Increased intracardiac vascular endothelial growth factor levels in patients with paroxysmal, but not persistent atrial fibrillation

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Aims
Although inflammation appears to play a pivotal role in the pathophysiology of atrial fibrillation (AF), the source of inflammation is unknown. We hypothesized that multilevel measurement of several inflammatory proteins in AF patients would help assess the extent and the source of inflammation.

Methods and results
Thirty-nine patients with paroxysmal AF, 33 with persistent AF, and 9 control patients with Wolff–Parkinson–White syndrome were enrolled. Peripheral, left atrial, coronary sinus, and pulmonary vein blood samples were obtained during catheterization. Serum levels of vascular endothelial growth factor (VEGF), interleukin-8 (IL-8), soluble intercellular adhesion molecule 1 (sICAM-1), and transforming growth factor-β1 (TGF-β1) were measured at the four sampled sites. Interleukin-8, sICAM-1, and TGF-β1 levels did not differ among groups at any of the sampled sites. Peripheral VEGF levels were higher in both paroxysmal and persistent AF patients than in controls ($P \leq 0.03$). Left atrial VEGF levels were higher in paroxysmal AF ($P = 0.05$), but not in persistent AF ($P = 0.32$), compared with controls. Coronary sinus and pulmonary vein VEGF levels did not differ significantly among groups.

Conclusions
Low levels of several inflammatory markers in both paroxysmal and persistent AF patients suggest that the inflammatory process is of low grade, if present. In the context of normal pulmonary vein VEGF levels, the heart itself is the most likely source of high left atrial VEGF levels in paroxysmal AF patients; however, this disorder appears to be a transient event in the natural history of AF.

Keywords
Atrial fibrillation • Inflammation • Vascular endothelial growth factor

Introduction
Atrial fibrillation (AF), the most common clinical arrhythmia, is associated with significant impairment of quality of life, and makes an important contribution to general morbidity and mortality.1,2 Both clinical and experimental studies postulate inflammation as a predisposing factor for AF, as well as for its complications.3 Observation of inflammatory infiltrates, myocyte necrosis, and fibrosis in atrial biopsies from patients with lone AF refractory to anti-arrhythmic drug therapy supports the hypothesis that AF is closely related to the inflammatory process.4 The contribution of inflammation to AF is also suggested by the high incidence of AF after cardiac surgery.5,6 Inflammatory cytokines have been recently described as facilitators of AF development and recurrence.7,8 However, several studies have failed to demonstrate elevated levels of C-reactive protein (CRP), interleukin-6 (IL-6), and interleukin-8 (IL-8) in AF patients.1,2,9–11

We hypothesized that multilevel intracardiac and extracardiac measurements of several inflammatory proteins would help assess the extent and source of inflammation in AF patients.
Methods

Patient selection
A total of 81 patients scheduled for electrophysiological study or catheter ablation procedures at the Rhythmology Department of the Louis Pradel Cardiologic Hospital in Lyon, France, between August 2008 and June 2010 were screened for participation in this study. The control group included nine patients with left-sided accessory pathway Wolff–Parkinson–White syndrome and without any history of AF. A total of 39 patients with paroxysmal AF and 33 patients with persistent AF were enrolled. Atrial fibrillation was defined as ‘paroxysmal’ when the arrhythmia was self-terminating within 7 days and ‘persistent’ when the AF episode persisted for >7 days or pharmacological or electrical cardioversion were required to terminate the arrhythmia.

Exclusion criteria included recent history of an acute cardiovascular or cerebrovascular event, major trauma or surgery, apparent ischaemic heart disease, malignancy, connective tissue or inflammatory disease, acute or chronic infection, and pulmonary, hepatic, or renal impairment. Patients under ongoing anti-inflammatory or steroid treatment were also excluded. A detailed medical and drug history was obtained for each patient enrolled. All patients underwent physical examination and routine laboratory tests. The left atrial area was assessed by transesophageal echocardiography prior to electrophysiological study or catheter ablation procedures. For each patient, stroke risk was evaluated according to current recommendations (CHA2DS2-VASc score). This score assigns one point for each of the following risk factors for patients with AF: congestive heart failure, hypertension, age 65–74 years, diabetes, female sex, and vascular disease. Two points are assigned for a history of stroke or transient ischaemic attack and age ≥75 years.

