours before the study, and to refrain from alcohol consumption, smoking or physical exercise at least 1 day in advance of the study. After resting in supine position for 15 minutes, their heart rate and blood pressure were measured and baseline arterial image was acquired from the right arm 2–5 cm above the antecubital fossa. When a suitable 2-dimensional longitudinal axis image of the vessel was obtained and digitally recorded, the position of the ultrasound probe was fixed and remained unchanged throughout the examination. Arterial diameter measurements were performed off-line as a distance between the near and far wall lumina-intima boundaries at end-diastole (onset of the R wave on the ECG). To accommodate for beat-to-beat flow velocity variations in AF, arterial diameters taken from 5 consecutive cardiac cycles were averaged for each measurement. The same method was applied in the healthy controls.

After determination of baseline arterial diameter (Dbase), a phymgomanometric cuff was placed on the forearm and inflated to ≥200 mmHg for 5 minutes. Hyperemic stimulus was produced by rapid cuff deflation. Digital recording of the brachial artery was resumed 30 s before and continued for 90 s after cuff deflation. Approximately 60 s after cuff deflation brachial artery was measured again to determine the diameter of the maximal endothelium-dependent dilation (Dmax). FMD was calculated using a formula: FMD = [(Dmax − Dbase) / Dbase] × 100 (%).

Endothelium-independent dilation (NMD), a measure of vascular smooth muscle vasoreactivity, was assessed 15 to 20 min after FMD to allow for the restoration of baseline conditions. Five min after sublingual administration of 0.4 mg of nitroglycerine, brachial artery diameter was measured to determine nitroglycerine-induced dilation (DNTG). NMD was calculated using a formula: NMD = [(DNTG−Dbase) / Dbase] × 100 (%).

Vascular studies were successful in all the participants. Inter- and intraobserver variations for baseline brachial artery measurements in our laboratory are 0.04 ± 0.03 mm and 0.02 ± 0.02 mm, respectively.

### Statistical analysis

Sample size was determined from a pilot study that included 15 patients with persistent lone AF and 15 healthy controls. Respective mean values and standard deviations (SD) of FMD were determined to be 5.5% ± 2.8% and 8.8% ± 3.3%. It was determined that a minimum of 21 cases should be included in each group to detect the difference in FMD means with a 90% power and type I error probability of 0.05.

Following a test of statistical normality (Kolmogorov-Smirnov test), continuous variables are presented as mean ± SD or median and interquartile range (IQR), depending on a distribution. Categorical variables are reported as counts (n) with percentages (%). To analyze statistical differences between the 2 study groups the Student’s t test, Mann-Whitney’s test or Pearson’s χ² test were used, as appropriate. The association of clinically significant variables with FMD was tested using a univariate linear regression analysis, and variables related to FMD (p < 0.1) were entered into a stepwise multivariate linear regression model. All the analyses were performed using SPSS statistical software, version 17.0. The statistical significance was set at a p value < 0.05 and 95% confidence intervals (CI) were used (2-sided).

### Results

The present study included 38 patients with persistent, lone AF (24 to 60 years old, 68.4% male), and 28 healthy control subjects (27 to 60 years old, 53.6% male). Clinical characteristics of the participants are presented in Table 1. There were no differences between AF patients and the controls with respect to age, gender and most clinical and echocardiographic characteristics (p > 0.05 for all). However, the AF patients had a higher resting heart rate (p = 0.018), body mass index (p = 0.027) and serum CRP levels (p = 0.007). Left atrial anteroposterior diameter was also greater in the AF patients compared with the controls (p < 0.001).

In the AF group, arrhythmia persisted from 2 to 44 weeks before the enrollment (median AF duration was 16 weeks). All the AF patients received heart rate controlling medications (27 patients received beta-blocker monotherapy, 7 patients received verapamil only and 4 patients received a combination of either a beta-blocker or verapamil with digoxin) (Table 1).

