A Cross-Sectional and Diurnal Study of Thrombogenesis Among Patients With Chronic Atrial Fibrillation

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OBJECTIVES
First, we sought to determine whether there is diurnal variation in hemostatic factors related to thrombogenesis and hypercoagulability among patients with chronic atrial fibrillation (AF). Second, we sought to determine whether levels of soluble thrombomodulin (sTM), a marker of endothelial function, or soluble P-selectin (sP-sel), an index of platelet activation, are altered in patients with AF as compared with subjects in sinus rhythm.

BACKGROUND
Atrial fibrillation is associated with an increased risk of stroke and thromboembolism and is known to confer a hypercoagulable state, with abnormalities of thrombosis, platelet activation and endothelial cell function. Many cardiovascular events, such as acute myocardial infarction, have thrombosis as an underlying process, and they undergo diurnal variation.

METHODS
Fifty-two patients (45 men, mean ± SD age 66 ± 6 years) with chronic AF, none of whom received antithrombotic therapy, were studied. Baseline levels of fibrinogen, sP-sel, sTM and von Willebrand factor (vWF) were compared to those levels in matched healthy control subjects in sinus rhythm. In a subgroup of 20 patients, five venous blood samples were collected through an indwelling cannula at 6-h intervals from 12 PM to 12 PM the following day and were analyzed for the same markers.

RESULTS
Patients with chronic AF had higher plasma sP-sel, sTM, vWF and fibrinogen levels as compared with control subjects in sinus rhythm. Significant correlations were found between fibrinogen and sP-sel in patients with AF (r = 0.567 [Spearman], p < 0.001) and in control subjects (r = 0.334, p = 0.016). There was no significant diurnal variation in plasma levels of sP-sel, sTM, vWF or fibrinogen over the 24-h study period (repeated measures analysis of variance, p = NS).

CONCLUSIONS
There is no circadian or diurnal variation in the hypercoagulable state seen in AF, as assessed by plasma fibrinogen and markers of platelet (sP-sel) and endothelial function (vWF and sTM). The persistent hypercoagulable state, together with the loss of diurnal variation in various hemostatic markers, in chronic AF may contribute to the high risk of stroke and thromboembolic complications in these patients. (J Am Coll Cardiol 2000;35: 1926–31) © 2000 by the American College of Cardiology

Atrial fibrillation (AF) is a common cardiac arrhythmia and is associated with a substantial risk of stroke and thromboembolism (1). This is probably because AF confers a hypercoagulable state, with abnormalities of hemostasis, thrombosis and platelet function (2–4). For example, in a cross-sectional study of 73 patients with chronic AF and 21 patients in sinus rhythm, Kumagai et al. (4) reported increased fibrin D-dimer levels, a marker of intravascular thrombogenesis, irrespective of the presence of underlying heart disease. The beneficial role of oral anticoagulation therapy in reducing the risk of stroke and thromboembolic in patients with nonvalvular AF has been confirmed by recent large-scale studies (5).

Recent research has identified two more plasma markers that may be of use in understanding the pathophysiology of thrombogenesis. Soluble thrombomodulin (sTM), another marker of endothelial cell damage, is increased in patients with systemic hypertension, peripheral artery disease and coronary artery disease, and raised levels predict adverse events even among patients receiving long-term anticoagulation (6–9). It has been suggested that increased levels of the soluble adhesion molecule, P-selectin, implies platelet activation (10,11), as may be the case in atherosclerosis.
compared with subjects in sinus rhythm. The aim of the study was to determine whether levels of sTM or soluble P-selectin (sP-sel) are altered in patients with AF as an underlying pathologic process, and some hemostatic factors have also been shown to demonstrate a circadian variation that may in part contribute to this diurnal incidence (24–26). Furthermore, paroxysmal AF, which is also associated with a risk of stroke, exhibits a unique circadian variation that differs from the well-known pattern in acute cardiovascular events (27). As the mechanisms of the increased thromboembolic risk in patients with AF have not been fully elucidated, abnormalities in indexes of hypercoagulability indicative of a prothrombotic state could account for this risk (1–4,28,29), which may demonstrate diurnal variation.

