High Prevalence of Antiphosphatidylinositol Antibodies in Young Patients With Cerebral Ischemia of Undetermined Cause

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Background and Purpose—Anticardiolipin antibodies (aCL) are associated with thrombotic phenomena including cerebral ischemia in young adults. Although aCL are directed to a neoepitope formed by phospholipid and β2-glycoprotein I (β2-GPI), immunoassays based on cardiolipin as target antigen are widely used. We previously demonstrated that 47% of aCL-negative systemic lupus erythematosus (SLE) patients had antiphospholipid antibodies (aPL) to epitopes other than cardiolipin, and we found an association between aPL to noncardiolipin antigens and thrombosis. We now assess the prevalence and clinical significance of noncardiolipin aPL in young adults with cerebrovascular disease of undetermined etiology.

Methods—Seventy-seven non-SLE patients, aged <51 years, with cerebral ischemia were studied. Specificity of aPL were characterized by ELISAs using 7 different phospholipids: cardiolipin (CL), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylglycerol (PG), phosphatidic acid (PA), phosphatidylethanolamine, and phosphatidylethanolamine.

Results—Thirty-four patients (44.1%), had aPL to 1 or more of the following antigens: 23.4% to CL, 18.2% to PS, 15.6% to PG, 14.3% to PA, and 28.6% to PI. Fifty-nine patients (76.6%) were aCL negative. Of these subjects 23.4% showed aPL to noncardiolipin epitopes. PI was the specificity with highest prevalence in all subgroups, and in 6 patients anti-PI antibodies were the only detectable aPL. The binding of aPL to the different antigens was β2-GPI dependent.

Conclusions—Our data demonstrate a high prevalence of aPL in young adults with cerebral ischemia of undetermined cause. PI was the specificity with highest prevalence, suggesting that anti-PI antibodies may be an immunological marker in young patients with cerebrovascular disease. (Stroke. 1998;29:1759-1764.)

Key Words: antibodies, anticardiolipin antibodies, antiphospholipid cerebral ischemia hemostasis young adults

Antiphospholipid antibodies are a heterogeneous family of closely related autoantibodies that are directed to a neoepitope formed by anionic phospholipids coupled to a plasma protein acting as cofactor and necessary for both immunological and functional properties of these antibodies. They include LA and aCL, which are detected by ELISA using CL as target antigen and are dependent on the cofactor β2-GPI.¹⁻³ Both LA and aCL have been reported in patients with SLE, malignant or inflammatory diseases, or without an underlying illness, and have been associated with relevant clinical manifestations such as thrombosis, recurrent fetal loss, and thrombocytopenia.⁴ Recent well-controlled clinical studies also demonstrated an association between aPL (LA or aCL) and cerebral ischemia in young adults.⁵⁻¹²

Using a panel of 7 different phospholipid antigens separately tested by ELISA, we demonstrated that 47% of SLE patients who were aCL negative had 1 or more aPL to an epitope different from CL, and a significant association was also demonstrated between aPL to non-CL antigens and thrombotic events after a mean follow-up period of 30 months.⁵ These data suggest that aPL to non-CL epitopes may predict thrombotic complications in patients with SLE, and ELISA methods based on CL as target antigen may not be sensitive enough to detect all aPL-positive subjects.

The aim of the present study was to assess the prevalence and to verify the possible clinical significance of aPL to phospholipid epitopes different from CL in a sufficiently large series of young non-SLE adults with cerebral ischemia of unknown etiology.

Subjects and Methods

Patients
Seventy-seven consecutive patients, 47 men and 30 women, aged 27 to 51 years (mean 36.5 years), with ischemic cerebrovascular events and no obvious causes of arterial thromboembolism, were included in the study between January 1992 and April 1997 after informed consent.
Selected Abbreviations and Acronyms

\[
\begin{aligned}
apL &= \text{antiphospholipid antibodies} \\
aCL &= \text{anticardio lipin antibodies} \\
CL &= \text{cardiolipin} \\
\beta2-GPI &= \beta2\text{-glycoprotein I} \\
LA &= \text{lupus anticoagulant} \\
OD &= \text{optical density} \\
PA &= \text{phosphatidic acid} \\
PC &= \text{phosphatidylcholine} \\
PE &= \text{phosphatidylethanolamine} \\
PG &= \text{phosphatidylglycerol} \\
PI &= \text{phosphatidylinositol} \\
PS &= \text{phosphatidylserine} \\
SLE &= \text{systemic lupus erythematosus} \\
TIA &= \text{transient ischemic attack}
\end{aligned}
\]

