# Nonvalvular atrial fibrillation: evidence for a prothrombotic state

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## Abstract

**Objective:** To determine whether patients with nonvalvular atrial fibrillation (NVAF) have prothrombotic changes compared with patients in sinus rhythm.

**Design:** Cross-sectional study. Hemostatic function compared in NVAF patients without prior embolic event (transient ischemic attack or embolic stroke) and control subjects without prior thrombotic stroke, and in NVAF patients with prior embolic event and control subjects with prior thrombotic stroke.

**Setting:** Internal medicine outpatient group practice and anticoagulation clinic in 2 teaching hospitals.

**Patients:** A total of 75 NVAF patients (50 without and 25 with prior embolic event) and 42 control patients (31 without and 11 with prior thrombotic stroke) recruited concurrently over 18 months during 1990–91.

**Outcome measures:** Platelet count, prothrombin time (PT), partial thromboplastin time (PTT), and plasma levels of hemoglobin, fibrinogen, von Willebrand factor antigen, factor VIII, fibrin D-dimer, antithrombin III, protein C, protein S, fibrinopeptide A and prothrombin fragment F1+2. All statistical analyses were performed after adjustments for age and sex.

**Results:** The NVAF patients without a prior embolic event had significantly higher mean hemoglobin and fibrinogen levels ($p < 0.001$ and $p = 0.05$, respectively) than the control subjects without prior thrombotic stroke. The 29 NVAF patients not taking warfarin (none had had an embolic event) had significantly lower mean protein C and protein S levels ($p = 0.012$ and $p < 0.001$, respectively) and a significantly higher fibrinopeptide A level ($p = 0.03$, after exclusion of outliers) than the control subjects without prior stroke. The NVAF patients with a prior embolic event had alterations in the hemostatic variables similar to those seen in the control patients with a prior thrombotic stroke. The latter had significantly higher fibrinogen, von Willebrand factor antigen and factor VIII levels ($p = 0.04$, 0.002 and 0.002, respectively) and significantly lower protein S levels ($p = 0.02$) than the control subjects without prior stroke.

**Conclusions:** NVAF patients without a history of an embolic event show evidence of a prothrombotic state compared with patients in sinus rhythm who have not had a thrombotic stroke. NVAF patients with a history of an embolic event show evidence of a prothrombotic state similar to that of patients in sinus rhythm who have had a thrombotic stroke. Prospective studies are needed to determine whether these abnormalities predict higher risk of stroke in individual NVAF patients.

## Résumé

**Objectif :** Déterminer si les patients atteints de fibrillation auriculaire non valvulaire (FANV) subissent des changements prothrombotiques comparativement aux patients en rythme sinusal.

**Conception :** Étude transversale. Comparaison de la fonction hémostatique chez des patients en FANV qui n'avaient pas subi d'embolie (accident ischémique transitoire ou embolie) auparavant et chez des sujets témoins qui n'ont pas subi de thrombose auparavant, ainsi que chez des patients en FANV qui ont déjà été victimes d'une embolie et des sujets témoins qui ont déjà été victimes d'une thrombose.

*Evidence*

*Études*

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**Contexte :** Pratique collective externe de médecine interne et clinique d'anticoagulation dans 2 hôpitaux d'enseignement.

**Patients :** Au total, 75 patients en FANV (50 qui n'avaient pas subi d'embolie auparavant et 25 qui en avaient subi une) et 42 patients témoins (31 qui n'avaient pas subi de thrombose auparavant et 11 qui en avaient subi une) recrutés en même temps sur une période de 18 mois en 1990–1991.

**Mesures des résultats :** Numération plaquettaire, temps de prothrombine, temps de céphaline et taux plasmatiques d'hémoglobine, de fibrinogène, d'antigène du facteur de von Willebrand, de facteur VIII, de D-dimères de la fibrine, d'antithrombine III, de protéine C, de protéine S, de fibrinopeptide A et de fragment F1+2 de la prothrombine. Toutes les analyses statistiques ont été effectuées après rajustement en fonction de l'âge et du sexe.