All participants provided written informed consent in accordance with the local ethics committee. The study protocol complies with the Declaration of Helsinki and was approved by the South-East II People’s Protection Committee and the Advisory Committee on Information Processing in Research in the Field of Health.

Blood sampling and laboratory analysis
Blood sampling was performed during routine cardiac catheterization, prior to any ablations or administration of heparin. Blood samples were obtained serially from the femoral vein and coronary sinus. The left atrium was accessed through trans-septal puncture and a third blood sample was taken from the left atrium via the trans-septal sheath. Blood samples from the left superior pulmonary vein were collected via the trans-septal sheath advanced into the proximal segment of the sampled vein.

Vacutainer (BD, Plymouth, UK) tubes (6.0 mL) containing EDTA were used. The tubes were stored and shipped on ice within 30 min after sampling.

Serum concentrations of soluble intercellular adhesion molecule-1 (sICAM-1) and transforming growth factor-β1 (TGF-β1) were measured using commercially available quantitative enzyme-linked immunosorbent assay kits (Human TGF-beta1 and Human sICAM-1/CD54 Quantikine ELISA kits, R&D Systems, Inc., Minneapolis, MN, USA). Lumexin methodology was used to measure serum levels of vascular endothelial growth factor (VEGF) and IL-8, with R&D System kits (Human VEGF and Human CXCL8/IL-8 Fluorokine Multi-analyte Profiling ELISA, R&D Systems, Inc.). The technician performing the analysis was not aware of the patient’s diagnosis or rhythm. The lower limits of detection were 4 pg/mL for VEGF, IL-8, and TGF-β1, and 30 ng/mL for sICAM-1.

Results

Patient clinical characteristics
Demographic and clinical data regarding patients and control subjects are summarized in Table 1.

There was no significant difference among the three groups in terms of sex ratio, smoking status, history of hypertension or diabetes, or statin treatment (all \( P > 0.10 \)). Atrial fibrillation patients were more likely to be treated with anti-aldosteronics and angiotensin converting enzyme inhibitors (ACEIs) or angiotensin II receptor blockers (ARBs) (\( P < 0.01 \)). They were also older (\( P < 0.01 \)) and had a higher body mass index (\( P < 0.01 \)). As expected, left atrial area as assessed by echocardiography in the parasternal long axis was higher in paroxysmal AF patients than in controls and in persistent AF than in paroxysmal AF patients (\( P = 0.001 \)). Persistent AF patients were more frequently in arrhythmia at the moment of blood draw compared with paroxysmal AF patients (\( P < 0.001 \)).

Twelve patients in the paroxysmal AF group (31%) and 13 (39%) in the persistent AF group had single or multiple prior ablation procedures (\( P = 0.26 \)). Mean duration of AF history was 95 ± 86 months in paroxysmal AF patients and 77 ± 58 months in persistent AF patients (\( P = 0.29 \)). None of the control patients presented AF during electrophysiological study or ablation procedure.

Inflammatory marker levels
Mean levels of inflammatory markers are presented in Table 2.

There were no significant differences among the three groups regarding IL-8, sICAM-1, or TGF-β1 levels at any of the sampling sites (all \( P > 0.05 \)). Similarly, CRP levels (measured in peripheral blood only) did not differ among the three groups (\( P = 0.60 \)).

