Premature Stroke in a Family With Lupus Anticoagulant and Antiphospholipid Antibodies

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Lupus anticoagulant and antiphospholipid antibodies are associated with thromboembolic phenomena in individuals both with and without systemic lupus erythematosus. A 32-year-old woman (the index case) with lupus anticoagulant, multiple cerebrovascular events, and a family history of premature stroke raised the possibility of a familial diathesis. Histories or interviews, examinations, and blood tests were obtained for 23 members of four generations of her family. Four individuals had suffered strokes and three more had suffered neurologic symptoms. Two living individuals who had suffered strokes, two individuals with neurologic symptoms, and five asymptomatic individuals had antiphospholipid activity in their blood. In addition, a cousin of the index case was found to have systemic lupus erythematosus and antiphospholipid activity. Elevated concentrations of von Willebrand factor antigen were found associated with some positive lupus anticoagulant assays, the highest concentrations in the two individuals with stroke. The characteristic presentation of the index case and her good response to treatment suggests that further studies of families in whom antiphospholipid antibodies may represent a risk factor for stroke is worthwhile. (Stroke 1990;21:66-71)

Since the original description of lupus anticoagulant (LAC) in patients with systemic lupus erythematosus (SLE), it has become apparent that antiphospholipid antibodies are responsible for the in vitro abnormality and that thrombosis is the associated clinical problem. Numerous associations are described, including recurrent abortion, vascular thrombosis, thrombocytopenia, valvular heart disease, and cerebrovascular disease. Antiphospholipid antibodies are also seen in patients without SLE, with similar clinical associations. These patients are increasingly described as having the antiphospholipid antibody syndrome.

We describe a Caucasian family in which a few members have a history of premature stroke and in which there is a more widespread occurrence of antiphospholipid antibody activity. Recently there have been reports of an association between antiphospholipid antibody activity and strokes in young patients, sometimes against the background of migraine. Since this syndrome may be detectable clinically before a major stroke occurs and since it seems possible to treat these patients effectively with aspirin or anticoagulants, it is important to define more clearly the patient profile and the clinical significance of the presence of antiphospholipid antibodies.

Subjects and Methods

A questionnaire was applied to all members of the pedigree who were studied, all of whom gave informed consent. In addition to a full medical history, all members were specifically questioned about a history of migraine-like episodes, possible transient ischemic attacks (TIAs), thrombosis, and any drug therapy. A smoking history was obtained, and all females were questioned as to their use of oral contraceptives and any history of miscarriages.

For the coagulation assays, blood was collected into 3.2% trisodium citrate (nine parts blood to one part anticoagulant). Platelet-poor plasma was obtained by centrifuging the samples at 3,000g for 15 minutes at 4°C. Plasma was stored at -70°C until tested. Activated partial thromboplastin times (APTTs) were determined using a Coag-a-mate coagulometer (General Diagnostics, Morris Plains, New Jersey) with Actin-FS (Dade-American Hospital Supply del Caribe, Inc., Aguada, Puerto Rico) as the first reagent. Testing was repeated using Automated APTT Reagent (Organon Technika Corp., Durham, North Caro-
The presence of LAC was tested using the kaolin clotting time (KCT) as described by Exner et al.\textsuperscript{17} LAC was diagnosed as present when the mixing curve was convex in the region of the left axis. Positive samples were screened for the presence of a heparin-like inhibitor with a thrombin clotting time, and the presence of factor VIII antibodies were excluded by a lack of the time-dependency shown by these inhibitors. The concentration of von Willebrand factor antigen (vWF.Ag) was measured by an electroimmunoassay method as described.\textsuperscript{18} Each assay was standardized by including a commercial reference standard (assayed reference plasma, Helena Laboratories, Beaumont, Texas). The range for vWF.Ag concentration in normal subjects in our laboratory is 45–145 µg/dl. Activity assays were also performed for antithrombin III and protein C.

