Markers of Thrombin and Platelet Activity in Patients With Atrial Fibrillation

Correlation With Stroke Among 1531 Participants in the Stroke Prevention in Atrial Fibrillation III Study

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Background and Purpose—Markers of thrombin generation and platelet activation are often elevated in patients with nonvalvular atrial fibrillation, but it is unclear whether such markers usefully predict stroke. Therefore, we undertook the present study to assess the relationship between prothrombin fragment F1.2 (F1.2), β-thromboglobulin (BTG), fibrinogen, and the factor V Leiden mutation with stroke in atrial fibrillation.

Methods—Specimens were obtained from 1531 participants in the Stroke Prevention in Atrial Fibrillation III study. The results were correlated with patient features, antithrombotic therapy, and subsequent thromboembolism (ischemic stroke and systemic embolism) by multivariate analysis.

Results—Increased F1.2 levels were associated with age (P<0.001), female sex (P<0.001), systolic blood pressure (P<0.006), and heart failure (P=0.001). F1.2 were not affected by aspirin use and were not associated with thromboembolism after adjustment for age (P=0.18). BTG levels were higher with advanced age (P=0.05), coronary artery disease (P=0.05), carotid disease (P=0.005), and heart failure (P<0.001), lower in regular alcohol users (P=0.05), and not significantly associated with thromboembolism. Fibrinogen levels were not significantly related to thromboembolism but were associated with elevated BTG levels (P<0.001). The factor V Leiden mutation was not associated with thromboembolism (relative risk 0.5, 95% CI 0.1 to 3.8).

Conclusions—Elevated F1.2 levels were associated with clinical risk factors for stroke in atrial fibrillation, whereas increased BTG levels were linked to manifestations of atherosclerosis. In this large cohort of patients with atrial fibrillation who were receiving aspirin, F1.2, BTG, fibrinogen, and factor V Leiden were not independent, clinically useful predictors of stroke. (Stroke. 1999;30:2547-2553.)

Key Words: atrial fibrillation • coagulation • fibrinogen • platelet activation • thrombin

Atrial fibrillation (AF) results in stasis of blood flow in the left atrial appendage, leading to thrombus formation and embolic stroke. Although AF is associated with a 6-fold increase in stroke, most patients with AF never suffer stroke, which suggests that factors in addition to the dysrhythmia contribute to the formation of atrial appendage thrombi. Hemostasis conditions favoring thrombosis could be important in the pathogenesis of AF-associated stroke, but this has not been firmly established.

Previous studies have demonstrated elevation of markers of thrombin and platelet activity in patients with AF. Prothrombin fragment 1+2 (F1.2) reflects in vivo thrombin generation, is reported to be elevated in AF, and is suppressed by anticoagulation in a dose-dependent manner. Elevation of β-thromboglobulin (BTG), a protein fragment released from α-granules during the second phase of platelet activation, has also been reported in AF patients. In addition, people with a genetic abnormality leading to activated protein C resistance (ie, factor V Leiden, G1691A mutation) have an increased risk of venous thrombosis, but this has not been systematically assessed as a predictor of stroke in patients with AF.

To explore hemostatic markers in patients with atrial fibrillation, we measured F1.2, fibrinogen, and BTG in participants in the Stroke Prevention in Atrial Fibrillation (SPAF) III study, correlating these markers with patient