It is unclear whether there is diurnal variation in these hemostatic factors related to thrombogenesis and hypercoagulability in patients with chronic AF that may perhaps relate to the occurrence of clinical events. It has also not been determined whether levels of sTM or soluble P-selectin (sP-sel) are altered in patients with AF as compared with subjects in sinus rhythm. The aim of the present study was to test both these hypotheses prospectively.

METHODS

Patients and control subjects. We recruited patients with chronic AF, which was seen on the electrocardiogram on at least two occasions at least six weeks apart, either by a general practitioner or at hospital or outpatient clinic review. Exclusion criteria were other acute causes of AF (e.g., thyrotoxicosis, pneumonia), acute cardiovascular or cerebrovascular events (e.g., myocardial infarction, congestive heart failure, stroke), use of aspirin or anticoagulant agents, inflammatory or connective tissue disease and chronic renal or hepatic disease. A random subgroup of 20 patients was admitted for 36 h for clinical assessment before initiation of anticoagulation therapy. Five venous blood samples were collected through an indwelling cannula at 6-h intervals from 12 PM to 12 PM the following day.

Control subjects were found among the healthy hospital staff and among those patients who were in the hospital for hernia repair, varicose veins or minor operations. All were free of diabetes and were without signs or symptoms of cardiovascular, neoplastic or connective tissue disease. Systolic and diastolic blood pressure were recorded in each subject after a minimum of 5 min of rest, and the subjects’ smoking status was determined. The project had the approval of the research Ethics Committee of the West Birmingham Health Authority, and written, informed consent was obtained from each participant.

Laboratory measures. Citrated plasma was obtained from venous blood by centrifugation at 2,500 rpm for 15 min at 4°C. Aliquots were stored at −70°C to allow batch analysis. Soluble P-selectin, sTM and von Willebrand factor (vWF) were measured by the enzyme-linked immunosorbent assay (ELISA) technique using commercial reagents (R&D Systems, Abingdon, United Kingdom; Diagnostica Stago, Asnieres-sur-Seine, France, and Dako-Patts, Ely, United Kingdom, respectively). The unit for vWF is IU/dl and was standardized by the reference vWF from the National Institute for Biological Standards and Controls (Hertfordshire, United Kingdom). Other indexes (ng/ml) were standardized by the recombinant product supplied by the manufacturer. Intra-assay coefficients of variation for all ELISA assays were <5%; interassay variances were <10%. Plasma fibrinogen (g/liter) was measured by the Clauss technique on a Pacific Hemostasis (Hunterville, North Carolina) coagulometer and with reagents from Alpha Laboratories (Eastleigh, Hants, United Kingdom).

Power calculation and statistics. Data were initially analyzed by using the Shapiro-Wilks test to determine normality of distribution. In the cross-sectional study, data for vWF, sTM and fibrinogen are presented as the mean value ± SD and analyzed by using the unpaired t test; however, sP-sel levels were nonparametrically distributed and expressed as the median value with interquartile range and analyzed using the Mann-Whitney U test. Correlations between indexes were performed by the Spearman rank correlation method. We hypothesized that sP-sel and sTM would be increased by one-half of a standard deviation among patients with AF as compared with control subjects. To prove this, we would need to recruit a minimum of 40 patients and 40 control subjects for a one-sided p value <0.05 with 80% power. Two other studies of diurnal variation recruited only 9 and 10 subjects, respectively (24,25). Therefore, we aimed to recruit at least 20 subjects. In the diurnal study, the results were analyzed by repeated measures analysis of variance. All statistical calculations were performed on a microcomputer using a commercially available statistical package (Minitab Release 12, Minitab Inc., State College, Pennsylvania). A p value <0.05 was considered statistically significant.
RESULTS

Cross-sectional data. Demographic data are presented in Table 1. There were no differences in age, gender, proportion of current smokers or blood pressure between the groups. Patients with AF had higher plasma sP-selectin, sTM, fibrinogen and vWF levels as compared with control subjects in sinus rhythm (Table 2). There were no significant differences for the measured levels of plasma sP-selectin, sTM, vWF and fibrinogen between patients with lone AF (n = 16) and patients with AF associated with underlying disease (n = 36) (Table 2).