**Résultats :** Les patients en FANV qui n'avaient pas subi d'embolie auparavant avaient des taux moyens d'hémoglobine et de fibrinogène beaucoup plus élevés ($p < 0{,}001$ et $p = 0{,}05$, respectivement) que les sujets témoins qui n'avaient pas subi de thrombose auparavant. Les 29 patients en FANV qui ne prenaient pas de warfarine (aucun d'entre eux n'avait subi d'embolie auparavant) avaient des taux moyens de protéine C et de protéine S beaucoup moins élevés ($p = 0{,}012$ et $p < 0{,}001$, respectivement) et un taux de fibrinopeptide A beaucoup plus élevé ($p = 0{,}03$, après exclusion des valeurs aberrantes) que les sujets témoins qui n'avaient pas subi d'attaque auparavant. Les patients en FANV qui avaient déjà subi une embolie présentaient des altérations des variables hémostatiques semblables à celles qu'on a constatées chez les sujets témoins qui avaient déjà subi une thrombose. Ces derniers présentaient des taux beaucoup plus élevés de fibrinogène, d'antigène du facteur de von Willebrand et de facteur VIII ($p = 0{,}04$, 0,002 et 0,002, respectivement) et des taux beaucoup moins élevés de protéine S ($p = 0{,}02$) que les sujets témoins qui n'avaient pas subi d'attaque auparavant.

**Conclusions :** Les patients en FANV qui n'ont pas subi d'embolie auparavant présentent des signes d'état prothrombotique comparativement aux patients en rythme sinusal qui n'ont pas subi de thrombose. Les patients en FANV qui ont déjà subi une embolie montrent des signes d'état prothrombotique semblable à celui des patients en rythme sinusal qui on déjà subi une thrombose. Des études prospectives s'imposent pour déterminer si ces anomalies permettent de prédire un taux plus élevé d'attaques chez certains patients en FANV.

Chronic nonvalvular atrial fibrillation (NVAF) is an independent risk factor for ischemic stroke, affected patients being at 5 times the risk of people in sinus rhythm.[1–3] In recent randomized placebo-controlled trials of antithrombotic therapy the rate of ischemic stroke was 5% per year on average among patients receiving placebo.[4–6]

Randomized clinical trials have demonstrated the efficacy of warfarin[4–8] and, less consistently, acetylsalicylic acid (ASA)[4,5] in reducing the incidence of stroke and systemic embolism in patients with NVAF, probably by either stabilizing or preventing the formation of atrial thrombus. Since many patients with NVAF are elderly or have specific contraindications to these medications, it would be useful to stratify them according to their risk for stroke, permitting better application of these preventive treatments to patients who are likely to benefit.

To date, some studies have shown that clinical factors such as recent congestive heart failure, hypertension, previous arterial thromboembolism and age are independent predictors of increased risk for embolic stroke among patients with NVAF,[3,9,10] as are echocardiographic evidence of left ventricular dysfunction and increased left atrial size.[11] Other studies have not found that these variables predict stroke.[1,12,13] Since the cause of stroke in NVAF is presumed to be embolism arising from a thrombus in the fibrillating left atrium, specific disturbances in the hemostatic system may exist in NVAF patients that would increase their risk of thrombus formation.

We conducted this study to determine whether NVAF patients have alterations in their clotting factors suggestive of hypercoagulability. We compared NVAF patients without prior embolic stroke with control subjects in sinus rhythm, and NVAF patients with prior embolic stroke with control subjects with prior thrombotic stroke.

A unique feature of our study was that we investigated

3 concurrent groups of patients with NVAF: those not taking warfarin, those taking warfarin who had no history of stroke and those taking warfarin who had had a stroke. Also, some of the hemostatic markers we measured have not been previously studied in NVAF patients.

## Methods

### *Patient selection*

Patients were recruited over 18 months during 1990–91 from an internal medicine outpatient group practice and an anticoagulation clinic located in 2 teaching hospitals. All patients had chronic NVAF, as demonstrated by 2 electrocardiograms showing atrial fibrillation at least 30 days apart in the 6 months preceding recruitment. As well, the apical beat was auscultated at enrolment to ensure that an irregular rhythm was present. Patients were included regardless of whether they were taking warfarin or had a history of an embolic event. An embolic event was defined as a history compatible with transient ischemic attack or embolic stroke that required medical attention, was diagnosed by a physician and occurred at least 6 months before study entry.