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Subjects and Methods

All patients were participants in the SPAF III study, which was performed at 20 clinical sites in the United States and Canada between 1993 and 1997; the design and main results have been reported previously. In brief, participants were adults with sustained or intermittent AF without mitral stenosis or prosthetic cardiac valves and were stratified as either low risk or high risk for stroke based on associated clinical features (Figure 1). Those with any of the 4 high-risk criteria were randomized to receive either adjusted-dose warfarin (target international normalized ratio [INR] 2 to 3) or fixed, low-dose warfarin (target INR 1.2 to 1.5) plus aspirin 325 mg/day alone. Primary thromboembolic events included all ischemic strokes and non–central nervous system (CNS) systemic cardiogenic emboli. Strokes were secondarily categorized by use of a clinical classification scheme as probably cardioembolic, probably noncardioembolic, or of uncertain cause by a central events committee whose members were unaware of treatment. The factor V Leiden G1691A mutation was assessed by restriction-enzyme digestion. The interassay coefficient of variation (CV) was 8%. The fibrinogen assay was performed according to the manufacturer’s specifications as previously described. The interassay coefficient of variation (CV) was 8%. The FPA assay was measured with a radioimmunoassay (Byk-Sangtec) according to the manufacturer’s instructions, with an interassay CV of 18%. The fibrinogen assay was performed according to the method of Clauss, with an interassay CV of 3%. BTG was measured with an ELISA (American Bioproducts); the interassay CV was 9%. The factor V Leiden G1691A mutation was assessed by genotyping with polymerase chain reaction amplification and restriction-enzyme digestion.

Data Analysis

Analyses for F1.2 were performed in the subgroup of patients who had not received warfarin in the prior 2 weeks. The upper limit of normal for F1.2 was chosen as 2.8 mmol/L. All analyses were done with the natural log transform for F1.2 and BTG levels because the distributions were heavily skewed (Figures 2 and 3). Differences in markers (transformed values) between independent groups were evaluated with 2-sample t tests and ANOVA for continuous marker levels and a χ² test for dichotomized levels. Changes over time in transformed values were evaluated with a paired t test. Forward and
backward stepwise linear regression analyses were used to identify features independently associated with marker levels. The relative risk of a thromboembolic event associated with a marker level was estimated with a Cox proportional hazards model (likelihood ratio test). Statistical significance was accepted at the 0.05 level (2-sided). Analyses were done with SPSS software.

**Results**

**Prothrombin Activation Fragment 1.2**

F1.2 levels were determined at study entry in 553 participants (mean age 70 years); none were taking warfarin, whereas 488 took aspirin, and 65 received no antithrombotic therapy (Table 1). F1.2 levels ranged from 0.5 to 7.2 nmol/L (excluding 2 patients with values >3 SDs above the transformed mean), with a median of 1.9 nmol/L. In 8%, F1.2 levels were ≤1.0 nmol/L, and 18% of F1.2 levels exceeded 2.8 nmol/L (Figure 2). The mean ln-transformed level (F1.2 ln) was 0.6 ± 0.4 nmol/L (anti-ln of mean = 1.8 nmol/L) and was identical for those taking versus those not taking aspirin. The influence of aspirin was further assessed by examination of 32 patients who had an initial measurement while receiving no antithrombotic therapy and then had a second determination while taking aspirin 325 mg/d; there was no significant difference in mean F1.2 ln levels (mean difference 0.05 nmol/L, P = 0.5). Among the 553 patients, 42 were recorded as having unsatisfactory venipuncture; mean F1.2 ln levels were identical with satisfactory venipunctures (P = 0.95), and these patients were included in all subsequent analyses.

Increased F1.2 levels were strongly associated with older age (P < 0.001) and female sex (P < 0.001) (Table 2). Women >75 years of age (mean age 81 years) had a mean F1.2 ln level of 0.9 versus 0.7 nmol/L for men older than 75 years (mean age 80 years, P = 0.003). Dense spontaneous echo contrast, present in 23 of the subset of 206 participants who underwent transesophageal echocardiography, was not associated with F1.2 levels (P = 0.8).