consent had been obtained. The study was approved by the institutional Ethics Committee. The patients were examined at the Department of Neurology of San Carlo Borromeo Hospital for ischemic stroke or TIA. Diagnosis of stroke or TIA was made according to the criteria of the World Health Organization and the Stroke Committee, respectively. Diagnosis of stroke was confirmed by CT and/or MRI performed within 24 hours after the neurological event in all cases. Patients with nonischemic diseases (eg, trauma, tumors, or intracerebral or subarachnoid hemorrhage) were excluded from the study. Subjects were diagnosed with stroke when clinical symptoms lasted for more than 24 hours and brain CT scan or MRI showed new isolated or multiple cerebral infarctions. Patients were diagnosed with TIA when symptoms resolved within 24 hours and no ischemic abnormalities were demonstrated by CT or MRI. According to these criteria 65 subjects (42 men and 23 women) were classified with stroke, and 12 (9 men and 3 women) were classified as TIA. No differences in age or sex were observed in these 2 groups. None of the patients under study had SLE according to the American Rheumatism Association criteria. Patients with other conditions known to be associated with aPL such as recent bacterial or viral infections, malignancies, non-SLE autoimmune disorders, or HIV infection were also excluded.

A total of 92 patients were initially enrolled. All patients underwent continuous-wave Doppler ultrasonography of the carotid arteries to assess possible causes of thromboembolism. Conventional selective cerebral angiography was also performed in 54 patients (59%) and transcranial Doppler ultrasonography in 32 (35%). An ulcerated, nonstenosing atherosclerotic plaque of internal carotid artery was observed in 3 patients (3.3%, 1 at the intracranial and 2 at the extracranial level), a stenosing (>50%) lesion of internal carotid artery at the extracranial level was found in 1 patient, whereas 1 additional patient had an arteriolar dissection of a posterior cerebral artery. These patients were excluded from the study.

All patients received cardiac evaluation including ECG and 2-dimensional echocardiography. Two cases (2.2%) had possible embolic heart disease: 1 with mitral valve prolapse and 1 atrial fibrillation. These 2 patients were excluded. Data on transesophageal echocardiography, available in 61 of the initially enrolled patients (66%), did not show any possible additional sources of cardiac embolization such as atrial or appendage thrombi or valvular vegetations.

Patients with congenital or acquired deficiency of natural coagulation inhibitors such as antithrombin III, protein C, or protein S, with resistance to activated protein C, or with hyperhomocysteinemia, which are conditions known to cause a prothrombotic tendency, were excluded. Of the initially enrolled 92 cases, an antithrombin III defect was found in 1 patient, protein C and protein S deficiencies were found in 2 (2.2%) and 1 patients, respectively, and 3 patients (3.3%) showed resistance to activated protein C. The screening test for hyperhomocysteinemia was performed in only 54 patients (59%) and gave a positive result in 1 additional subject.

Routine laboratory assessment and collection of historical data with particular attention to cerebrovascular risk factors were performed in all 77 cases who remained in the study. Five patients (6.5%) had hypertension (systolic blood pressure >140 mm Hg and diastolic pressure >90 mm Hg), 10 (13.0%) were smokers (>20 cigarettes daily), 12 (15.6%) had hypercholesterolemia (total cholesterol >6 mmol/L, high density lipoprotein <1 mmol/L), 9 (11.7%) had hypertriglyceridemia (>1.9 mmol/L), and none had obesity, diabetes mellitus, or increased alcohol intake.

Eight patients were taking oral contraceptives (26.6% of female population). Four cases (5.2%) had a previous clinical cerebral event as also confirmed by multiple lesions at different stages of evolution at CT scan. Three patients (3.4%) reported a history of migraine. In 4 patients (5.2%) phlebographically documented episodes of deep vein thrombosis were reported, and 1 patient had thrombocytopenia. Five patients had a history of fetal loss (16.6% of female population). None of the patients had previous peripheral artery occlusion or coronary events.