Patients were excluded if they were taking ASA, dipyridamole or estrogen because these medications can affect clotting parameters. Patients with a history of rheumatic fever or rheumatic heart disease, valvular heart disease, cardiomyopathy, ventricular aneurysm and any previously known hypercoagulable state such as antithrombin III deficiency, protein C or S deficiency, active cancer or myeloproliferative disorder were excluded because these conditions could predispose to the formation of blood clots independent of the presence of atrial fibrillation. Also excluded were patients with active kidney or liver disease because these conditions could affect the blood levels of various clotting factors through altered synthesis or clearance.

There were 2 control groups: patients in sinus rhythm without a history of thrombotic stroke and patients in sinus rhythm with a history of thrombotic stroke. These participants were recruited concurrently with the NVAF patients from the same group practice. None of the control subjects had a history of atrial fibrillation. The thrombotic stroke patients had had a nonhemorrhagic cerebrovascular accident at least 6 months before study entry. The same exclusion criteria as those for NVAF patients applied to the control groups. Because ASA use was an exclusion criterion, we were able to recruit only a small number of thrombotic stroke patients over the study period.

Informed consent was obtained from all participants. The study protocol was approved by the hospitals' ethics committees.

### *Laboratory investigation*

All study participants had blood samples drawn by venipuncture by the same study nurse, who used a 20-gauge butterfly needle and a 2-syringe technique. After discarding the contents of the first syringe, a portion of the remaining fresh sample was sent for immediate determination of the hemoglobin concentration, platelet count, prothrombin time (PT) and partial thromboplastin time (PTT). The remainder of the sample was distributed into tubes containing ACD (acid-citrate-dextrose), EDTA (ethylenediaminetetraacetic acid), adenosine and heparin for prothrombin fragment F1+2 determination (ratio of 1:6 with plasma); thrombin inhibitor, EDTA and aprotinin for fibrinopeptide A (FPA) determination (ratio of 1:10 with plasma); and citrate-containing tubes for the remaining tests. All tubes were immediately placed on ice, and, within 30 minutes after venipuncture, plasma fractions were obtained by centrifugation at 4°C for 30 minutes at 2500 rpm. The plasma aliquots were stored at –70°C until they were used.

Standard laboratory methods were used to determine the hemoglobin concentration, platelet count, PT and PTT. Factor VIII was assayed with the use of the 1-stage clotting assay.[14] Fibrinogen was measured with a functional assay, as described by Clauss.[15] Measurement of the von Willebrand factor antigen was by enzyme-linked immunoassay with a kit from Diagnostica Stago (Asnières sur Seine, France). Levels of fibrin D-dimer (fibrin degradation fragment) were determined with the use of a latex agglutination assay using reagents from Ortho Diagnostic Systems (Johnson & Johnson Co., Raritan, NJ). Antithrombin III and protein C levels were measured by means of chromogenic activity assay according to the manufacturer's instructions; kits were from Organon Teknika (antithrombin III; AKZO Nobel, Scarborough, Ont.) and Diagnostica Stago (protein C). Protein S immunoassay was performed according to the Laurell technique[16] with the use of antibody purchased from Biopool Canada (Burlington, Ont.). Plasma concentrations of the prothrombin fragment F1+2 were determined by means of double antibody radioimmunoassay,[17] and plasma FPA levels were measured by means of radioimmunoassay with a kit provided by Byk-Sangtec (Dietzanbach, Germany). Table 1 gives the normal laboratory values for the hemostatic markers studied.

### *Statistical analysis*

For continuous variables, 2-tailed *t*-tests were used to measure the differences in means between groups. Descriptive statistics for categorical variables are reported as percentages. For categorical variables, the $\chi^2$ test or

Fisher's exact test was used to test the differences in proportions between groups. One-way analysis of variance was used to measure the significance of differences in hemostatic variables between groups after adjustment for age and sex.

Alterations in some of the hemostatic markers we measured have been previously described in stroke patients. Hence, for markers unaffected by warfarin, we compared results for NVAF patients who had no prior embolic event with those for control patients who had no prior thrombotic stroke, and we compared results for NVAF patients who had a prior embolic event with those for control patients who had a prior thrombotic stroke. For hemostatic markers known to be affected by warfarin,[18] results for NVAF patients who were not taking warfarin (none of whom had had an embolic event) were compared with those for control subjects who had no prior thrombotic stroke. All NVAF patients with a history of an embolic event were taking warfarin; hence, for markers affected by warfarin, we were unable to compare results for these patients with those for control subjects who had a history of thrombotic stroke.

