Patent Foramen Ovale, Cardiac Valve Thickening, and Antiphospholipid Antibodies as Risk Factors for Subsequent Vascular Events

The PICSS-APASS Study

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Background and Purpose—We sought to estimate risk of recurrent stroke/TIA/death in the subgroup of the Patent Foramen Ovale in the Cryptogenic Stroke Study (PICSS) cohort with patent foramen ovale (PFO) and antiphospholipid antibodies (aPL) and to estimate risk of recurrent stroke/TIA/death in aPL-positive patients who have thickened left-side heart valves (VaT). PFO is associated with cryptogenic ischemic stroke. Also, the presence of aPL is associated with ischemic cerebrovascular disease.

Methods—Combined data from 2 major substudies of the Warfarin Aspirin Recurrent Stroke Trial (WARSS) were evaluated. PICSS subjects were included if they were enrolled in the Antiphospholipid Antibodies and Stroke Study (APASS) and underwent a baseline aPL test (lupus anticoagulant, anticoagulant antibodies, or both) within 1 month of the stroke. All patients in PICSS underwent transesophageal echocardiography for PFO as well as VaT, which was performed blinded to aPL status and treatment arm (325 mg/day aspirin or adjusted dose warfarin; target international normalized ratio, 1.4–2.8). The primary outcome event was the 2-year risk of recurrent stroke/TIA/death and was evaluated using Cox proportional hazards model. Because there was no treatment effect, warfarin and aspirin groups were combined to increase power. For the combined end point, power to detect HR of 2 was 47.8% for the PFO and aPL-positive group, and 75.3% for the valve thickening and aPL-positive group, assuming 2-sided type I error of 0.05.

Results—Five hundred twenty-five subjects were tested for the combined presence of PFO and aPL and were available for evaluation. The primary outcome event rate was 23.9% (HR, 1.39; 95% CI, 0.75–2.59) in the PFO-positive/aPL-positive group, compared to 13.9% (HR, 0.83; 95% CI, 0.44–1.56) in the PFO-positive/aPL-negative group, and 19.9% (HR, 1.16; 95% CI, 0.68–1.90) in the PFO-negative/aPL-positive group. Five hundred forty-five subjects tested for combined presence of aPL and left-side cardiac VaT were available for evaluation. The primary event rate was 22.6% (HR, 1.65; 95% CI, 0.88–3.09) in the VaT-positive/aPL-positive group, compared to 19.4% (HR, 1.50; 95% CI, 0.82–2.75) in the VaT-positive/aPL-negative group, and 20.2% (HR, 1.63; 95% CI, 0.81–3.25) in the VaT-negative/aPL-positive group.

Conclusions—The combined presence of aPL either with a PFO or with left-side cardiac VaT did not significantly increase risk of subsequent cerebrovascular events in this PICSS/APASS cohort of patients. (Stroke. 2009;40:2337-2342.)

Key Words: anti-phospholipid antibodies ■ cardiac valve thickening ■ patent foramen ovale ■ stroke recurrence risk ■ stroke risk factors

Patent foramen ovale (PFO) is associated with cryptogenic ischemic stroke, which accounts for ≈20% to 40% of all ischemic strokes. Case-control studies have consistently shown this association, especially in patients younger than 55 years of age, although prospective cohort or population-based studies have not. Similarly, the presence of antiphospholipid antibodies (aPL) is associated with ischemic cerebrovascular disease. Many case-control studies and prospective cohort studies have shown an association between aPL and initial stroke, but the relationship to recurrent or subsequent stroke is more uncertain. If paradoxical embolism is responsible for the majority of
strokes in patients with PFO, then hypercoagulable states that increase the risk of deep vein thrombosis may be overrepresented in PFO patients with a stroke. Therefore, the association of stroke with the combined presence of PFO and aPL is of interest.

Left-side cardiac valve thickening, which is easily diagnosed by transesophageal echocardiogram (TEE), has been suspected to be a risk factor for ischemic stroke. Moreover, Libman Sacks endocarditis is associated with aPL in some patients and may be an important mechanism of stroke. Little is known about stroke recurrence when these risk factors occur in combination. Hence, we studied the risk of recurrent stroke and death associated with aPL and PFO, as well as aPL and thickened left-side heart valves.

