Background and Purpose—Previous studies have produced conflicting results regarding the putative association between anticardiolipin antibodies (aCL) and infarction in the general stroke population. These inconsistencies may be a function of sample size and methodological differences among the studies. The purpose of the present study, the largest case-control study of this issue to date, was to assess aCL status as an independent risk factor for ischemic stroke in a multiethnic, urban population.

Methods—We obtained aCL titers in 524 hospitalized acute stroke patients and 1020 community controls enrolled in the Minorities Risk Factors and Stroke Study. The results were interpreted as negative (<22.9 IgG phospholipid [GPL] or 10.9 IgM phospholipid [MPL] units), low positive (22.9 to 30.0 GPL or 10.9 to 15.0 MPL units), or high positive (>30.0 GPL or 15.0 MPL units). Odds ratios (ORs) were adjusted for age, sex, race/ethnicity, history of diabetes, hypertension, atrial fibrillation, coronary artery disease, and current cigarette smoking.

Results—A positive aCL titer was present in 11% (111/1020) of controls and 34% (180/524) of cases. The adjusted OR for any positive aCL titer was 4.0 (95% CI, 3.0 to 5.5). For any positive IgG aCL titer this value was 3.9 (95% CI, 2.8 to 5.5), and for any positive IgM aCL titer it was 3.4 (95% CI, 2.1 to 5.5). There were no significant differences in ORs associated with high- or low-positive IgG or IgM aCL titers.

Conclusions—In the largest study of its kind to date, aCL antibodies were demonstrated to be independent stroke risk factors across the 3 ethnic groups studied, conferring a 4-fold increased risk of ischemic stroke. IgG and for the first time IgM aCL were each shown to be associated with increased stroke risk. The prevalence of these antibodies and the stroke risk associated appear greater than previously reported. (Stroke. 1999;30:1561-1565.)

Key Words: antibodies, anticardiolipin ■ antibodies, antiphospholipid ■ risk factors ■ stroke, ischemic

Although the association between anticardiolipin antibodies (aCL) and thrombosis is well recognized, their role in stroke pathogenesis, and the extent to which they are independent stroke risk factors in the general stroke population, remain to be determined. While there have been many descriptive reports detailing the clinical characteristics of aCL-related stroke, controlled studies examining the relationship between aCL and stroke have been limited and have produced conflicting results.1-9 We conducted the largest case-control study reported to date to determine the extent to which these antibodies are independently associated with stroke in a multiethnic population.

Subjects and Methods

Subjects
We assessed aCL status in the subset of patients with ischemic stroke and controls enrolled in the Minorities Risk Factors and Stroke Study (MRFASS). Details regarding subject recruitment, data collection, and demographic characteristics of the population have been published previously.10 Briefly, patients were included in the present study if they were admitted to the hospital within 5 days of onset of symptoms of ischemic stroke and either the patient or a proxy gave informed consent. The control group comprised residents of the hospital catchment area who were aged at least 45 years and had no history of prior stroke. Self-identification determined the classification of race/ethnicity. Historical, demographic, and clinical information was obtained by a study nurse from chart review and interview of patients and family. Stroke diagnoses were made according to a previously published algorithm10 by a study neurologist who examined the patient and reviewed pertinent diagnostic tests but was blinded to the results of any antiphospholipid testing. Blood samples
were obtained the morning after admission for patients and the morning of the study visit for controls.

Specimens
Serum specimens were centrifuged and frozen within 2 hours of collection and stored at −70°C until aCL enzyme-linked immunosorbent assays were performed with standardized, commercially available kits (REAADS).

The IgG and IgM isotype results were reported as IgG phospholipid (GPL) and IgM phospholipid (MPL) units, whereby 1 unit is equal to 1 μg/mL of IgG or IgM. Titers were categorized as negative (≤22.9 GPL or 10.9 MPL units), low positive (22.9 to 30.0 GPL or 10.9 to 15.0 MPL units), or high positive (>30.0 GPL or 15.0 MPL units).

Data Analysis
Odds ratios (ORs) with 95% CIs were calculated by logistic regression analysis, with adjustment for age, sex, race/ethnicity, current cigarette smoking, and history of hypertension, diabetes mellitus, atrial fibrillation, and coronary artery disease (defined as previous myocardial infarction, angina, or a coronary revascularization procedure). All first-order interactions between historical stroke risk factors and aCL status were examined. Fisher’s exact test and χ² analysis were used for comparison of categorical variables, and the t test was used for comparison of continuous variables. All analyses used procedures of the Statistical Analysis System, version 6.12 (SAS Institute).