Plasma for coagulation studies was obtained from blood samples collected before starting anticoagulant therapy and within 12 hours after diagnosis into plastic tubes containing 0.129 mol/L trisodium citrate (9:1, vol:vol). Samples for serum preparation were collected into plastic tubes without anticoagulant at the moment of entry in the study and at 1 and 2 months after the index episode.

The control group consisted of 178 normal volunteers who regularly donate blood at our institution. One hundred-seven were men and 71 were women. Their age range was 25 to 51 years (mean 34.7 years). All normal subjects routinely received a complete physical examination, an ECG, a standard chest roentgenogram, and a complete assessment of biochemical and hemostatic parameters. All subjects who were positive for aCL antibodies, other hematicologic abnormalities, or cardiovascular diseases, except hypertension, were not included in the control group. No statistically significant differences were demonstrated in cerebrovascular risk factor incidence between the patient and control groups. Ten subjects (5.6%) had hypertension (systolic blood pressure >140 mm Hg and diastolic pressure >90 mm Hg), 21 (11.8%) were smokers (>20 cigarettes daily), 29 (16.3%) had hypercholesterolemia (total cholesterol >6 mmol/L, high density lipoprotein <1 mmol/L), 18 (10.1%) had hypertriglyceridemia (>1.9 mmol/L), and none had obesity, diabetes mellitus or increased alcohol intake. Six of 71 normal women were taking oral contraceptives (9.8%), a statistically significant smaller proportion than that found in the patient group (P=0.04, χ² test).

Laboratory Studies

Detection of Lupus Anticoagulant

Three different methods were used for LA detection: activated partial thromboplastin time, kaolin clotting time (KCT) and dilute Russell viper venom time (dRVVT). Activated partial thromboplastin time was determined with an automated coagulometer (ACL 3000, Instrumentation Laboratory) employing cephalin with microwenized silica (Instrumentation Laboratory). Kaolin clotting time was measured according to the method of Exner et al. dRVVT was determined with a commercially available kit (LA-SCREEN, Gradi
d-silica (Instrumentation Laboratory). The tests were considered positive if prolonged results were not corrected by the addition of an equal volume of normal plasma to the test plasma. For the dRVVT confirmatory test (LA-CONFIRM, Gradi-

Patients were considered to have LA if at least 2 tests gave positive results. Guidelines recommended in 1995 by the Standardization Committee of the International Society of Thrombosis and Hemostasis were followed. The results of coagulation studies for LA detection of patients enrolled before 1995 were reexamined to validate LA diagnosis according to the above-mentioned criteria.

Detection and Characterization of Antiphospholipid Antibodies

Detection, characterization of specificity to phospholipid antigens, and immunoglobulin isotype of aPL antibodies were performed by
ELISA using a panel of 7 different commercially available phospholipids as previously described. The following phospholipids were tested: CL, PS, PI, PG, PA, PC, and PE, all from Sigma. Briefly, the wells of microtiter plates were coated with 30 µL of CL at a concentration of 40 µg/mL in ethanol overnight at 4°C. Plates were then blocked to prevent nonspecific binding with 100 µL of 10% FCS in PBS for 2 hours at room temperature. The wells were then washed 6 times with PBS, and 100 µL of a 1:100 dilution of patients and control sera in PBS/10% FCS was added in triplicate in each well. The plates were subsequently incubated for 1 hour at room temperature, and after 6 more washes with PBS, 100 µL of a 1:1,000 dilution of peroxidase-labeled goat anti-human IgG, IgM, or IgA (KPL Laboratories) was added. After 1 more incubation at room temperature for 1 hour, the plates were washed as before, and 100 µL of substrate (500 g/LABTS in H2O2, KPL Laboratories) was added and sufficient time allowed for the reaction to occur. The absorbance was then measured at 405 nm using a Titertek Multiscan MC photometer (Flow Laboratories).

