Stroke

Antiphospholipid Antibodies and Stroke in Young Women * Editorial Comment

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Antiphospholipid Antibodies and Stroke in Young Women

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Background and Purpose—Antiphospholipid antibodies have been associated with ischemic stroke in some but not all studies.

Methods—We performed a population-based case-control study examining antiphospholipid antibodies (anticardiolipin antibodies and lupus anticoagulants) using stored frozen sera and plasma in 160 cases and 340 controls enrolled in the Stroke Prevention in Young Women study. We evaluated for the presence of anticardiolipin antibody (IgG, IgM, and IgA isotypes) by an enzyme-linked immunosorbent assay and for the lupus anticoagulant using several phospholipid-dependent coagulation tests (activated partial thromboplastin time, dilute Russell's viper venom time) with mixing studies. If mixing studies were prolonged, confirmatory tests were performed.

Results—A positive anticardiolipin antibody level of any isotype was seen in 43 cases (26.9%) and 62 controls (18.2%) ($P=0.03$), lupus anticoagulant in 29 cases (20.9%) and 38 controls (12.8%) ($P=0.03$), and either anticardiolipin antibody or lupus anticoagulant in 61 cases (42.1%) and 86 controls (27.9%) ($P=0.003$). After adjustment for age, current cigarette smoking, hypertension, diabetes, angina, ethnicity, body mass index, and high-density lipoprotein levels, the relative odds of stroke for women with anticardiolipin antibody immunoreactivity of any isotype or a lupus anticoagulant was 1.87 (95% confidence interval, 1.24 to 2.83; $P=0.0027$).

Conclusions—The results from this study support the importance of antiphospholipid antibodies as an independent risk factor for stroke in young women. (***Stroke***. 2002;33:2396-2401.)

Key Words: antibodies, antiphospholipid ◼ anticoagulants, lupus ◼ cerebrovascular disorders ◼ thrombosis ◼ women

Antiphospholipid antibodies (aPLs) are a group of antibodies directed against phospholipids or phospholipid-protein complexes. These antibodies have been linked to a clinical syndrome consisting of thrombosis, thrombocytopenia, and recurrent fetal loss. Much work has been done in efforts to determine whether any antibody characteristics are associated with a greater thrombotic risk, and several have been suggested.[1,2] The presence of a lupus anticoagulant (LA), an aPL that is detected with a phospholipid-dependent coagulation test, appears to be associated with a greater thrombotic risk for both venous and arterial thrombosis.[3]

Cerebral ischemia associated with aPL is the most common arterial manifestation.[4,5] However, the importance of aPL as a cardiovascular risk factor is controversial. A number of case-control studies have found an association between aPL and incident ischemic stroke,[6–14] but others have not.[15–17] Several prospective studies have also found an association

between aPL and stroke and myocardial infarction.[18–20] Many studies evaluated only for anticardiolipin antibodies (aCL) using an enzyme-linked immunosorbent assay technique. Thus, an important association between the more "high-risk" aPL, the LA, could have been missed. In addition, prior studies[8,13] have suggested that aPLs are more strongly associated with stroke in young adults. We evaluated the association between several types of aPLs (aCL and LA) and the risk of stroke in women enrolled in a population-based case-control study, the Stroke Prevention in Young Women Study.

Methods

The Stroke Prevention in Young Women Study is a population-based case-control study initiated to study risk factors for ischemic stroke

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in young women. The study population included all residents of Maryland except the far western panhandle, Washington DC, and the southern portions of both Pennsylvania and Delaware. Cases and controls were recruited for the study between February 26, 1992, and January 1, 1996.

Cases were women 15 to 44 years of age with a first cerebral infarction, identified by discharge surveillance at all 59 hospitals in the study area and through direct referral by regional neurologists. The methods for discharge surveillance, chart abstraction, case adjudication, and assignment of probable and possible underlying causes have been previously described.[21,22] Recruitment within 1 year of stroke was required for participation. Of 291 cases who were both eligible and identified within the 1-year time window, 227 could be contacted and agreed to participate; 160 had a serum sample available for aCL testing, and 139 had a platelet-poor plasma sample available for LA testing.

Controls were women without a history of stroke, frequency matched by age and geographic region of residence to the cases, identified by random digit dialing. Of 450 eligible controls, 392 agreed to participate, 340 had a serum sample, and 298 had a platelet-poor plasma sample available for testing.

Blood was not available for all cases and controls for each assay for several reasons. Every case and control subject did not agree to having blood drawn, and some who did agree did not have blood drawn for technical reasons. In some cases, some blood was obtained, but the line clotted before all tubes could be successfully obtained. There was a priority system for the order in which the tubes were drawn. The tube for the LA testing was always drawn last to avoid any contamination of heparin in the line.