A $p$ value of less than 0.05 was considered to be significant.

## Results

Seventy-five patients with chronic NVAF were recruited during the study period: 29 were not taking warfarin and had no history of an embolic event, 21 were taking warfarin but had no history of an embolic event, and 25 were taking warfarin and had had an embolic event. There were 42 control subjects in sinus rhythm: 31 without a history of thrombotic stroke and 11 with such a history.

The demographic and clinical characteristics of the patients are shown in Table 2. Compared with the control subjects who had no prior stroke, the mean age of the NVAF patients was significantly higher (72.7 [standard deviation (SD) 8.9] years v. 65.0 [SD 11.8] years; 95% confidence interval [CI] for difference 3.3–12.5), as was the proportion of men (66.7% v. 38.7%; 95% CI for difference 8%–48%). Because several of the hemostatic markers studied may be affected by age and sex,[19,20] all subsequent analyses were adjusted for these 2 characteristics. With regard to comorbid conditions, there were no significant differences between the groups in the proportion of those with hypertension or diabetes mellitus. However, coronary artery disease was more common among the NVAF patients than among the control subjects without prior stroke (25.3% v. 6.5%; 95% CI for difference 6%–32%). Medication use was similar in the patient groups except that digoxin use was significantly higher in the NVAF group than in the control group without prior stroke (80% v. 0%; 95% CI for difference 71%–89%), whereas the use of other vasodilators for hypertension was most common in the control group without prior stroke, and the use of lipid-lowering agents was most common in the control group with prior stroke.

Table 3 summarizes the hemostatic markers unaffected by warfarin in the NVAF patients without a prior embolic

**Table 1: Normal laboratory hematologic values**

| Variable | Normal values |
|---|---|
| Hemoglobin, g/L | |
| Males | 140–180 |
| Females | 120–160 |
| Platelet count, $\times 10^9$/L | 150–400 |
| Fibrinogen, g/L | 2.0–4.0 |
| Von Willebrand factor antigen, µ/mL | 0.5–1.5 |
| Factor VIII, µ/mL | 0.5–1.5 |
| Fibrin D-dimer, ng/mL | < 200 |
| Antithrombin III, µ/mL | 0.75–1.25 |
| Protein C, µ/mL | 0.75–1.30 |
| Protein S, µ/mL | 0.70–1.30 |
| FPA,* mean (and SD†), nmol/L | |
| < 50 yr | 1.10 (1.00) |
| 50–69 yr | 1.15 (0.73) |
| ≥ 70 yr | 1.64 (1.27) |
| Prothrombin fragment F1+2, mean (and SD), nmol/L | |
| < 50 yr | 0.574 (0.176) |
| 50–69 yr | 0.703 (0.196) |
| ≥ 70 yr | 0.900 (0.428) |

*FPA = fibrinopeptide A.
†SD = standard deviation.

event and in the control subjects without a prior thrombotic stroke. The hemoglobin and fibrinogen levels were significantly higher ($p < 0.001$ and $p = 0.05$, respectively) in the NVAF group than in the control group. The values for the other markers listed in the table did not differ significantly between the 2 groups.

Table 4 summarizes the hemostatic markers affected by warfarin in the NVAF patients not taking warfarin and in the control subjects without a prior stroke. Both the protein C and the protein S levels were significantly lower ($p = 0.012$ and $p < 0.001$, respectively) in the NVAF group than in the control group. The FPA levels were significantly higher ($p = 0.03$) in the NVAF group than in the control group when all outliers with values greater than 10 nmol/L were removed from the analysis (this level has been associated with venipuncture artifact[21]). In contrast, the prothrombin fragment F1+2 levels, with and without removal of outliers as described above, were significantly lower ($p = 0.002$ and $p < 0.001$, respectively) in the NVAF group than in the control group. The differences in F1+2 levels were in a direction opposite to that hypothesized. There were no significant differences between the 2 groups in the PT or the PTT, both expressed as ratios of mean patient result over laboratory control (data not shown).

The hemostatic markers unaffected by warfarin did not differ significantly between the NVAF patients with a history of an embolic event (all of these patients were taking warfarin) and the control subjects with prior thrombotic stroke (Table 5).