Results
There were 524 ischemic stroke patients and 1020 controls included in the study. Demographic and risk factor information for cases and controls are presented in Table 1. Cases were older and more likely to be white, non-Hispanic, and male. With the exception of current cigarette smoking, traditional stroke risk factors were more prevalent in cases. Seven cases and 1 control had a previous history of deep vein thrombosis. A history of collagen vascular disease was present in 2 cases and 2 controls. Since age, sex, race/ethnicity, and traditional stroke risk factors and prevalence of aCL positivity were virtually identical for cases with and without a history of prior stroke, all infarction patients were considered together in subsequent analyses.

The distribution of each of the aCL titers is positively skewed and appears to be log-normal, as has been described by previous investigators. 1 The median IgG aCL titer in cases was 9.9 GPL units (interquartile range, 5.5 to 16.6; maximum, 125.7), while in controls it was 8.7 (interquartile range, 5.1 to 13.8; maximum, 92.5). The median IgM aCL titer in cases was 3.1 (interquartile range, 2.1 to 5.3; maximum, 102.5), while in controls it was 3.0 (interquartile range, 2.0 to 4.7; maximum, 43.7). Table 2 categorizes cases and controls by aCL status. A positive aCL titer (IgG or IgM) was present in 11% (111/1020) of controls and 34% (180/524) of cases. Excluding atrial fibrillation did not substantially alter this OR and any OR for each of the positive aCL titer categories (versus negative titers) presented in Table 3. There was no statistically significant difference in the adjusted OR associated with a high- versus low-positive titer of either IgG or IgM.

TABLE 1. Demographic and Clinical Characteristics of Stroke Patients and Controls

| Stroke risk factors (%) | Cases (n=524) | Controls (n=1020) | P  
|-------------------------|--------------|------------------|-----
| Race/ethnicity          |              |                  |     
| White (%)               | 243 (46)     | 343 (34)         | 0.001 
| Black (%)               | 180 (34)     | 410 (40)         |     
| Hispanic (%)            | 97 (19)      | 260 (25)         |     
| Stroke risk factors (%) |              |                  |     
| History of hypertension | 342 (65)     | 452 (44)         | 0.001 
| History of diabetes mellitus | 179 (34) | 129 (13)         | 0.001 
| History of coronary artery disease | 119 (23) | 91 (9)           | 0.001 
| History of atrial fibrillation | 66 (13) | 10 (1)           | 0.001 
| Current smoking         | 64 (12)      | 155 (15)         | 0.11  

TABLE 2. Distribution of aCL Titers for Cases and Controls

<table>
<thead>
<tr>
<th>IgG</th>
<th>Cases (n=524)</th>
<th>Controls (n=1020)</th>
</tr>
</thead>
</table>
|     |              |                  | P  
| Low (+) | 66 (13) | 37 (4)           |     
| High (+) | 62 (12) | 37 (4)           |     

<table>
<thead>
<tr>
<th>IgM</th>
<th>Cases (n=524)</th>
<th>Controls (n=1020)</th>
</tr>
</thead>
</table>
|     |              |                  | P  
| Low (+) | 27 (5) | 25 (2)           |     
| High (+) | 39 (7) | 17 (2)           |     

TABLE 3. ORs for aCL Titer Categories Adjusted for Age, Sex, Race, and Risk Factors

<table>
<thead>
<tr>
<th>IgG</th>
<th>Total</th>
<th>White</th>
<th>Black</th>
<th>Hispanic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Any (+)</td>
<td>3.9 (2.8–5.5)</td>
<td>4.6 (2.7–87.6)</td>
<td>3.8 (2.4–6.1)</td>
</tr>
<tr>
<td></td>
<td>Low (+)</td>
<td>3.5 (2.2–5.6)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>High (+)</td>
<td>4.3 (2.7–7.0)</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IgM</th>
<th>Total</th>
<th>White</th>
<th>Black</th>
<th>Hispanic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Any (+)</td>
<td>3.4 (2.1–5.5)</td>
<td>3.8 (2.0–7.5)</td>
<td>2.5 (1.1–6.0)</td>
</tr>
<tr>
<td></td>
<td>Low (+)</td>
<td>2.3 (1.2–4.5)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>High (+)</td>
<td>5.1 (2.6–9.9)</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

