Enhanced Thrombogenesis but Not Platelet Activation Is Associated With Transcatheter Closure of Patent Foramen Ovale in Patients With Cryptogenic Stroke

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Background and Purpose—No studies have yet determined whether antiplatelet or anticoagulant therapy is the more appropriate treatment after transcatheter closure of patent foramen ovale (PFO) in patients with cryptogenic stroke. The objective of this study was to prospectively evaluate the presence, degree, and timing of activation of the platelet and coagulation systems after transcatheter closure of PFO in patients with cryptogenic stroke.

Methods—Twenty-four consecutive patients (mean age, 44 ± 10 years; 11 men) with previous cryptogenic stroke who had undergone successful transcatheter closure of PFO were included in the study. Prothrombin fragment 1 + 2 (F1 + 2) and thrombin–antithrombin III (TAT) were used as markers of coagulation activation, and soluble P-selectin and soluble CD40 ligand were used as markers of platelet activation. Measurements of all hemostatic markers were taken at baseline just before the procedure and at 7, 30, and 90 days after device implantation.

Results—F1 + 2 and TAT levels increased from 0.41 ± 0.16 nmol/L and 2.34 ± 1.81 ng/mL, respectively, at baseline to a maximal value of 0.61 ± 0.16 nmol/L and 4.34 ± 1.83 ng/mL, respectively, at 7 days, gradually returning to baseline levels at 90 days (P < 0.001 for both markers). F1 + 2 and TAT levels at 7 days after PFO closure were higher than those obtained in a group of 25 healthy controls (P < 0.001 for both markers). Levels of soluble P-selectin and soluble CD40 ligand did not change at any time after PFO closure.

Conclusions—Transcatheter closure of PFO is associated with significant activation of the coagulation system, with no increase in platelet activation markers. These findings raise the question of whether optimal antithrombotic treatment after PFO closure should be short-term anticoagulant rather than antiplatelet therapy. (Stroke. 2007;38:100-104.)

Key Words: coagulation ■ cryptogenic stroke ■ patent foramen ovale ■ platelets ■ transcatheter closure

Many studies have associated cryptogenic stroke with the presence of patent foramen ovale (PFO) in patients ≤55 years old, with paradoxical embolism being the pathophysiological mechanism suggested in these cases.1 Even though there is no definite evidence that PFO is associated with an increased risk for recurrent stroke among medically treated patients with cryptogenic stroke and that no prospective studies to date have shown the superiority of PFO closure compared with medical treatment in the prevention of stroke recurrences, transcatheter PFO closure has been used increasingly in the past several years as the treatment for these patients.2 However, neurological event recurrences still occur after PFO closure, with a reported annual event rate ranging from 0.7% to 3.6%.3–13 Also, most of these events occur within the year after PFO closure, and a significant proportion of them (20% to 66%) occur within the first weeks after the procedure.3–10 Antiplatelet treatment with aspirin instead of anticoagulation has been used extensively after transcatheter closure of PFO,3–13 and the addition of clopidogrel to aspirin has been increasingly observed.6,11 However, there is no biological basis supporting this approach, and no clinical studies have evaluated which is the more adequate antithrombotic treatment (antiplatelet versus anticoagulant) after device implantation.

Platelet and coagulation activation can be detected by several biological markers. Thus, soluble P-selectin (sP-selectin) and soluble CD40 ligand (sCD40L) have been well validated as markers of platelet activation,14,15 and prothrombin fragment 1 + 2 (F1 + 2) and thrombin–antithrombin III (TAT), as markers of coagulation system activation,16 The aim of this study was to prospectively determine the presence, degree, and timing of activation of the platelet and coagula-
tion systems after transcatheter closure of PFO in patients with cryptogenic stroke, as assessed by measuring the serum markers of platelet (sP-selectin, sCD40L) and coagulation (F1+2, TAT) activation.