Correlations with clinical variables. Among patients with AF, there was a significant correlation between plasma fibrinogen and sP-selectin levels ($r = 0.567$ [Spearman], $p < 0.001$). Among the control subjects, the correlation between plasma fibrinogen and sP-selectin was also significant ($r = 0.334$, $p = 0.016$). There were no other significant correlations between various indexes in patients or control subjects.

Diurnal study. We studied 20 patients with chronic AF who were not receiving antithrombotic therapy. Demographic data for these patients are summarized in Table 3.

<table>
<thead>
<tr>
<th>Table 1. Demographic Data for Total Study Group</th>
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<tr>
<td>Control Subjects (n = 60)</td>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Male gender</td>
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<td>Smokers</td>
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<td>Systolic blood pressure (mm Hg)</td>
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<td>Diastolic blood pressure (mm Hg)</td>
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<td>Known hypertension (&gt;160/90 mm Hg)</td>
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<td>Lone AF</td>
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<td>Coronary artery disease</td>
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<td>Previous thromboembolic stroke</td>
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<td>Diabetes mellitus</td>
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<td>Peripheral vascular disease</td>
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Data are presented as the mean value ± SD or number (%) of patients or subjects. AF = atrial fibrillation.

<table>
<thead>
<tr>
<th>Table 2. Plasma Levels of Soluble Adhesion Molecule P-Selectin, Soluble Thrombomodulin, von Willebrand Factor and Fibrinogen in Patients With Chronic Atrial Fibrillation and in Control Subjects</th>
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<tbody>
<tr>
<td>a) Patients With AF Versus Healthy Control Subjects in Sinus Rhythm</td>
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<tr>
<td>Control Subjects (n = 60)</td>
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<tr>
<td>Soluble P-selectin (ng/ml)</td>
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<td>Soluble thrombomodulin (ng/ml)</td>
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<tr>
<td>von Willebrand factor (IU/dl)</td>
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<td>Fibrinogen (g/liter)</td>
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b) Lone AF Compared With Atrial Fibrillation Associated With Underlying Disease

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<tr>
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<tr>
<td>Patients With Lone AF (n = 16)</td>
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</tr>
<tr>
<td>Soluble P-selectin (ng/ml)</td>
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<td>Soluble thrombomodulin (ng/ml)</td>
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<tr>
<td>von Willebrand factor (IU/dl)</td>
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<tr>
<td>Fibrinogen (g/liter)</td>
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Data are presented as the mean value ± SD (analyzed by the unpaired t test), except for soluble P-selectin levels, which are expressed as the median value (interquartile range), analyzed using the Mann-Whitney U test. AF = atrial fibrillation.
Table 3. Demographic Data for Diurnal Study Group (n = 20, All With Atrial Fibrillation)

| Age (years) | 73 ± 7 |
| Male gender | 16 (80%) |
| Smokers | 3 (15%) |
| Systolic blood pressure (mm Hg) | 144 ± 22 |
| Diastolic blood pressure (mm Hg) | 82 ± 12 |
| Hypertension only | 5 (25%) |
| Lone AF | 10 (50%) |
| Coronary artery disease | 2 (10%) |
| Previous thromboembolic stroke | 1 (5%) |
| Diabetes mellitus | 1 (5%) |
| Peripheral vascular disease | 1 (5%) |

Data are presented as the mean value ± SD or number (%) of patients.

AF = atrial fibrillation.

There was no significant diurnal variation in plasma levels of sP-sel, sTM, vWF or fibrinogen over the five sampling points (12 PM, 6 PM, 12 AM, 6 AM and 12 PM) over the 24-h period (Table 4).