The markers for the 2 control groups are summarized in Table 6. The levels of fibrinogen, von Willebrand factor antigen and factor VIII were significantly higher ($p = 0.04$, 0.002 and 0.002, respectively) in the group with a history of thrombotic stroke than in those without such a history. The protein S level and the prothrombin fragment F1+2 level (after removal of outliers) were significantly lower ($p = 0.02$ and 0.005, respectively) in the group with a history of thrombotic stroke. For the F1+2 level, the difference was in a direction opposite to that hypothesized. The mean PT and PTT, and the mean hemoglobin, fibrin D-dimer, antithrombin III and FPA levels did not differ significantly between the 2 groups (data not shown).

**Table 2: Demographic characteristics, comorbid conditions and medication use of patients with nonvalvular atrial fibrillation (NVAF) and control subjects in sinus rhythm with or without a history of thrombotic stroke**

| | Group; no. (and %) of patients* | | |
|---|---|---|---|
| | | Control patients | |
| Variable | NVAF patients *n = 75* | Without history of stroke *n = 31* | With history of stroke *n = 11* |
| **Demographic characteristics** | | | |
| Age, mean (and SD), yr | 72.7 (8.9)§ | 65.0 (11.8) | 65.0 (14.0) |
| Sex, % male | 66.6‖ | 38.7 | 63.6 |
| **Comorbid conditions** | | | |
| Coronary artery disease | 19 (25.3)¶ | 2 (6.5) | 0 |
| Hypertension | 28 (37.3) | 16 (51.6) | 6 (54.6) |
| Diabetes mellitus | 11 (14.7) | 2 (6.5) | 2 (18.2) |
| **Medication use** | | | |
| ACE inhibitors† | 7 (9.3) | 3 (9.7) | 2 (18.2) |
| Beta-blockers | 18 (24.0) | 9 (29.0) | 2 (18.2) |
| Calcium-channel blockers | 17 (22.7) | 5 (16.1) | 0 |
| Digoxin | 60 (80.0)** | 0 | 0 |
| Diuretics | 27 (36.0) | 11 (35.5) | 1 (9.1) |
| Insulin | 1 (1.3) | 0 | 0 |
| Lipid-lowering drugs | 1 (1.3) | 0 | 2 (18.2)¶ |
| Nitrates | 8 (10.7) | 1 (3.2) | 0 |
| NSAIDs‡ | 6 (8.0) | 3 (9.7) | 0 |
| Oral hypoglycemic agents | 7 (9.3) | 0 | 1 (9.1) |
| Other vasodilators | 1 (1.3)†† | 5 (16.1) | 0 |

*Unless otherwise stated.
†ACE = angiotensin-converting-enzyme.
‡NSAIDs = nonsteroidal anti-inflammatory drugs.
§$p = 0.002$, compared with control patients without prior stroke.
‖$p = 0.008$, compared with control patients without prior stroke.
¶$p = 0.02$, compared with control patients without prior stroke.
**$p = 0.001$, compared with control patients without prior stroke.
††$p = 0.012$, compared with control patients without prior stroke.

The markers studied did not differ significantly between the NVAF patients with a history of an embolic event and those without such a history (data not shown).

The expected effects of warfarin were found in the NVAF patients taking warfarin; namely, higher mean PT and PTT, and lower mean protein C, protein S, prothrombin fragment F1+2 and FPA levels than those for all of the other patient groups (data not shown).

## Discussion

In our study population, we found that NVAF patients showed evidence of a prothrombotic state in comparison with control subjects. Those without a history of an embolic event had significantly higher mean hemoglobin and fibrinogen levels than control subjects without a history of thrombotic stroke. The mean von Willebrand factor antigen and factor VIII levels were also higher in these NVAF patients, but after adjustment for age and sex the differences were not statistically significant. When comparing the NVAF patients who had had an embolic event with control subjects who had had a thrombotic stroke, we found no significant difference in any of the hemostatic variables measured. We also found that in the NVAF patients not taking warfarin (none had had an embolic event) the mean protein C and protein S levels were significantly lower and the FPA level was significantly higher than the levels in the control group without a history of thrombotic stroke; these differences were similar in magnitude to those seen between the control subjects with a history of thrombotic stroke and the control subjects without such a history.