Methods and Patients

The Patent Foramen Ovale in Cryptogenic Stroke Study (PICSS) and Antiphospholipid Antibodies and Stroke Study (APASS) were both collaborative studies with the Warfarin Aspirin Recurrent Stroke Study (WARSS). Both PICSS and the APASS studies relied on the WARSS for patient recruitment, as well as follow-up. Patients were included in the present post hoc analysis if they had a TEE test as part of the PICSS study, and also if they had tests for aPL status as part of the APASS study. Patients undergoing TEE were systematically evaluated for the presence of a PFO as well as thickened left-side cardiac (mitral or aortic) valves. WARSS was a double-blind multicenter trial comparing adjusted dose warfarin (international normalized ratio, 1.4–2.8) vs aspirin (325 mg/day) for prevention of stroke in patients with noncardioembolic ischemic stroke. Patients were followed-up for 2 years for occurrence of stroke or death. Details of the WARSS methodology and the results have been published previously. Briefly, patients were eligible for WARSS if they had an ischemic stroke within 30 days, were aged 30 to 85 years, had a moderate, mild, or no deficit (rating on Glasgow outcome scale ≤3), and had no contraindication to warfarin therapy. Patients were excluded if baseline international normalized ratio was >1.4. stroke was attributable to a procedure, or attributable to a high-grade carotid stenosis or cardioembolic source such as atrial fibrillation. At each WARSS center, the diagnostic stroke patients were asked to undergo TEE. PICSS also included patients from WARSS who underwent TEE for clinical reasons. Thus, the PICSS cohort included cryptogenic stroke patients as well as patients with other known stroke etiologies. The APASS study was a prospective study of the effect of aPL positivity on subsequent thromboembolism among patients in the WARSS study. Forty-four of the 48 WARSS centers participated in APASS. Blood was drawn for aPL testing within 1 month of the ischemic event. Institutional Review Board approvals were obtained at each site for the PICSS, APASS, and WARSS studies.

TEE Protocol

All patients underwent TEE according to a predetermined protocol, and the procedure is described in detail in the PICSS study. Biplane or multiplane probes were used and the tapes were analyzed by a single reader (Dr S. Homma) who was blinded to treatment, outcomes, and aPL status of the patients. The TEE protocol emphasized determination of cardioembolic sources including valve leaflet abnormalities with thickening. Patients were considered to have valve thickening if either the aortic or the mitral valve thickness was ≥2 mm. Presence of PFO was determined by agitated saline contrast study at rest and after Valsalva maneuver or cough. Presence of ≥1 microbubbles in the left atrium within 3 cardiac cycles was considered positive for presence of PFO.

Antiphospholipid Antibodies Assays

The blood for testing antiphospholipid antibodies was drawn at baseline before the patients were randomized for treatment in WARSS. This was within 30 days of stroke onset. All participating centers received video training of the procedure for drawing blood, storing, processing, and shipping of aPL samples. The tests were conducted at a central laboratory. Patients were tested once at baseline for presence of the lupus anticoagulant (LA) and for the presence of anticardiolipin antibodies (aCL). Tests for LA included a sensitive activated partial thromboplastin time (Diagnostic Stago Inc), dilute Russell viper venom test (American Diagnostics Inc), and a hexagonal phase confirmatory test (StaClot LA; Diagnostic Stago Inc). An LA test result was positive if the results of either the activated partial thromboplastin time or dilute Russell viper venom test was positive, or if the StaClot test was positive. Testing for aCL was performed using commercial enzyme-linked immunosorbent assay technique (Corgenix Inc).

Cutoff values for positive results were as follows: IgM >12 mcg/dL, IgG >21 mcg/dL, and IgA >23 mcg/dL. Patients were classified as aPL-positive if they tested positive at baseline blood draw for aCL alone (any isotype), LA alone, or both aCL and LA combined. Conversely, patients were aPL-negative if both LA and aCL tests were negative.

Assessment of Outcomes and End Points

Two groups of patients were evaluated separately: PFO and aPL, and thickened left-side heart valves and aPL. The primary end point was a composite of ischemic stroke, TIA, or death from any cause. Patients were followed-up for 2 years or until an end point occurred. The diagnosis of stroke was based on a new lesion on CT scan or MRI scan. When imaging was negative, a clinical syndrome lasting >24 hours was required for the diagnosis.

Statistical Analysis

The null hypotheses were: (1) the added presence of aPL positivity does not affect the risk of ischemic stroke, TIA, or death in stroke patients with PFO; and (2) the added presence of aPL positivity does not affect the risk of ischemic stroke, TIA, and death in stroke patients with left-side cardiac valve thickening.

In the WARSS study, there was no significant difference in the outcome of stroke or death at 2 years in the aspirin group compared to the warfarin group. Thus, for this analysis, the 2 groups were combined to increase power. The analysis addresses potential confounding because the aPL-positive and the aPL-negative groups were not randomly divided in the WARSS treatment groups. The variables tested included age, sex, hypertension, history of diabetes, history of cardiac disease, smoking status, obesity, alcohol consumption, and sedentary lifestyle. Variables were out of balance when the difference in means or proportions was significant at P<0.1 level. The means of 2 groups are compared with 2-sided t test and the proportions of 2 groups are compared with χ² test.