Patients and Methods

Study Population

From June 2003 to August 2005, a total of 47 patients ≤55 years old were diagnosed with cryptogenic stroke or transient ischemic attack in 2 neurology departments. The diagnosis of cryptogenic stroke was established after a systematic etiological work-up, including brain computed tomography and/or magnetic resonance imaging, routine blood tests, a detailed coagulation study (including protein C, protein S, antithrombin III, antiphospholipid antibodies, factor V Leiden, and prothrombin variant G20210A), 12-lead ECG, echocardiography, 24-hour Holter ECG, extracranial Doppler ultrasonography with frequency analysis and B-mode imaging, and cerebral computed tomography and/or magnetic resonance angiography. Definite causes of stroke included significant large-artery atherosclerosis (≥50% stenosis), lacunar stroke, cardioembolic causes, complex atheromas of the aortic arch, nonatherosclerotic arteriopathies, and coagulopathies. Transesophageal echocardiography with contrast study was performed in all cases, and 26 patients were diagnosed as having a PFO. Twenty-four of these 26 patients underwent (on the decision of the neurologist responsible for the patient) PFO transcatheter closure and formed the study population. All procedures were performed at least 3 months after the neurological event, via a femoral approach, under general anesthesia and with transesophageal echocardiography guidance. Full anticoagulation with sodium heparin (100 U/kg) was used during the procedure in all cases. The Amplatzer PFO occluder (AGA Medical Corp) was the device implanted in all patients. Patients were treated with aspirin 325 mg before the procedure and continued with the same treatment throughout the study period. Patients treated with anticoagulants and those who developed any vascular access complication were excluded. Clinical follow-up and device implantation were kept on ice for a maximum of 2 hours before centrifugation at 2000×g for 15 minutes. Plasma and serum were pipetted into plastic vials in aliquots and stored at −70°C until analysis. Enzyme immunoassays were used for determining laboratory levels of F1+2, TAT, (Stago), sP-selectin (R&D Systems), and sCD40L (R&D Systems). All of the same measurements were performed in a group of 25 healthy subjects matched for age and sex (control group). Intra-assay coefficients of variation for all ELISAs were <5%, and interassay variences were <10%.

Statistical Analysis

Categorical data are expressed as percentages, and continuous variables are expressed as mean±SD. Categorical variables of the study and control groups were compared with the χ² test, and continuous variables were compared with Student t test. An ANOVA for repeated measures was performed to test for equal means at different times. Statistical significance was assumed with a P value <0.05. A commercially available statistical package (SAS Institute) was used for all analyses.

Results

Baseline demographics of the study population are shown in Table 1, and anatomic and procedural features are listed in Table 2. The PFO was successfully closed with 1 device in all cases, and no complication occurred during the procedure. At 3 months’ follow-up, no thromboembolic events, device thromboses, or any other complications were observed.

Results of Coagulation and Platelet Activation Assessment

The results of coagulation system activation as assessed by F1+2 and TAT variations are shown in the Figure. Mean baseline levels of F1+2 and TAT were 0.41±0.16 nmol/L and 2.34±1.81 ng/mL, respectively. The ANOVA for repeated measures showed a significant change in both F1+2 and TAT levels after PFO closure (P<0.001 for both markers). Thus, F1+2 levels had increased by 64% by day 7 (95% CI, 39% to 90%) and gradually decreased to 15% higher than baseline by day 30 (95% CI, 17% to 73%), with a complete return to baseline values by day 90. TAT levels increased by 140% from baseline to day 7 (95% CI, 89% to 191%) but had returned completely to baseline levels at days 30 and 90. Mean values of F1+2 and TAT of the control group were 0.40±0.13 nmol/L and 1.96±0.94 ng/mL, respectively, with no differences compared with the baseline values of both groups.

Table 1. Clinical Characteristics of the Study Population (n=24)

<table>
<thead>
<tr>
<th>Age, mean±SD, y (range)</th>
<th>44±10 (23–55)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, n (%)</td>
<td>13 (54)</td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>4 (17)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>2 (8)</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Dyslipidemia, n (%)</td>
<td>5 (21)</td>
</tr>
<tr>
<td>Family history of cardiovascular events, n (%)</td>
<td>9 (37)</td>
</tr>
<tr>
<td>Use of oral contraceptives, n (%)</td>
<td>2 (8)</td>
</tr>
<tr>
<td>History of migraine, n (%)</td>
<td>6 (25)</td>
</tr>
<tr>
<td>Prior stroke, n (%)</td>
<td>4 (17)</td>
</tr>
<tr>
<td>Type of event, n (%)</td>
<td></td>
</tr>
<tr>
<td>Stroke</td>
<td>17 (71)</td>
</tr>
<tr>
<td>Transient ischemic attack</td>
<td>7 (29)</td>
</tr>
<tr>
<td>Months since stroke/transient ischemic attack, mean±SD</td>
<td>6±5</td>
</tr>
</tbody>
</table>