Values in parentheses are 95% CI.
antibodies, although low-positive IgM aCL had an OR approximately half that of high-positive titers. Only the first-order interaction between history of diabetes mellitus and any positive aCL titer was significant (OR = 0.42; 95% CI, 0.20 to 0.89), suggesting that aCL antibodies may be of less importance in diabetics. There was no evidence of an interaction between race/ethnicity and the effect of aCL status on stroke risk. We also examined logistic regression models within each race/ethnic group finding similar statistically significant associations for any IgG aCL, any IgM aCL, and any IgG or IgM aCL in each group (Table 3).

To further examine the effect of age on aCL titer, we divided our subjects into 3 age categories (<60, 60 to 72, >72 years), corresponding approximately to tertiles among the controls, and found a trend toward an increase in titer in the oldest tertile in both cases and controls. However, in multivariable modeling the stroke risk associated with a positive aCL titer did not change with age.

Because of the suggestion that there may be an increased risk of stroke associated with aCL in young women with increased serum lipid levels (S. Kittner, MD, oral communication), we examined the subset of our patients (n = 438) and controls (n = 1017) for whom serum lipid data were available. We did not obtain serum lipid samples in patients admitted >48 hours after stroke onset because of the possibility that the event would affect lipid levels. We found no relationship between either LDL or total cholesterol level and the effect of aCL status in the group as a whole, among either sex, or among those aged 60 years or younger of either sex.

The stroke subtype distribution was similar among aCL-positive and aCL-negative patients in our study. Among the 180 aCL-positive patients, 11% of strokes were classified as atherothrombotic, 31% were cardioembolic, 26% were lacunar, 6% had other known etiologies, and 27% were considered cryptogenic. Among the 344 aCL-negative patients, 12% were atherothrombotic, 20% were cardioembolic, 37% were lacunar, 8% had other etiologies identified, and 24% were cryptogenic. Of the 131 patients with cryptogenic infarctions, 48 (37%) were aCL positive. Transesophageal echocardiography was obtained in 22 of the cryptogenic infarction patients, of whom 9 (41%) were aCL positive. These percentages are very similar to those found in patients with known stroke subtypes. There were 10 aCL-positive patients with cryptogenic infarction who had no other stroke risk factors.

Discussion

The prevalence of elevated aCL titers among unselected stroke patients in previous studies has ranged from 1% to 38% and from 0% to 12% in controls. We defined positive titers consistent with the recommendations of the manufacturer of the assay kits, which were based on the clinical performance of its assay on healthy and diseased populations, including systemic lupus erythematosus patients with and without a history of thrombosis. This may correspond to a higher titer than has been used in many previous studies, although the frequencies of positive titers we report are toward the higher end of the ranges previously reported. In our study an elevated titer of IgG or IgM (or both) is independently associated with increased stroke risk and is only slightly affected by adjustment for known stroke risk factors. The magnitude of this association is similar across the 3 ethnic groups studied.

Previous studies have varied in regard to population studied, method of antibody determination, and definition of positive titer (Table 4). The majority of prior controlled studies have demonstrated an association between either IgG aCL alone or a combination of aCL isotypes and stroke. Each was a case-control study that considered patients with either first ischemic stroke (cerebrovascular accident) or a combination of transient ischemic attack and cerebrovascular accident. The earlier studies were relatively small and did not adjust for other stroke risk factors. The Antiphospholipid Antibodies in Stroke Study (APASS) was the largest previous study but was still not adequately powered to consider aCL isotypes separately or to address the issue of titer. We report a higher rate of aCL positivity among cases, despite a less inclusive definition, than APASS reported, as well as a higher risk-adjusted OR than the 2.3 (95% CI, 1.1 to 14.9) reported by APASS. A smaller, more recent Italian study considered only IgG aCL, finding a prevalence of positive titers and risk-adjusted OR (4.9: 95% CI, 1.05 to 22.9) similar to what we report but with much wider CIs.