Reported event rates were actuarial estimates from the Kaplan–Meier curves that adjust for censoring. Differences among groups in the 2-year risk of the composite end point were calculated. A Cox proportional hazards model was used for calculating the HR and the 95% CI after adjusting for covariates that had P<0.2 in univariate analysis.

For the combined end point, power to detect a HR of 2 for the aPL-positive and PFO-positive group with the sample size 525 was 47.8% after adjustment of age, history of stroke, heart disease status, BMI, current smoking, sedentary lifestyle, and diabetes, assuming 2-sided type I error of 0.05. The power to detect a HR of 2 for the thickened left-side heart valve (VaT)-positive/aPL-positive group with the sample size 545 was 75.3% after adjustment of age, gender, history of stroke, heart disease status, BMI, current smoking, sedentary lifestyle, and diabetes, assuming 2-sided type I error of 0.05.
Results
The APASS study enrolled a total of 1770 patients and the PICSS study enrolled 630 patients. Subjects enrolled in both those studies formed the combined PICCS/APASS cohort, which was used for evaluation in the present study.

Presence of PFO and aPL
There were 525 subjects from the APASS/PICSS cohort available for evaluation. The baseline characteristics of the patients with and without PFO are described in Table 1. PFO was present in 175 and there were 350 subjects who did not have a PFO. The subjects were divided into 4 subgroups based on presence or absence of both PFO and aPL. The primary outcome event rates did not statistically differ in the various groups compared to the PFO-negative/aPL-negative group (Figure 1).

Hypertension, diabetes mellitus, and sedentary lifestyle as risk factors were more prevalent in the group without PFO and consequently were adjusted for in the multivariate analysis. The HR for combined risk of TIA, ischemic stroke, or death in the 3 groups were as follows: PFO-positive/aPL-positive group 1.39 (95% CI, 0.75–2.59), PFO-negative/aPL-positive group was 1.14 (95% CI, 0.68–2.01), and 0.83 (95% CI, 0.44–1.56) for the PFO-positive/aPL-negative group, all of which were not statistically significant (Table 3). This did not change even after adjusting for the variables, which were statistically different in the various subgroups.

Presence of Valve Thickening and aPL
The baseline characteristics of the patients with and without left-side cardiac valve thickening are described in Table 2. Of 545 subjects available for evaluation, 313 had left-side cardiac VaT, whereas 232 did not. Those with valve thickening were older, more likely to have diabetes mellitus, and more likely to be current smokers. Based on combined presence or absence of VaT and aPL, the subjects were divided into 4 subgroups. The primary outcome event rates did not differ significantly in the different subgroups when compared to the VaT-negative/aPL-negative group. The HR for the risk of TIA, ischemic stroke, or death in the various subgroups were: 1.65 (95% CI, 0.88–3.09) for VaT-positive/aPL-positive group, 1.50 (95% CI, 0.82–2.75) for the VaT-positive/aPL-negative group, and 1.63 (95% CI, 0.81–3.25) for the VaT-negative/aPL-positive group (Table 3). This did not change even after adjusting for the variables, which were statistically different in the various subgroups.

Table 1. Patient Characteristics of the PFO/aPL Combination Group

<table>
<thead>
<tr>
<th>Variables</th>
<th>Entire Group, N=525</th>
<th>No PFO/aPL, N=250</th>
<th>PFO Present, N=275</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, yr, ±SD</td>
<td>59.2±12.1</td>
<td>60.3±11.7</td>
<td>59.48±11.1</td>
</tr>
<tr>
<td>Male (%)</td>
<td>282 (53.7%)</td>
<td>81 (55.5%)</td>
<td>101 (49.5%)</td>
</tr>
<tr>
<td>HTN, n=519</td>
<td>317 (61.1%)</td>
<td>125 (61.9%)</td>
<td>100 (70.4%)</td>
</tr>
<tr>
<td>Diabetes, n=524</td>
<td>146 (27.9%)</td>
<td>51 (34.9%)</td>
<td>59 (28.9%)</td>
</tr>
<tr>
<td>Heart disease</td>
<td>98 (18.7%)</td>
<td>27 (18.5%)</td>
<td>40 (19.6%)</td>
</tr>
<tr>
<td>Current smoker, n=522</td>
<td>141 (27%)</td>
<td>41 (28.5%)</td>
<td>55 (27%)</td>
</tr>
<tr>
<td>Sedentary lifestyle, n=522</td>
<td>189 (36.2%)</td>
<td>61 (42.4%)</td>
<td>82 (40.2%)</td>
</tr>
<tr>
<td>Obesity, n=520</td>
<td>264 (50.8%)</td>
<td>77 (53.1%)</td>
<td>96 (47.3%)</td>
</tr>
</tbody>
</table>

| PFO/aPL−n=108               |                      |                   |
|                            | 62 (27%)             | 22 (20%)          |
| PFO/aPL+n=67               |                      |                   |
|                            | 9 (9.9%)             | 6 (6.7%)          |