Nonfasting blood samples were obtained at a median time after stroke of 87 days; 30.4% were obtained within 30 days of stroke. After processing, serum and plasma samples were stored at $-70°C$ and not thawed until the aPL assays were performed.

aCL assays were performed on serum samples according to the manufacturer's instructions with a commercially available kit (Sanofi for IgG and IgM isotypes and Reaads Medical Products for IgA isotype). The suggested manufacturer's cut points were used as follows for IgG, IgM, and IgA isotypes: negative, >20 U; low positive, >20 to 40 U; moderate positive, >40 to 60 U; and high positive, >60 U. LA testing was performed on platelet-poor plasma samples with commercially available reagents. The activated partial thromboplastin time (Diagnostica Stago) and dilute Russell's viper venom time (DRVVT; American Diagnostics) were performed on all samples. Mixing studies using a 50:50 mixture of patient and normal plasma were performed if the initial tests were abnormal. If the mixing studies were also abnormal, the StaclotLA and the DRVVT Confirm test were performed to confirm the presence of LA. The manufacturers' cut points were used as follows: normal activated partial thromboplastin time mixing study if correction to normal range; normal DRVVT mixing study if ratio of DRVVT to DRVVT 50:50 mix was <1.3; normal StaclotLA for <5.6 seconds; and normal DRVVT Confirm test if DRVVT to DRVVT Confirm ratio was ≤1.41. Assays were run in duplicate, and the results were averaged. For the aCL assays, the mean within-assay within-pair coefficient of variation was 16%.

Potential confounders in the association between aPL and stroke included age, race, poverty status, hypertension, diabetes mellitus, angina or myocardial infarction, current smoking status, body mass index, total cholesterol, high-density lipoprotein (HDL) cholesterol, fever, oral contraceptives, and plasma homocyst(e)ine levels. Hypertension, diabetes mellitus, and angina or myocardial infarction were determined by asking the study participant (or a proxy if the participant was unable to answer) if a physician had told her that she had the condition. Similarly, age, race, current smoking status, fever, oral contraceptive use, and poverty status were determined by the subject or proxy report. The number of cigarettes smoked per day was determined for current smokers. Poverty status, defined as 200% of the 1993 Federal Poverty Income Guideline, was based on total family income and the number of household members. The poverty threshold was raised to 200% of federal guidelines because of the high cost of living in Maryland and Washington, DC. Body mass

Frequency of aPL Immunoreactivity: Anticardiolipin Isotypes and LA

Type of aPL	Cases, n (%)	Controls, n (%)
aCL IgG	22/160 (14)*	28/340 (8)
aCL IgM	1/160 (0.6)	3/340 (0.9)
aCL IgA	23/160 (14)	39/340 (11)
aCL, any isotype (with/without LA)	43/160 (27)†	62/340 (18)
LA (with/without aCL)	29/139 (21)‡	38/298 (13)
aCL, any isotype or LA	61/125 (42)§	86/308 (28)
aCL (without LA)	20/110 (18)	35/259 (14)
LA (without aPL)	15/115 (13)	23/271 (8)
aPL and LA	11/119 (9)	14/280 (5)

*Relative OR, 1.78; 95% CI, 0.99–3.20; P=0.055.
†Relative OR, 1.65; 95% CI, 1.06–2.57; P=0.026.
‡Relative OR, 1.80; 95% CI, 1.06–3.06; P=0.028.
§Relative OR, 1.87; 95% CI, 1.24–2.83; P=0.0027.

index was based on self-report and calculated as weight in kilograms divided by the square of height in meters.

Total cholesterol and HDL cholesterol were measured according to standard practice. Total cholesterol was considered high at ≥240 mg/dL. HDL cholesterol was considered low at ≤35 mg/dL.

We used t tests to compare means and χ^2 tests to compare proportions. All probability values were 2 sided. Effect modification was sought by examining the probability value for an interaction term in a logistic regression model. Adjusted odds ratios (ORs) derived from logistic regression were used to determine whether individual or aggregate types of aPL were associated with an increased risk for stroke after controlling for important confounders. A potential confounder was considered important if adjustment for that factor altered the unadjusted estimate by ≥10%.[23]

Results

The 160 cases included in the analysis were compared with the 67 for whom stored serum or plasma was unavailable. There were no significant differences in medical record–derived age, race, hypertension, diabetes mellitus, coronary artery disease, smoking status, or adjudicated stroke origin.

