Culprit Factors for the Failure of Well-Conducted Warfarin Therapy to Prevent Ischemic Events in Patients With Atrial Fibrillation
The Role of Homocysteine

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Background and Purpose—In patients with atrial fibrillation (AF), oral anticoagulant therapy (OAT) is effective in reducing stroke and embolism. However, despite OAT, ischemic events do occur in some patients. Studies specifically addressing the identification of risk factors for ischemic events during well-conducted OAT are not available. In this study, we prospectively investigated the role of classic risk factors and homocysteine levels in the occurrence of ischemic complications in 364 AF patients on OAT.

Methods—The quality of anticoagulation levels and the occurrence of bleeding and thrombotic events were recorded.

Results—During follow-up (859 patient years) 21 patients had ischemic complications (rate 2.4/100 patient-years). Homocysteine plasma levels were higher in these patients than in patients without ischemic complications during OAT (P<0.01), whereas no difference was observed in relation to the quality of OAT. The presence of a history of previous ischemic events, hypertension, and homocysteine plasma levels over the 90th percentile were all associated with an increased risk of ischemic events during OAT (odds ratio [OR]=7, 4.5, and 4.7, respectively). The coexistence of these risk factors markedly increased the risk (OR=13.1; 95% CI, 3.7 to 45.7; P=0.001).

Conclusion—In conclusion, our results indicate that AF patients with multiple risk factors may not be sufficiently protected by OAT, even when this is well conducted. (Stroke. 2005;36:2159-2163.)

Key Words: atrial fibrillation ■ homocysteine ■ oral anticoagulant therapy ■ stroke
Florence. Patients’ demographic and clinical data were collected. A computerized program (PARMA System, Instrumentation Laboratory) was used for the routine management of OAT. At each follow-up visit, OAT was monitored by prothrombin time expressed as international normalized ratio (INR), determined by capillary blood test (Thrombotest, Nycomed Pharma AS). During each follow-up visit, INR, dose prescription, hospital admissions, intercurrent illnesses, bleeding, and thrombotic events were recorded. Patients who missed check-ups for >2 months were contacted (personally or through their family or general practitioner), and the reason for interrupting treatment monitoring was recorded. In the case of death, additional information about its cause was requested. When this information was lacking, the National Register of causes of death and autopsy results (if available) were consulted. Data were censored after the first major complication, after the cessation of OAT, or when a patient stopped being monitored by our anticoagulation clinic. A software program, kindly provided by Dr Fritz R. Rosendaal (University Hospital, Leiden, the Netherlands), was used for the assessment of the quality of anticoagulation by determining the percentage time spent at different INR levels.

The occurrence of all types of bleeding and ischemic complications was recorded. INR was defined as temporally related to the adverse event when it was obtained at the time of the event or during the preceding 8 days. Bleeding was classified as major when fatal, intracranial (documented by imaging), ocular causing blindness, articular, or retroperitoneal, when surgery or transfusion of >2 blood units were required or when hemoglobin was reduced by >2 g/dL. Stroke was defined as a syndrome characterized by rapidly developing clinical symptoms and/or signs of focal and at times global loss of brain function, lasting >24 hours, and with no apparent cause other than vascular. Ischemic stroke was defined as a stroke with either a normal brain computed tomography or evidence of a recent infarction in the clinically relevant area of the brain on a computed tomography or magnetic resonance scan within 3 weeks of the event, whereas previous TIA was diagnosed when neurological defects lasted <24 hours. Peripheral embolism was diagnosed when proved with angiography or thrombectomy.

The presence of traditional cardiovascular risk factors and characteristics associated with ischemic complications in nonvalvular AF was assessed on the basis of patients’ interview, echocardiography, and hospital records. Hypertension was defined in the presence of blood pressure >130/80 mm Hg and/or an antihypertensive treatment and diabetes was defined according to American Diabetes Association criteria. Coronary artery disease was defined on the basis of a history of myocardial infarction or stable and unstable angina. Impaired left ventricular function was defined as a recent diagnosis of congestive heart failure or a fractional shortening <25% by transthoracic echocardiography. Exclusion criteria for patients were a significant occlusion (>70%) of the carotid arteries or the presence of renal failure (serum creatinine >1.3 mg/dL).

All of the patients underwent an ECG and transthoracic echocardiography. At enrollment, venous blood was collected from the basilic vein after overnight fasting. Total homocysteine (free and protein bound) was determined in plasma by high-performance liquid chromatography, and C677T MTHFR polymorphism, vitamin B12, vitamin B6, and folates were determined as described previously. All of the subjects gave their informed consent, and the investigation was approved by the institutional committee on human research.