Figure 1. Kaplan–Meier event-free survival curves for the patient group with PFO and aPL.
Results of aPL Testing
The aPL tests included testing for LA as well as aCL; 312 of the 525 patients tested were negative for both the LA and the aCL, whereas the remaining 213 were classified as aPL-positive (40.6%). Of these, 34 were positive for both LA and the aCL (6.5%), and the rest were positive for either the LA or the aCL test (34%).

Discussion
In this post hoc, exploratory analysis from the PICSS and APASS studies, we did not detect a significant increase in the risk of ischemic stroke/TIA/death in ischemic stroke patients with presence of PFO and aPL either individually or in combination compared to those without PFO or aPL. Similarly, the study failed to show a significant risk of recurrent stroke/TIA/ death in ischemic stroke patients who had presence of VaT and aPL either separately or in combination compared to patients with neither VaT nor aPL.

PFO are associated with cryptogenic stroke, and the mechanism is postulated to be paradoxical embolism, with clots forming and originating in the venous circulation and traveling to the arterial side via a right-to-left shunt. Systemic hypercoagulable states can potentially facilitate this process by increased formation of clots. In the PELVIS study, cryptogenic stroke patients were found to have increased evidence of pelvic vein thrombosis. The study did not look for hypercoagulable states, but did add to the notion that paradoxical embolism may be important in at least some patients with cryptogenic stroke.

The mechanisms of thromboembolic phenomena associated with aPL are heterogeneous, including immune-mediated coagulopathy and that resulting from left-side cardiac valvular lesions of thickening and Libman Sacks endocarditis. The cardiac valve thickening and endocarditis may be manifestations of the same pathological process, which is a subendothelial deposition of immunoglobulins including aCL and complements. Krause et al found significant associations between cardiac valve thickening and several nervous system manifestations, such as epilepsy, migraine, and strokes in patients with primary antiphospholipid antibody syndrome. Interestingly, they defined valve thickening to be present if it was >5 mm, whereas our study defined it to be >2 mm.

The study patients were part of the larger WARSS study and were randomized to receive aspirin or warfarin. It is conceivable that the effect of combination of aPL/PFO and aPL/VaT on patient outcome events could have been affected by the treatment. However, we did not find any significant treatment effect when comparing warfarin vs aspirin ($P<0.36$ for the aPL/PFO group and $P<0.88$ for the aPL/VaT group).

Table 2. Patient Characteristics of the Valve Thickening-aPL Combination Group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Entire Group, n=545</th>
<th>No Valve Thickening/aPL-Negative, n=143</th>
<th>No Valve Thickening/aPL-Positive, n=89</th>
<th>Valve Thickening/aPL-Negative, n=180</th>
<th>Valve Thickening/aPL-Positive, n=133</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>59.3±12.1</td>
<td>54.4±11.2</td>
<td>54.9±11.2</td>
<td>62.1±11.1</td>
<td>63.7±11.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male</td>
<td>294 (53.9%)</td>
<td>73 (51.1%)</td>
<td>58 (65.2%)</td>
<td>88 (48.9%)</td>
<td>75 (56.4%)</td>
<td>0.07</td>
</tr>
<tr>
<td>HTN, n=539</td>
<td>330 (61.2%)</td>
<td>82 (57.8%)</td>
<td>47 (54.7%)</td>
<td>111 (62.0%)</td>
<td>90 (68.2%)</td>
<td>0.17</td>
</tr>
<tr>
<td>Diabetes, n=544</td>
<td>151 (27.8%)</td>
<td>35 (24.7%)</td>
<td>15 (16.9%)</td>
<td>51 (28.4%)</td>
<td>50 (37.6%)</td>
<td>0.006</td>
</tr>
<tr>
<td>Heart disease</td>
<td>105 (19.3%)</td>
<td>26 (18.2%)</td>
<td>12 (13.5%)</td>
<td>34 (18.9%)</td>
<td>33 (24.8%)</td>
<td>0.20</td>
</tr>
<tr>
<td>Current smoker, n=522</td>
<td>147 (27.1%)</td>
<td>47 (32.9%)</td>
<td>30 (34.1%)</td>
<td>39 (21.8%)</td>
<td>31 (23.5%)</td>
<td>0.05</td>
</tr>
<tr>
<td>Sedentary lifestyle, n=542</td>
<td>197 (36.4%)</td>
<td>49 (34.3%)</td>
<td>28 (32.2%)</td>
<td>67 (37.4%)</td>
<td>53 (39.9%)</td>
<td>0.63</td>
</tr>
<tr>
<td>Previous stroke, n=507</td>
<td>70 (13.8%)</td>
<td>13 (9.9%)</td>
<td>14 (17.5%)</td>
<td>25 (14.7%)</td>
<td>18 (14.3%)</td>
<td>0.44</td>
</tr>
<tr>
<td>Obesity, n=520</td>
<td>274 (50.7%)</td>
<td>69 (49.3%)</td>
<td>48 (53.9%)</td>
<td>86 (48.0%)</td>
<td>71 (53.8%)</td>
<td>0.68</td>
</tr>
</tbody>
</table>
Table 3. Outcome Event Rates in Various Subgroups

