Autoantibodies Against the Fibrinolytic Receptor, Annexin A2, in Cerebral Venous Thrombosis

Gabriela Cesarman-Maus, MD, PhD; Carlos Cantú-Brito, MD, PhD; Fernando Barinagarrementeria, MD; Rosario Villa; Elba Reyes, PhD; Jorge Sanchez-Guerrero, MD; Katherine A. Hajjar, MD; Ethel Garcia Latorre, PhD

Background and Purpose—Cerebral venous thrombosis (CVT) may be a manifestation of underlying autoimmune disease. Antibodies against annexin A2 (anti-A2Ab) coincide with antiphospholipid syndrome, in which antiphospholipid antibodies (aPLA) are associated with thrombosis in any vascular bed. Annexin A2, a profibrinolytic receptor and binding site for β2-glycoprotein-I, the main target for aPLA, is highly expressed on cerebral endothelium. Here we evaluate the prevalence of anti-A2Ab in CVT.

Methods—Forty individuals with objectively documented CVT (33 women and 7 men) and 145 healthy controls were prospectively studied for hereditary and acquired prothrombotic risk factors, classical aPLA, and anti-A2Ab.

Results—One or more prothrombotic risk factors were found in 85% of CVT subjects, (pregnancy/puerperium in 57.5%, classical aPLA in 22.5%, and hereditary procoagulant risk factors in 17.5%). Anti-A2Ab (titer >3 SD) were significantly more prevalent in patients with CVT (12.5%) than in healthy individuals (2.1%, P <0.01, OR, 5.9).

Conclusions—Anti-A2Ab are significantly associated with CVT and may define a subset of individuals with immune-mediated cerebral thrombosis. (Stroke. 2011;42:501-503.)

Key Words: cerebral venous thrombosis ■ annexin A2 ■ antiphospholipid syndrome ■ thrombophilia

Cerebral venous thrombosis, a multifactorial disease that preferentially affects young adults and children, occurs without recognizable risk factors in up to 20% of subjects.1 Antiphospholipid antibodies (aPLA) are detected in 6% to 25% of cases.1,2 Though more frequent in antiphospholipid syndrome than in the general population, cerebral venous thrombosis (CVT) is still a rare event (0.7%/1000 patients).3 We have previously found that antibodies against annexin A2 (anti-A2Ab) are significantly more prevalent in patients with antiphospholipid syndrome–related thrombosis (22.6%) than in healthy individuals (2.1%, P<0.001), patients with non-autoimmune thrombosis (0%, P=0.017), or patients with lupus but without thrombosis (6.3%, P<0.001).4 Here we examined the prevalence of anti-A2Ab and other prothrombotic risk factors in consecutive patients with CVT.

Methods

Subject Populations
We studied 185 adults, 40 consecutive patients with CVT and 145 with no prior history of thrombosis, who met standard criteria for blood donation. Consent was obtained per internal review board–approved protocols. CVT was documented by magnetic resonance image (100%) and by cerebral angiography (35%). Blood samples were collected at 2 to 6 months following the thrombotic event. aPLA were considered positive if aCL (>5 SD), anti-β2-glycoprotein-I (anti-b2GPI; >3 SD), or a positive lupus anticoagulant was present.

Coagulation and Antibody Assays
Sera were evaluated for anti-A2Ab (IgG and IgM) by enzyme-linked immunosorbent assay as previously described.4 Lupus anticoagulant was determined using dRVVT (American Diagnostica, Inc.), and anti-cardiolipin (aCL) and anti-β2GPI antibodies by enzyme-linked immunosorbent assay.4 Functional protein C, S, and AT (Stago kits) and the PCR/Mnl-1 restriction enzyme assay for factor V Leiden mutation were determined as described.5

Statistical Analyses
Descriptive statistics were used to define the subject’s characteristics. Categorical variables were compared using χ² or Fisher’s exact test. Probability value was set at <0.05, two tailed. Analysis was conducted using SPSS version 17 for Windows.

Results
Among patients studied, 57.5% recovered fully, while 30%, 7.5%, and 5% had mild, moderate, and severe sequelae, respectively, at discharge.
Prothrombotic risk factors are shown in Table 1. Nine patients with CVT (22.5%) had at least 1 positive aPLA titer, and 1 fulfilled diagnostic criteria for systemic lupus erythematosus. Among patients with CVT, 12.5% (IgG:7.5%; IgM:5%) were positive for anti-A2Ab (>3SD) compared to 2.1% (IgG:1.4%; IgM:0.7%; P<0.01) of healthy controls; OR 5.9 (with wide 95% CI, 1.3 to 25.8), Table 2. Concomitant risk factors for individuals with anti-A2Ab are depicted in Table 3.