In addition to age and sex, other independent correlates of elevated F1.2 levels were systolic blood pressure.
Aspirin-Treated AF Patients*

TABLE 4. F1.2 Levels and Thromboembolic Events in

<table>
<thead>
<tr>
<th>Feature</th>
<th>Expected Difference in F1.2 ln, nmol/L</th>
<th>P</th>
<th>RR of F1.2 &gt;2.8 nmol/L</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (per decade)</td>
<td>0.3</td>
<td>&lt;0.001</td>
<td>1.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Female sex</td>
<td>0.3</td>
<td>&lt;0.001</td>
<td>1.8</td>
<td>0.02</td>
</tr>
<tr>
<td>Systolic BP &gt;160 mm Hg</td>
<td>0.3</td>
<td>0.006</td>
<td>1.7</td>
<td>0.09</td>
</tr>
<tr>
<td>Hx heart failure</td>
<td>0.3</td>
<td>0.001</td>
<td>1.9</td>
<td>0.04</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>-0.2</td>
<td>0.01</td>
<td>0.8</td>
<td>0.6</td>
</tr>
</tbody>
</table>

BP indicates blood pressure; Hx, history of.

*The following variables were tested: age, sex, body mass index, serum cholesterol, current tobacco smoking, alcohol use, carotid bruit/surgery, diabetes, hypertension, heart failure, coronary artery disease, duration of AF, intermittent vs sustained AF, systolic hypertension, prior stroke/transient ischemic attack, aspirin use, and hormone replacement therapy.

†Based on a 70-year-old male in an ln-transformed F1.2 model.

>160 mm Hg (P=0.006), a history of congestive heart failure (P=0.001), and the absence of diabetes (P=0.01), with the independent contribution of each of these features being of similar magnitude (Table 3). Postmenopausal hormone replacement therapy (n=46) was not independently associated with F1.2 (P=0.1). The 5 features independently associated with increased F1.2 levels (Table 3) predicted 23% of the variability in measured levels (ie, adjusted r²=23%).

When an additional 263 participants who took aspirin or aspirin plus low, inefficacious doses of warfarin and who also had F1.2 levels measured 3 months after study entry but not before were considered, 21 thromboembolic events occurred in 726 patients over the subsequent 2 years (rate of 2.1% per year). F1.2 levels measured at study entry or at the 3-month follow-up were higher in those with subsequent thromboembolic events, but differences were small and of marginal statistical significance (Table 4). When analysis was restricted to cardioembolic events, the differences increased (relative risk [RR] associated with F1.2 >2.8 nmol/L=3.0, P=0.03; Table 4). Adjustment for age resulted in a relative risk of thromboembolism associated with F1.2 >2.8 nmol/L of 1.9 (95% CI 0.8 to 4.9; P=0.18). Multivariate analysis to assess the predictive value of F1.2 levels when considered with other clinical predictors of thromboembolic events could not be performed owing to the small number of events.

To assess the relationship between F1.2 levels and thromboembolic events among high-risk AF patients, the last measured F1.2 levels preceding the primary thromboembolic event (a median of 57 days, range 6 to 289 days) in 21 high-risk AF patients assigned to combination therapy were compared with 126 high-risk participants without thromboembolism matched on the basis of age, sex, body surface area, and interval between study entry and sample collection. Mean F1.2 in those with stroke and controls (0.8 versus 0.6 nmol/L, respectively; P=0.12). The frequency of F1.2 >2.8 nmol/L was similar in those with stroke and controls (RR=1.1, 95% CI 0.4 to 3.3).

β-Thromboglobulin

BTG levels were measured at study entry or at the initial 3-month follow-up visit in 1338 participants (Table 1); 646 were taking aspirin, 467 were taking adjusted-dose warfarin, 160 were taking both warfarin and aspirin, and 65 were not taking antithrombotic therapy. BTG levels ranged from 1.5 to 266 ng/mL, with a median of 26 ng/mL (Figure 3). Mean BTG was 3.3±0.8 ng/mL and was not affected by antithrombotic therapy (P=0.5) or by the exclusion of patients recorded as having unsatisfactory venipuncture (n=113, mean BTG=3.5 ng/mL). Serial measurement of BTG at 3 and 12 months after entry during treatment with aspirin was done in 252 participants; the mean BTG difference was −0.08±1.0 ng/mL, with an intermeasurement correlation coefficient of 0.25.