Other investigators have examined the relation between NVAF and markers of coagulation. Levels of fib-

**Table 3: Hemostatic markers unaffected by warfarin in NVAF patients without a history of an embolic event* and control subjects without a history of thrombotic stroke**

| | Group; mean value (and SD)† | | | |
|---|---|---|---|---|
| Marker | NVAF patients without history of event *n = 50* | Control subjects without history of stroke *n = 31* | Difference in means (and 95% CI) | Adjusted *p* value‡ |
| Hemoglobin, g/L | 149 (13) | 134 (15) | 15 (9, 21) | < 0.001 |
| Platelet count, × $10^9$/L | 230 (98) | 233 (49) | –3 (–35, 29) | 0.35 |
| Fibrinogen, g/L | 3.7 (0.8) | 3.2 (1.1) | 0.5 (0.1, 1.0) | 0.05 |
| Von Willebrand factor antigen, µ/mL | 1.8 (0.6) | 1.5 (0.6) | 0.3 (0.02, 0.5) | 0.26 |
| Factor VIII, µ/mL | 1.6 (0.7) | 1.3 (0.3) | 0.3 (0.02, 0.5) | 0.11 |
| Fibrin D-dimer, % of subjects with level < 200, 200–500 and > 500 ng/mL respectively | 90, 6, 4 | 87, 7, 6 | – | 0.12 |
| Antithrombin III, µ/mL | 1.00 (0.15) | 1.00 (0.13) | 0 (–0.06, 0.06) | 0.78 |

*Embolic event = transient ischemic attack or embolic stroke.
†Unless otherwise stated.
‡Determined by analysis of variance (ANOVA), for difference between groups after adjustment for age and sex.

**Table 4: Hemostatic markers affected by warfarin in NVAF patients not taking warfarin and in control subjects without a history of thrombotic stroke**

| | Group; mean value (and SD) | | | |
|---|---|---|---|---|
| Marker | NVAF patients not taking warfarin *n = 29* | Control subjects without history of stroke *n = 31* | Difference in means (and 95% CI) | Adjusted *p* value* |
| Protein C, µ/mL | 0.89 (0.18) | 1.10 (0.24) | –0.21 (–0.31, -0.10) | 0.012 |
| Protein S, µ/mL | 0.83 (0.14) | 0.98 (0.17) | –0.15 (–0.23, -0.07) | < 0.001 |
| FPA, nmol/L | | | | |
| All subjects | 10.30 (16.70) | 7.40 (17.80) | 2.90 (–5.80, 11.60) | 0.57 |
| Excluding outliers† | 4.00 (2.40) *n = 23* | 2.90 (2.30) *n = 29* | 1.10 (–0.19, 2.40) | 0.03 |
| Prothrombin fragment F1+2, nmol/L | | | | |
| All subjects | 1.30 (0.50) | 1.80 (0.99) | –0.50 (–0.89, –0.10) | < 0.001 |
| Excluding outliers† | 1.20 (0.48) *n = 23* | 1.72 (0.97) *n = 29* | –0.52 (–0.90, –0.12) | 0.002 |

*Determined by ANOVA, for difference between groups after adjustment for age and sex.
†All values of FPA > 10 nmol/L and the corresponding F1+2 values were considered outliers and removed for this analysis (see reference 21).

rinogen,[22] von Willebrand factor antigen,[22,23] factor VIII,[22] fibrin D-dimer[22–26] and thrombin–antithrombin III complex[23–25] were found to be higher in NVAF patients, with or without a history of stroke, than in control subjects. Most previous studies did not include a comparison group of control patients with a history of thrombotic stroke.

We found that the patients with NVAF in our study who were not taking warfarin had significantly lower levels of protein C and protein S than the control subjects without a history of thrombotic stroke. Decreases in these levels have been linked to arterial and venous thromboembolism. Gustaffson and associates[22] found a lower mean protein C level in NVAF patients than in control subjects with or without a history of stroke. Takano and collaborators[27] found that patients with acute cardiogenic cerebral embolism of various causes had significantly lower protein C levels than age-matched control subjects. Consumption of protein C during intracardiac thrombus formation could explain this finding. Alternatively, a preexisting protein C deficiency may have contributed to the development of cardiogenic embolism. To our knowledge, protein S levels have not been previously studied in patients with NVAF.