Four previous studies reported no significant association between aCL and stroke. Each had important methodological differences from the above studies. One followed a cohort of patients (the placebo group in the Warfarin Reinfarction Study) who had suffered a myocardial infarction. Samples for aCL were obtained an average of 1 month after myocardial infarction, and subjects were followed a mean of 3.2 years. Conceivably, some of the subjects categorized as positive could have had had an elevated titer transiently in response to the myocardial infarction. Another was a nested case-control study within the Physicians Health Study, a prospective randomized trial of aspirin and beta carotene in 22,071 men. In that study cerebral infarctions occurred an average of 3 years after samples for aCL were obtained, and assays were performed after an average of 8 years of frozen storage (possibly affecting the likelihood of obtaining a positive result). These negative results are of interest because, although other interpretations are possible, they suggest that aCL titers obtained at a random time point may not predict future stroke, conceivably because an individual’s aCL titer changes over time. A British case-control study included intracerebral hemorrhage as well as infarction patients and actually found higher mean titers of IgG aCL among cases than controls, although this was offset by higher titers of other aCL isotypes in controls than in cases. The most prevalent elevated titers were of the IgA isotype, not studied by most other investigators, and the definitions of aCL positivity differed from most others as well, making comparison with other studies problematic. The most recent study failed to find an association between aCL and stroke risk, but it included only 151 of 379 eligible infarction patients.

None of the 3 previous studies that considered the IgM isotype individually found an association with stroke, but 2 of these also did not find an association between IgG aCL and stroke and differ from other studies methodologically, as discussed above. The other had a small sample size and was
underpowered to detect an effect that may be less robust than the effect of IgG aCL. In summary, the accumulated evidence to date favors an association between IgG and IgM aCL and increased stroke risk, and methodological differences may partially explain apparently conflicting previous results. Although ours is the largest controlled study of aCL in stroke to date, therefore producing risk estimates with narrower CIs and providing the most convincing evidence of a significant association, demonstration of association does not prove causation. It has been suggested that aCL (IgM in particular) could be related to an acute phase response to stroke. It is unlikely that the elevated aCL antibodies identified in the present study resulted from the index stroke, since blood samples were obtained within 5 days of onset and APASS7 demonstrated no relation between aCL positivity and time from onset within this time frame. Additionally, Camerlingo et al8 reported a nearly identical 26% positivity rate for IgG aCL in samples drawn within 6 hours of stroke onset. Nevertheless, the case-control design of our study leaves open this possibility, which can best be excluded by a prospective study with serial aCL determinations that would track seropositivity over time. It is unlikely that the elevated aCL antibodies identified in the present study resulted from the index stroke, since blood samples were obtained within 5 days of onset and APASS7 demonstrated no relation between aCL positivity and time from onset within this time frame. Additionally, Camerlingo et al8 reported a nearly identical 26% positivity rate for IgG aCL in samples drawn within 6 hours of stroke onset.

We propose that a distinction be drawn between patients with primary antiphospholipid syndrome (characterized by recurrent atherothrombotic events, fetal loss, livedo reticularis, and thrombocytopenia) or patients with secondary antiphospholipid syndrome (ie, those who have these manifestations in the setting of systemic lupus erythematosus) and patients with other risk factors for vascular disease who are found to have positive serology for aCL but do not demonstrate these other clinical features. The “aCL syndrome” group may represent a distinct, albeit small, group with a thrombogenic, immune-mediated disorder but who have few traditional stroke risk factors. The latter group are likely at increased risk of the common types of stroke, perhaps only transiently, but cannot be said to suffer from a distinct syndrome. In both groups antiphospholipid antibodies may predispose to thromboembolism, but in the former they may represent the sole causative factor, while in the latter they may be one of many predisposing (and interactive) factors.

aCL are the best characterized and intensively studied of a heterogeneous family of antibodies directed toward negatively charged phospholipids or phospholipid-binding plasma proteins. An association between anti–β2-glycoprotein I (β2-GPI) antibodies and thrombosis has been reported.13 We did not measure anti–β2-GPI antibodies or assess the role of β2-GPI in aCL binding to cardiolipin. The significance of β2-GPI remains controversial, and a better understanding of its role will help to clarify the relationship between aCL and thrombogenicity. Recent studies have demonstrated that antibodies directed against anionic or neutral phospholipids are also associated with stroke13,14 and may be present in patients with thrombotic syndromes but