For the antiphospholipid antibody assays, antibodies to cardiolipin and phosphatidylserine were detected using an enzyme-linked immunosorbent assay (ELISA). Forty-five microliters of a 50 µg/ml solution of either cardiolipin or phosphatidylserine (Sigma Chemical Co., St. Louis, Missouri) in ethanol was applied to each well and evaporated to dryness overnight at 4° C under N\textsubscript{2}. Two hundred microliters of phosphate-buffered saline (PBS) containing 10% fetal calf serum (FCS) was then added to each well, and the plate was incubated for 2 hours. The wells were then washed three times with PBS, and 50 /µl of either test or control sera diluted 1:40 in PBS containing 10% FCS was then added to each well, and the plate was incubated for 2 hours. The wells were then washed three times with PBS, and 50 µl of either test or control sera diluted 1:40 in PBS containing 10% FCS were then added and incubated for 1 hour. All samples were run in duplicate. The wells were then washed and developed with alkaline phosphatase-conjugated goat antihuman IgG or IgM in a standard ELISA. The normal ranges for phospholipid concentrations were established using 50 normal controls. Any result >3 standard deviations (SDs) greater than the mean of the normal controls was regarded as positive. Results are expressed in arbitrary units (AUs); 1 AU is 1 SD greater than the mean of the normal controls, and therefore any value of >3 AU is positive. The range of reactivity for our anticardiolipin assay has been validated against the standards circulated as part of the Kingston Antiphospholipid (KAPS) international study group,\textsuperscript{19} and their cutoff level for low positives (<20 units) is 2 SDs above the mean for our normal controls (2 AU).

The presence of antinuclear antibodies was assayed using a standard fluorescence method employing rodent liver as the substrate.\textsuperscript{20} Fluorescence at a dilution of 1:20 or greater was regarded as positive. The presence of double-stranded deoxyribonucleic acid (DNA) antibodies were sought using the \textit{Crithidia luciliae} fluorescence assay,\textsuperscript{21} with a sample giving fluorescence at a dilution of 1:10 or greater being positive.

Case Studies

Index Case

A 32-year-old woman (III 5 in Figure 1) presented in the 32nd week of her first pregnancy with weakness and numbness in her left arm. Three and a half months previously she had noticed the onset of daily right-sided supraorbital headaches associated with nausea and photophobia, lasting a few hours at a time. One month before admission she suffered episodes of numbness and tingling in her left arm that had increased in frequency until a few days before admission, when the numbness and tingling became continuous. Shortly before presentation she had also noticed visual blurring in her right eye and periods of confusion and had suffered two amnesic spells.

Her medical history was unremarkable. She had taken an oral contraceptive, which she had stopped 6 months before becoming pregnant because of migrainous-type headaches that had then resolved. She had no risk factors for cerebrovascular disease other than a positive family history of stroke occurring at an early age in three close relatives.

On examination, the index case was mentally alert. She had mild weakness and subjective loss of pinprick sensation in her left arm. Deep tendon reflexes were increased on the left side; both plantar reflexes were downgoing. There was a right upper temporal...
quadratic field defect associated with a small ischemic area in the right retina. Her blood pressure was 115/75 mm Hg, and there was a grade II/VI systolic murmur at the left sternal edge. Her uterus was compatible with a 32-week pregnancy.

Hemoglobin concentration was 111 g/l, leukocyte count was 7.5 x 10^9/l, platelet count was 151 x 10^9/l, and erythrocyte sedimentation rate was 49 mm/hr. Prothrombin time and partial thromboplastin time were within normal limits, but KCT revealed the presence of LAC. Tests for the presence of antinuclear antibody and anti-DNA antibody were negative. Anticardiolipin antibody concentrations were elevated, IgG at 19.3 AU and IgM at 4.2 AU. A test for the presence of IgG phosphatidylserine antibody was also strongly positive at 16.6 AU. VDRL was positive, with a negative confirmatory test (biologic false-positive). Echocardiogram was normal, and carotid Doppler studies showed normal blood flow. Magnetic resonance imaging showed multiple small right-hemispheric white matter hyperintensities on T2-weighted images.

In the absence of clinical evidence of vasculitis and in view of the presence of LAC and a high concentration of anticardiolipin antibodies, it was felt that her illness was thromboembolic in nature and she started on 80 mg aspirin/day. Within 48 hours after the start of aspirin therapy, her neurologic episodes and headaches ceased and there was gradual resolution of the neurologic deficit in her left arm. Labor was induced at 38 weeks, resulting in the delivery of a healthy 3,050-g infant. The index case’s mother, aged 64 years (II 3 in Figure 1) died at age 60 years following several strokes that had occurred during 20 years. The maternal grandmother of the index case (II in Figure 1) covered members of four generations, with detailed histories and results of laboratory studies on members of three generations.