DISCUSSION

In the present study, we confirmed previous observations of increased vWF and fibrinogen levels in patients with chronic AF as compared with healthy control subjects in sinus rhythm (2), and we provide new evidence of a hypercoagulable or thrombogenic state in chronic AF, with increased levels of the endothelial cell product, sTM, as well as a new marker of platelet activation—soluble adhesion molecule P-selectin. Importantly, this hypercoagulable state does not appear to be subject to any significant diurnal variation, nor is it related to whether or not the patient had lone AF or not, suggesting that chronic AF confers a constant prothrombotic state per se over the 24-h day, which was independent of underlying heart disease or etiology (3).

Endothelial dysfunction in AF. The increase in vWF levels parallels the increased levels of sTM, another index of endothelial dysfunction (6,30–32). The latter molecule is of interest because thrombomodulin is a constitutive membrane protein that is an important regulator of activated thrombin, converting thrombin from a procoagulant (cleaving fibrinogen) to an anticoagulant by altering its substrate specificity, so that it activates protein C (30). However, thrombomodulin probably needs to be cleaved for soluble forms to be found in the circulation. In vitro experiments suggest that the presence of sTM in tissue culture supernatant is probably the result of damage to the endothelial cells (31). Unlike vWF, however, levels of sTM appear to be independent of the inflammatory cytokines interleukin-1 and tumor necrosis factor (6,32). Our finding of high sTM levels in patients with chronic AF in the present study is in contrast to a recent preliminary report from a much smaller cohort of patients with chronic AF in whom this marker may not have been elevated (33). The precise mechanism for the increased markers of endothelial dysfunction or activation in cardiovascular disorders is uncertain, but may nevertheless include a cytokine-mediated increase in synthesis, increased secretion from stored pools, increased synthesis de novo or release from damaged endothelial cells. In the case of AF, abnormalities in blood flow may be partly responsible, resulting in flow abnormalities and adding to endothelial disturbance in the pulmonary vasculature.

Platelet activation in AF. The presence of platelet activation has been recognized in chronic AF, which has usually been indicated by high plasma levels of betathromboglobulin (28), although this finding is controversial (34,35). In the present study, we have measured levels of sP-sel, which is a new marker of platelet activation (10,11). Our sP-sel data therefore complement those of Pongratz et al. (35), who found increased expression of membrane-bound P-selectin on platelets of patients with AF. Like Pongratz et al. (35), we interpret this as further evidence of inappropriate platelet activation in these patients, which is correlated with plasma fibrinogen, an established index of hemorheology and clotting. Increased platelet activation, in combination with endothelial dysfunction and abnormal hemostatic factors, is thus in keeping with the hypercoagulable state in AF (3,4).

Table 4. Diurnal Changes in Markers of Thrombogenesis in Chronic Atrial Fibrillation*

<table>
<thead>
<tr>
<th>Time Points</th>
<th>12 PM</th>
<th>6 PM</th>
<th>12 AM</th>
<th>6 AM</th>
<th>12 PM</th>
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</thead>
<tbody>
<tr>
<td>Soluble P-selectin (ng/ml)</td>
<td>113 (82–168)</td>
<td>107 (71–139)</td>
<td>104 (85–139)</td>
<td>105 (74–149)</td>
<td>114 (100–157)</td>
</tr>
<tr>
<td>Soluble thrombomodulin (ng/ml)</td>
<td>49 ± 19</td>
<td>48 ± 15</td>
<td>46 ± 13</td>
<td>50 ± 13</td>
<td>51 ± 17</td>
</tr>
<tr>
<td>von Willebrand factor (IU/dl)</td>
<td>134 ± 33</td>
<td>135 ± 27</td>
<td>134 ± 27</td>
<td>133 ± 31</td>
<td>135 ± 27</td>
</tr>
<tr>
<td>Fibrinogen (g/liter)</td>
<td>2.62 ± 0.57</td>
<td>2.69 ± 0.56</td>
<td>2.74 ± 0.59</td>
<td>2.73 ± 0.66</td>
<td>2.89 ± 0.60</td>
</tr>
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</table>

*p Value for Main Effect

There was no significant variation in these data (repeated measures analysis of variance) for diurnal changes in patients with atrial fibrillation (all p = NS).