**Statistical Analysis**

The STATA statistical software package (STATA Corporation; version 7.0) was used for data processing. Data are expressed as median and range because of their skewed distribution. Preliminary statistical analysis was performed using Wilcoxon signed rank test or Fishers exact test (categorical data). The nonparametric Mann–Whitney test was used for comparison between individual groups. The independent effect of various possible risk factors, sex, and age were investigated by performing the incidence rate ratio. For the analysis of ischemic events in relation to the time of the event, Cox regression analysis was used with homocysteine plasma levels above the 90th percentile for our population (>23.1 μmol/L) as the independent variables. We analyzed the interval from the beginning of OAT to the occurrence of ischemic events (uncensored observations) or to the cessation of warfarin therapy (censored observation) in order to estimate the probability of ischemic events as a function of time, according to the method of Kaplan–Meier. The probability of embolic events was compared among the groups with use of the log-rank test.

To evaluate the association between the occurrence of embolic events during OAT either with classic or with emerging risk factors, a univariate analysis was initially performed. Logistic regression analysis was used to determine the multivariate predictors of adverse events. All odds ratios (ORs) are given with their 95% CIs, and a value of P<0.05 was chosen for statistical significance.

**Results**

We studied 364 AF patients referred to our anticoagulation clinic for the management of OAT (Table 1). Patients were followed up for 859 patient-years (pt/ys). Patients were assessed for the quality of anticoagulant treatment: time spent within, above, and below the intended therapeutic range was 70, 14, and 16%, respectively. Total mortality rate during follow-up was 1.7×100 pt/ys. Three patients died of cerebral bleeding (0.35×100 pt/ys), 1 patient (0.1×100 pt/ys) had a fatal stroke, 8 patients (0.9×100 pt/ys) had cardiovascular death, and 3 patients (0.35×100 pt/ys) died of cancer. Eight major bleeding events were recorded (rate 0.9×100 pt/ys), in particular, 2 patients had gastrointestinal bleeding, and 6 patients had cerebral bleeding (3 of which were fatal). Patients who had bleeding complications were 6 years older than patients without bleeding (mean age, 79.1±3.8 years).
We analyzed the characteristics of patients who developed thrombotic events during OAT. They showed a higher prevalence of arterial hypertension \((P=0.01)\) and of a positive history for ischemic events with respect to the other patients \((P<0.001; \text{Table 1})\). The univariate analysis confirmed these data \((\text{Table 2})\). In relation to hypertension, patients showed a similar intensity of treatment; in particular, the percentage of patients taking \(<3\) or \(\geq 3\) antihypertensive drugs was not different from those without events \((P=0.32)\). No difference was found in relation to the presence of diabetes mellitus, left ventricular dysfunction, or coronary artery disease.

Homocysteine plasma levels were significantly higher in patients with an ischemic event before starting OAT with respect to patients without ischemic event \([15.4 (7.3 to 54.1)\) and \(13.9 (1.9 to 49)\), respectively; \(P=0.01)\). Homocysteine was also significantly higher \((P=0.01)\) in patients who developed ischemic events during OAT than in patients who did not \((\text{Table 1})\). Homocysteine plasma levels above the 90th percentile for our population \((>23.1 \ \mu\text{mo/L})\) were significantly more frequent in patients with ischemic events during OAT than in the other patients \((33.3\% \text{ versus } 9.6\%; \ P<0.002)\). Univariate analysis of different risk factors for ischemic events is reported in \(\text{Table 2}\). A survival analysis with the Kaplan-Meier curves confirms that the overall risk of ischemic events was significantly higher among patients with elevated homocysteine levels \((P=0.006; \text{Figure 1})\).

There were no differences in homocysteine plasma levels with respect to the presence or absence of hypertension \([14.8 [1.9 to 54.1] \text{ versus } 14.3 [6.7 to 38.6] \ P=0.3)\), diabetes \((14.8\%\) and \(14.8\%\) \(P=0.3)\), and obesity \((21.8\%\) and \(21.8\%\) \(P=0.3)\).

### Table 2. ORs for Ischemic Events During OAT According to Hypertension, Previous Ischemic Event, and Hyperhomocysteinemia (Univariate and Multivariate Analysis)

<table>
<thead>
<tr>
<th>Analysis</th>
<th>OR (95% CI)</th>
<th>(P) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Univariate</strong></td>
<td></td>
<td></td>
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<tr>
<td>Hypertension</td>
<td>4.5 (1.3–15.6)</td>
<td>0.01</td>
</tr>
<tr>
<td>Previous ischemic event</td>
<td>7 (2.3–21.4)</td>
<td>0.001</td>
</tr>
<tr>
<td>Hyperhomocysteinemia &gt;90th percentile</td>
<td>4.7 (1.7–12.6)</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Multivariate</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>4.8 (1.3–17.3)</td>
<td>0.016</td>
</tr>
<tr>
<td>Previous ischemic event</td>
<td>6.1 (1.9–19.2)</td>
<td>0.002</td>
</tr>
<tr>
<td>Hyperhomocysteinemia &gt;90th percentile</td>
<td>4.2 (1.4–12.0)</td>
<td>0.007</td>
</tr>
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</table>

73.1±8.2 years; \(P=0.02\). No difference was recorded in relation to the quality of OAT between these patients and all of the other AF patients.