The influence of antithrombotic therapies on BTG levels was further explored by comparison of BTG levels obtained at entry with those from the 3-month follow-up on a different therapy. Among those who initially received no antithrombotic therapy and were then given aspirin (n=32), there was no significant change in mean BTG (mean difference −0.009±0.7, P=0.95, 95% CI for relative change at mean BTG=−42% to 41%). For those who were initially taking adjusted-dose warfarin who subsequently were given aspirin alone or aspirin plus low-dose warfarin (n=164), there was also no significant change in mean BTG (mean difference...
0.06±0.8, P=0.35, 95% CI for relative change at mean BTG$_\text{ln}$ −48% to 52%.

By multivariate analysis, BTG levels were associated with age (P=0.006), heart failure (P<0.001), and coronary artery disease (P=0.05) (Table 5), but together these accounted for only 2% of the observed variability (ie, adjusted $r^2$=2%). In addition, carotid bruit/surgery and regular alcohol consumption were independently associated with higher and lower levels of BTG, respectively (Table 5). Antithrombotic therapy, sex, mitral regurgitation, mitral annular calcification, and dense spontaneous echo contrast were not associated with BTG levels. There was no association between BTG and F1.2 levels (P=0.2, $r=0.05$).

When the patients who were assigned to adjusted-dose warfarin were excluded, 40 thromboembolic events occurred over 2 years among 1004 patients who had BTG levels measured (annualized rate of 3.0% with aspirin or combination therapy). BTG levels were not predictive of thromboembolic events either when analyzed as a continuous variable (P=0.8) or when those with BTG levels $>42$ ng/mL were compared with others (RR=1.0, 95% CI 0.5 to 2.1). The restriction of the analysis to events classified as cardioembolic (n=24) did not appreciably alter these results.

**Fibrinogen Levels**

Fibrinogen levels were measured in 621 participants within 6 months of study entry; the mean level was 319±70 mg/dL after 8 participants with extreme values (3 <50 mg/dL, 5 >640 mg/dL) were excluded. Features independently associated with fibrinogen levels included hypertension (P=0.003), diabetes (P=0.03), current tobacco smoking (P=0.02), and heart failure (P=0.003); age was marginally significant in this model (P=0.07) (Table 6). Treatment with aspirin, warfarin, or hormone replacement therapy was not associated with fibrinogen levels. The 5 associated features (Table 6) accounted for 5% of the variability (ie, adjusted $r^2=5$%).

When patients who were assigned to aspirin or aspirin plus low, inefficacious doses of warfarin (n=461) were considered, 23 thromboembolic events occurred within 2 years...
(annualized rate 3.4%). Fibrinogen levels were not significantly associated with thromboembolic events ($P=0.5$ for continuous data). The relative risk of a thromboembolic event associated with fibrinogen levels $>362$ mg/dL was likewise not statistically significantly increased, although confidence intervals were wide: $RR=2.1$, 95% CI 0.9 to 4.9, $P=0.11$; after adjustment for hypertension, $RR=1.9$, 95% CI 0.8 to 4.5, $P=0.17$. Multivariate analysis to assess the predictive value of fibrinogen levels when considered with clinical predictors of thromboembolism could not be performed owing to the limited number of events.

Factor V Leiden

Among 752 low-risk AF patients, the factor V Leiden mutation was present in 6% (n=46). During follow-up while patients were receiving aspirin, the occurrence of 29 thromboembolic events (annualized rate of 1.9%) was not associated with the mutation (RR 0.5, 95% CI 0.1 to 3.8, $P=0.5$). The presence of the factor V Leiden mutation was independently associated with higher levels of F1.2 (an increase of $\approx 40\%$; $P<0.001$).

Discussion

We sought to identify markers of coagulation and platelet activity that were independently associated with stroke in AF patients. These selected markers, which were assessed in the setting of a multicenter clinical trial, were not useful predictors of stroke when analyzed with age and other clinical features.

