Coagulation Factors and the Increased Risk of Stroke in Nonvalvular Atrial Fibrillation

Claes Gustafsson, MD, Margareta Blomback, MD, Mona Britton, MD, Anders Hamsten, MD, and Jan Svensson, MD

We studied whether hemostatic abnormalities contribute to the increased risk of stroke in patients with nonvalvular atrial fibrillation. Hemostatic function was studied in four age-matched groups: 20 patients with nonvalvular atrial fibrillation and a previous ischemic stroke, 20 patients with nonvalvular atrial fibrillation without a previous stroke, 20 stroke patients with sinus rhythm, and 40 healthy controls. Both groups with nonvalvular atrial fibrillation had significantly higher concentrations of von Willebrand factor, factor VIII:C, fibrinogen, D-dimer (a fibrinolytic product), β-thromboglobulin, and platelet factor 4; a significantly higher fibrinogen/antithrombin ratio; and significantly higher spontaneous amidolytic activity than the healthy controls. Prekallikrein levels were significantly lower in both groups with nonvalvular atrial fibrillation. Stroke patients with sinus rhythm had normal hemostatic function, normal concentrations of platelet-related factors, and a slightly increased concentration of fibrinopeptide A compared with the healthy controls. Both groups with nonvalvular atrial fibrillation differed from the stroke patients with sinus rhythm as they did from the healthy controls. No difference in hemostatic function was seen between the nonvalvular atrial fibrillation patients with and without a previous ischemic stroke. Thus, alterations in hemostatic function may contribute to the increased risk of stroke in patients with nonvalvular atrial fibrillation.

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Nonvalvular atrial fibrillation (NVAF) afflicts 2–4% of 70-year-old people, and its prevalence increases with age. NVAF is an important risk factor for stroke. In the Framingham Study, NVAF was associated with a 5–6 times higher incidence of stroke compared with an age-, sex-, and blood pressure–matched control group without NVAF.2

Left atrial thrombosis causing stroke by arterial embolism has been thought to be the main pathogenetic mechanism in the association between NVAF and stroke. However, the precise mechanism is difficult to determine in individuals with NVAF. As pointed out in recent studies, a considerable proportion of strokes are probably due to atherothrombosis.3-4 Generalized atherosclerosis might be the common cause of both NVAF and stroke, thereby explaining the association. If this is true, it can be assumed that there are higher concentrations of fibrinogen, factor VIII:C, and von Willebrand factor (vWF:Ag) due to more pronounced atherosclerosis or as a sign of hypercoagulation5-7 in NVAF patients.

Prophylactic measures to diminish the risk of stroke in patients with NVAF are much needed. Anticoagulant treatment with coumarin derivatives might be the best choice if embolism were the predominant cause. However, many NVAF patients are too old for such treatment or have other contraindications to this medication. Since atherothrombosis involving platelet aggregation is a plausible mechanism of stroke in many patients with NVAF, treatment with acetylsalicylic acid might be an alternative to anticoagulant therapy. We studied several aspects of hemostatic function in NVAF patients, in stroke patients with sinus rhythm, and in healthy controls to investigate possible disturbances in the blood coagulation and fibrinolytic systems that might increase the risk of stroke among NVAF patients. The second aim of our study was to investigate whether NVAF patients with stroke differed in their hemostatic function from NVAF patients without a previous stroke and if factors could be found that identify high-risk NVAF patients who might benefit from prophylactic treatment.
Table 1. Prevalence of Risk Factors in Patients With Nonvalvular Atrial Fibrillation and Stroke Patients With Sinus Rhythm

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>With previous stroke (n = 20)</th>
<th>Without stroke (n = 20)</th>
<th>Sinus rhythm (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>35</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>65*</td>
<td>65*</td>
<td>10</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>40</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>15</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Alcohol overconsumption</td>
<td>5</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Smoking</td>
<td>35</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

Data are %. *p<0.001 different from sinus rhythm by χ² test.