Data are presented as the median value (interquartile range) for soluble P-selectin and the mean value ± SD for the other markers.
Diurnal variation in markers of thrombogenesis. Previ-
ous studies in subjects without AF have suggested diurnal or
circadian variation in certain clotting factors. For example,
the study by Jafri et al. (25) reported that circadian changes
occurred in beta-thromboglobulin (p < 0.05) and platelet
factor-4 (p < 0.06, NS) in nine normal healthy subjects. In
another small study involving only 10 normal healthy
subjects, Bridges et al. (24) demonstrated that significant
circadian variation occurred with tissue plasminogen activa-
tors (p < 0.001), plasminogen activator inhibitor (p < 0.04)
and 11-dehydro-thromboxane B2 (p < 0.005), with mea-
surements taken at 4-h intervals from 12 PM to 8 AM the
following day. These findings are thus consistent with the
clinical observations of a diurnal pattern to acute vascular
events. Nevertheless, in the present study, we report that the
hypercoagulable state in chronic AF does not seem to
undergo any significant diurnal or circadian rhythm. This
may be a reflection of the high risk of stroke and thrombo-
embolism associated with chronic AF, suggesting that such
patients may be at a constant high prothrombotic risk
throughout the day, necessitating adequate antithrombotic
therapy. Although there appears to be a diurnal pattern to
the onset of stroke in general (17), we are not aware of any
published studies specifically investigating whether there is
a diurnal variation in stroke onset among patients with AF.
Nevertheless, preliminary observations from an ongoing
project in our unit do not suggest a significant diurnal
pattern to the onset of stroke in patients with AF. The
absence of a diurnal variation in the hypercoagulable state in
patients with AF would be in keeping with this.

Clinical study implications. Although the use of anti-
thrombotic therapy may reduce the risk of thrombosis, even
administration of anticoagulation with a constant infusion
of intravenous heparin is associated with a diurnal variation
in the intensity of anticoagulation (36). If chronic AF did
show significant diurnal variation in the hypercoagulable or
prothrombotic state, there will be periods when anticoagu-
lation intensity may be insufficient to provide prophylaxis
against thromboembolism, and other periods when antico-
agulation exceeds what was needed therapeutically, with a corre-
sponding increase in the risk of bleeding. These problems
are highly clinically relevant when considering thromboprophylaxis for patients with AF. Our finding of a constant
hypercoagulable state in chronic AF, therefore, provides
further reassurance that anticoagulation with warfarin, aim-
ing for a consistent target International Normalized Ratio of
2.0 to 3.0, is likely to provide adequate anticoagulation
“cover” for each 24-h period.

Study limitations. This study is limited by its case-
controlled, cross-sectional design and the association of AF
with other pathologic processes, such as hypertension and
coronary artery disease, or risk factors for atherosclerosis. It
has previously been shown that the hypercoagulable state in
AF is independent of underlying etiology or associated heart
disease (2–4). In addition, hypertension and coronary artery
disease would result in relatively smaller changes in these
markers as compared with those seen in the present study of
AF. However, the main objective of our study was to assess
the circadian or diurnal variation in the hypercoagulable state
in AF, rather than to reproduce many previous analyses of
the effects of heart disease on the markers of hypercoag-
ulability, which are suggestive of a continuum that exists
between health, “statistically” increased hemostatic abnor-
malities as a prethrombotic or hypercoagulable state and
“overtly” increased clotting in acute thrombosis (or some-
times in acute extravascular fibrin formation) which follows
injury or operation (2–4). We also accept that the present
study was neither designed nor adequately powered to
specifically compare differences in the hypercoagulable state
between patients with lone AF and patients with AF
associated with underlying disease; however, previous data
(3) suggest that the hypercoagulable state in AF is indepen-
dent of underlying etiology and structural heart disease.

Conclusions. We suggest that there is no circadian or
diurnal variation in the hypercoagulable state seen in AF, as
assessed by plasma fibrinogen and markers of platelet
(sP-sel) and endothelial function (vWF and sTM). The
persistent hypercoagulable state, together with the loss of
diurnal variation in various hemostatic markers, in chronic
AF may contribute in part to the high risk of stroke and
thromboembolic complications in these patients.

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