Twenty-one patients \((\text{rate } 2.4\times100 \text{ pt/ys})\) had ischemic complications during follow-up: 11 patients had stroke \((1\text{ fatal})\), 7 had TIA, and 3 had peripheral embolism. No difference in ischemic complications was recorded in relation to the quality of OAT or in relation to age between these patients and the other patients who had no ischemic complications \((P=0.1)\). Warfarin dosage per week was similar between patients with or without ischemic events during OAT \([18.7 \text{ mg (5 to 51.25 mg) and 22.5 mg (1.5 to 72.5 mg), respectively; } \ P=0.2\). Two patients had both ischemic and bleeding complications during follow-up.

We analyzed the cumulative probability (Kaplan–Meier curves) of ischemic event in relation to homocysteine levels. Solid line, Group 1, patients on OAT with ischemic event and homocysteine >90\(^\text{th}\) percentile. Dotted line, Group 2, patients on OAT with ischemic event and homocysteine <90\(^\text{th}\) percentile. Group 1 vs group 2 \(P=0.006\).
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patients with a history of arterial hypertension and diabetes. Patients with previous stroke and a lower proportion of the ISCOAT patients were younger, with only 16% of their population showed different clinical characteristics and was at lower risk of stroke than our population. In particular, the copresence of arterial hypertension, which confers a 7-fold risk of developing a new event.

Moreover, in our population, ≈60% of patients were affected by arterial hypertension, which is also associated with an increased risk of stroke. Hypertension has been considered an underlying factor in ≈70% of strokes, and it is well documented that the treatment of hypertension reduces cardiovascular morbidity and mortality. In our patients, hypertension was associated with a >4-fold risk of developing ischemic complications on OAT, although all of the hypertensive patients were also on blood pressure lowering treatment. Although data on blood pressure control for all of the patients are not available, no differences were found in relation to the number of antihypertensive drugs used between patients with and without ischemic events during follow-up.

Previous reports have identified hyperhomocysteinemia as a possible risk factor for stroke both in sinus rhythm and in AF patients. Moreover, other authors have outlined the role of hyperhomocysteinemia in stroke recurrence and in the presence of left atrial thrombus in AF patients with acute stroke. Our results show that the concentration of this toxic amino acid was significantly higher in AF patients with a history of previous stroke before starting OAT. A concentration of homocysteine above the 90th percentile for our population confirmed these parameters as independent risk factors for the occurrence of ischemic complications in AF patients on OAT (Table 2).

We additionally analyzed patients who had the copresence of the 3 above-mentioned risk factors. When multiple risk factors were present, the ORs for the occurrence of ischemic events on OAT were higher than in the presence of a single risk factor. In particular, the copresence of arterial hypertension, history of previous ischemic events, and homocysteine above the 90th percentile for our population markedly increased the risk (OR, 13.1; Table 3).

The serum levels of vitamin B6, vitamin B12, and folic acid tended to be lower in patients with ischemic events during OAT than in the other patients, but this difference did not reach statistical significance. The prevalence of MTHFR C677T (C677T) genotype was similar in the 2 groups. A multivariate analysis adjusted for the history of previous thrombotic events, arterial hypertension, and homocysteine above the 90th percentile for our population confirmed these parameters as independent risk factors for the occurrence of ischemic complications in AF patients on OAT (Table 2).

The results of this prospective study show that AF patients carrying additional risk factors for thromboembolism had a high rate of ischemic events even when OAT was well conducted. In our population we recorded 2.4×100 pt/yr rate of ischemic events during OAT. The ISCOAT study found a lower incidence of stroke in AF patients on OAT. However, their population showed different clinical characteristics and was at lower risk of stroke than our population. In particular, the ISCOAT patients were younger, with only 16% of patients with previous stroke and a lower proportion of patients with a history of arterial hypertension and diabetes. In contrast, ≈40% of our patients had suffered from a previous ischemic event before starting OAT, and the classic risk factors for stroke were present in a higher percentage.

In our study, patients were followed up in an anticoagulation clinic, and the quality of anticoagulation was good, reaching ≈70% of time spent within the intended therapeutic range. The rate of bleeding complications was relatively low (0.9×100 pt/yr), which would, thus, confirm the safety of OAT if well conducted, as has been reported previously.

