Prothrombotic Activity Is Increased in Patients With Nonvalvular Atrial Fibrillation and Risk Factors for Embolism*

Hiroshi Inoue, MD, FCCP; Takashi Nozawa, MD; Ken Okumura, MD; Lee Jong-Dae, MD; Akihiko Shimizu, MD; and Katsusuke Yano, MD

Study objectives: The aim of this study was to investigate whether risk factors for embolism would promote thrombus formation in patients with nonvalvular atrial fibrillation (NVAF).

Methods: Hemostatic markers for platelet activity (ie, platelet factor-4 and β-thromboglobulin [TG]), thrombotic status (ie, prothrombin fragments 1 and 2), and fibrinolytic status (ie, d-dimer) were determined in 246 patients with NVAF (mean age, 66.1 years) and 111 control subjects without NVAF (68.3 years).

Results: The β-TG level was higher in NVAF patients than in control subjects. D-dimer levels were higher in NVAF patients having risk factors (mean ± SE d-dimer level, 158.6 ± 9.2 ng/mL) than in those without risk factors (mean d-dimer level, 92.1 ± 6.7 ng/mL; p < 0.01) and in control subjects (mean d-dimer level: control subjects with risk factors, 31.0 ± 7.4 ng/mL; control subjects without risk factors, 15.0 ± 6.4 ng/mL; p < 0.01). NVAF (odds ratio [OR], 3.94; 95% confidence interval [CI], 1.87 to 8.30; p = 0.0003) and age of ≥ 75 years (OR, 5.68; 95% CI, 2.87 to 11.23; p < 0.0001) emerged as predictors of elevated levels of d-dimer, and only NVAF (OR, 10.30; 95% CI, 5.67 to 18.72; p < 0.0001) emerged as a predictor of elevated levels of β-TG.

Conclusions: NVAF patients whose conditions were complicated with risk factors for embolism could be in the prothrombotic state. Advanced age is a strong predictor of the prothrombotic state in NVAF patients.

Key words: atrial fibrillation; embolism; hemostatic markers; risk factors

Abbreviations: AF = atrial fibrillation; CI = confidence interval; F1 + 2 = prothrombin fragments 1 and 2; NVAF = nonvalvular atrial fibrillation; OR = odds ratio; PF = platelet factor; TG = thromboglobulin

It is well-established that patients with nonvalvular atrial fibrillation (NVAF) are at risk for embolism, especially when their conditions are complicated with risk factors for embolism.1–3 The risk factors include a history of embolism or transient ischemic attack, congestive heart failure, hypertension, and diabetes mellitus.4 Prior studies5–8 have shown that hypercoagulability was present in patients with NVAF by determining the levels of hemostatic markers. However, results concerning the activation of platelet function were controversial.9

One clinical study10 failed to show that higher levels of BP would further promote the hypercoagulable state in NVAF patients by increasing the degree of hemostatic abnormalities. The Framingham Offspring Study11 indicated that the prothrombotic profile associated with NVAF could be explained by the risk factors of patients and by the presence of cardiovascular disease. However, results concerning the activation of platelet function were controversial.9

One clinical study10 failed to show that higher levels of BP would further promote the hypercoagulable state in NVAF patients by increasing the degree of hemostatic abnormalities. The Framingham Offspring Study11 indicated that the prothrombotic profile associated with NVAF could be explained by the risk factors of patients and by the presence of cardiovascular disease. However, level of von Willebrand factor, a marker of endothelial dysfunction, was increased in NVAF patients who conditions were complicated with risk factors.12 We hypothesized that patients with NVAF whose condi-
tions were complicated with the well-known risk factors for embolism would be in the hypercoagulable state. To validate our hypothesis, we related levels of hemostatic markers to well-established risk factors for embolism in patients with NVAF and in control subjects without NVAF.

**Materials and Methods**

**Study Subjects**

We conducted a prospective, cooperative study from 1999 to 2001 to determine the clinical efficacy of measuring hemostatic markers to identify patients with NVAF who would be at increased risk for embolic complications. The study protocol was approved by the ethics committee of each institution, and consent was obtained from each subject. The primary end point of our prospective study included clinically evident cerebral infarction, transient ischemic attack, or embolism of the peripheral arteries. However, the relation of clinical risk factors to levels of hemostatic markers was analyzed in the present study.

Patients with chronic or paroxysmal atrial fibrillation (AF) were consecutively enrolled into the study. Patients with mitral stenosis or mechanical heart valves were excluded from the study. Those patients having infections, renal failure, liver dysfunction, and neoplastic diseases that could potentially affect d-dimer levels were also excluded from the study. Chronic AF was confirmed electrocardiographically on at least two separate occasions (at ≥ 4 weeks). Paroxysmal AF was defined as the occurrence of AF documented electrocardiographically at least once in the preceding 12 months. AF needed to have lasted at least 1 h and to have terminated spontaneously or as a result of therapy with antiarrhythmic drugs.