Subjects and Methods

Twenty consecutive patients with electrocardiographically verified chronic atrial fibrillation, no mitral stenosis on echocardiography, and a previous ischemic stroke were matched for sex, age (±2 years), and duration (±2 years) of NVAF with 20 patients with chronic NVAF but no previous stroke and 20 patients with sinus rhythm and stroke. Mean age in all three groups was 77 (range 62–84) years. Mean duration of NVAF in the two former groups was 7.1 and 6.3 years, respectively (difference not significant). No patient had malignant disease, severe alcohol habituation, or was receiving anticoagulant treatment with coumarin. No patient had a recent history of an acute infection or an inflammatory disease. Cardiovascular risk factors for stroke in the three groups are presented in Table 1. As expected, congestive heart failure was significantly more common in the two groups of NVAF patients by χ² test.

The control group consisted of 40 sex- and age-matched healthy subjects randomly selected from the population register. Mean age in this group was 77 (range 62–84) years. No control had a history, physical signs, or electrocardiographic findings indicative of a previous thromboembolic event, ischemic heart disease, hypertension, atrial fibrillation, diabetes, malignancy, ongoing infection, or inflammatory disease.

All participants fasted for 12 hours before the blood sampling. Venipuncture technique and preparation of the plasma samples for analysis have been described. The blood tests were chosen to reflect the different parts of the hemostatic system. In the two stroke groups, blood was sampled at least 12 (mean 25, range 12–46) months after the event to avoid the influence of acute-phase reactions. Patients receiving acetylsalicylic acid stopped medication 10 days before the blood samples were drawn.

Prothrombin complex concentration (Normotest) was measured using reagents from Nycomed, Oslo, Norway.

The concentration of the acute-phase reactant orosomucoid was determined by quantitative electroimmunoassay. The concentration of β-thromboglobulin was determined according to the method of Ludlam et al using reagents from Amersham International, Amersham, England. The concentration of platelet factor 4 was measured according to the manufacturer, Abbott Laboratories, North Chicago, Illinois.

The concentration of factor VIII:C was analyzed with both one-stage and two-stage clotting assays. The concentration of vWF:Ag, formerly factor VIII R:Ag was determined by quantitative electroimmunoassay. The vWF:Ag/factor VIII:C ratio was calculated, with a value of >1.0 indicating hypercoagulability. Antithrombin concentration was measured with a heparin cofactor assay using a synthetic chromogenic peptide substrate (S-2238) method. Fibrinogen concentration was analyzed by a polymerization test. The fibrinogen/antithrombin ratio was calculated as an index of hypercoagulability. Protein C concentration was determined using an enzyme-linked immunosorbent assay (ELISA) method using reagents from Boehringer Mannheim, Mannheim, Germany.

Tissue plasminogen activator (tPA) activity was determined by a fibrin plate method, and tPA capacity was measured as the difference in tPA activity before and after 10 minutes of venous stasis at 100 mm Hg (between the systolic and diastolic blood pressures).

Plasminogen activator inhibitor (PA inhibitor) and α2-antiplasmin concentrations were analyzed with synthetic chromogenic substrate (S-2251) methods. Prekallikrein concentration and kallikrein inhibiting activity were measured using synthetic chromogenic peptide substrate (S-2302) methods, as was spontaneous amidolytic activity (SPA) using substrate S-2288. The concentration of fibrinopeptide A (FPA) was determined using a radioimmunoassay as modified by Kockum and Frebelius. The concentrations of D-dimer and related cross-linked fibrin derivatives were measured using an ELISA method and reagents from Mabco, Brisbane, Australia; this analysis was possible in only 13 NVAF stroke and 11 NVAF control patients.

Informed consent was obtained from all participants, and the study protocol was approved by the regional ethical committee.

The significance of differences in hemostatic variables among and between groups was tested using one-way analysis of variance and two-tailed t tests. Individual values for PA inhibitor activity were subjected to logarithmic transformation due to skewness before statistical analysis and significance testing. Correlation between variables was evaluated using the Pearson correlation coefficient. The levels of significance considered were 0.1%, 1%, 5%, and not significant (NS).

Results

Platelet counts were significantly lower and β-thromboglobulin concentrations were significantly higher in both NVAF groups than in the healthy controls.
Platelet factor 4 concentrations were significantly higher in the NVAF group with stroke than in the healthy controls (Table 2). Platelets were thus activated in the patients with NVAF, possibly more so in those with stroke. Furthermore, β-thromboglobulin levels were higher in both NVAF groups than in the stroke patients with sinus rhythm.