However, despite the high-clinical quality of OAT, 21 patients experienced ischemic complications. The analysis of risk factors associated with these events on OAT revealed the high incidence of previous ischemic events in these patients, which confers a 7-fold risk of developing a new event.

<p>| TABLE 3. ORs for Ischemic Events During OAT According to the Contemporary Presence of Multiple Risk Factors (Univariate Analysis) |</p>
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<th>Analysis</th>
<th>OR</th>
<th>95% CI</th>
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<tr>
<td>Hypertension + previous ischemic event</td>
<td>7.1</td>
<td>2.8–18.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Hypertension + hyperhomocysteinemia (&gt;90th percentile)</td>
<td>6.6</td>
<td>2.2–19.3</td>
<td>0.001</td>
</tr>
<tr>
<td>Hypertension + previous ischemic event + hyperhomocysteinemia (&gt;90th percentile)</td>
<td>13.1</td>
<td>3.7–45.7</td>
<td>0.001</td>
</tr>
</tbody>
</table>

1.9 to 37.3] versus 14.6 [6.2 to 54.1]; P=1), or ischemic heart disease (15.1 [8.5 to 34.5] versus 14.5 [1.9 to 54.1]; P=0.2).

Discussion
The results of this prospective study show that AF patients carrying additional risk factors for thromboembolism had a high rate of ischemic events even when OAT was well conducted. In our population we recorded 2.4×100 pt/yr rate of ischemic events during OAT. The ISCOAT study found a lower incidence of stroke in AF patients on OAT. However, their population showed different clinical characteristics and was at lower risk of stroke than our population. In particular, the ISCOAT patients were younger, with only 16% of patients with previous stroke and a lower proportion of patients with a history of arterial hypertension and diabetes. In contrast, ≈40% of our patients had suffered from a previous ischemic event before starting OAT, and the classic risk factors for stroke were present in a higher percentage.

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Moreover, in our population, ≈60% of patients were affected by arterial hypertension, which is also associated with an increased risk of stroke. Hypertension has been considered an underlying factor in ≈70% of strokes, and it is well documented that the treatment of hypertension reduces cardiovascular morbidity and mortality. In our patients, hypertension was associated with a >4-fold risk of developing ischemic complications on OAT, although all of the hypertensive patients were also on blood pressure lowering treatment. Although data on blood pressure control for all of the patients are not available, no differences were found in relation to the number of antihypertensive drugs used between patients with and without ischemic events during follow-up.

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It is well known that the pathogenesis of stroke in individual patients is often difficult to elucidate. Approximately 30% of strokes remain cryptogenic despite adequate evaluation, and strokes occurring in AF patients are not necessarily caused by cardioembolism. Optimal preventive therapy is determined on the basis of the results of randomized clinical trials, which have demonstrated that AF patients have a lower risk of stroke occurrence on warfarin (at dose adjusted to maintain INR between 2 and 3) than on aspirin. The use of a higher target INR for secondary prevention of stroke occurring during well-conducted OAT has been proposed. Unfortunately, this practice is associated with an increase in the risk of major bleeding and, therefore, is not recommended. The association of warfarin and low-dose aspirin could be a therapeutic strategy, which might be useful to evaluate in these patients. Adding low-dose aspirin to OAT at an adjusted dose has been demonstrated to be safe without a serious increase in bleeding risk. Additional studies with combination therapy would be useful to better define the role of this treatment. It is to be hoped that additional studies will be undertaken to investigate the possibility of new combinations of antithrombotic drugs (such as aspirin plus clopidogrel) to additionally decrease the rate of thrombotic events in AF patients.

In our population, the MTHFR polymorphism seems not to be associated with an increased risk of ischemic events. It is interesting to note that vitamin serum levels were slightly, even if not significantly, lower in patients with ischemic
events during OAT than in patients without ischemic events. The possible role of homocysteine reduction by vitamin supplementation as adjunctive preventive strategy in high-risk AF patients should be investigated. Until now, studies so far undertaken on the efficacy of homocysteine reduction by vitamin supplementation have failed to produce reliable results.28,29 In fact, the Vitamin Intervention for Stroke Prevention28 trial was conducted on a population without the necessary statistical strength to demonstrate convincingly the failure of vitamin supplementation to reduce the risk of stroke.

In conclusion, our study indicates that, in addition to hypertension and a history of previous ischemic events, hyperhomocysteinemia enables the identification of a group of patients at high risk of ischemic complications during well-conducted OAT. These patients should be the object of special consideration for the correction of their modifiable risk factors and for the best possible clinical management of antithrombotic treatment.

References
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