Case 2

The index case’s mother, aged 64 years (II 3 in Figure 1), had suffered episodes of numbness of her lips and left arm with occasional loss of consciousness at age 35 years, diagnosed as epilepsy and treated with antiepileptics without relief. At age 45 years, case 2 had suffered a right-sided hemiplegia with aphasia, and a computed tomogram of her head at that time showed a large left-sided frontoparietal infarction; the results of carotid Doppler blood flow studies were normal. Case 2 continues to have periods of “absence,” unaffected by the administration of aspirin. In addition, she has had three first-trimester miscarriages, deep-vein thrombosis, and transient thrombocytopenia. Current investigations show a decreased platelet count of 153 x 10^9/l, tests negative for antinuclear factor (ANF) and antiDNA antibodies, and a normal KCT but strongly positive antiphospholipid antibody assays (Table 1).

In view of the strong family history of stroke and the presence of antiphospholipid antibody activity in mother and daughter, a study of all other available family members was undertaken. The pedigree (Figure 1) covers members of four generations, with detailed histories and results of laboratory studies on members of three generations.

Results

The maternal grandmother of the index case (1 I in Figure 1) died at age 60 years following several strokes that had occurred during 20 years. The mother of the index case (II 3, or case 2) has had a stroke, three miscarriages, deep-vein thrombosis, and an episode of thrombocytopenia. She had a negative KCT test for LAC but serology positive for anticardiolipin and antiphosphatidylserine antibodies. II 3, or case 2, had nine siblings, of whom one brother (II 2 in Figure 1) died at age 52 years following several strokes. Of his four children, one (III 3 in Figure 1) had a positive test for LAC and was pregnant at the
time of testing; the other three children had normal serology. Three of these four children were asymptomatic. One of the three seronegative children, a 38-year-old woman (III 2 in Figure 1), has classical migraine and episodes suggestive of TIAs, which were exacerbated by oral contraceptives. Seven of case 2’s other eight siblings are alive and well; one brother (II 5 in Figure 1) died at age 49 years of carcinoma. Blood was obtained from six of case 2’s remaining seven siblings. Three were negative for all tests. Two (II 8 and II 9 in Figure 1) had positive results on the KCT assay for LAC but negative antiphospholipid antibody assays; the third (II 7 in Figure 1) had positive results for LAC, ANF, anticardiolipin antibodies, and antiphosphatidylserine antibodies. One of his two children, III 6 in Figure 1, has SLE and is being treated with steroids and azathioprine. She had positive results for LAC and antiphosphatidylserine antibodies. Her sister, III 7 in Figure 1, had had vertiginous episodes while taking oral contraceptives and had positive results for LAC, anticardiolipin antibodies, and antiphosphatidylserine antibodies and a low platelet count. A child of one of case 2’s siblings (III 10 in Figure 1) had negative results for LAC and antiphospholipid antibodies. Four members of the fourth generation, all grandchildren of case 2’s brother who died of stroke (II 2 in Figure 1), have been tested; all four are normal. No individual who tested positive had taken drugs recognized as causing antiphospholipid activity.

It is of interest that in this pedigree, those individuals testing positive for LAC showed a paradoxical response to the two APTT reagents. In the presence of LAC, the second reagent usually exhibits increased sensitivity to antiphospholipid activity, resulting in a prolonged APTT. In the affected members of this family, APTT with this second reagent were shorter than APTT with the first reagent.

In an attempt to identify an independent marker of endothelial cell damage in these subjects, plasma levels of vWF.Ag were assayed. Of the 19 family members studied, 10 had results above the normal range and eight of the 10 had other serologic abnormalities (either positive LAC or antiphospholipid antibody assays, Table 1). One of these eight (III 3) was pregnant, which could account for her elevated vWF.Ag concentration, but the greatest elevations (244 and 212 μ/dl) were seen in the index case and case 2, both of whom had had strokes but were not acutely ill at the time of testing. The APTT might be shortened, at least in part, by increased concentrations of coagulation factors such as vWF.Ag.

Antithrombin III and protein C activities were normal in all members of this family, both symptomatic and asymptomatic.

Discussion

The index case, a young patient with migraine, multiple cerebrovascular events, and LAC, who fits a clinical profile described by this and other centers,13,16,22 and her similarly affected mother pointed to the possibility of a familial diathesis.