The concentrations of several coagulation factors (factor VIII:C, vWF:Ag, and fibrinogen and the fibrinogen/antithrombin ratio) were significantly elevated, indicating an increased risk of thrombosis in the two NVAF groups compared with the healthy controls (Table 3). The same differences were seen relative to the stroke patients with sinus rhythm.

Factor VIII:C, vWF:Ag, fibrinogen, and antiplasmin are considered to reflect an acute-phase reaction, but no correlation with stroke was seen for the other acute-phase reactant, orosomucoid (data not shown). In the NVAF group without stroke, α2-antiplasmin concentration was increased (Table 4). The concentration of prekallikrein was significantly decreased in both NVAF groups, indicating an activation of the kallikrein system compared with the healthy controls (Table 4). Concentrations of D-dimer, the cross-linked fibrin derivative, were significantly increased in both NVAF groups, indicating fibrin formation and secondary fibrinolysis relative to the healthy controls. SPA, indicating increased proteolytic activity, was also significantly higher in the NVAF groups than in the healthy controls. These latter findings are consistent with activation of both the coagulation and the fibrinolytic systems. Compared with the stroke patients with sinus rhythm, both NVAF groups had significantly increased SPA concentrations. Furthermore, the two NVAF groups had significantly lower prekallikrein concentrations.

The results of the coagulation (Table 3) and fibrinolytic (Table 4) analyses as well as analysis of the platelet-related factors (Table 2) in the stroke patients with sinus rhythm were similar to those in the healthy controls. The only significant difference was a slightly increased FPA concentration (Table 3).

No significant difference in platelet activation was seen between the NVAF patients with stroke and the

### TABLE 2. Platelet Counts and Concentrations of Related Factors in Patients With Nonvalvular Atrial Fibrillation, Stroke Patients With Sinus Rhythm, and Healthy Controls

<table>
<thead>
<tr>
<th>Factor</th>
<th>With previous stroke (n=20)</th>
<th>Without stroke (n=20)</th>
<th>Sinus rhythm (n=20)</th>
<th>Healthy controls (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count (10^9/l)</td>
<td>Mean</td>
<td>First-third quartiles</td>
<td>Mean</td>
<td>First-third quartiles</td>
</tr>
<tr>
<td>Platelet factor 4 (ng/ml)</td>
<td>6.6±</td>
<td>0.9-9.0</td>
<td>3.9</td>
<td>1.5-5.8</td>
</tr>
<tr>
<td>β-Thromboglobulin (ng/ml)</td>
<td>42.8±</td>
<td>25.8-49.0</td>
<td>36.0±</td>
<td>31.0-42.0</td>
</tr>
</tbody>
</table>

*§p<0.05, 0.001, respectively, different from healthy controls by two-tailed t test.

### TABLE 3. Concentrations and Activities of Blood Coagulation Factors and Their Inhibitors in Patients With Nonvalvular Atrial Fibrillation, Stroke Patients With Sinus Rhythm, and Healthy Controls

<table>
<thead>
<tr>
<th>Factor</th>
<th>With previous stroke (n=20)</th>
<th>Without stroke (n=20)</th>
<th>Sinus rhythm (n=20)</th>
<th>Healthy controls (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor VIII:C</td>
<td>Mean</td>
<td>First-third quartiles</td>
<td>Mean</td>
<td>First-third quartiles</td>
</tr>
<tr>
<td>One-stage clotting (IU/ml)</td>
<td>1.92±</td>
<td>1.80-2.14</td>
<td>1.86±</td>
<td>1.48-2.29</td>
</tr>
<tr>
<td>Two-stage clotting (IU/ml)</td>
<td>1.88±</td>
<td>1.54-2.19</td>
<td>1.81±</td>
<td>1.57-2.10</td>
</tr>
<tr>
<td>von Willebrand factor (IU/ml)</td>
<td>1.81±</td>
<td>1.29-2.19</td>
<td>1.83±</td>
<td>1.22-2.27</td>
</tr>
<tr>
<td>von Willebrand factor/factor VIII:C ratio</td>
<td>0.95</td>
<td>0.72-1.28</td>
<td>0.98</td>
<td>0.74-1.14</td>
</tr>
</tbody>
</table>

*†p<0.001, 0.01, 0.05, respectively, different from healthy controls by two-tailed t test.
NVAF patients without stroke, although there was a tendency toward more pronounced changes in the former relative to the healthy controls (Table 2). Neither were there any significant differences between the two NVAF groups in the mean values of the coagulation (Table 3) or fibrinolytic (Table 4) variables.