Stroke cases were classified as having a probable, possible, or undetermined origin as previously described.[12,13] Among the 160 stroke patients, 63 (39%) had at least 1 probable cause: large-artery atherosclerosis, 16; cardioembolism, 14; lacune, 6; and other causes, 27. Forty-seven (29%) had no probable cause but at least 1 possible cause: large-artery atherosclerosis, 7; cardioembolism, 18; lacune, 0; and other causes, 21. Fifty (31%) were indeterminate. "Other causes" of stroke included hematologic disorders and nonatherosclerotic vasculopathy (eg, vasculitis and dissection), migraine, oral contraceptive use, drug abuse, and stroke associated with the postpartum state. Hematologic disorders and nonatherosclerotic vasculopathy could be listed in the probable or possible category, depending on the strength of the evidence, whereas the other conditions were included in the possible category only.

The Table shows the frequency of positive values for aCL isotypes and LA in cases and controls. Anticardiolipin IgG antibody was the isotype most strongly associated with stroke (relative OR, 1.78; 95% CI, 0.99 to 3.20; P=0.055). aCL of any isotype was seen in 43 cases (26.9%) and 62 controls (18.2%) (P=0.03), LA in 29 cases (20.9%) and 38 controls

(12.8%) ($P=0.03$), and either aCL or LA in 61 cases (42.1%) and 86 controls (27.9%) ($P=0.003$). The unadjusted OR for either aCL or LA was 1.87 (95% CI, 1.22 to 2.83). The unadjusted OR was 1.36 (95% CI, 0.70 to 2.61) for blacks and 2.22 (95% CI, 1.30 to 3.79) for whites, but there was no statistically significant interaction by race. There was no evidence for effect modification by age, current cigarette smoking, hypertension, diabetes, angina, ethnicity, body mass index, and HDL levels, and after adjustment for these factors, the relative OR of stroke for women with aCL immunoreactivity of any isotype or a LA was 1.87 (95% CI, 1.24 to 2.83; $P=0.0027$). After adjustment for age, current cigarette smoking, hypertension, diabetes, angina ethnicity, body mass index, and HDL levels, the relative OR of stroke for women with aCL immunoreactivity of any isotype or LA was 1.87 (95% CI, 1.24 to 2.83; $P=0.0027$). There was no evidence of a dose-response relationship between any of the anticardiolipin isotypes and the risk of stroke. For all isotypes combined, there were 59 subjects in the low positive group, 39 in the moderate positive group, and 4 in the high positive group. The ORs for the low, moderate, and high positive groups were 1.6 (95% CI, 0.9 to 2.8), 1.4 (95% CI, 0.7 to 2.9), and 2.3 (95% CI, 0.3 to 16.7), respectively.

As expected, compared with controls, cases of stroke were more likely to be hypertensive; have diabetes mellitus, higher cholesterol levels, and body mass indexes; and smoke more cigarettes. None were important confounders because none altered the unadjusted aPL risk estimate by $\geq10\%$. Similarly, poverty status, plasma homocyst(e)ine history of fever, and oral contraceptive use also failed to alter the unadjusted aPL risk estimate by $\geq10\%$.

Discussion

We report the first population-based study of aPL and stroke in young women. Both aCL and LA were tested, unlike many population-based studies in which only aCL could be tested because banked serum was used and plasma was unavailable for testing. Consistent with prior studies evaluating several types of aPL, we found anticardiolipin IgG and LA to be associated with a higher stroke risk than other anticardiolipin isotypes. The highest risk for stroke was seen when any anticardiolipin isotype or LA was present, underscoring the importance of evaluating for LA. The magnitude of the increased stroke risk seen in this study was modest and does not support the speculation made by older studies that aPLs are more strongly associated with stroke risk in young compared with older populations.

Case-control studies of stroke in young people have been uniformly positive.[6,8,12,13] Some[7,9–11,14] but not all[15–17] case-control studies among older adults have found aPLs to be associated with ischemic stroke. The ORs found in positive studies of unselected stroke patients have ranged from 2.3[7] to 6.7[24] or higher.[9] Case-control studies have been criticized because of the difficulty of establishing the temporal relationship between positive antibody levels and stroke. However, studies that obtained blood within 7 days of the event[7] or even within 6 hours of onset[24] have had positive findings. Although these time periods may be too short to allow the development of measurable IgG levels because of a primary

or an amnestic immune response,[25] they do not preclude the possibility of antibodies induced by a recent prior febrile illness. Infection-associated cerebral infarction is common[26] and has been associated with higher levels of aCL of the IgG isotype in 1 study.[15,27] Recent animal studies have shown that thrombosis-producing aPL can be induced with a viral peptide immunization.[28] We did not find an interaction between fever, a proxy for infection, and aPL in this study.