Peripheral levels of VEGF were higher in both paroxysmal and persistent AF patients than in controls (\( P = 0.03 \) and 0.02, respectively) (Figure 1). In the left atrium, VEGF levels were higher in the paroxysmal AF group than the control group (\( P = 0.05 \)); however, there was no significant difference between persistent AF patients and controls (\( P = 0.32 \)) (Figure 1). Although not statistically significant (\( P > 0.10 \)), coronary sinus levels of VEGF were higher in paroxysmal AF patients compared with both controls and persistent AF patients (22.2 ± 8.7 pg/mL in paroxysmal AF patients vs. 9.5 ± 7.7 pg/mL in control patients and 9.8 ± 7.9 pg/mL in persistent AF patients) (Figure 1).

Pulmonary vein levels of VEGF did not differ significantly among the three groups (\( P = 0.73 \)).
Intersite vascular endothelial growth factor gradients

In patients with paroxysmal AF, no significant difference was observed between peripheral and left atrial or coronary sinus and left atrial levels of VEGF (both \( P > 0.05 \)); however, both left atrial and peripheral levels of VEGF were significantly higher than pulmonary vein levels (both \( P < 0.01 \)). In patients with persistent AF, peripheral levels of VEGF were higher than left atrial levels (\( P = 0.05 \)); however, no significant difference was observed among left atrial, coronary sinus, and pulmonary vein levels of VEGF (\( P > 0.05 \)).

Vascular endothelial growth factor predictors

Neither gender, the presence of hypertension or diabetes, current smoking status, anti-aldosteronics, statins, treatment with ACEI or

Table 1 Clinical characteristic of study groups

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 9)</th>
<th>Paroxysmal atrial fibrillation (n = 39)</th>
<th>P value</th>
<th>Persistent atrial fibrillation (n = 33)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>43 ± 18</td>
<td>56 ± 9</td>
<td>&lt;0.01</td>
<td>58 ± 8</td>
<td>0.46</td>
</tr>
<tr>
<td>Men (n, %)</td>
<td>6 (67%)</td>
<td>32 (82%)</td>
<td>0.46</td>
<td>28 (85%)</td>
<td>0.46</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.2 ± 3.5</td>
<td>27.1 ± 3.4</td>
<td>&lt;0.01</td>
<td>29.2 ± 5.2</td>
<td>0.12</td>
</tr>
<tr>
<td>Hypertension (n, %)</td>
<td>2 (22%)</td>
<td>16 (41%)</td>
<td>0.12</td>
<td>19 (58%)</td>
<td>0.05</td>
</tr>
<tr>
<td>Diabetes (n, %)</td>
<td>0 (0%)</td>
<td>5 (13%)</td>
<td>0.05</td>
<td>3 (9%)</td>
<td>0.46</td>
</tr>
<tr>
<td>Smokers (n, %)</td>
<td>1 (11%)</td>
<td>12 (31%)</td>
<td>0.42</td>
<td>11 (33%)</td>
<td>0.29</td>
</tr>
<tr>
<td>History of AF (months)</td>
<td>N/App</td>
<td>95 ± 86</td>
<td>0.01</td>
<td>77 ± 58</td>
<td>0.02</td>
</tr>
<tr>
<td>Anti-aldosteronics (n, %)</td>
<td>0 (0%)</td>
<td>2 (5%)</td>
<td>0.02</td>
<td>7 (21%)</td>
<td>0.16</td>
</tr>
<tr>
<td>Statins (n, %)</td>
<td>0 (0%)</td>
<td>9 (23%)</td>
<td>0.16</td>
<td>10 (30%)</td>
<td>0.16</td>
</tr>
<tr>
<td>ACEI/ARB (n, %)</td>
<td>0 (0%)</td>
<td>8 (21%)</td>
<td>&lt;0.01</td>
<td>17 (52%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Left atrial surface (cm²)</td>
<td>15.7 ± 2.5</td>
<td>20.6 ± 3.7</td>
<td>0.001</td>
<td>25.2 ± 6.1</td>
<td>0.001</td>
</tr>
<tr>
<td>AF during blood sampling (n, %)</td>
<td>N/App</td>
<td>8 (21%)</td>
<td>24 (73%)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Prior ablation procedure (n, %)</td>
<td>N/App</td>
<td>12 (31%)</td>
<td>13 (39%)</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>CHA2DS2VASc score</td>
<td>0.63 ± 0.26</td>
<td>1.33 ± 0.21</td>
<td>0.34</td>
<td>1.21 ± 0.21</td>
<td>0.34</td>
</tr>
</tbody>
</table>