A total of 509 patients were enrolled in the prospective study. Of these patients, 246 had not received anticoagulation treatment with warfarin, and they constituted the study group. One hundred eleven age-matched and sex-matched patients with normal sinus rhythm who did not have the above exclusion criteria were randomly selected from outpatients and served as control subjects. Clinical risk factors, including diabetes mellitus, hypertension, New York Heart Association functional status, and prior cerebral infarction or transient ischemic attack were determined from medical records and routine laboratory findings.

**Determination of Hemostatic Markers**

Hemostatic markers for coagulation and fibrinolysis are important indexes to evaluate prothrombotic status. In addition to fibrin generation, platelet activation has an important role in the process of thrombogenesis. Therefore, the levels of the following hemostatic markers were determined: platelet factor (PF) 4 (normal value, < 20 ng/mL) and β-thromboglobulin (TG) (normal value, <50 ng/mL) as indexes of platelet activation; prothrombin fragments 1 and 2 (F1 + 2) (normal value, 1.4 nmol/L) as a marker of thrombotic activity; and d-dimer (normal value, < 150 ng/mL) as an index of active fibrinolysis. Blood samples were obtained from the antecubital vein using the two-syringe technique. The preparation of blood samples and the determination of hemostatic markers at the central laboratory were performed as in our previous studies.

**Statistical Analysis**

The data are presented as the mean ± SE. The χ² test was used to compare the categoric variables. To determine the effects of risk factors and NVAF itself on hemostatic markers, patients with NVAF and control subjects were divided into two groups (ie, those with at least one risk factor and those without any risk factors). For a comparison of the continuous variables among the four groups, one-way analysis of variance was employed, and multiple comparisons were made with the Bonferroni t test using a statistical software package (SPSS, version 8.0J; SPSS, Chicago, IL). Multivariate logistic regression analysis then was performed to identify independent predictors of elevated levels of hemostatic markers using the block entry of variables. Results of multivariate analysis are expressed as odds ratios (ORs) with 95% confidence intervals (CIs). A p value of < 0.05 was considered to be significant.

**Results**

The clinical characteristics of patients with NVAF and control subjects in sinus rhythm are summarized in Table 1. Control subjects were selected from our patients, and therefore hypertension was more frequently seen in control subjects than in patients with NVAF. This could contribute to the higher proportion of control subjects having at least one risk factor compared with patients with NVAF (Table 1), although congestive heart failure (New York Heart Association functional class II or higher) was more frequently observed in patients with NVAF than in control subjects. Antiplatelets (ie, aspirin or ticlopidine) were more frequently administered to patients with NVAF than to control subjects.

**Platelet Activation**

PF4 and β-TG levels did not differ between NVAF patients with at least one risk factor and those without any risk factors and between control subjects with at least one risk factor and those without any risk factors (Table 2). Patients with NVAF had higher levels of markers of platelet activation com-

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>NVAF Patients (n = 246)</th>
<th>Control Subjects (n = 111)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>66.1 ± 0.7</td>
<td>68.3 ± 0.9</td>
<td>NS</td>
</tr>
<tr>
<td>Male gender</td>
<td>61.8</td>
<td>63.0</td>
<td>NS</td>
</tr>
<tr>
<td>Chronic AF</td>
<td>48.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 75 yr old</td>
<td>22.0</td>
<td>26.1</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension</td>
<td>40.2</td>
<td>65.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>13.8</td>
<td>18.9</td>
<td>NS</td>
</tr>
<tr>
<td>Prior cerebral infarction</td>
<td>11.8</td>
<td>6.3</td>
<td>NS</td>
</tr>
<tr>
<td>CHF</td>
<td>15.4</td>
<td>5.4</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>≥ 1 risk factor</td>
<td>64.6</td>
<td>52.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Antiplatelet therapy</td>
<td>66.3</td>
<td>46.8</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

*Values given as mean ± SE or %, unless otherwise indicated. NS = not significant; CHF = congestive heart failure.
Coagulation and Fibrinolysis

In Table 2, the levels of markers for coagulation and fibrinolysis are summarized. F1 + 2 levels were higher in both control subjects and NVAF patients whose conditions were complicated with at least one risk factor than in those without any risk factors, but mean values did not exceed the upper limit of the normal range. The difference was significant only between NVAF patients without any risk factors and both control subjects and NVAF patients with at least one risk factor.

D-dimer levels were higher in NVAF patients having at least one risk factor than in the other three groups (p < 0.01). Control subjects without any risk factors showed the lowest levels of d-dimer, but the difference did not reach the significance level when compared with control subjects with risk factors and NVAF patients without any risk factors.

