Atrial Fibrillation and the Prothrombotic State in the Elderly
The Rotterdam Study

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Background and Purpose—Atrial fibrillation (AF) is a major cause of stroke among the elderly. Evidence for a prothrombotic state in AF is controversial, and there is a lack of studies among the elderly. We studied the relationships between AF and 3 prothrombotic plasma markers—von Willebrand factor (vWf; a marker of endothelial damage/dysfunction), soluble P-selectin (sP-sel; a marker of platelet activation), and fibrinogen—in a matched case-control study nested within a large community-based study of an elderly population.

Methods—We identified 162 elderly participants (mean±SD age, 78±8 years; 51% male) in the Rotterdam Study with documented AF and matched each case by age and sex to 2 population controls. vWf and sP-sel were measured by enzyme-linked immunosorbent assay; fibrinogen was measured with the Clauss method. We used conditional logistic regression analysis to assess the relationships between the markers and AF, adjusting for potential confounders.

Results—There were no significant relationships between either fibrinogen (P=0.8) or sP-sel (P=0.6) and AF. However, a positive linear relationship between vWf level and presence of AF remained significant after adjustment for potential confounders among women (odds ratio [OR], 1.17; 95% CI, 1.02 to 1.34) per 10-IU/dL increase in vWf but not among men (OR, 1.06; 95% CI, 0.96 to 1.17).

Conclusions—We observed a positive relationship between AF and plasma vWf (or endothelial damage/dysfunction) in our elderly population, which was most apparent among women. Fibrinogen and sP-sel levels were unrelated to AF. The prothrombotic state of AF may be subject to sex differences, but longitudinal studies are needed to determine the relationship between these plasma markers and stroke risk. (Stroke. 2003;34:413-417.)

Key Words: atrial fibrillation ■ fibrinogen ■ selectins ■ von Willebrand factor

Atrial fibrillation (AF), the most common sustained cardiac arrhythmia, is a major cause of morbidity and mortality through an increased risk of thromboembolic stroke.1-3 The prevalence of AF and the thromboembolic risk associated with the arrhythmia increase with advancing age, making AF a particular problem among the elderly.4 The mechanisms behind cerebral thromboembolism in AF are incompletely understood,5 but abnormal levels of prothrombotic plasma markers have been found in AF patients compared with healthy control subjects,6-9 leading to suggestions of a generalized prothrombotic state in AF. Although it has recently been suggested that it may be the presence of additional cardiovascular disease among AF patients rather than AF itself that is associated with the observed changes in prothrombotic markers,10 the prothrombotic state in AF has not been adequately studied among the elderly.

To investigate the presence of a prothrombotic state in AF among the elderly, we studied the relationships between AF and 3 prothrombotic plasma markers—von Willebrand factor (vWf; a marker of endothelial damage/dysfunction), soluble P-selectin (sP-sel; a marker of platelet activation), and fibrinogen (the precursor to insoluble fibrin and an important rheological factor)—in a matched case-control study nested within a large community-based study of an elderly population.

Methods

Study Population
The Rotterdam Study is a population-based prospective cohort study of the occurrence and progression of chronic diseases of the elderly and its risk factors. The study primarily addresses neurological, cardiovascular locomotor, and ophthalmological diseases. The design of the study has been described previously.11 In brief, between 1990 and 1993, 7983 inhabitants of Ommoord, a suburb of Rotterdam, ≥55 years of age were extensively interviewed in their homes and examined at a specially equipped research center to allow collection of baseline data, including a resting 12-lead ECG. Two
hundred four cases of AF were identified among 6808 participants for whom an ECG was available for analysis; ECGs were missing for 1175 subjects usually because of technical or logistical problems. Stored plasma samples were available for analysis for 162 of the AF cases. Each case was matched on the basis of sex and age within 5 years with 2 controls without AF from the cohort for whom plasma was available for analysis.

**Baseline Examination**

Information on current health status, medical history, and smoking behavior was obtained from a computerized questionnaire. Participants were classified as current or nonsmokers. Blood pressure was measured twice on the right upper arm with a random-zero mercury sphygmomanometer in patients in the sitting position. Systolic and diastolic blood pressures were calculated as the average of 2 consecutive measurements. Hypertension was defined from the World Health Organization criteria of the time as systolic blood pressure of ≥160 mm Hg, diastolic blood pressure of ≥90 mm Hg, or the use of blood pressure–lowering drugs prescribed for hypertension. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Serum total cholesterol was measured with an automated enzymatic method. Diabetes was defined as the use of antidiabetic medication or a preload or postload serum glucose level of ≥11.1 mmol/L.