A vascular study revealed similar median baseline brachial artery diameters in both AF patients and the controls (Table 2). An absolute increase in arterial diameter after cuff deflation was observed in all healthy subjects, but in 4 of the 38 AF patients (10.5%) no endothelium-dependent dilation occurred (Table 2). Maximal endothelium-dependent diameter change (Dmax−Dbase) was considerably greater in healthy subjects than in AF patients (p = 0.001) (Table 2).

On the other hand, arterial dilation was observed in all the subjects after nitroglycerine application, and there was no difference in the absolute diameter change (DNTG−Dbase) or median NMD value (p > 0.05 for both) as presented in Table 2.
Clinical characteristics of the study participants

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>AF group (n = 38)</th>
<th>Control group (n=28)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), ȗ ± SD</td>
<td>45.3 ± 11.4</td>
<td>43.1 ± 13.2</td>
<td>0.970</td>
</tr>
<tr>
<td>Gender (male), n (%)</td>
<td>26 (68.4)</td>
<td>15 (53.6)</td>
<td>0.219</td>
</tr>
<tr>
<td>Resting heart rate (bpm), ȗ ± SD</td>
<td>74.0 ± 8.5</td>
<td>69.8 ± 5.8</td>
<td>0.018</td>
</tr>
<tr>
<td>Body mass index (kg/m²), ȗ ± SD</td>
<td>23.2 ± 1.6</td>
<td>22.2 ± 2.1</td>
<td>0.027</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg), ȗ ± SD</td>
<td>122.5 ± 11.6</td>
<td>126.3 ± 9.1</td>
<td>0.704</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg), ȗ ± SD</td>
<td>76.2 ± 4.7</td>
<td>78.8 ± 4.9</td>
<td>0.639</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L), ȗ ± SD</td>
<td>4.6 ± 0.8</td>
<td>4.5 ± 0.5</td>
<td>0.340</td>
</tr>
<tr>
<td>Triglycerides (mmol/L), ȗ ± SD</td>
<td>1.2 ± 0.4</td>
<td>1.1 ± 0.2</td>
<td>0.710</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/L), ȗ ± SD</td>
<td>4.3 ± 0.5</td>
<td>4.2 ± 0.8</td>
<td>0.908</td>
</tr>
<tr>
<td>C-reactive protein (mg/L), ȗ (min – max)</td>
<td>1.9 (1.4–3.5)</td>
<td>1.3 (1.1–1.9)</td>
<td>0.007</td>
</tr>
<tr>
<td>Current smokers, n (%)</td>
<td>6 (15.8)</td>
<td>4 (14.3)</td>
<td>0.866</td>
</tr>
<tr>
<td>LAD (cm), ȗ ± SD</td>
<td>4.0 ± 0.4</td>
<td>3.4 ± 0.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LV EDD (cm), ȗ ± SD</td>
<td>5.0 ± 0.5</td>
<td>4.9 ± 0.5</td>
<td>0.217</td>
</tr>
<tr>
<td>LV ESD (cm), ȗ ± SD</td>
<td>3.4 ± 0.5</td>
<td>3.2 ± 0.4</td>
<td>0.345</td>
</tr>
<tr>
<td>LVEF (%), ȗ ± SD</td>
<td>59.5 ± 8.1</td>
<td>61.5 ± 4.5</td>
<td>0.262</td>
</tr>
</tbody>
</table>

AF – atrial fibrillation; LAD – left atrial anteroposterior dimension; LV EDD – left ventricular end-diastolic dimension; LV ESD – left ventricular end-systolic dimension; LVEF – left ventricular ejection fraction; BMP – beat per minute.