Multivariate Logistic Analysis

Table 3 shows a summary of multivariate logistic analysis of clinical variables related to elevated levels of β-TG (ie, ≥ 50 ng/mL) and d-dimer (ie, ≥ 150 ng/mL). For this analysis, NVAF, age of ≥ 75 years, gender, hypertension, cerebral infarction and transient ischemic attack, diabetes mellitus, congestive heart failure, and antiplatelet therapy were included as explanatory variables. NVAF and older age were independently predictive of elevated d-dimer levels. However, only NVAF emerged as an independent predictor of elevated β-TG level. When logistic regression analysis was confined to NVAF patients, only age of ≥ 75 years emerged as an independent predictor of elevated levels of d-dimer (OR, 7.96; 95% CI, 3.98 to 15.92; p < 0.0001), but none emerged as a predictor of elevated levels of β-TG.

Discussion

Major Findings

The major findings of the present study were as follows. First, NVAF patients with at least one risk factor for embolism had elevated levels of d-dimer compared with NVAF patients without any risk factors and with control subjects. However, NVAF patients without any risk factors did not have elevated d-dimer levels compared with control subjects having at least one risk factor. These results might be consistent with the current guidelines for the antithrombotic treatment of NVAF patients. That is, NVAF patients with risk factors including older age (ie, ≥ 75 years) need anticoagulation therapy for the prevention of embolism, however, those without any risk factors need not receive anticoagulation therapy.

Table 2—Effect of Risk Factors on Hemostatic Markers*

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>PF4, ng/mL</th>
<th>β-TG, ng/mL</th>
<th>F1 + 2, nmol/L</th>
<th>D-dimer, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk factor (−)</td>
<td>17.7 ± 3.1</td>
<td>46.3 ± 5.5</td>
<td>0.82 ± 0.05</td>
<td>31.0 ± 7.4</td>
</tr>
<tr>
<td>Risk factor (+)</td>
<td>14.7 ± 1.9</td>
<td>43.9 ± 3.3</td>
<td>1.04 ± 0.04</td>
<td>79.1 ± 10.3</td>
</tr>
<tr>
<td>NVAF patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk factor (−)</td>
<td>23.1 ± 2.1</td>
<td>77.0 ± 4.9</td>
<td>0.79 ± 0.06</td>
<td>92.1 ± 6.7</td>
</tr>
<tr>
<td>Risk factor (+)</td>
<td>21.6 ± 1.5</td>
<td>74.5 ± 3.3</td>
<td>0.98 ± 0.05</td>
<td>155.6 ± 9.2</td>
</tr>
</tbody>
</table>

*Values given as mean ± SE. The study groups were divided into subjects or patients with at least one risk factor and those without any risk factors. (−) = without; (+) = with.
†p < 0.05 vs NVAF patients with and without a risk factor.
‡p < 0.01 vs NVAF patients with a risk factor.
§p < 0.01 vs NVAF patients with and without a risk factor.
¶p < 0.01 vs NVAF patients without a risk factor.
Second, NVAF patients had elevated levels of markers of platelet activation compared with control subjects. Risk factors did not affect the levels of these markers in NVAF patients or in control subjects. Logistic regression analysis revealed that NVAF and aging were independent predictors of elevated levels of d-dimer, but other risk factors did not emerge as predictors. For the elevation of β-TG levels, only NVAF was an independent predictor, but well-established risk factors were not predictive of platelet activation.

Hemostatic Markers and NVAF

Previous studies consistently have shown that NVAF patients had enhanced fibrinolytic activity. This suggests that NVAF patients would be in the prothrombotic state and could explain the higher prevalence of embolism in NVAF patients compared with subjects who are in sinus rhythm. However, the results of platelet activation were controversial. The present result that levels of markers of platelet activation were increased in NVAF patients is consistent with results of Lip et al and Sohara et al.

Previous investigators have tried to relate risk factors to the prothrombotic state seen in NVAF patients. Kistler et al determined F1 + 2 levels in 125 patients selected from patients enrolled in the Boston Area Anticoagulation Trial (BAATF). They found that F1 + 2 levels increased along with the aging of patients with NVAF, a finding that is consistent with the results of the present study. Our NVAF patients aged ≥ 75 years had higher levels of F1 + 2 than those aged < 75 years (1.26 ± 0.09 vs 0.82 ± 0.04 nmol/L, respectively; p < 0.01). However, the mean F1 + 2 value of older NVAF patients did not exceed the upper limit of the normal range. Kistler et al found that warfarin suppressed F1 + 2 levels, irrespective of patients’ ages, but that aspirin did not affect F1 + 2 levels. Other investigators did not find elevated levels of F1 + 2 in patients with NVAF compared with healthy control subjects.