The ELISAs for the detection of aPL antibodies to the other phospholipids (PS, PI, PG, PA, PC, and PE) were performed in a similar way except that coating of the wells was carried out with 40 µL of a 50 µg/mL solution of the phospholipid molecules in chloroform/ethanol 1:4 (vol:vol). A sample was considered positive when the OD was greater than 5 SDs above the mean of normal control values and highly positive when greater than 7 SDs. All positive cases were confirmed in at least 3 samples collected at a 1-month interval. Three cases of the 92 initially enrolled patients, who gave discordant results (ie, positivity in the first 2 samples and negativity in the third), were excluded from the study. These patients were subsequently found to have other causes of cerebral thromboembolism (2 had carotid atherosclerotic lesions and 1 had mitral valve prolapse). The majority of patients who tested negative for the different phospholipid epitopes at the first evaluation were not further tested. However, a sample of 10 cases who were negative at the first evaluation were retested 2 more times at 1 month. Negativity to all phospholipids was confirmed and none of these cases tested positive at the subsequent evaluation.

Calibration samples kindly provided by Dr N. Harris (University of Louisville, Ky, USA) were used for quantitative measurement of IgG and IgM aCLs. Samples that tested positive for aCL by our criteria were also moderately or highly positive when calculated by the calibration curve obtained with Dr Harris' standard sera (>20 GPL and >10 MPL units for IgG and IgM aCLs, respectively).

To verify that aPL bound to the wells was specific for the different phospholipids and not specifically adsorbed to the plastic surface, experiments were carried out in which coating of the plates was performed by omitting phospholipids and by using solvent alone (ethanol or chloroform/ethanol). The ELISA was then performed as described above. OD of 10 samples that had previously tested highly positive for different aPL of different isotypes showed negligible absorbance values in these conditions.

Finally, to assess whether aPL binding to the different antigens was dependent on the presence of the cofactor β2-GPI, experiments were performed in which FCS, which contains β2-GPI, was substituted with 30% purified bovine albumin (Biotest AG) used at a wide range of dilutions in the different steps of the ELISA in 10 selected samples that were highly positive for aPL to the different antigens. Substitution of FCS with bovine albumin resulted in OD values that were not significantly different than those of the control group in all the samples, suggesting that β2-GPI was necessary for aPL binding to all phospholipid epitopes tested.

Statistical analysis was performed by Student's t and χ2 tests as appropriate.

## Results

Prevalence of aPL to the panel of phospholipid epitopes tested in young adults with cerebral ischemia of uncertain etiology is shown in Table 1.

Of the entire series, 34 (44.1%) were positive for aPL to 1 or more phospholipids. A significant number of patients had aPL to anionic phospholipids CL (23.4%), PS (18.2%), PG (15.6%), PA (14.3%), and PI (28.6%), which was the specificity with highest prevalence. A smaller number of cases showed aPL with specificity to neutral (zwitterionic) phospholipids PC (6.5%) and PE (10.4%).

In 17 cases (22.1% of all cases and 50% of positive cases) positivity only for 1 phospholipid was demonstrated. PI was the specificity that showed the highest prevalence also in this subgroup (Table 2). Nine of these patients (11.7%) had only 1 immunoglobulin isotype, whereas in 8 (10.4%) 2 isotypes were observed. aPL isotype was IgG in 14 cases (18.2%) and IgM in 3 cases (3.9%). None of these patients had IgA isotype. In 6 patients (7.8% of total and 17.6% of positive cases) aPL with PI specificity was the only detectable aPL.

In contrast, the remaining 17 cases showed positivity for multiple phospholipids and more than 1 isotype was demonstrated. The results of these patients are reported in Table 3. This subgroup of patients also showed higher prevalence for aPL to negatively charged phospholipids, whereas that for neutral phospholipids was less prominent. PI was the specificity with highest prevalence and IgG was also the most common isotype in these subjects.

Fifty-nine patients (76.6%) were aCL negative. Of these subjects, 18 (23.4%) showed positivity for aPL to epitopes other than cardiolipin. Prevalence and isotypes of aPL with specificities to non-CL phospholipids of these subjects are reported in Table 4. Sixteen cases (20.8% of all cases and 47% of positive cases) had aPL to an isolated phospholipid, and the antibody belonged to a single isotype, whereas in only 2 patients more than 1 specificity and multiple isotypes were demonstrated (Table 4). PI was the specificity with highest prevalence also in this subgroup of patients.

