Value of Immunologic Testing in Stroke Patients  
A Prospective Multicenter Study

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Background and Purpose  
The aims of this prospective and multicenter study were to determine the frequency of antiphospholipid and antinuclear antibodies in an unselected ischemic and hemorrhagic stroke population and to evaluate the clinical significance of these autoantibodies. 

Methods  
Over a 1-year period, we collected plasma from 481 consecutive patients with ischemic or hemorrhagic stroke attending four different hospitals. Blood (10 mL) was drawn from each subject into a citrated glass tube. Plasma was obtained immediately by centrifugation and was stored at −70°C until use. Concentrations of IgM and IgG antiphospholipid antibodies were measured at room temperature in normal (not heat-treated) plasma by standardized enzyme-linked immunosorbent assay. All sera were treated by indirect immunofluorescence on mouse liver and kidney sections for antinuclear antibodies.

Results  
A total of 481 patients (325 men, 156 women) 16 to 90 years in age (mean age, 61 years) were studied. Anticardiolipin antibodies were present in 5 of 481 (1.04%) patients. One patient was IgG positive and four patients were IgM positive. Of 481 patients, 35 (7.2%) were positive for antinuclear antibodies. Anti-DNA antibodies were not demonstrable in any patient.

Conclusions  
The frequency of anticardiolipin antibodies in a heterogeneous stroke population is possibly lower than reported. The routine screening of anticardiolipin and antinuclear antibodies in a stroke population is of questionable value. 

Key Words  
• antibodies, antinuclear  •  antibodies, antiphospholipid  •  cerebrovascular disorders

More than 100 causes of stroke are recorded. Anticardiolipin antibodies (aCL) and lupus anticoagulant (LA) belong to the group of circulating antiphospholipid antibodies (aPL). Recently, a pathogenic role for aPL antibodies was recognized in cerebral ischemia. This association was reported both in patients with autoimmune disorders such as systemic lupus erythematosus (SLE) and in the primary antiphospholipid syndrome. aPL are found in 30% to 60% of patients with SLE; 50% to 70% of these patients also have LA. Furthermore, some patients present with stroke as the initial manifestation of a systemic disease with or without aPL. Determination of antinuclear antibodies (aNA) and aPL in stroke patients has thus become standard in many departments of neurology. Nevertheless, in non-SLE patients, the relationship between aPL and stroke has not yet been well established. Reports in the literature are based on different stroke populations and use different techniques and methods, so results are contradictory. On the other hand, current tests do not distinguish between those persons at risk of the clinical events and those not at risk. It is not generally recommended to treat individuals in whom aPL are detected and who have not suffered previous thrombosis.

To assess the value of this approach, we performed a prospective study of 481 patients with ischemic or hemorrhagic stroke in whom aNA and aCL estimation had been done routinely.

Subjects and Methods  
Over a 1-year period (February 1989 to January 1990), we collected plasma from 481 consecutive patients with stroke attending four different hospitals. The samples were collected within a week of the stroke. The inclusion criteria were (1) the presence of focal neurological deficit and (2) evidence of stroke (ischemic or hemorrhagic) on the cerebral computed tomogram (CT) or magnetic resonance imaging (MRI). When an ischemic lesion was suspected and the initial radiological test was negative, a second CT or MRI was performed in a week. Cerebrovascular disease was separated into four subtypes according to clinical and radiological findings: (1) hemorrhagic stroke, with the presence of hematoma demonstrated on CT or MRI; (2) lacunar stroke, in patients with clinical and CT lacunar infarction in the territory of a deeply perforating artery; (3) embolic stroke, if cardiac abnormalities that are conventionally accepted as potential sources of emboli were demonstrated by two-dimensional echocardiography or electrocardiogram and if other etiology was absent; and (4) the absence of hematoma, lacunar infarction, potential source of emboli, and presence of atherosclerotic vascular disease (thrombotic stroke). Because the clinical diagnosis of ischemic stroke is based on a constellation of findings, not one of which is individually diagnostic, the final diagnosis was made by the neurological investigator at each clinical center.
Clinical data were obtained at the time of plasma collection from the patients by interview and from chart review.