British investigators determined the effects of the degree of BP on the hypercoagulable state in 82 patients having chronic NVAF who had not received antithrombotic therapy. Levels of d-dimer, von Willebrand factor, and fibrinogen were all higher in NVAF patients than in control subjects who were in sinus rhythm. However, the systolic, diastolic, or mean BP did not affect the levels of these three markers. In the present study, hypertension did not emerge as an independent predictor of elevated levels of hemostatic markers, although control subjects with hypertension had higher levels of F1 + 2 and d-dimer than did those without hypertension (data not shown). Hypertension is known to confer a 1.6-fold increase in the risk of stroke, but mechanisms other than hypertension might contribute to the prothrombotic state in NVAF patients.

NVAF patients having left atrial thrombi or left atrial dysfunction could have more elevated levels of hemostatic markers than those not having atrial thrombi or dysfunction. Actually, β-TG and d-dimer levels were higher in NVAF patients with left atrial thrombi than in those without thrombi, and levels were also higher in NVAF patients with decreased flow velocity in the left atrial appendage than in those with normal flow velocity. However, we did not determine the echocardiographic findings systematically in the present study. Risk factors including higher age, prior stroke, heart failure, and diabetes mellitus were related to elevated levels of von Willebrand factor, and therefore could promote thrombus formation in the cardiovascular system. These findings seem to be consistent with the present result, which indicated that NVAF patients whose conditions were complicated with at least one risk factor had elevated levels of d-dimer. Patients with NVAF alone had similarly elevated levels of d-dimer as those with NVAF with complications in a previous study. This finding may not be consistent with the present study in which NVAF patients without any risk factors did not have elevated d-dimer level compared with control subjects, although our NVAF patients without risk factors did not strictly represent patients with NVAF alone.

The Framingham Offspring Study indicated that the prothrombotic profile associated with NVAF could be explained by the risk factors of the subjects and the presence of cardiovascular disease, but not by NVAF itself. This was not consistent with the present study in which AF itself was an independent predictor of elevated levels of d-dimer and β-TG.

Methodological Considerations

The present study had several limitations. First, subjects receiving antiplatelet therapy, mostly aspirin, were included in the present study, since many previous studies indicated that antiplatelet therapy did not affect levels of markers of platelet activation, F1 + 2, and d-dimer, although aspirin decreased β-TG levels significantly in a previous report.

Second, paroxysmal AF was present in about half of NVAF patients. Cardiac rhythm was not strictly identified in all patients at the time of the collection of blood samples. The frequency and duration of AF episodes were not determined in detail in the present study. The levels of hemostatic markers did not differ significantly between patients with paroxysmal AF and those with...
chronic AF in the present study, and the data were therefore pooled into one group and were analyzed in the present study. However, Lip et al.7 showed that levels of hemostatic markers in patients with paroxysmal AF were intermediate between control subjects in sinus rhythm and patients with chronic AF.

Third, prior cerebral infarction or transient ischemic attack, the strongest predictor (OR, 3.4)1 for embolism in patients with NVAF, did not emerge as an independent predictor of elevated hemostatic markers in the present study. In contrast, only age ≥ 75 years emerged as a predictor of elevated levels of d-dimer in patients with NVAF. We do not have any plausible explanation for this finding, but the selection of patients with NVAF, the relatively small number of study subjects or patients with prior cerebral infarction, and other factors could possibly contribute to this finding.

Finally, control subjects who were in sinus rhythm consisted of patients having various cardiovascular diseases other than NVAF. Therefore, the present study could not make a strict comparison of NVAF patients with healthy subjects, but did make a comparison of NVAF patients with patients having cardiovascular diseases. Approximately 80% of control subjects had at least one risk factor for embolism, and this might hamper the present results concerning the relation between risk factors and levels of hemostatic markers. However, the presence of risk factors did not promote the activation of platelet function, coagulation, and fibrinolysis in subjects who are in sinus rhythm. Logistic regression analysis revealed that NVAF and age were independent predictors of elevated levels of d-dimer.

**Clinical Implication**

The present study aimed to relate risk factors for embolism to levels of hemostatic markers, but did not investigate the mechanisms for the activation of hemostatic markers. Although the elevation of markers of platelet activation and fibrinolysis suggests only a reflection of thrombosis rather than a causal role,17 the present study demonstrated that NVAF patients having at least one risk factor could be in the prothrombotic state and need anticoagulation therapy with warfarin. However, NVAF patients without any risk factors had a level of d-dimer that was similar to that of control subjects having at least one risk factor who might not require intensive anticoagulation with warfarin.

**REFERENCES**