Table 2

Results of endothelium-dependent and endothelium-independent dilatation of the brachial artery

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AF group (n = 38)</th>
<th>Control group (n = 28)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline arterial diameter – Dbase (mm), ȗ (min – max)</td>
<td>4.10 (3.95–4.30)</td>
<td>3.83 (3.51–4.02)</td>
<td>0.150</td>
</tr>
<tr>
<td>Maximal endothelium dependent diameter change – Dmax–Dbas (mm), ȗ (min – max)</td>
<td>+0.20 (0.11–0.30)</td>
<td>+0.29 (0.22–0.35)</td>
<td>0.001</td>
</tr>
<tr>
<td>Subjects without endothelium-dependent dilatation, n (%)</td>
<td>4 (10.5)</td>
<td>/</td>
<td>0.077</td>
</tr>
<tr>
<td>Diameter change after nitroglycerine – DNTG–Dbase (mm), ȗ (min – max)</td>
<td>+0.52 (0.46–0.58)</td>
<td>+0.53 (0.47–0.55)</td>
<td>0.165</td>
</tr>
<tr>
<td>NMD, ȗ (min – max)</td>
<td>13.35 (12.27–14.60)</td>
<td>13.65 (12.85–14.90)</td>
<td>0.222</td>
</tr>
</tbody>
</table>

Values are presented as median (interquartile range) and n (%); Dbase – baseline arterial diameter; Dmax – maximal endothelium-dependent dilatation; DNTG – nitroglycerine-induced dilation; AF – atrial fibrillation; NMD – endothelium-independent dilatation.

Figure 1 shows the median FMD values for AF patients and healthy subjects. As presented in Figure 1 endothelium-dependent dilatation was significantly better in healthy subjects (median value 8.85%, IQR 5.80%–12.50%) in comparison with AF patients (median value 5.00%, IQR 2.87%–7.50%) – p < 0.001.

The results of the regression analysis of clinical and echocardiographic FMD determinants of all study participants and AF patients are presented in Table 3.

In the univariate analysis of all the study participants, AF presence, resting heart rate, smoking, left atrial diameter, CRP and total cholesterol levels were predictive of FMD. In the multivariate analysis, the only independent FMD determinants were AF presence, smoking and total cholesterol levels (all p < 0.001).

The results of the univariate analysis for the AF patients revealed that AF duration, left atrial dimension, diastolic blood pressure, smoking, CRP and total cholesterol levels were predictive of FMD (all p < 0.05). In the multivariate analysis, the strongest FMD predictor in AF patients was arrhythmia duration (p < 0.001), followed by smoking (p = 0.013) and total cholesterol levels (p = 0.045).
Discussion

In the present study we demonstrated that systemic endothelial function, assessed by brachial artery FMD, was significantly impaired in the patients with sustained lone AF in comparison with the healthy individuals, whilst endothelial-independent dilation was preserved. In the present study population, AF is an independent predictor of lower FMD in patients with AF.

These findings are in agreement with the results of previous studies. The first to report on the presence of systemic endothelial dysfunction in AF were Takahashi et al. 13 who demonstrated impaired endothelium-dependent dilation, assessed by venous occlusion plethysmography in a group of AF patients. This study prompted interest into noninvasive evaluation of systemic endothelial function in AF, resulting in the publication of several trials, showing that the FMD technique could be reliably utilized for endothelial function assessment in AF 18, 21-25. These trials invariably demonstrated impaired FMD in the AF patients in comparison with the healthy subjects 18, 24, 25, as well as an improvement in endothelial function with the restoration of sinus rhythm 21-23. The implication of these findings was that AF presence could be regarded as a risk factor for systemic endothelial dysfunction. However, most of these trials have been conducted in patients with underlying comorbidities, most often hypertension, coronary artery disease and diabetes, which are recognized risk factors for endothelial damage. There have been a few studies that enrolled a relatively small subset of predominantly older patients with idiopathic AF that also confirmed impaired FMD 18, 21, 22.