This is borne out by the family investigation, which revealed other symptomatic members with a variety of laboratory abnormalities indicating antiphospholipid activity and asymptomatic members with similar serologic abnormalities, together with one family member with SLE and another positive for ANF. Raised concentrations of vWF.Ag in some family members suggests the chronic release of von Willebrand factor from damaged endothelium. Alternatively, the elevated levels may represent an additional hereditary abnormality in this family. The clinical symptoms and the results of laboratory tests are, however, not in complete concordance (Table 1). All but one symptomatic family member had one or more laboratory abnormalities. A number of asymptomatic members had one or more positive tests; this may be explained by the propensity of serologic markers in autoimmune disease to occur in asymptomatic family members,23 or it may indicate that the laboratory abnormalities are markers for some other as-yet undefined factor that promotes thromboembolic events. The presence of antiphospholipid antibodies and LAC in this family in both symptomatic and asymptomatic members argues for a causal association rather than their being the result of thromboembolic events. The raised concentrations of vWF.Ag in both symptomatic and asymptomatic family members, usually together with other laboratory abnormalities, argues for a perturbation of endothelial cells, perhaps mediated by the antiphospholipid antibodies as has been suggested.24,25 This also supports a causal relation between antiphospholipid activity and the neurologic symptoms. It is notable that the highest levels of vWF.Ag were found in the two patients with the most severe symptoms. The lack of concordance between the various tests themselves is not unexpected, having been noted in a large phenothiazine-treated psychiatric population investigated by us, as well as in patients symptomatic for cerebrovascular disorders.26 The observed discrepancy between the APTT results and those for KCT suggest that the latter is a more sensitive test for the presence of LAC.

The patient with SLE (III 6) whose father (II 7) was positive for ANF and antiphospholipid antibodies and whose sister (III 7) was also positive for antiphospholipid antibodies raises an additional point of interest. The original descriptions of LAC, as the name implies, and the associated thromboembolic phenomena were in patients with SLE. There have also been reports of the same clinical and serologic associations in patients who clearly do not have SLE, as well as in patients with illnesses bearing many of the features of SLE but who did not meet criteria sufficient for such a diagnosis to be made. This suggests a spectrum of diseases between the so-called antiphospholipid antibody syndrome and a subgroup of patients with SLE who have these antibodies. Additionally, observations that family members of
SLE patients may have either LAC27 or antiphospholipid antibodies28 suggests that such antibody activity in the blood of patients without SLE may in some cases reflect a genetic susceptibility to SLE. Studies of family members are necessary to examine the possibility that these antibodies are a part of the wider lupus diathesis. Mackie et al27 also noted LAC activity in the spouses of some SLE patients, suggesting the possibility of transmissible or environmental agents. Although we did not extensively study the spouses in this family, we tested the husbands of the index case and case 1; both men were negative for LAC and antiphospholipid activity. There have been a few reports of the familial occurrence of LAC-like activity in asymptomatic patients without SLE.29-31 Jacobson et al32 reported brothers who presented at 39 and 41 years of age with stroke and evidence of both LAC and antiphospholipid antibodies, but tests of the rest of the family were negative.

This family is of interest for several reasons. First, a high incidence of stroke occurring at a young age is associated with a familial incidence of LAC and/or antiphospholipid antibodies. Although the pattern of inheritance is unclear, the genetic susceptibility to the development of various autoimmune conditions has been associated with certain class II human lymphocyte antigen alleles,33 and a similar association may underlie the familial pattern of disease seen in this family. Second, although it is too early to be certain in the younger members, the presence of such antiphospholipid activity does not appear to be invariably associated with manifestations of thromboembolic disease, suggesting either that such activity is not in itself harmful but merely acts as a marker for another process, or that an additional and as-yet unidentified cofactor is required for disease expression. It is notable that the index case and her cousin (III 7) both developed migraine while taking oral contraceptives, a combination recognized as a risk factor for cerebrovascular events,34 and that the index case developed major problems while pregnant, as pregnancy itself can cause a hypercoagulable state. Third, the elevated concentrations of vWF.Ag in the two individuals who have had strokes as well as in several individuals with antiphospholipid activity suggests a chronic endothelial disturbance. Indeed, elevated vWF.Ag levels have been reported in SLE in association with thromboembolic phenomena.35 Last, a cousin (III 2) of the index case has recurrent migraines and possible TIAs, yet she has neither LAC nor any serologic abnormalities.

These observations are important because the clinical picture is that of multiple small events rather than a single catastrophic one, and the rapid and sustained response to aspirin in the index case, which is in accord with our experience in some other patients with antiphospholipid-related cerebrovascular syndromes,18 suggests that early identification of such patients is well worthwhile. Furthermore, the study of families with a high incidence of premature stroke may uncover more such groupings and help clarify the pathogenetic mechanisms involved.

References

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