AF, atrial fibrillation; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; N/App, not applicable.

Table 2 Inflammatory marker levels in the three study groups

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 9)</th>
<th>Paroxysmal atrial fibrillation (n = 39)</th>
<th>P value</th>
<th>Persistent atrial fibrillation (n = 33)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF (pg/mL)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Femoral vein</td>
<td>15.4 ± 10.8</td>
<td>24.1 ± 3.5</td>
<td>0.03</td>
<td>25.8 ± 3.9</td>
<td>0.02</td>
</tr>
<tr>
<td>Left atrium</td>
<td>9.4 ± 3.9</td>
<td>25.2 ± 4.5</td>
<td>0.05</td>
<td>15.0 ± 4.2</td>
<td>0.32</td>
</tr>
<tr>
<td>Coronary sinus</td>
<td>9.5 ± 7.7</td>
<td>22.2 ± 8.7</td>
<td>0.77</td>
<td>9.8 ± 7.9</td>
<td>0.81</td>
</tr>
<tr>
<td>Pulmonary vein</td>
<td>6.7 ± 3.5</td>
<td>2.7 ± 0.8</td>
<td>0.15</td>
<td>8.8 ± 0.7</td>
<td>0.72</td>
</tr>
<tr>
<td>IL-8 (pg/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Femoral vein</td>
<td>7.0 ± 4.9</td>
<td>6.9 ± 0.6</td>
<td>0.21</td>
<td>8.0 ± 1.4</td>
<td>0.67</td>
</tr>
<tr>
<td>Left atrium</td>
<td>7.7 ± 3.4</td>
<td>7.8 ± 0.7</td>
<td>0.17</td>
<td>8.0 ± 1.7</td>
<td>0.37</td>
</tr>
<tr>
<td>sICAM-1 (ng/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Femoral vein</td>
<td>161.4 ± 15.8</td>
<td>178.5 ± 8.3</td>
<td>0.36</td>
<td>179.7 ± 7.0</td>
<td>0.25</td>
</tr>
<tr>
<td>Left atrium</td>
<td>160.1 ± 15.5</td>
<td>174.4 ± 8.1</td>
<td>0.43</td>
<td>173.2 ± 7.1</td>
<td>0.46</td>
</tr>
<tr>
<td>TGF-β1 (ng/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Femoral vein</td>
<td>25.0 ± 10.0</td>
<td>15.9 ± 1.8</td>
<td>0.63</td>
<td>22.3 ± 3.2</td>
<td>0.87</td>
</tr>
<tr>
<td>Left atrium</td>
<td>13.1 ± 3.3</td>
<td>15.7 ± 2.5</td>
<td>0.88</td>
<td>15.5 ± 2.0</td>
<td>0.41</td>
</tr>
<tr>
<td>CRP (μg/mL)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Peripheral blood</td>
<td>9.6 ± 7.0</td>
<td>3.6 ± 0.9</td>
<td>0.10</td>
<td>6.6 ± 2.4</td>
<td>0.60</td>
</tr>
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</table>

\(^a\) Paroxysmal atrial fibrillation vs. control.