Until recently, all published prospective studies of aPL and stroke were negative.[29,30] However, these studies were limited in statistical power and suffered from technical limitations. The most recent, a prospective case-control study of men enrolled in the Honolulu Heart Program,[18] found an independent association between aPL and stroke and myocardial infarction. The overall risk factor–adjusted OR for aCL of the IgG isotype for stroke was 2.2 ($P<0.001$). The association became statistically significant as early as 10 years after the baseline measurement and appeared strongest after 15 years of follow-up. Although all cases and controls were also free of angina at baseline, it was not possible to examine the issue of subclinical disease. In addition, LA was not evaluated in this study.

The mechanisms by which aPL could lead to stroke and other thrombotic manifestations are multiple and varied. Effects on platelets, coagulation proteins, and endothelial cells by aPL have been demonstrated and support the plausibility of aPL-associated thrombosis. For example, aPL from patients with thrombosis, thrombocytopenia, or fetal loss can induce a dose-dependent increase in the activation and aggregation of human platelets.[31] This effect appears to be mediated through binding to phosphatidylserine,[32] the most common phospholipid in cell membranes, or β2-glycoprotein 1, a glycoprotein aPL cofactor. aPL can interfere with the protein C pathway by inhibiting thrombin formation, interfering with thrombomodulin expression, and inhibiting the degradation of activated protein C (activated protein C resistance) (reviewed elsewhere[33]). Interestingly, aPL-related activated protein C resistance does not appear to be related to a mutation in the coagulation factor V gene.[34,35] Passive and active immunization of normal laboratory mice with either aPL or with β2GP1 results in the induction of an experimental antiphospholipid syndrome, including thrombocytopenia, placental infarction and fetal loss, myocardial infarction, and neurological dysfunction.[36,37]

This study has several limitations. An inherent problem for case-control studies is that a potential "risk factor," in this case aPL, may be a consequence rather than a predictor of disease, in this case stroke. Women were included within 1 year of stroke onset. Serum and plasma were collected at variable time points for each subject temporally distant from the stroke onset. Thus, this study cannot distinguish whether the increased presence of aPL in the stroke group is a contributing cause or a consequence of the stroke. However, a temporal association between the presence of aPLs and stroke has already been demonstrated in other studies.[7,8] In addition, recall bias is a potential limitation of all historically ascertained exposures in case-control studies. This could be the case for an exposure such as fever, but it unlikely to be the case for oral contraceptive use.[38] Another limitation is in the

clinical interpretation of our data. The association of first stroke with aPL in this study is only modest, with an OR of 1.7 to 1.8, which is more consistent with a predisposing factor such as hypertension or diabetes rather than a definite origin. Even the presence of an aCL of moderate to high titer or LA cannot be considered to be the cause of stroke, and a full evaluation for other causes is always indicated. In terms of treatment, studies evaluating the risk for recurrent stroke associated with aPLs have largely been retrospective or case series.[4,5,39] Even if the presence of aPL doubled the incidence of the combined end point of vascular death, nonfatal myocardial infarction, or recurrent stroke among young adults with first stroke from 2.6% per year[40] to 5.2% per year, data on the differential effectiveness of antiplatelet and anticoagulation regimens in this young population are needed for making treatment recommendations. Furthermore, recently established criteria for the antiphospholipid syndrome requires that a moderate- to high-titer aCL or a LA be present in association with a thrombotic event, thrombocytopenia, or unexplained fetal loss on at least 2 occasions at least 6 weeks apart.[41] In this study, aCL and LA were measured on only 1 occasion. Whether the presence of the antiphospholipid syndrome conveys a higher stroke risk or higher recurrent stroke risk in young women or any other population remains to be seen. Optimal treatment may well differ, depending on whether aPL is present in isolation or whether other elements of the antiphospholipid syndrome are present.

In conclusion, our findings support an association of modest strength between aPLs and stroke in young women. Taken together with the results from other studies, this suggests that aPL has an effect on stroke risk across the age range and in both sexes. Given the modest effect seen in this and other studies, it is important to stress the importance of a complete evaluation for other causes in patients with aPL and stroke. It is important for future studies of aPL and stroke to consider the impact of persistently positive aPL assays and other clinical evidence of the antiphospholipid syndrome on stroke risk. Treatment recommendations for stroke prevention in aPL-positive stroke patients await data on the relationship between aPL and the risk of recurrent stroke and the comparative risk/benefit of antiplatelet and anticoagulant treatment.

