Is the Hypercoagulable State in Atrial Fibrillation Mediated by Vascular Endothelial Growth Factor?

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Background and Purpose—Tissue factor (TF; an initiator of coagulation) and vascular endothelial growth factor (VEGF; a marker of angiogenesis) are involved in the hypercoagulable state associated with malignancy. We investigated their roles in chronic atrial fibrillation (AF), a condition also associated with increased risk of stroke and thromboembolism, as well as a prothrombotic or hypercoagulable state.

Methods—We studied 25 patients with AF (20 men; mean±SD age, 62±13 years) who were compared with 2 control groups in sinus rhythm: 30 healthy control subjects (17 men; mean age, 60±9 years) and 35 patient control subjects with coronary artery disease (CAD; 27 men, mean age, 60±12 years). Plasma levels of TF, VEGF, and the VEGF receptor sFlt-1 were measured by enzyme-linked immunosorbent assay.

Results—VEGF, sFlt-1, and TF were significantly different between the 3 groups, with abnormal levels in AF and CAD patients compared with control subjects (P<0.001, P=0.022, and P=0.008, respectively). Among the AF patients, TF levels were significantly correlated with VEGF (Spearman’s r=0.65, P<0.001) and sFlt (r=0.54, P=0.006) levels. Only TF and VEGF levels were significantly correlated in CAD patients (r=0.39, P=0.02). There were no significant correlations among the healthy control subjects.

Conclusions—Patients with chronic AF have high TF levels, in keeping with the prothrombotic state associated with this arrhythmia. The relationships between TF and VEGF and its receptor sFlt-1 in AF suggest a possible role for VEGF in the hypercoagulable state found in AF, as seen in malignancy and atherosclerosis. (Stroke. 2002;33:2187-2191.)

Key Words: angiogenesis ■ atrial fibrillation ■ thrombosis

Atrial fibrillation (AF) is a common arrhythmia with an increasing prevalence with age, rising from 0.5% at 50 to 59 years of age to ≈9% at 80 to 89 years of age.1 AF confers a significant morbidity and mortality, with a 5-fold increase in the risk of thromboembolic stroke. Indeed, the attributable risk of stroke associated with AF increases from 1.5% at 50 to 59 years of age to 23.5% at 80 to 89 years of age, which is important clinically in that the treatment of patients with anticoagulation introduces new risks (eg, hemorrhage) to those of increasing age.2

The pathophysiology of thromboembolism is multifactorial, but increasing evidence points to the fulfilment of Virchow’s triad (ie, abnormalities of blood flow, blood constituents, and vessel wall abnormalities) for thrombogenesis in AF, resulting in this arrhythmia conferring a prothrombotic or hypercoagulable state.3 Malignant disease is also associated with a hypercoagulable state.4–6 Tumor cells have been shown to produce tissue factor (TF), plasminogen activators, and other factors that can upset the normal homeostasis and balance between activation and inhibition of the coagulation and fibrinolytic systems.7 These activities play a role in tumor cell growth and metastasis. In addition to alterations in the coagulation cascade, platelet turnover has also been found to be increased in cancer patients.8 Not only do activated platelets influence the hypercoagulable state, but they also produce growth factors such as vascular endothelial growth factor (VEGF).9 In cancer, VEGF can lead to the upregulation of TF mRNA and its subsequent expression at the cell membrane.9 In separate studies, plasma levels of TF and VEGF have been shown to be increased in atherosclerosis, the pathophysiology of which involves a tendency to thrombosis and angiogenesis.10–14

We hypothesized raised levels of TF and VEGF in AF and a possible relationship between the 2 indexes. We tested this hypothesis in a case-control study of patients with AF, comparing them with patients with coronary artery disease (CAD) in sinus rhythm and healthy control subjects in sinus rhythm.

Patients and Methods
We studied 25 patients (20 men, 5 women; mean age, 64.8±12.1 years) with chronic AF recruited from the outpatient department and wards of our hospital. All patients had a history of AF of >6 weeks and were anticoagulated with warfarin (international normalized ratio, 2.0 to 3.0). The West Birmingham ethics committee passed the protocol, and informed consent was obtained.
Results from the AF patients were compared with 2 control groups in sinus rhythm: 30 “healthy control” subjects (17 men, 13 women; mean age, 59.9±8.8 years) and 35 “disease control” patients (27 men, 8 women; mean age, 60.4±12.1 years) attending for elective day case coronary angiography with documented CAD. All healthy control subjects were recruited from hospital staff and preoperative clinics for minor procedures, including hernia repairs and cataract surgery. These healthy control subjects were “healthy” by virtue of careful clinical history and examination, as well as basic blood screening tests. Disease control patients had a history of stable angina and documented CAD (>50% stenosis in ≥1 vessels at cardiac catheterization). For this study, we excluded any patients with conditions that may influence the indexes measured, eg, recent (<6 weeks) myocardial infarction, unstable angina, or stroke; we also excluded patients with any history of malignancy, connective tissue disease, infection/inflammation, use of steroids, hormone replacement therapy, etc.