\(^b\) Persistent atrial fibrillation vs. control.
Increased intracardiac VEGF levels in paroxysmal AF

**Discussion**

The main findings of the present study are that (i) patients with paroxysmal or persistent AF and no concomitant inflammatory disease did not have high CRP, IL-8, TGF-β1, or sICAM-1 levels; (ii) patients with paroxysmal AF had high peripheral and left atrial, but similar pulmonary vein levels of VEGF compared with non-AF controls; (iii) although patients with persistent AF had high peripheral levels of VEGF, their left atrial, coronary sinus, and pulmonary vein levels of VEGF were not significantly elevated compared with non-AF controls.

**Could inflammation in atrial fibrillation have been overestimated?**

Although a large amount of data suggest a link between inflammation and AF, several studies failed to show elevated levels of CRP, IL-6, or IL-8 in AF patients.1,2,9–11 Furthermore, studies assessing the efficacy of anti-inflammatory therapy in AF prevention have yielded conflicting results,13,14 suggesting that the role of inflammatory processes in the pathophysiology of AF might be overestimated. Our study did not demonstrate any significant difference in CRP, IL-8, or sICAM-1 levels among the three patient groups. These results suggest that the high inflammatory marker levels reported in AF patients could be due to associated co-morbidities rather than to AF per se.

Inflammation and pro-thrombotic state seem to be related in AF patients. However, we did not find any significant correlation between age, smoking, hypertension, diabetes, or CHA2DS2VASc score and any of the inflammatory markers. The low-risk profile of our cohort could explain the absence of high inflammatory marker levels and the relatively low risk of stroke (mean CHA2DS2VASc score 1.33 ± 0.21 in paroxysmal AF and 1.21 ± 0.21 in paroxysmal or persistent AF patients and persistent AF patients were considered as a single group, left atrial levels of VEGF were significantly negatively correlated with ACEI or ARB therapy (Spearman’s rank correlation coefficient $r = -0.25$, $P = 0.03$). In multivariate analysis, the presence of AF independently predicted elevated peripheral levels of VEGF ($r = 0.31$, $P = 0.04$), whereas there was no significant association between AF and left atrial, coronary sinus, or pulmonary vein levels of VEGF (all $P > 0.05$).

Patients with both paroxysmal and persistent AF were then divided according to stroke risk as assessed by CHA2DS2VASc score into low-intermediate (CHA2DS2VASc $= 0–1$, $n = 46$) and high (CHA2DS2VASc $\geq 2$, $n = 26$) stroke risk groups. We did not find any significant differences between peripheral or left atrial VEGF levels between patients considered at low-intermediate and high stroke risk according to CHA2DS2VASc score (peripheral VEGF levels were 21.31 pg/mL in low-intermediate stroke risk group and 27.73 pg/mL in high stroke risk group, left atrial levels of VEGF were 16.42 pg/mL in low-intermediate stroke risk group and 23.19 pg/mL in high stroke risk group, both $P > 0.1$).

**Figure 1** Mean levels of vascular endothelial growth factor in the femoral vein, left atrium, coronary sinus, and pulmonary vein in the three groups. *P < 0.05 vs. control.
persistent AF). This further supports the hypothesis that associated co-morbidities and not AF itself induces the inflammatory and pro-thrombotic states reported in AF patients, as previously stated by Lip and colleagues.15

The source of vascular endothelial growth factor in atrial fibrillation patients
In paroxysmal AF patients, the combination of normal pulmonary vein and high left atrial VEGF levels suggests the heart itself as the most likely source of high left atrial VEGF levels. Accumulated evidence indicates that VEGF and VEGF receptor mRNA are expressed in heart tissue.16,17 Ogi et al.18 recently reported high VEGF levels in the atrial tissue of AF patients compared with non-AF controls.