In the NVAF stroke group, men had lower concentrations than women of antithrombin and protein C (p<0.05, data not shown). No association was seen between low concentrations of antithrombin and high factor VIII:C and low concentrations of prekallikrein. Men with NVAF and stroke also had lower α2-antiplasmin and prekallikrein concentrations than women (p<0.05 and p<0.01, respectively; data not shown).

Discussion

Our first aim was to investigate whether there are any disturbances in the blood coagulation and fibrinolytic systems that might explain the increased risk of stroke in NVAF patients. Our data show that NVAF patients both with and without a previous stroke have multiple disturbances of the coagulation system that may contribute to their increased risk of stroke. In contrast, disturbances of the fibrinolytic enzyme system seem to be less pronounced.

Thus, the concentrations of vWF:Ag and fibrinogen were significantly elevated in NVAF patients. A raised fibrinogen concentration has been reported to be a predictor of myocardial infarction, stroke, and death. A correlation between high vWF:Ag levels and atherosclerosis, or recurrence of serious stroke, was found by Mettinger. Other authors have found no such correlation in healthy subjects later suffering cardiovascular events. Uchiyama et al found increased vWF:Ag levels in patients with thromboembolic but not embolic (rheumatic valvular heart disease) stroke.

Regarding the vWF:Ag/factor VIII:C ratio, we found values just below 1.0 in both NVAF groups, which does not support the hypothesis of a prothrombotic state in these patients since values of >1.0 indicate hypercoagulation due to consumption of factor VIII:C. The discrepancy might be explained by a very slow, continuous activation of the coagulation system, which leads to an unaltered vWF:Ag/factor VIII:C ratio in the NVAF patients, confirmed by their almost-normal FPA concentrations. The significantly lower prekallikrein concentration is consistent with an activation of the kallikrein system.

β-Thromboglobulin concentrations in both NVAF groups and platelet factor 4 concentration in the NVAF group with stroke were significantly increased, indicating platelet activation. Shah et al studied groups of patients with thromboembolic and cardioembolic stroke and showed higher concentrations of β-thromboglobulin in both groups but increased platelet factor 4 concentration only in thromboembolic stroke patients compared with matched controls.

Increased β-thromboglobulin levels have also been reported in studies on undifferentiated ischemic stroke. The high concentrations of vWF:Ag and factor VIII:C and low concentrations of prekallikrein in the NVAF groups are consistent with the findings in previous studies of ischemic cerebrovascular disease. The fibrinolytic system seemed to be less involved in this elderly population with stroke than in young patients with stroke or myocardial infarction. Somewhat surprisingly, our stroke patients with sinus rhythm had normal platelet and hemostatic functions compared with age-matched healthy controls. This is in contrast to the findings of the previous studies discussed above, which, however, did not separate NVAF patients and those with sinus rhythm. It seems that, at least in the elderly, altered the altered hemostatic function that may explain the increased risk of stroke in NVAF patients.
static function is mainly confined to patients with NVAF.

Our second aim was to look for hemostatic risk factors for stroke among the patients with NVAF. However, no clear differences were found between NVAF patients with and without stroke. Thus, it does not seem possible to use these hemostatic variables to select high-risk patients for prophylactic treatment.

These NVAF patients were elderly but nevertheless are comparable with our earlier reported stroke patients with NVAF.3 Inherited coagulation disorders in these patients seem less probable as an explanation of their prothrombotic state. The findings may contribute to left atrial thrombus formation with secondary embolism as well as to atherothrombotic stroke. Clearly, however, prospective studies of hemostatic function in relation to the risk of stroke in NVAF patients would be desirable.

References
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