Blood (10 mL) was drawn from each subject into a citrated glass tube; plasma was obtained immediately by centrifugation and stored frozen at −70°C until use. All laboratory tests were performed within 2 months after the index event. Concentrations of aCL were measured at room temperature in normal (not heat-treated) plasma by standardized enzyme-linked immunosorbent assay as devised by Gharavi et al.12 Results of aCL assay were expressed according to the standardization workshop. The IgG and IgM isotype results were assessed in GPL and MPL units; one unit is equal to 1 μg/mL IgG or IgM.13 They were interpreted as negative (<10 GPL or MPL units), low positive (10 to 20 GPL or MPL units), moderate positive (21 to 40 GPL or MPL units), or high positive (>40 GPL or MPL units). All positive results were repeated at least once, and a second blood sample was checked 2 months later. All sera were tested by indirect immunofluorescence on mouse liver and kidney sections for aNA at a dilution of 1:10. Positive sera were further tested on HEp2 cells for aNA patterns and on Crithidia luciliae for ds-DNA antibodies. All tests were performed in a blinded fashion in the same laboratory (Lupus Laboratory, St Thomas’ Hospital, London).

Results

Four hundred eighty-one patients (325 male, 156 female) were enrolled. Their mean age was 61 years (range, 16 to 90). The frequency of the different subtypes of cerebrovascular disease was as follows: thrombotic, 77%; embolic, 9.2%; hemorrhagic, 11.8%; and lacunar infarction, 2%.

Of 481 patients, 5 (1.04%) were positive for aCL. Only 1 patient was IgG-positive (high titer), and 4 patients were IgM-positive (low titers). All aCL-positive patients had thrombotic stroke (the Table). A second blood sample was tested 2 months later in each positive patient, and all patients were aCL-positive at this time.

Of the 481 patients, 35 (7.2%) were positive for aNA. Of these, 27 patients had thrombotic stroke, 3 patients had hemorrhagic stroke, and 5 patients had embolic stroke. Anti-DNA antibodies were not demonstrable in any patient. Of the 35 positive patients, 16 reacted on HEp2 nuclei at a dilution of 1:40. The patterns of fluorescence were nuclear in 7 cases, speckled membrane in 3, homogeneous in 2, and speckled in 4. In 1 patient, aNA titer was 1:320; in 2 patients, 1:80; in 4 patients, 1:20; and in 28 patients, less than 1:20. Only 2 of 5 patients, one with high titer of IgG and another with low titer of IgM aCL, had no other vascular risk factors; nevertheless, the first patient developed SLE several months later and was positive for anti-DNA antibodies at that time. Both patients had a thrombotic stroke. aPL and aNA did not appear to play a clear role in the pathogenesis of stroke in any other patients.

Discussion

Initially, the association between aPL and thrombosis in patients without SLE had been derived largely from case reports and small case series. Compelling evidence has emerged that links aPL to thrombosis in SLE.6,8,9,14–17 Although some studies suggest that aPL may be associated with stroke and vascular thrombosis in patients without systemic disease,18–22 a clear association in non-SLE patients has not been well established.

Differences in the methods used to detect aPL have contributed to the wide range of reported frequencies: 17% to 100% for LA,29,30 and 11% to 58% for aCL in SLE patients with thromboembolic complications19,28 and 0% to 60% for LA29,30 and 2% to 41% for aCL-LA31,32 in non-SLE patients with thrombosis. Variability of methods for aCL testing is well recognized.33–35

In patients with SLE, the compiled results of different series suggest that in these patients a statistically significant association exists between aPL and a history of venous or arterial thrombotic complications.36 It is not clear, however, to what extent factors such as follow-up time, duration or severity of disease, and immunosuppressive or anticoagulant treatment may have influenced these results. In addition, these patients have other factors for developing vascular thrombosis.