### Discussion

As opposed to United States and European series where CVT is rare, it comprises 8% of individuals (166 of 2045) with Mexican Mestizo ancestry at the National Neurology and Neuropsychiatry Institute’s Stroke Registry. Nutritional deficiency may account for this high incidence.2

Although the prevalence of known prothrombotic risk factors was similar to other series, the factor V Leiden mutation was not associated with CVT. Interestingly, anti-A2Ab was strongly associated with CVT independently of classical aPLAs. A limitation to our cross-sectional design is that the stability of anti-A2Ab titers over time is unknown.

Annexin A2 localizes fibrinolytic activity to the cell surface and is also the high affinity receptor for β2GPI, the main target antigen for pathogenic aPLAs.6 Upon binding to endothelial cells, aPLAs induce nuclear factor kappa B (NF-κB) translocation, possibly by signaling through toll-like receptors in complex with A2.7 Cultured human cerebral endothelial cells express higher amounts of A2 and generate more plasmin (P<0.0001) when compared with those from skin, lung, iliac artery or vein, aorta, and coronary artery. Blockade of A2 inhibits tPA-induced cerebral endothelial cell plasmin generation, suggesting a key role for A2 in maintaining cerebral vascular patency.8 Of related interest, A2 polymorphisms are a risk factor for stroke in sickle cell disease.9

Recent studies10,11 confirm our previous finding that anti-A2Ab are significantly associated with antiphospholipid syndrome–related thrombosis and that patient-derived anti-A2Ab promote thrombosis by blocking endothelial cell surface fibrinolysis, and by inducing tissue factor expression.4 A2 may offer a novel therapeutic target, as current efforts at preventing cellular activation by aPLAs are being sought through inhibition of binding of aPLA to β2GPI, inhibition of antibody/β2GPI complex binding to cell surfaces, downregulation of intracellular signaling and proteasome inhibition to suppress NF-κB activation.12

In conclusion, anti-A2Ab are significantly associated with CVT in our patient population, a finding which requires confirmation in an independent sample. Anti-A2Ab may play a role in the pathogenesis of CVT, offer novel therapeutic strategies, and define a new subset of individuals with immune-mediated thrombosis.

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### Disclosures

None.

### References


### Table 1. Prothrombotic Risk Factors

<table>
<thead>
<tr>
<th>Patient Characteristic</th>
<th>Healthy Control</th>
<th>CVT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>145</td>
<td>40</td>
</tr>
<tr>
<td>Median age range</td>
<td>36.7 (22–63)</td>
<td>28.2 (14–61)</td>
</tr>
<tr>
<td>Female/male</td>
<td>145 (100%)/0</td>
<td>33 (82.5%)/7 (17.5%)</td>
</tr>
<tr>
<td>Concomitant non-cerebral thrombosis</td>
<td>-</td>
<td>7 (17.5%)</td>
</tr>
<tr>
<td>No identifiable risk factors</td>
<td>-</td>
<td>5 (12.5%)</td>
</tr>
<tr>
<td>Aquired risk factors for CVT</td>
<td>-</td>
<td>6 (15%)</td>
</tr>
<tr>
<td>Puerperium</td>
<td>-</td>
<td>19 (47.5%)</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>-</td>
<td>4 (10.0%)</td>
</tr>
<tr>
<td>Oral contraceptives</td>
<td>-</td>
<td>1 (2.5%)</td>
</tr>
<tr>
<td>Anabolics</td>
<td>-</td>
<td>1 (2.5%)</td>
</tr>
<tr>
<td>aPLA</td>
<td>-</td>
<td>9 (22.5%)</td>
</tr>
<tr>
<td>Protein S deficiency, family history of VTE</td>
<td>-</td>
<td>1 (2.5%)</td>
</tr>
</tbody>
</table>

### Table 2. Prevalence of Anti-A2Ab (>3 SD)

<table>
<thead>
<tr>
<th></th>
<th>Healthy Control</th>
<th>CVT</th>
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<tbody>
<tr>
<td>Number</td>
<td>145</td>
<td>40</td>
</tr>
<tr>
<td>IgG</td>
<td>2 (1.38%)</td>
<td>3 (7.5%)</td>
</tr>
<tr>
<td>IgM</td>
<td>1 (0.68%)</td>
<td>2 (5.0%)</td>
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<tr>
<td>IgG and IgM</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IgG or IgM</td>
<td>3 (2.07%)</td>
<td>5 (12.5%)</td>
</tr>
</tbody>
</table>

*P<0.01.*


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