Table 2. Anatomic and Procedural Features

<table>
<thead>
<tr>
<th>PFO diameter, mean±SD, mm</th>
<th>2.9±1.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous right-to-left shunt, n (%)</td>
<td>9 (38)</td>
</tr>
<tr>
<td>Degree of shunt, n (%)</td>
<td></td>
</tr>
<tr>
<td>Small (&lt;20 microbubbles)</td>
<td>4 (17)</td>
</tr>
<tr>
<td>Large (≥20 microbubbles)</td>
<td>20 (83)</td>
</tr>
<tr>
<td>Atrial septal aneurysm, n (%)</td>
<td>9 (37)</td>
</tr>
<tr>
<td>Eustachian valve, n (%)</td>
<td>7 (29)</td>
</tr>
<tr>
<td>Amplatzer PFO device size, n (%)</td>
<td></td>
</tr>
<tr>
<td>25 mm</td>
<td>21 (88)</td>
</tr>
<tr>
<td>35 mm</td>
<td>3 (12)</td>
</tr>
<tr>
<td>Residual postprocedural shunt (immediate), n (%)</td>
<td>10 (41)</td>
</tr>
<tr>
<td>Residual shunt at 3 months’ follow-up, n (%)</td>
<td>1 (4)</td>
</tr>
</tbody>
</table>
markers in the study group. Both F1+2 and TAT levels at day 7 after PFO closure were significantly higher than those of the control group (P<0.001 for both markers). The upper normal limits (mean±2SDs, control group) for F1+2 and TAT levels were 0.66 nmol/L and 3.84 ng/mL, respectively, and 50% of the study patients had F1+2 and/or TAT levels above the upper normal limits at day 7 after PFO closure. None of the clinical (age, sex, cardiovascular risk factors, number of events, and time since stroke), echocardiographic (atrial septal aneurysm, eustachian valve, diameter of PFO, and severity of shunt), or procedural (size of the device and residual shunt) variables were correlated with the degree of increase in both F1+2 and TAT levels.

The results of platelet activation as assessed by sP-selectin and sCD40L levels are shown in the Figure. The ANOVA for repeated measures showed no significant changes in sP-selectin (baseline, 36±19 ng/mL, P=0.579) and sCD40L (baseline, 247±93 pg/mL, P=0.245) at any time after PFO closure. Mean values of sP-selectin and sCD40L of the control group were 32±10 ng/mL and 194±148 pg/mL, respectively, with no differences at any time with the values of sP-selectin and sCD40L obtained in the study group (P>0.10 for both markers).

Discussion

The results of this study demonstrate that transcatheter closure of PFO with the Amplatzer PFO device induces significant activation of the coagulation system, as assessed by F1+2 and TAT levels, which reached maximal levels 7 days after the procedure, gradually returning to baseline by day 90. On the other hand, implantation of the device was not associated with any increase in platelet activation, as assessed by sP-selectin and sCD40L levels.

Experimental studies have demonstrated that the Amplatzer PFO occluder is partially endothelialized 1 month after implantation and completely covered by neendothelial cells at 3 months. Thus, the device is exposed to circulating blood during the first weeks after PFO closure and is potentially much more thrombogenic during this period of time. The results of this study show that enhanced thrombin generation is the main hemostatic effect associated with transcatheter closure of PFO, most likely related to the deposit of fibrin at the interface between blood and the device, and these results are comparable to those obtained after transcatheter closure of atrial septal defects. All patients included in the present study were prescribed aspirin treatment, and one might wonder whether this could have influenced the results regarding platelet activation. It is well known that aspirin exerts its antithrombotic effect by inhibiting platelet aggregation, and many studies have demonstrated the absence of any effect of aspirin on platelet activation. Also, increased platelet activation, despite aspirin treatment, has been shown in many prothrombotic disorders.

No studies to date have determined the most appropriate antithrombotic treatment after transcatheter closure of PFO, and the choice of antithrombotic treatment after this procedure has been empirically determined, with aspirin the therapy most frequently used in these cases. Thromboembolic events during antiplatelet treatment occur in 1.6% to 8.2% of patients within the year after PFO closure.