In recent years, different studies have reported an association of aPL in stroke patients without systemic disease. Thus, a recent study found that prevalence of aCL in stroke was 2%, concluding that routine screening of aCL in stroke is of questionable value.31 On the other hand, another recent study32 found that 29 of 68 (43%) patients were positive for aPL. In this study, however, 59% of the aPL patients had other vascular risk factors, 66% had headache, and 11% had mitral valve prolapse.33 Czlonkowska et al36 found 32% of aCL in patients who had their first transient ischemic attack (TIA) or ischemic stroke before 50 years of age. All aCL-positive patients had other vascular risk factors in that study. Ferro et al36 found that 6 of 33 patients were positive for lupus anticoagulant. Of these patients, 2 had SLE, and no data are available about associated vascular risk factors. In two previous reports,39,40 we found that prevalence of aPL in stroke patients was 4% and 6.8%, respectively, but 50% of these patients had other vascular risk factors and several had SLE.
In the present study, of 481 patients with ischemic and hemorrhagic stroke, 5 (1.04%) were aCL-positive, but only 2 had no other vascular risk factors. Furthermore, one patient developed SLE several months later.

In a previous report, we found no significant clinical or paraclinical differences between these patients and those without aPL. In that study, aCL-positive patients were predominantly male and not necessarily young. Levine and Welch reviewed this topic and found some typical features in aPL-positive patients, including a relatively young age, a female preponderance, and the presence of TIAs or stroke. Most of their patients had other definite stroke risk factors.

The Antiphospholipid Antibodies in Stroke Study (APASS) group has recently shown that aCL appear to be an independent risk factor for first ischemic stroke. They found a frequency of 9.7%, which is higher than ours. One possible explanation is that the APASS group studied only first ischemic stroke and we studied any type of stroke (first or consecutive, ischemic or hemorrhagic). Unfortunately, the differentiation between first or consecutive stroke was not included in the present study.

A recent cross-sectional study found no association between the presence of aCL and ischemic heart disease. In a prospective study of 62 patients without SLE who were younger than 45 years of age and who survived their first myocardial infarction, 13 patients were found to have elevated aCL titers. After a follow-up of 36 to 64 months, the presence of aCL was a significant risk factor for recurrent cardiovascular events. By contrast, aCL were measured in a study of 597 patients surviving an acute infarct. Although an increased proportion of survivors had aPL, the presence of these antibodies was not a risk factor for subsequent coronary or cerebrovascular thrombosis. Likewise, a case control study in a prospective cohort in 15 008 male physicians showed that having an aCL level above the 95th percentile is an important risk factor for deep venous thrombosis or pulmonary embolus but not for ischemic stroke in healthy adult men, although a modest effect cannot be excluded.

With regard to aNA, the APASS group found that these antibodies and anti-DNA antibodies were elevated in 58 of 106 (54.7%) and 10 of 61 (16.4%) patients, respectively. In our study, 35 of 481 (7.2%) patients were positive for aNA. Anti-DNA antibodies were not demonstrable in any patient. The APASS findings should be interpreted with caution because the group retrospectively analyzed a highly selected population.

According to Levine and Welch, (1) nearly all patients with stroke associated with aPL have had coexisting risk factors and (2) no consistent correlations exist with the level of aPL, isotype or specificity, and onset of thrombotic event. An association between aPL and stroke does not mean a cause-and-effect relationship. Thus, the population of patients with cerebrovascular disease and aPL may be heterogeneous with respect to pathogenesis and course of the disease. The presence of aPL may be an important risk factor for ischemic stroke in patients with SLE, but it does not appear to be a strong risk factor for ischemic stroke in a non-SLE population.

Thus, according to this study, the frequency of aCL in stroke population is possibly lower than reported. The routine screening of aCL and aNA in an unselected stroke population is of questionable value; thus, further investigations are needed to establish the role of aCL in stroke.

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