Blood Samples and Analysis
Citrated plasma samples were taken from subjects and immediately placed on ice before being centrifuged at 3000 rpm for 20 minutes at 4°C. They were then stored at −70°C until analysis. Samples were analyzed by enzyme-linked immunosorbent assays (ELISA) for VEGF and sFlt-1 (K+D Systems) and for TF (Axis-Shield). The lower limits of detection by ELISA were 10 pg/mL for VEGF and TF and 0.1 ng/mL for sFlt. The interassay and intra-assay variabilities were <5% and <10%, respectively, for all assays.

Power Calculation
We have previously reported increased TF in patients with atherosclerosis compared with healthy control subjects of 1 SD (P<0.0001). Using this as a benchmark, we hypothesized increased TF in AF patients of 0.6 SD between the group with a power (1−β) of 0.8 and P<0.05. To achieve this, 19 subjects per group were required. However, to improve power and because we also intended to compare VEGF levels, we recruited a minimum of 25 subjects per group.

Statistical Analysis
Statistical analyses were performed with the SPSS 10.0 for Windows. Continuous data were subjected to the Ryan-Joiner test to assess distribution. Parametric results are expressed as mean±SD, and differences between groups were compared by 1-way analysis of variance. Noncategorical data were compared by the χ2 test. Nonparametric results are expressed as median (interquartile range), and comparisons were made by use of the Mann-Whitney and Kruskal-Wallis tests. Because the distribution of VEGF, sFlt-1, and TF did not normalize after log transformation, intergroup comparisons were performed by use of the Mann-Whitney test rather than Tukey’s test. Correlations were examined with Spearman’s rank correlation. The level of significance was taken at P<0.05.

Results
Patient and control subject characteristics are shown in Table 1. The AF patients had a higher incidence of treated hypertension, but mean blood pressure readings were similar. Plasma levels of VEGF, sFlt-1, and TF were significantly different among the 3 subject groups (see Table 2). Levels of VEGF were higher in AF (Mann-Whitney test, P<0.001) and CAD (P=0.001) patients compared with healthy control subjects. There was no significant difference in levels between the 2 patient groups (P=0.103), although a trend was toward the highest median VEGF levels in the AF group.

Similarly, higher levels of TF were found in AF (P=0.016) and CAD (P=0.004) patients compared with healthy control subjects. Levels of sFlt-1 were significantly lower in the AF patients compared with CAD patients (P=0.036) and healthy control subjects (P=0.017).

In the AF patients, levels of TF correlated significantly with VEGF and sFlt-1 (Spearman’s r=0.65, P<0.001 and r=0.54, P=0.006, respectively; Figure 1a and 1b). However,
in the CAD patients, only TF correlated with VEGF (Spearman’s \( r = 0.39, P = 0.02 \); Figure 2). There were no significant correlations in healthy control subjects.

**Discussion**

We have shown that patients with chronic AF have raised plasma levels of TF and VEGF compared with healthy control subjects that are comparable to those in CAD patients. Both AF and CAD are associated with thrombogenesis, and levels of TF have been reported to be raised in coronary artery disease.\(^{10}\) However, to the best of our knowledge, levels of TF have not previously been investigated in AF patients.

Previous studies have examined hemostatic markers in AF in an attempt to elucidate the underlying pathophysiology of thromboembolism in this common arrhythmia. That AF is associated with a hypercoagulable state has been known for some time now,\(^{3}\) with abnormalities in coagulation factors and platelets.\(^{15-20}\) It is therefore surprising to find that levels of TF have not been studied in AF. Certainly, TF is an essential component of the coagulation pathway. It acts as a cofactor to factor VIIa, which has poor activity in the absence of TF, and the TF–factor VIIa complex then activates factor IX and X, triggering the coagulation cascade. The possibility therefore arises that if elevated TF were a marker for hypercoagulability in chronic AF, the inhibition of this protein could provide a therapeutic approach for thromboprophylaxis in such patients.