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Editorial Comment

aPL and Stroke in Young Women: More Fuel for the Stroke Risk Fire

Antiphospholipid antibodies (aPL) are a heterogeneous family of immunoglobins that have been thought to be associated with thrombogenic states for many years. Whereas several mechanisms of action have been suggested, convincing evidence of a specific etiopathologic mechanism with regard to stroke remains elusive, partly due to the heterogeneity of aPL. Nevertheless, the association between these antibodies, or markers of their hematologic effects, and ischemic stroke has been demonstrated in numerous case-control[1] and, more recently, prospective cohort[2] studies. In this article, Brey and colleagues[3] extend these observations to young women, confirming findings from previous, less rigorous studies. In the present study, 160 women aged 15 to 44 years with first cerebral infarction, identified by discharge surveillance at 59 hospitals in the region surrounding Baltimore, Maryland, and through direct referral by neurologists in the region, were compared with 340 age-matched controls recruited by random-digit dialing. After adjusting for potential confounders such as race, oral contraceptive use, and hypertension history, the relative odds for stroke in women with an anticardiolipin antibody or lupus anticoagulant approached 2, with a fairly narrow CI that excluded both 1 and 3. The magnitude of this result, while demonstrating a statistically significant association between aPL and ischemic stroke, is on the low end of the range of odds ratios (ORs) reported in previous studies, demonstrating a positive relationship between these antibodies and stroke. As the authors point out, this may be related to their inclusion of stroke patients up to 1 year from stroke onset; it is possible that an individual's aPL status could change from positive to negative during the intervening time. The controls had relatively high frequencies of aPL positivity (more than 1 in 10 controls had evidence of a lupus anticoagulant and almost 1 in 5 had evidence of aCL), much higher than what is generally reported in the general population. This raises concern over the specificity of aPL and the cutoff values for normal, as the assays may be overly sensitive, detecting "immunological noise." This may be another factor contributing to the relatively low ORs demonstrated.

Because of the time interval between stroke onset and blood draw, it is not possible in this study to exclude the possibility that, rather than contributing to the cause of, or risk for, stroke, the presence of aPL is the consequence of the stroke. However, data from other studies, in which blood sampling occurred immediately following or preceding stroke, make this an unlikely explanation for the current findings.

The relatively low ORs reported are somewhat surprising because in a population of young women, one expects a lower prevalence of atherosclerosis and a higher proportion of stroke related to the aPL syndrome than in the general stroke population. However, this does not appear to be the case. As the authors suggest, an OR of 2 is more consistent with a predisposing factor than with a specific etiology. One of the limitations of this study is its inability to distinguish an individual with an isolated aPL from one with the hallmarks of a more complete aPL syndrome. Perhaps examining the clinical data, if available, on patients' platelet counts (for

thrombocytopenia), syphilis serology (for a false-positive VDRL), antinuclear antibody titers, previous miscarriages, and previous thrombo-occlusive events may give insight into the actual proportion of stroke cases that had supporting evidence of the aPL syndrome. Also, although we are told of no relationship to several individual risk factors including HDL, aPL data related directly to LDL and total cholesterol and cardiac disease are not provided. There may be a relationship between the number of stroke risk factors present and aCL.[4]

The association of aPL, measured in a variety of ways, and stroke has now been well documented in several different populations, of different ages and both genders, using a variety of observational methods, yet several key questions remain: Do these antibodies play a direct role in the stroke process or are they merely an epiphenomenon? Are they merely markers of a thrombogenic state or active participants? How do they come to be present in the stroke patient; is there a common inciting agent that accounts for their occurrence or is their etiology in stroke patients a varied list that includes infections, medications, immunizations, malignancies, and autoimmune diseases? Current data are merely suggestive. Is there an identifiable subset of these antibodies (eg, cofactor dependent) that are more specifically associated with stroke? Again, current data suggest but do not prove this.[2,5] Does their presence tell us anything about a patient's risk for recurrent events and, perhaps most importantly from a clinical perspective, should we be treating patients who harbor these antibodies differently from those who do not? Current data fail to demonstrate an association with recurrent stroke.[6,7] Initial results from the WARSS cohort do not support the superiority of warfarin over aspirin for recurrent stroke prevention in patients with aPL, and previous smaller studies do not provide convincing evidence of the effectiveness of any specific therapy.[6] Epidemiologic investigations have brought us to our current state of knowledge and continue to add fuel to the fire of the aPL story. A greater understanding of the biologic mechanisms underlying the demonstrated associations is needed to truly understand the nature of the relationship between aPL and stroke and to rationally guide therapy.

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