Furthermore, almost half of these events occur within the first month after the procedure, suggesting a relation with the
prothrombotic status generated by the presence of a nonendothelialized device at the atrial level.\textsuperscript{3,5,7} Windecker et al\textsuperscript{2} retrospectively compared medical treatment (antiocoagulant or antiplatelet) with percutaneous closure followed by aspirin therapy in 308 patients with PFO and cryptogenic stroke. At 4 years' follow-up, patients who had undergone PFO closure tended to have a lower risk of cerebrovascular events compared with those treated medically (7.8\% versus 22.2\%). However, the risk of recurrence tended to be higher in the PFO closure group within the first year of follow-up (4.6\% versus 3.7\%), suggesting an excess of thromboembolic events related to the PFO device itself. Also, many cases of device thrombosis have been reported within the first months after septal closure device placement (including atrial septal closure devices), with an incidence of up to 7\%, depending on the device.\textsuperscript{25,26} Importantly, thrombus formation occurs in the left atrial side of the device in most cases,\textsuperscript{25} and its presence has been associated with a higher risk of thromboembolic events.\textsuperscript{26} Interestingly, most of the patients who experienced device thrombosis were receiving antiplatelet treatment, and most of them were successfully treated with heparin or warfarin. Thrombus formation has been reported for all types of commercially available septal closure devices, including the Amplatzer device,\textsuperscript{25} even though a lower incidence of device thrombosis has been suggested for this device.\textsuperscript{26} The results of the present study suggest that short-term anticoagulation (1 to 3 months) might be the most appropriate antithrombotic treatment after transcatheter PFO closure. Anticoagulation would be more efficient in reducing the enhanced thrombogenic status associated with the PFO device, and once device endothelialization is completed and no residual shunt is observed, anticoagulant treatment could probably be switched to antiplatelet therapy or even no antithrombotic treatment. Some authors have empirically suggested the addition of clopidogrel to aspirin for reducing antithrombotic treatment after transcatheter PFO closure.\textsuperscript{9} However, no prospective clinical data support such an approach, nor do the findings of the present study favor the implementation of additional antiplatelet treatment in these cases. Moreover, this antiplatelet combination has been recently discouraged for ischemic stroke or transient ischemic attack patients (class III, level of evidence A).\textsuperscript{27} In any case, prospective studies should be done to determine the most appropriate and cost-effective antithrombotic treatment for these cases. In addition to the thromboembolic events potentially related to the PFO closure device, both the presence of residual shunt and the occurrence of atrial fibrillation after PFO closure might also be associated with an increased risk of thromboembolic events. Atrial fibrillation has been seen in \approx 10\% of patients within the first weeks after PFO closure,\textsuperscript{28} and it has been shown that the prothrombotic milieu associated with such an arrhythmia is mainly dependent on activation of the coagulation system, for which anticoagulant therapy has been demonstrated more efficient than antiplatelet therapy in preventing thromboembolic events.\textsuperscript{29,30} The presence of a residual shunt after PFO closure has also been associated with an increased risk of stroke recurrence.\textsuperscript{9} In fact, up to 20\% of deep venous thromboses have been demonstrated by magnetic resonance imaging or angiography in patients with cryptogenic stroke and PFO.\textsuperscript{31,32} and cases of pulmonary embolism have been reported within days after PFO closure.\textsuperscript{12} Thus, and in the absence of additional clinical data, anticoagulant therapy should especially be considered in the high-risk group of patients with significant residual shunt after PFO closure.

Despite the absence of clinically relevant arrhythmias in our study population, no Holter studies have been performed to detect silent paroxysmal arrhythmias after PFO closure, and we therefore cannot rule out the potential influence of undetected silent arrhythmias, especially atrial fibrillation, on hemostatic markers. Although cardiac catheterization induces a mild activation of the coagulation system, this returns to normal values within 24 hours after the procedure, and any influence of the procedure (venous puncture, insertion of catheters) on the final results seems quite unlikely.\textsuperscript{33,34}

In conclusion, transcatheter PFO closure induces a significant transient activation of the coagulation system, with no detectable effect on activation of the platelet system. These results raise the question of whether the optimal antithrombotic therapy after PFO closure should be short-term anticoagulation rather than antiplatelet treatment and underline the importance of carrying out prospective and adequately powered, randomized trials to determine the most appropriate antithrombotic therapy after PFO closure for the prevention of recurrent cerebrovascular events.

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**Disclosures**

None.

**References**

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