In our study, we noted higher fibrinogen levels in the NVAF patients than in the control subjects. In prospective population-based studies, higher baseline levels of fibrinogen, even within the normal range, were predictive of future stroke and myocardial infarction.[28,29] In follow-up studies of patients with angina pectoris[30] and peripheral vascular disease[31] fibrinogen was a strong independent predictor of acute coronary syndromes and death from coronary disease. Fibrinogen appears to be a reliable marker of a prothrombotic state.

Compared with the control subjects, we found higher hemoglobin levels in the NVAF patients, even in those who were taking warfarin. Because we had made adjustments for age and sex, we could not attribute this finding to differences in these characteristics between the groups.

**Table 5: Hemostatic markers unaffected by warfarin in NVAF patients with a history of an embolic event and control subjects with a history of thrombotic stroke**

| Variable | Group; mean value (and SD) | | Difference in means (and 95% CI) | Adjusted *p* value* |
|---|---|---|---|---|
| | NVAF patients with history of event *n = 25* | Control subjects with history of stroke *n = 11* | | |
| Hemoglobin, g/L | 141 (12) | 143 (17) | –2 (–13, 9) | 0.48 |
| Platelet count, × $10^9$/L | 253 (82) | 242 (77) | 11 (–45, 66) | 0.94 |
| Fibrinogen, g/L | 3.8 (0.9) | 4.0 (0.9) | –0.2 (–0.8, 0.4) | 0.28 |
| Von Willebrand factor antigen, µ/mL | 2.1 (0.6) | 2.3 (1.2) | –0.2 (–0.9, 0.5) | 0.12 |
| Factor VIII, µ/mL | 1.6 (0.5) | 1.7 (0.7) | –0.1 (–0.6, 0.4) | 0.08 |
| Fibrin D-dimer, % of subjects with level < 200, 200–500 and > 500 ng/mL respectively | 92, 4, 4 | 94, 4, 2 | – | |
| Antithrombin III, µ/mL | 1.00 (0.14) | 1.00 (0.13) | 0 (–0.09, 0.09) | 0.74 |

*Determined by ANOVA, for difference between groups after adjustment for age and sex.

**Table 6: Hemostatic markers in control subjects**

| Marker | Control group; mean value (and SD) | | Difference in means (and 95% CI) | Adjusted *p* value* |
|---|---|---|---|---|
| | With history of stroke *n = 11* | Without history of stroke *n = 31* | | |
| Fibrinogen, g/L | 4.0 (0.9) | 3.2 (1.1) | 0.8 (0.16, 1.43) | 0.04 |
| Von Willebrand factor antigen, µ/mL | 2.3 (1.2) | 1.5 (0.6) | 0.8 (0.06, 1.53) | 0.002 |
| Factor VIII, µ/mL | 1.7 (0.7) | 1.3 (0.3) | 0.4 (–0.05, 0.85) | 0.002 |
| Protein C, µ/mL | 0.91 (0.23) | 1.10 (0.24) | –0.19 (–0.35, –0.03) | 0.08 |
| Protein S, µ/mL | 0.86 (0.09) | 0.98 (0.17) | –0.12 (–0.20, –0.04) | 0.023 |
| Prothrombin fragment F1+2, nmol/L | | | | |
| All subjects | 1.5 (1.43) | 1.8 (0.99) | –0.30 (–1.20, 0.60) | 0.46 |
| Excluding outliers† | 1.04 (0.27) *n = 7* | 1.72 (0.97) *n = 29* | –0.68 (–1.10, –0.30) | 0.06 |

*Determined by ANOVA, for difference between groups after adjustment for age and sex.
†As described in Table 4.

Elevated hemoglobin levels increase the risk for[32,33] and size of[34] cerebral infarction, probably through enhanced thrombus formation[35] and slowing of cerebral blood flow because of increased blood viscosity.[36]

The NVAF patients not taking warfarin had significantly higher FPA levels than the control subjects after we removed outliers with technically inadequate blood samples. Elevated FPA levels have previously been found in conditions associated with procoagulant activation, such as deep vein thrombosis,[37] angina pectoris and myocardial infarction,[38] and cerebral infarction.[39] Although our FPA results are consistent with a relative prothrombotic state in the NVAF patients as compared with the control subjects, the mean values for both groups were higher than the age-specific laboratory reference range. It is thus difficult to interpret the importance of this finding.