Ten-second 12-lead ECGs were recorded at the research center with an ACTA Gnosis IV ECG recorder (ESAote), stored digitally, and analyzed with the Modular ECG Analysis System (MEANS). The computer program has been shown to be highly reliable at ECG diagnosis. ECGs that the MEANS program was not able to interpret because of poor ECG quality or pacemaker activity on the ECG were evaluated separately. Visual coding of these ECGs to determine the presence of a rhythm disorder was possible in 52 poor-quality ECGs. Because pacemaker implantation is often necessary in AF, information on the underlying rhythm disorder was obtained from general practitioner files for 25 subjects with pacemaker activity on the ECG. Of these participants, 12 had AF as the underlying rhythm disorder. Left ventricular hypertrophy (LVH) was diagnosed by the MEANS program with an algorithm taking into account QRS voltages with an age-dependent correction and regularization. A history of myocardial infarction (MI) was defined as a self-reported MI with hospital admission or the presence of MI on the ECG. A positive report of MI was confirmed by a review of the medical records of general practitioners and specialists for the presence of MI.

Peripheral venous blood samples were taken at the research center, with no stasis or minimal stasis applied if needed, and collected in CTAD collection tubes (0.11 mol/L citrate, 15 mmol/L theophylline, 3.7 mmol/L adenine plasma, and 0.198 mmol/L dipyridamole). Samples were stored at −80°C until laboratory analysis. Measurements of sP-sel and vWF were performed with enzyme-linked immunosorbent assay (ELISA) with reagents from R&D Systems and Dako-Patts, respectively. The unit for vWF, IU/dL, was standardized by reference vWF from the National Institute for Biological Standards and Controls. Intra-assay coefficients of variation for all ELISA assays were <5%; interassay variences were <10%. Plasma fibrinogen (g/L) was measured with the Clauss technique on a Pacific Hemostasis coagulometer with bovine thrombin from Alpha Laboratories. Because of natural sample wastage over time, 1 control sample was unavailable for all analyses, and an additional 10 samples produced unreliable results on fibrinogen analysis (concentration, <1 g/L) and thus were excluded from subsequent statistical analysis for this marker.

**Statistical Analysis**

Patient characteristics were compared between cases and matched controls by use of a χ² test for categorical variables and Student’s t test for continuous variables. Because fibrinogen and sP-sel were not normally distributed, we undertook analyses using the natural log transformation of these variables because this technique would result in a more normal distribution. The associations between AF and the 3 prothrombotic plasma markers were examined by calculating crude odds ratios (ORs) with their 95% confidence intervals (CIs) through conditional logistic regression. Stratified analyses were performed according to sex.

We undertook 2 separate approaches to examine the true relationships between AF and our 3 markers by removing the effects of possible confounding factors. First, indexes of blood pressure (systolic blood pressure, diastolic blood pressure, history of hypertension, LVH), history of MI, smoking status, serum cholesterol, diabetes, and BMI were considered potential confounding factors if a univariate value of P<0.25 was found for the association with any prothrombotic marker and for AF. These potential confounding factors were tested in the conditional logistic regression model for AF with the relevant prothrombotic markers and were kept in the final conditional logistic regression model if P<0.05 was met. If any index of blood pressure was significant, we entered all other blood pressure indexes into the final model because these markers represent different characteristics of hypertension, although diastolic and systolic blood pressures were never together in the models.

Second, we restricted our investigation to those free of cardiovascular disease (other than AF). Participants were considered free of cardiovascular disease if they had no previous history of MI, no hypertension, no LVH on the ECG, and no diabetes. Cases of AF without these additional features were considered “lone” AF cases. Because the absence or presence of cardiovascular disease was not a matching variable, we performed an unmatched logistic regression analysis in this group. An additional justification for this procedure was that the estimates of both the matched and unmatched analysis did not differ substantially. The estimates of the unmatched analysis were generally closer to unity.

### Results

#### Whole Group

The clinical features of the AF cases and non-AF controls are outlined in Table 1. Compared with the control group, the AF group had a lower mean systolic blood pressure and a lower mean serum cholesterol level. There were no significant differences with regard to mean age and sex (as expected