F1.2 levels were independently associated with advanced age, female sex, systolic blood pressure, and heart failure and were not influenced by aspirin use. Advanced age and female sex have been consistently associated with higher F1.2 levels.\textsuperscript{17,21,23–25} Thus, patient features predictive of a high risk of stroke in AF according to the SPAF analysis\textsuperscript{14} (ie, older women, systolic blood pressure $>160$ mm Hg, and heart failure) were independently associated with increased levels of F1.2.

Previous case-control studies suggest that AF patients have increased F1.2 levels compared with those in sinus rhythm.\textsuperscript{8,10} This issue cannot be addressed directly by the present study, because all participants had AF. The geometric (ie, antilog of the mean of ln-transformed values) mean F1.2 in young and middle-aged healthy cohorts has been reported as 0.5 to 0.7 nmol/L\textsuperscript{21,25} compared with 1.8 nmol/L in these elderly AF patients. However, it is difficult to compare F1.2 levels directly between studies owing to differences in specimen collection, processing, and analysis and in the reporting of results.\textsuperscript{22,26} A higher frequency of systolic hypertension and heart failure could explain higher observed levels of F1.2 in cohorts of AF patients.

F1.2 levels measured near study entry were higher in participants who subsequently suffered thromboembolic events, but differences were only marginally statistically significant (Table 4) and were even less so after adjustment for age. Hence, F1.2 does not appear to be a useful independent predictor of stroke in AF patients, although the analyses did not have sufficient power to definitively exclude such a relationship. Too few events were observed to reliably compare F1.2 levels with INR as predictors of stroke during anticoagulation.

The range and SD of BTG levels from this cohort were wider than those from most other studies.\textsuperscript{5,7,8,27–32} The correlation coefficient comparing 2 BTG levels from the same individual was only 0.25, and the relatively weak relationships with patient features accounted for only 3% of the observed large variance. Taken together, these findings suggest that BTG levels in this clinical trial cohort were either not assessed accurately or were inherently unstable.

BTG levels were associated with age, as found in several previous studies,\textsuperscript{29,33,34} and with coronary artery disease and carotid bruit/surgery, both of which are manifestations of atherosclerosis. Regular alcohol consumption was an independent predictor of reduced stroke risk in these AF patients\textsuperscript{55} and also was independently associated with lower BTG levels. Antithrombotic therapy with aspirin had no detectable effect on BTG levels, consistent with most previous studies,\textsuperscript{3,28,36} with exceptions.\textsuperscript{27} BTG levels did not predict stroke in this cohort of AF patients, but this finding must be considered in light of the caveats regarding the intraindividual variability.

The presence of the mutation for factor V Leiden was not associated with thromboembolism. The cohort for this assessment was restricted to low-risk AF patients, who may have fewer cardioembolic relative to noncardioembolic strokes than high-risk patients\textsuperscript{14,15}; all were given aspirin, and confidence intervals were wide. Whether the factor V Leiden mutation is associated with formation of stasis-precipitated left atrial appendage thrombi in high-risk AF patients is an important, unresolved issue.\textsuperscript{37}

Several potential limitations may apply. The participants, on average, had lower rates of thromboembolism (2% to 3% per year) than most published cohorts of AF patients, because all received some type of antithrombotic therapy. The limited number of events resulted in wide confidence intervals that did not exclude clinically important associations and prohibited multivariate analysis to adjust for other influences.

We were unable to identify hemostatic markers that were independently predictive of subsequent stroke in AF patients. The markers assessed in the present study were associated with advanced age, hypertension, heart failure, and other features associated with an increased stroke risk in AF. Whether the hemostasis abnormalities contribute directly to stroke or are epiphenomena is unclear. The potential role of prothrombotic diatheses as contributors to the formation of atrial thrombi in patients with AF remains elusive.

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References


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