Antiphospholipid-related clinical events were also present in aCL-negative aPL-positive patients. These features are summarized in Table 5. Two patients who had multiple strokes both had an isolated anti-PI antibody.

### Table 1. Prevalence of aPL to the Panel of 7 Phospholipid Epitopes Tested in Young Adults With Cerebral Ischemia

<table>
<thead>
<tr>
<th>Specificity</th>
<th>CL</th>
<th>PS</th>
<th>PC</th>
<th>PE</th>
<th>PI</th>
<th>PG</th>
<th>PA</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases</td>
<td>18</td>
<td>14</td>
<td>5</td>
<td>8</td>
<td>22</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>%</td>
<td>23.4</td>
<td>18.2</td>
<td>6.5</td>
<td>10.4</td>
<td>28.6</td>
<td>15.6</td>
<td>14.3</td>
</tr>
</tbody>
</table>

Percentage values refer to the total number of patients studied (77 cases).

### Table 2. Prevalence and Isotype Distribution of aPL to the Panel of 7 Phospholipid Epitopes Tested in the Patients Who Were Positive for Only 1 Phospholipid Epitope (17 Cases)

<table>
<thead>
<tr>
<th>Specificity</th>
<th>CL</th>
<th>PS</th>
<th>PC</th>
<th>PE</th>
<th>PI</th>
<th>PG</th>
<th>PA</th>
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</thead>
<tbody>
<tr>
<td>No. of cases</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>%</td>
<td>1.3</td>
<td>2.6</td>
<td>2.6</td>
<td>5.2</td>
<td>7.8</td>
<td>0</td>
<td>2.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Isotype</th>
<th>IgM</th>
<th>IgG</th>
<th>IgG</th>
<th>IgM</th>
<th>IgG</th>
<th>IgM</th>
<th>IgG</th>
</tr>
</thead>
</table>

*In the cases who had PI specificity, aPL was IgG in 5 cases and IgM in 1 case; percentage values refer to the total number of patients studied (77 cases).
Forty-three patients (55.8%) tested negative for all phospholipid antigens.

Detection of LA was performed in only 66 patients (86%), and it was found in only 3 (4.5%). aPL specificities and isotypes of LA positive subjects did not show a definite pattern. Two patients had aPL to negatively charged phospholipids CL, PS, PI, PG, and PA of IgG, IgM, or IgA isotypes, whereas in 1 patient IgGs to neutral phospholipids PC and PE were also observed.

Finally, no statistically significant differences in age, sex, diagnosis (stroke or TIA), or cerebrovascular risk factors were observed between patients who had aPL to a single phospholipid and 1 or 2 isotypes and those with aPL to multiple phospholipids and multiple isotypes.

### Table 3. Prevalence and Patterns of aPL Reactivity and Isotype Distribution in Patients Who Had Multiple Positivity to the Panel of 7 Phospholipid Epitopes Tested

<table>
<thead>
<tr>
<th>Specificity and Isotypes</th>
<th>CL</th>
<th>PS</th>
<th>PC</th>
<th>PE</th>
<th>PI</th>
<th>PG</th>
<th>PA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient No.</td>
<td>IgG</td>
<td>IgM</td>
<td>IgA</td>
<td>IgG</td>
<td>IgM</td>
<td>IgA</td>
<td>IgG</td>
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<tr>
<td>1</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<td>0</td>
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<td>2</td>
<td>++</td>
<td>+</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
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<td>3</td>
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<td>+</td>
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<tr>
<td>Total</td>
<td>12</td>
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<td>10</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>%</td>
<td>15.6</td>
<td>5.5</td>
<td>5.2</td>
<td>13.0</td>
<td>5.2</td>
<td>2.6</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Samples were considered positive (+) or highly positive (+++) when OD was greater than 5 and 7 SD, respectively, above the mean of normal control values (see “Subjects and Methods”); percentage values refer to the total number of patient studied (77 cases).