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>AF Cases (n=162)</th>
<th>Controls (n=324)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Percentages</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>51</td>
<td>51</td>
<td>1.0</td>
</tr>
<tr>
<td>Current smoker</td>
<td>22</td>
<td>20</td>
<td>0.6</td>
</tr>
<tr>
<td>Diabetes</td>
<td>19</td>
<td>16</td>
<td>0.4</td>
</tr>
<tr>
<td>Hypertension</td>
<td>23</td>
<td>27</td>
<td>0.4</td>
</tr>
<tr>
<td>LVH</td>
<td>12</td>
<td>8</td>
<td>0.2</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>21</td>
<td>14</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>Mean (SD)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>78 (8)</td>
<td>77 (8)</td>
<td>0.6</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>140 (23)</td>
<td>145 (22)</td>
<td>0.02</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>75 (13)</td>
<td>73 (11)</td>
<td>0.08</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>6.1 (1.2)</td>
<td>6.4 (1.4)</td>
<td>0.004</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.4 (3.70)</td>
<td>25.7 (3.37)</td>
<td>0.07</td>
</tr>
<tr>
<td>LN Fibrinogen, g/L</td>
<td>0.80 (0.29)</td>
<td>0.79 (0.30)</td>
<td>0.8</td>
</tr>
<tr>
<td>vWF, IU/dL</td>
<td>144 (32)</td>
<td>138 (32)</td>
<td>0.08</td>
</tr>
<tr>
<td>LN sP-sel, ng/mL</td>
<td>3.39 (0.32)</td>
<td>3.41 (0.33)</td>
<td>0.6</td>
</tr>
</tbody>
</table>

P values obtained by chi-square test for categorical variables and Student’s t test for continuous variables. LN indicates natural log transformation.
from the matching process), presence of hypertension, diabetes mellitus, previous MI, LVH (by ECG criteria), and smoking status. Furthermore, there were no significant differences in natural log–transformed fibrinogen, vWF, or natural log–transformed sP-sel levels between lone AF cases and controls. In the logistic regression model (Table 2), no significant associations were found between natural log–transformed fibrinogen or natural log–transformed sP-sel and AF in our conditional logistic models. However, a significant positive association was found between vWF and AF (OR, 1.10; 95% CI, 1.02 to 1.18). When we stratified cases and controls according to sex, we found significant positive associations between vWF levels and AF among women (adjusted OR, 1.17; 95% CI, 1.02 to 1.34 per 10 IU/dL increase) but not men (adjusted OR, 1.06; 95% CI, 0.96 to 1.17; Table 2). Indeed, women with vWF levels in the fourth quartile of the range were 4.4 times as likely to be in AF than those with vWF levels in the lowest quartile, after adjustment for potential confounders (OR, 4.42; 95% CI, 1.35 to 14.5). In men, this result could not be found (OR, 1.77; 95% CI, 0.74 to 4.20; Table 2). No significant relationship was found between natural log–transformed sP-sel or natural log–transformed fibrinogen and AF among individual sexes. The proportion of men in the study >75 years of age was 54%, whereas 72% of women were >75 years of age. However, secondary analysis of the data by age stratification did not reveal any effect of age on the relationships between vWF, natural log–transformed sP-sel, or natural log–transformed fibrinogen and AF (data not shown).

Lone AF
We identified 66 cases with lone atrial fibrillation and 158 controls without cardiovascular disease. Table 3 summarizes the characteristics of this subpopulation by presence of lone atrial fibrillation. No significant differences were found in age, sex, smoking status, systolic blood pressure, cholesterol levels, BMI, and levels of vWF, natural log–transformed sP-sel, and natural log–transformed fibrinogen between lone AF cases and controls. In the logistic regression model (Table 4), no significant associations were found between prothrombotic markers and lone AF.

Discussion
Among an elderly community-based population, the presence of AF was significantly associated with increased vWF levels in women but not men. Indeed, after adjustment for potential confounding variables, the association between vWF and AF in women was more pronounced, becoming significant for the whole group despite the lack of a significant relationship in men alone. However, AF was not associated with increased fibrinogen or sP-sel levels among the whole group or among individual sexes, and the presence of lone AF was not associated with significantly altered vWF, sP-sel, or fibrinogen levels compared with controls free of cardiovascular disease.

Several previous studies, including those from our group, have described associations between AF and abnormal prothrombotic plasma markers, including fibrinogen, vWF, and
sP-sel, suggesting AF itself to be the cause of a prothrombotic state.6–9 However, these studies have usually compared AF cases with healthy controls and have failed to adequately adjust for the confounding presence of concomitant cardiovascular disease among AF cases. Furthermore, cases in such studies have been derived from a secondary care setting, and thus there may be important additional noncardiovascular differences between cases and controls. The Framingham Offspring Study addressed these problems in a community-based setting by comparing 47 subjects with prevalent AF (mean age, 62.0 years; 75% male) with 167 subjects without AF but matched for age, sex, BMI, and smoking. Their controls have the same characteristics, in the absence of AF. LN indicates natural log transformation.

The most important differences between our present study and the Framingham study are our larger sample size, older age group (with a higher proportion of women), and inclusion of a marker of platelet activation, sP-sel. These factors have allowed us, in an age group at greater risk of thromboembolic stroke, to more closely examine the relationship between AF and 3 potential mechanisms of thrombus formation: platelet activation, endothelial damage or dysfunction, and fibrinogen (as a direct contributor to both fibrin deposition and abnormal blood flow dynamics). In keeping with the results from the Framingham study,10 the present study found no association between AF and fibrinogen or the platelet marker sP-sel. However, our finding of a significant relationship between AF and vWF, after adjustment for possible confounders, may be important because the relationship appears more pronounced among women than men.