<table>
<thead>
<tr>
<th>Event rate, N (%)</th>
<th>No PFO/APL-Negative, N=146</th>
<th>No PFO APL-Positive, N=204</th>
<th>PFO/APL-Negative, N=108</th>
<th>PFO/APL-Positive, N=67</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted HR (95% CI)</td>
<td>39 (19.1%)</td>
<td>29 (19.9%)</td>
<td>15 (13.9%)</td>
<td>16 (23.9%)</td>
</tr>
<tr>
<td>1.14 (0.68–1.90) P=0.91</td>
<td>0.83 (0.44–1.56) P=0.22</td>
<td>1.39 (0.75–2.59) P=0.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No VaT, N=232</td>
<td>No VaT/APL-Positive, N=89</td>
<td>VaT/APL-Negative, N=180</td>
<td>VaT/APL-Positive, N=133</td>
<td></td>
</tr>
<tr>
<td>Event rate, N (%)</td>
<td>22 (15.4%)</td>
<td>18 (20.2%)</td>
<td>35 (19.4%)</td>
<td>30 (22.6%)</td>
</tr>
<tr>
<td>Unadjusted HR (95% CI)</td>
<td>1.63 (0.81–3.25) P=0.36</td>
<td>1.50 (0.82–2.75) P=0.34</td>
<td>1.65 (0.88–3.09) P=0.12</td>
<td></td>
</tr>
</tbody>
</table>

Outcome event rates are composite of ischemic stroke/TIA or death in the different study groups (compared to no PFO/aPL negative and no VaT/aPL-negative groups, respectively. Cox proportional hazard ratio.

This study has an important limitation in that it is a post hoc, retrospective analysis and thus should only be used for hypothesis generation. The WARSS study did not systematically evaluate patients for hypercoagulable states (eg, proteins C and S and antithrombin III). Similarly, the patients were not evaluated for presence of subclinical deep venous thrombosis. Nonetheless, although there did seem to be a trend toward increased risk of the predetermined end point among the group of patients with combined PFO and aPL, as well as the VaT-positive/aPL-positive group, this probably did not reach statistical significance because of the small sample size. Had the sample size been twice as large, the difference in end points achieved would have been statistically significant. Our study had reasonable power to detect an association for the patients with valve thickening but had limited power in the PFO subset. In addition, the patient population of WARSS was typically older and included all noncardiogenic stroke subtypes, a population in which traditional stroke risk factors such as hypertension and diabetes typically are more prevalent. PFO and, to a lesser extent, VaT have typically been shown to be associated with cryptogenic stroke patients of younger age. Also, contrary to more recent recommendations, the aPL studies in APASS were performed only once at baseline and were not systematically repeated again at 3 months. Finally, we only tested for aPL, whereas other hypercoagulable states such as APC resistance and protein C and S deficiencies also may be important in patients with presumed paradoxical embolism.

In conclusion, the PICSS-APASS cohort study could not demonstrate significant excess risk of ischemic stroke recurrence, TIA, or death among ischemic stroke patients attributable to the presence of PFO and a single baseline aPL positivity either separately or together. Similarly, the presence of left-side cardiac valve thickening and a single baseline aPL, either individually or together, did not increase the risk of stroke recurrence or death in this cohort. Because this study is a post hoc, retrospective subset analysis, it should only be used for hypothesis generation. Further prospective studies with a larger number of subjects are needed to confirm or refute these observations.

References

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