### Table 4. Prevalence and Isotype Distribution of aPL to Phospholipid Epitopes in Patients Who Were aCL Negative

<table>
<thead>
<tr>
<th>Specificity</th>
<th>PS</th>
<th>PC</th>
<th>PE</th>
<th>PI</th>
<th>PG</th>
<th>PA</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases (%)</td>
<td>4 (5.2)</td>
<td>2 (2.6)</td>
<td>4 (5.2)</td>
<td>8 (10.4)</td>
<td>1 (1.3)</td>
<td>3 (3.4)</td>
</tr>
<tr>
<td>IgG, n (%)</td>
<td>3 (3.9)</td>
<td>1 (1.3)</td>
<td>4 (5.2)</td>
<td>7 (9.1)</td>
<td>0 (0)</td>
<td>2 (2.6)</td>
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<tr>
<td>IgM, n (%)</td>
<td>1 (1.3)</td>
<td>1 (1.3)</td>
<td>0 (0)</td>
<td>2 (2.6)</td>
<td>1 (1.3)</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>IgA, n (%)</td>
<td>0 (0)</td>
<td>1 (1.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

All events occurred before the index cerebral ischemic event.

### Table 5. Clinical Features Other Than the Index Cerebral Ischemic Event That Occurred in Patients Negative for aCL but Positive for Non-CL aPL Antibodies and aPL Specificity and Isotype Distribution

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Clinical Event</th>
<th>Specificity</th>
<th>Isotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Deep vein thrombosis</td>
<td>PS, PI</td>
<td>IgG</td>
</tr>
<tr>
<td>2</td>
<td>Deep vein thrombosis</td>
<td>PE</td>
<td>IgG</td>
</tr>
<tr>
<td>3</td>
<td>Fetal loss</td>
<td>PS</td>
<td>IgG</td>
</tr>
<tr>
<td>4</td>
<td>Fetal loss</td>
<td>PS, PE, PI</td>
<td>IgG</td>
</tr>
<tr>
<td>5</td>
<td>Multiple ischemic strokes</td>
<td>PI</td>
<td>IgG</td>
</tr>
<tr>
<td>6</td>
<td>Multiple ischemic strokes</td>
<td>PI</td>
<td>IgG</td>
</tr>
</tbody>
</table>

Discussion

The close association between cerebrovascular disease (TIA or stroke) and aCL or LA has been observed in well-controlled prospective studies, particularly in the young.\cite{10-12}

We recently demonstrated that a significant proportion of patients with SLE who were aCL negative had aPL to phospholipids other than CL, and a significant association was prospectively demonstrated between positivity for aPL to non-CL antigens and thrombosis, suggesting that aPL to non-CL epitopes may be clinically relevant.\cite{13} The results of the present study agree with our previous data. By using a panel of 7 different phospholipids, separately tested by ELISA, we demonstrated an overall high prevalence of aPL.
positivity in young patients with cerebrovascular disease without evidence of SLE. The prevalence of aCL alone was comparable to that reported by Love and Santoro,7 but was higher than that found by Nencini.10 This may be because of the different methods used (homemade ELISA versus radioimmunoassay) and to differences in the calculation of normal values. In addition, those with PI specificity had the highest prevalence, suggesting that anti-PI may be a common immunological marker in young patients with cryptogenic cerebral ischemia.

Similar to findings in SLE patients,13 a consistent proportion (23%) of patients with cerebrovascular disease and no SLE who were aCL negative had aPL to non-CL phospholipids. ELISA methods currently used in the clinical setting, withCL as the only target antigen, may not be sensitive enough to detect all patients with aPL and may therefore underestimate the size of the aPL-positive population at risk for thromboembolic phenomena. In the aCL-negative sub-group, aPL with PI specificity was the most commonly observed, agreeing with previous reports25,26 which first demonstrated aPL to anionic phospholipids other than CL, and particularly to PI, in patients with LA and thrombosis. Falcón et al27,28 also showed that anti-PI was the main marker of antiphospholipid syndrome in some patients with autoimmune diseases and thrombotic complications including cerebrovascular events; in some of these patients anti-PIs were the only aPL observed. Also, in our series anti-PI was the only detectable aPL in 6 of 34 aPL-positive patients, and PI was the specificity with highest prevalence in the subgroup of subjects who had aPL to only 1 epitope.