The role of TF in the promotion of the hypercoagulable state in malignancy has been extensively demonstrated,\(^{21}\) and raised levels are also found in CAD.\(^{22-26}\) In cancer, TF expression and activity have been closely associated with VEGF levels.\(^{9,27}\) We are aware of only 1 study to date that has investigated levels of VEGF in AF.\(^{28}\) Although this study showed differences in levels of VEGF before cardioversion compared with healthy control subjects, serum samples were used and the results were expressed as mean ± SD. In our hands, VEGF has a nonparametric distribution; furthermore, increasing evidence suggests that VEGF should be measured through the use of plasma samples.\(^{8,29}\) Indeed, measurement of serum VEGF would significantly overestimate the true levels of free VEGF because some VEGF is released from platelets when blood clots and thus serum VEGF levels may (artifactually) rise significantly over time after clotting occurs.

In our study, plasma levels of VEGF were markedly higher in AF patients compared with healthy control subjects in sinus rhythm, suggesting an influence of the arrhythmia itself on VEGF expression. Only 5 of the patients also had CAD, so this is unlikely to account for the higher VEGF levels seen in the CAD group. Moreover, there is a significant difference between the prevalence of hypertension in the 2 groups of patients, and raised plasma VEGF levels have been reported in uncontrolled (blood pressure >160/90 mm Hg) essential hypertension.

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**Table 2. Plasma Levels of VEGF, sFlt-1, and TF in AF Patients Compared With CAD Patients and Healthy Control Subjects**

<table>
<thead>
<tr>
<th></th>
<th>AF Patients</th>
<th>CAD Patients</th>
<th>Healthy Control Subjects</th>
<th>Kruskal-Wallis Test</th>
<th>P, Kruskal-Wallis Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF, pg/mL</td>
<td>560* (120–1400)</td>
<td>130* (100–400)</td>
<td>80 (23.8–176.3)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>sFlt-1, ng/mL</td>
<td>1.0* (0.23–35.0)</td>
<td>11.0 (3.6–24.0)</td>
<td>20.5 (8.4–41.3)</td>
<td>0.022</td>
<td></td>
</tr>
<tr>
<td>TF, pg/mL</td>
<td>80* (43–130)</td>
<td>120* (32–360)</td>
<td>18 (10–95)</td>
<td>0.008</td>
<td></td>
</tr>
</tbody>
</table>

All values are expressed as median (interquartile range) and analyzed by Kruskal-Wallis test for a comparison of the 3 groups.

*Mann-Whitney \( P < 0.05 \) vs healthy control subjects.

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**Figure 1.** Relationships between plasma levels of TF and (a) VEGF and (b) soluble sFlt-1 in AF patients.

**Figure 2.** Relationship between TF and VEGF in CAD patients.
hypertension. However, the mean blood pressure in the AF group at the time of inclusion was fairly well controlled (<140/90 mm Hg), being broadly similar to that of the CAD group, and there were no marked differences in drugs used between the 2 groups apart from nitrates and aspirin.

Of particular interest are the significant correlations found between TF and the angiogenic markers VEGF and sFlt-1. This relationship is supported by previous work in malignancy and CAD, which have shown relationships between the immunohistochemical distribution of VEGF and TF and their mRNA expression. The source of each protein is still unclear, and which has the greatest effect over the other in these conditions is still to be elucidated. Endothelial damage or dysfunction and platelet activation are known to occur in AF, and it may be that endothelial and/or platelet activation produces the increased levels of TF and VEGF. Furthermore, atherogenesis and plaque rupture, for example, are known to upregulate TF, whereas hypoxia is known to upregulate VEGF; thus, the possibility arises that AF may cause tissue hypoxia, and because AF patients frequently have atherosclerosis or hypertension, some of the observed associations may not be too surprising.

This study is limited by its cross-sectional design but was adequately powered to undertake the main analyses in relation to patients with AF. Our AF patients were also taking warfarin, but there is no evidence to suggest that warfarin influences the levels of the indexes measured. Furthermore, a cross-sectional design allows us only to explore associations; no causality is implied because only a prospective cohort study with large numbers of subjects with AF can confirm the natural history of the indexes measured in the short, medium, and long term in relation to interventions (cardioversion, introduction of antithrombotic therapy, etc), as well as morbidity and mortality.

In summary, we have shown raised levels of TF and VEGF in AF patients that are correlated to each other. The possibility that TF is involved in the hypercoagulable state in AF is perhaps unsurprising, but the role that VEGF appears to play is unexpected in this condition in that there does not appear to be any direct role for angiogenesis or vascular permeability in AF, in contrast to malignancy and CAD. Further studies are needed to elucidate the extent of VEGF influence on the hypercoagulable state in AF and the endothelium, monocytes, and platelets in these patients as potential sources of the increased VEGF and TF levels.

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