<table>
<thead>
<tr>
<th>Author</th>
<th>aCL</th>
<th>Population</th>
<th>n (Case/Control)</th>
<th>% Stroke aCL (+)</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kushner2 (1990)</td>
<td>IgG/M/A</td>
<td>CVA/TIA*</td>
<td>45/53</td>
<td>29</td>
<td>8.3†</td>
<td>1.75–39.6†</td>
</tr>
<tr>
<td>Hess3 (1991)</td>
<td>IgG/M</td>
<td>CVA/TIA</td>
<td>110/122</td>
<td>8 (IgG)</td>
<td>5.3 (IgG)†</td>
<td>1.13–25.3†</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9 (IgM)</td>
<td>1.4 (IgM)‡</td>
<td>0.54–3.75†</td>
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<tr>
<td>Chakravarty4 (1991)</td>
<td>IgG/M</td>
<td>1st CVA/ICH</td>
<td>100/100</td>
<td>18 (IgG)</td>
<td>∞†§</td>
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<td></td>
<td></td>
<td></td>
<td>3 (IgM)</td>
<td></td>
<td></td>
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<tr>
<td>Sletnes5 (1992)</td>
<td>IgG/M</td>
<td>CVA in post-MI cohort</td>
<td>44/550</td>
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<td>1.14 (IgM)‡#</td>
<td>0.82–1.59</td>
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<td>Ginsburg6 (1992)</td>
<td>IgG</td>
<td>CVA</td>
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<td>1.35†#</td>
<td>0.60–3.08</td>
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<td>APASS7 (1993)</td>
<td>IgG/M/A</td>
<td>1st CVA</td>
<td>255/255</td>
<td>10</td>
<td>2.3#</td>
<td>1.12–4.91</td>
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<tr>
<td>Muir8 (1994)</td>
<td>IgG/M/A</td>
<td>CVA/ICH</td>
<td>262/226</td>
<td>38</td>
<td>1.7 (IgG)†</td>
<td>0.94–3.14†</td>
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<td>1.6 (IgM)†</td>
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<td>0.69 (IgA)‡#</td>
<td>0.46–1.04†</td>
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<tr>
<td>Camerlingo8 (1995)</td>
<td>IgG</td>
<td>1st CVA</td>
<td>100/100</td>
<td>26</td>
<td>4.9#</td>
<td>1.05–22.9</td>
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<tr>
<td>Metz9 (1998)</td>
<td>IgG/M/A</td>
<td>CVA</td>
<td>151/109</td>
<td>8</td>
<td>0.96†</td>
<td>0.39–2.36†</td>
</tr>
</tbody>
</table>

CVA indicates cerebrovascular accident; TIA, transient ischemic attack; ICH, intracerebral hemorrhage; and MI, myocardial infarction.

*Patients with cardiac disease excluded.
†As calculated from data in text.
‡Nonsignificant.
§No controls aCL (+).
||Overall; data not provided for stroke alone.
#Adjusted for stroke risk factors.
¶For highest tertile 1.0 GPL.
with no evidence of abnormal antibodies on the more widely used antiphospholipid antibody assays. This suggests that the magnitude of the clinical significance of these moieties may be underestimated if only 1 (or even several) of the conventional assays is used.

The practical significance of the demonstrated association between elevated aCL titers and stroke with regard to patient diagnosis and management is not clear. There is no current rationale for screening a healthy population for their presence as a prophylactic strategy especially if, as suggested above, aCL status is of limited temporal importance. Further research is needed to determine the consistency of aCL status over time. There is as yet no convincing evidence that the presence of aCL at the time of initial stroke increases the risk of recurrence in an unselected stroke population.15–17 Recom-
mendations that the best prophylactic regimen in these patients is high-intensity anticoagulation are based on small retrospective analyses and are probably most relevant to younger patients with evidence of prothrombotic tendencies and little other risk of stroke. Ongoing studies should help to determine the importance of aCL as risk factors for recurrent stroke and the possible benefit of anticoagulation relative to the use of antiplatelet agents in secondary stroke prevention.10,18

In summary, our data from the largest case-control study to date demonstrate an association between both IgG and IgM aCL and increased stroke risk. This relationship is evident even for low-positive titers. This is the first such study to demonstrate this relationship specifically for the IgM isotype. The magnitude of the association (adjusted OR, ≈4.0) appears to be greater than previously recognized and pertains to all 3 of the race/ethnic groups studied.

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References

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