In contrast, our study is the first to demonstrate impaired FMD in relatively young patients (mean age 45 years) with lone AF and low cardiovascular risk profile, which is of great importance considering that aging and the presence of various atherosclerotic risk factors could adversely affect endothelial function 26, 27. Nevertheless, two well recognized risk factors for endothelial damage, i.e. smoking 28, 29 and serum cholesterol levels 30, were independent predictors of lower FMD in the present study. Besides the influence of these established risk factors, we documented that AF is an independent predictor of reduced FMD. This observation is in line with a previously published trial that found AF presence to predict lower FMD even after adjustments for various comorbidities 18. Another noteworthy finding is an independent inverse relationship of AF duration and FMD which may indicate that the development of endothelial dysfunction in AF is time-dependent and that longer arrhythmia duration may be associated with a more profound endothelial damage.

Interestingly, our findings also revealed an inverse association in the univariate analysis of the left atrial dilation and FMD in the AF patient group. It could be inferred that there are similar underlying pathophysiologic processes linking left atrial remodeling with systemic endothelial dysfunction.

The precise pathophysiologic mechanisms behind systemic endothelial dysfunction in AF have not been fully elucidated. Under physiologic conditions, endothelial NO production is regulated by laminar shear stress 31. In AF, irregular heart beats produce turbulent blood flow and oscillating shear stress in systemic vessels with a negative influence on NO production and endothelial NO synthase expression 12, which is further supported by findings of reduced plasma nitrite/nitrate levels in AF 32. In our study, heart rate was inversely related to FMD in the univariate analysis, possibly reflecting an unfavorable effect of changed hemodynamics in AF. An interesting hypothesis proposes that AF-induced damage to the endocardium of the left atrium may contribute to systemic endothelial dysfunction by reducing circulating nitroso-compounds that serve as endogenous NO donors to systemic vessels 12, 31; further supporting the concept that endothelial dysfunction is a systemic phenomenon in AF patients 18. Other factors such as activation of renin-angiotensin system 34, neurohumoral activation 34 and heightened inflammatory 36 and oxidative stress 37 could be also implicated in the development of endothelial dysfunction in AF, particularly with longer arrhythmia duration. In keeping with the association of AF and inflammation, CRP levels in AF patients in the present study were significantly higher than in healthy individuals and CRP was inversely related to CRP levels in AF patients
with FMD in the univariate analysis. However, an independent association of CRP and FMD was not confirmed in the multivariate analysis. Thus, we concluded that elevated CRP levels were contributory, but not crucial for FMD impairment in our patients.

Study limitations

There is a concern about the influence of a relatively small sample size of the present study on the interpretation of the results. However, both patient and control groups were sufficiently homogenous and the differences in main findings between the groups were substantial enough to allow the conclusion that sample size did not impose significant limitations. The other concern is about the possible shortcomings of the FMD technique to accurately evaluate endothelial function in AF. To minimize the effect of beat-to-beat flow variations on endothelial function assessment, we adopted a modified FMD technique that has been shown to correlate with other markers of endothelial damage in AF. Furthermore, physical activity was not evaluated and it was recognized that regular exercise improves endothelial function. On the other hand, AF-related symptoms may impose limitations on physical activity, thus exerting a negative influence on the endothelium in AF patients. Additionally, the results of the present study could have been strengthened should we have correlated FMD with other established indices of endothelial damage and the left atrial volume instead of the left atrial anteroposterior diameter. Finally, it must be recognized that the influence of medications given to AF patients (beta-blockers, verapamil, digoxin and warfarin) were not controlled in our investigation. Nevertheless, there were no reports on the negative effects of these medications on endothelial function.

Conclusion

Our findings confirm that sustained AF is associated with systemic endothelial impairment even in relatively young patients with no cardiovascular disorders or risk factors. AF is an independent contributor to lower flow-mediated dilation and prolonged arrhythmia duration may confer the risk for more profound endothelial damage. These findings merit further research to clarify clinical relevance and potential therapeutic implications, particularly in thromboembolic risk stratification and prevention of the AF-related thrombembolism.

REFERENCES


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