It is unclear why there should be an independent association between AF and vWF in women but not in men or in cases of lone AF compared with healthy controls. However, the case-control design of this study has potential for survival effects: If coexisting AF and raised prothrombotic markers reduce survival, the combination would be seen less frequently in our elderly population, and the association thus would be underestimated. Thus, it is also possible that sex differences in this survival effect, such as an increased early mortality in men with raised vWF, might lead to apparent sex differences in the relationship between AF and vWF in our cross-sectional study. Of note, in an earlier study sampled from the same community population, AF was significantly associated with dementia and impaired cognitive function in women but not in men14; differences in antithrombotic therapy and coincident cardiovascular disorders were suggested as possible explanations. We chose fibrinogen, vWF, and sP-sel as prothrombotic markers because they appear to be unaffected by antithrombotic therapy.7,15,16 However, because of the design and methods of the Rotterdam Study, we had insufficient information regarding heart failure (or impaired left ventricular function) and peripheral vascular disease in our population to adjust for these parameters and therefore cannot exclude confounding by these factors. Furthermore, we did not take into account the possible presence of other cardiovascular disease among the control group, which may have lead to underestimation of the possible confounding factors within our population rather than identifying confounding factors using the available literature. In view of the high prevalence of cardiovascular disease among the control group, including higher systolic blood pressure and serum cholesterol than in AF cases, the control group may well have been far from healthy, which may have lead to underestimation of the relationship between AF and our markers.

We also considered that the age and condition of the plasma samples, some of which had been in storage for up to 10 years and thus were at risk of deterioration,17 might have affected the relationships between AF and our prothrombotic markers. Indeed, because 10 samples produced unreliable fibrinogen values, the remaining samples used may also have deteriorated from their original state, which may explain why we found no significant relationship between this marker and any clinical feature on univariate analysis (data not shown), despite the known association between fibrinogen and cardiovascular disease. However, we did observe the expected strong association between sP-sel and smoking (P<0.001; data not shown) and between vWF and MI, diabetes mellitus, current smoking, LVH, and peripheral vascular disease (data not shown), suggesting that sample deterioration is unlikely to have significantly affected these 2 markers and is thus unlikely to have altered the relationship between either sP-sel or vWF and AF.

### TABLE 4. ORs With Their 95% CI Calculated by Logistic Regression Describing the Relationship Between Prothrombotic Plasma Factors and Lone AF: The Rotterdam Study 1990–1993

<table>
<thead>
<tr>
<th>Marker</th>
<th>All</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>vWF*</td>
<td>1.02 (0.93–1.12)†</td>
<td>0.96 (0.86–1.07)†</td>
<td>1.19 (1.00–1.43)†</td>
</tr>
<tr>
<td>LN fibrinogen</td>
<td>0.7 (0.3–1.9)†</td>
<td>1.3 (0.4–4.6)†</td>
<td>0.2 (0.02–1.2)†</td>
</tr>
<tr>
<td>LN sP-sel</td>
<td>0.9 (0.4–2.2)†</td>
<td>0.8 (0.3–2.6)†</td>
<td>1.0 (0.2–4.2)†</td>
</tr>
</tbody>
</table>

*For vWF, ORs are presented per 10 IU/dL increase. †Adjusted for age and sex, if applicable. **Additionally adjusted for serum cholesterol level, systolic blood pressure, and BMI. ***Additionally adjusted for systolic blood pressure. ****Additionally adjusted for serum cholesterol level and diastolic blood pressure.
With the above caveats in mind, we must consider that raised vWF in AF, especially among women, might have implications for the pathogenesis of thromboembolic disease in this group. Raised plasma vWF levels have previously been found to independently predict presence of left atrial appendage thrombus in patients with AF and correlate with severity of ultrastructural changes to the left atrial appendage endocardium in mitral stenosis. Furthermore, increased left atrial appendage endocardial expression of vWF has been described in AF and appears to correlate with the presence of adherent platelet thrombus in the overloaded left atrial appendage. If the relationship between AF and raised vWF is indeed stronger among women, it may represent, in part, a potential mechanism of the apparent excess stroke risk conferred by AF among women compared with men and thus warrants further investigation. Indeed, even though we found no relationships between sP-sel or fibrinogen and AF, the cross-sectional nature of our analysis does not allow us to examine whether plasma fibrinogen, sP-sel, or vWF levels might relate to risk of subsequent stroke and thromboembolism in AF, a question that we intend to address in a future longitudinal study among the same cohort.

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References
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