The prothrombin fragment F1+2 is an in vivo marker of thrombin generation. Elevated levels have been observed in patients with antithrombin III and protein C deficiencies,[40] disseminated intravascular coagulation and venous thromboembolic disease.[41] In contrast, we found that the NVAF patients in our study had significantly lower F1+2 levels than the control subjects, but both groups had levels higher than the laboratory reference range. Possible explanations include venipuncture artifact and inapplicability of the laboratory reference ranges to our study population.

When we compared the NVAF patients who had had an embolic event with the control subjects who had had a thrombotic stroke, we found no difference between the 2 groups in the markers measured. Furthermore, when compared with the control subjects who had no history of thrombotic stroke, control subjects with such a history had the same pattern of abnormalities in hemostatic markers as did the NVAF patients without a history of an embolic event: namely, higher fibrinogen, factor VIII and von Willebrand factor antigen levels, and lower protein C and protein S levels. The prothrombin fragment F1+2 levels were significantly lower in the control subjects with a history of thrombotic stroke than in those without such a history, but again they were higher in both groups than the laboratory normal values.

Our finding of altered hemostatic function in the control subjects with a history of thrombotic stroke has previously been described. Studies have shown elevated levels of fibrin D-dimer, thrombin–antithrombin III complex,[25–27,42,43] plasmin–$a_2$-antiplasmin complex,[25,42] fibrinogen[42] and FPA,[42,43] and decreased levels of antithrombin III[27,42] and protein C[27] in various populations of patients with stroke. It is uncertain whether these abnormalities are a consequence of stroke or a predisposing cause of stroke.[44]

In summary, our study demonstrates that, compared with control subjects without a history of thrombotic stroke, NVAF patients without a history of an embolic event show evidence of a prothrombotic state. It also demonstrates that NVAF patients with a history of an embolic event have alterations in their hemostatic system similar to those in control patients who have had a thrombotic stroke. Our study design was unique in that we divided our NVAF patients into 2 groups — those with and those without a history of an embolic event — and we used corresponding comparison groups. In order to investigate hemostatic markers affected by warfarin, we were able to include a group of NVAF patients not taking warfarin because, at the time of patient recruitment, results from randomized clinical trials showing a clear benefit of warfarin in reducing embolic events in NVAF patients were not yet available. Although many of the alterations we found were within normal limits, they were still significantly higher than those in the control groups, and they were in a range consistent with results from other studies demonstrating a relation between abnormalities of these factors and future thrombotic events.

Our study has certain limitations that may have influenced the validity of the results. The small samples accord limited power to eliminate real differences between groups for variables that are not significantly different for the groups compared. The control group was not matched by age or sex to the NVAF patients; the results were, however, adjusted for age and sex. The cross-sectional design prevents us from drawing any conclusions regarding the causal nature of the relation between atrial fibrillation and the hemostatic abnormalities observed, and from determining whether any of these abnormalities are predictive of stroke in NVAF patients.

The clinical significance of our findings needs to be clarified in a larger, prospective study involving NVAF patients to determine whether these thrombotic abnormalities are predictive of embolic events in individual patients. If confirmed, this information would enhance our understanding of the increased stroke rate among NVAF patients and could prove useful in making decisions about anticoagulation therapy for NVAF patients.

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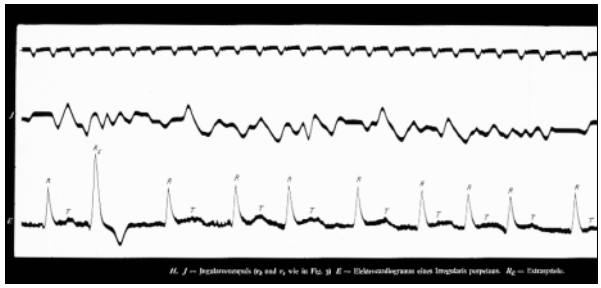
## Historic electrocardiographic recording from 1908



**Electrocardiographic recordings of atrial fibrillation came shortly after the string galvanometer was invented in 1901. This image is the first published electrocardiographic recording of atrial fibrillation showing F waves. The upper tracing is the timer, the middle tracing is the jugular-venous pulse recording, and the lower tracing is the electrocardiogram. (Hering HE. Das Elektrocardiogramm des Irregularis perpetuus. *Deutsches Archiv für Klinische Medizin.* 1908;94:205-8).**