A number of pathological conditions, such as oxygen deficiency, inflammation, or, according to recent reports, pulsatile mechanical stretch,19 are known to induce VEGF secretion. Low levels of several inflammatory markers in both paroxysmal and persistent AF patients suggest that the inflammatory process is of low grade, if present. Therefore, it seems unlikely that this process could account for the high peripheral VEGF levels observed in these patients. High VEGF levels have been reported in the myocardium and peripheral blood following myocardial infarction and unstable angina.20,21 Despite the absence of evidence for accelerated angiogenesis in paroxysmal AF patients, we found high left atrial levels of VEGF in this population. Histopathological studies report a high incidence of atrial ischaemia, in up to 17%, and isolated atrial infarctions in >20% of autopsied patients with a history of AF.22 A transient increase in hypoxia inducible factor α gene expression, a known trigger for VEGF secretion, has been reported in cardiac myocytes in the early onset of AF.23 On the other hand, the inverse relationship is also possible, with ischaemia favouring AF occurrence.

The fibrillating atrium leading to an irregular blood flow may induce pulsatile vascular stretch and impaired blood rheology. Both phenomena could trigger VEGF secretion, as well as its release from smooth muscle and circulating blood cells24–26 in AF patients, regardless of the clinical form of the arrhythmia. Pulsatile mechanical stretch, recently postulated as a potent trigger for VEGF secretion from cardiac myocytes,17,19 could be responsible for the high intracardiac VEGF levels observed in paroxysmal AF patients.

Progressive left atrial remodelling affects cardiac secretion of vascular endothelial growth factor (and the inverse)
Our results suggest that left atrial secretion of VEGF is a transient event in the natural history of AF. Vascular endothelial growth factor secretion has been previously shown to stimulate the fibrotic process within the atrial tissue, probably through the induction of angiogenesis.27,28 Several studies have emphasized that patients with persistent AF present more important fibrotic changes compared with patients with paroxysmal AF.29,30 The spreading of cardiac fibrosis could account for progressive cardiac stiffening in this setting, thereby reducing the degree of pulsatile stretch and subsequently diminishing VEGF levels.

Despite the important role that TGF-β1 plays in the genesis of fibrosis in patients with AF, we did not find a significant difference in TGF-β1 levels between patients with and without AF. The larger use of ACEI or ARB in AF patients (35% in AF patients vs. 0% in control patients) might have mitigated the differences between the groups. TGF-β1 expression is known to be induced by angiotensin II31 and blockade of the angiotensin II type 1 receptor is known to suppress TGF-β1 upregulation.32 Furthermore, the larger use of ACEI or ARB could also have mitigated even higher VEGF levels in AF patients.

Our results suggest that in the context of persistent AF the damaged and remodelled atrial tissue becomes less able to secrete and release VEGF. Meanwhile, VEGF inhibition has been associated with extensive apoptotic changes in the vasculature of neonatal mice.33 The low intracardiac VEGF levels observed in persistent AF patients might signify the loss of this protective mechanism of endothelial cells, subsequently leading to progressive endothelial and atrial endocardial damage. High VEGF levels observed in paroxysmal AF patients may participate in the progressive spread of atrial fibrosis, which, in turn, would diminish VEGF secretion by reducing atrial responsiveness to pulsatile mechanical stretch. Later, the resulting low intracardiac VEGF levels could contribute to progressive endothelial dysfunction.

Limitations
This study is limited by its cross-sectional design, which only permitted associations to be observed; cause–effect relationships could not be established. The small number of patients in the control group may have lowered the study’s statistical power. Control patients were also significantly younger than AF patients. However, there was no correlation between age and VEGF levels whatever the sampled site or the studied group. Additionally, one could argue that high VEGF levels might be due to coexisting cardiovascular conditions; however, there was no significant difference among the three groups regarding the proportion of hypertensive or diabetic patients.

Conclusions
Low levels of several inflammatory markers in both paroxysmal and persistent AF patients suggest that the inflammatory process is of low grade, if present. In the context of normal pulmonary vein VEGF levels, the heart itself is the most likely source of high left atrial VEGF levels in paroxysmal AF patients; however, this disorder appears to be a transient event in the natural history of AF.

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References
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