Patients with cerebral ischemia exhibited 2 patterns of aPL reactivity: half showed positivity for a single phospholipid and had only 1 or 2 immunoglobulin isotypes, whereas the other half had positivity to multiple epitopes and isotypes. The clinical significance of this is unknown since no statistically significant differences were found in age, sex, risk factors, or diagnosis (TIA or stroke) between the 2 groups. This observation supports the hypothesis that aPLs are a family of closely related but heterogeneous autoantibodies that may have different biological and pathogenic properties. Large prospective studies demonstrating a clear relationship between aPL to non-CL antigens and thrombosis are still lacking. The alternative hypothesis cannot be ruled out that the addition of non-CL antigens merely increases the sensitivity of ELISA assay but that non-CL aPL may not be specific for thrombosis. However, the observation of different aPL-related clinical features in our aCL-negative aPL-positive patients reinforces the hypothesis that aPL with nonCL specificity may have clinical significance.

Similar to our previous work, aPL with specificity for neutral phospholipids PE and PC was demonstrated in a significant number of young patients with cerebral ischemia. Both Pengo et al29 and Gharavi et al30 previously failed to demonstrate aPL with specificity for PC; however, anti-PEs were identified by other authors.30,31 It has been postulated that these aPL recognize nonbilayer phase (hexagonal) phospholipids.32 The biological importance of anti-PE is therefore questionable, since phospholipid molecules are arranged in lamellar (bilayer) phase in plasma membranes. However, Staub et al33 demonstrated in a patient with strong LA activity and severe thrombotic episodes anti-PE antibodies as the only aPL, suggesting that antibodies to neutral phospholipids may occasionally prolong in vitro–dependent coagulation tests and may also be associated with clinical features.

Recent experimental data demonstrated that aCLs detectable by ELISA require the presence of the cofactor β2-GPI for their interaction with CL.34,35 and various studies also reported antibodies that bind β2-GPI in the absence of phospholipids, thus suggesting that β2-GPI itself might be the target antigen of aCL.36,37 However, “true” aPLs directed to phospholipids in the absence of β2-GPI have also been demonstrated.38,39 The association between anti–β2-GPI antibodies and thrombosis has been observed by some authors.40–42 Our data suggest that the presence of both phospholipids and β2-GPI is necessary for aPL reactivity, since the absence of the cofactor in the ELISA system significantly reduced aPL binding. Data demonstrating that aPL recognize a cryptic epitope expressed only when β2-GPI is bound to anionic phospholipids43 are consistent with this hypothesis. However, the possibility remains that the different aPLs found in our series reflect cross-reactivity of a single antibody to multiple epitopes with different affinity of the phospholipids for β2-GPI. Purification of the aPL by affinity chromatography and tests of binding properties to the different phospholipid antigens are needed.

We found that only a few young patients with cerebral ischemia were positive for LA, a prevalence lower than those reported by other authors7,10 but comparable to that of Hart et al.44 These discrepancies are probably related to methodological problems in the laboratory diagnosis of LA,3 and LA detection may be misleading in the identification of young patients with cerebral ischemia and aPL. aPL reactivity, as assessed by our ELISAs, did not show a consistent pattern in LA-positive subjects. The anticoagulant activity of aPL has been associated with the presence of IgG aPL with PS specificity.45 This finding suggests that at least some of the anti-PS found in our series may be those responsible for LA reactivity.

Finally, if we consider the entire series of the patients with cerebral ischemia that we initially examined, a low incidence of other potential etiologies for cerebral thromboembolism such as atherosclerosis, arterial dissection, and cardioembolism was found when compared with data previously reported in the literature.46 This may result from an incomplete diagnostic evaluation of our patients and particularly from the limited number of subjects submitted to cerebral angiography, transcranial Doppler ultrasonography, or transesophageal echocardiography. Therefore, our data need to be validated by a prospectively designed study that includes these diagnostic tools in all patients.

References


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Antiphospholipid Antibodies and Cerebral Ischemia


High Prevalence of Antiphosphatidylinositol Antibodies in Young Patients With Cerebral Ischemia of Undetermined Cause
Vincenzo Toschi, Adele Motta, Carlo Castelli, Maria Luisa Paracchini, David Zerbi and Andrea Gibelli

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