Antiphospholipid Antibodies and Subsequent Thrombo-occlusive Events in Patients With Ischemic Stroke

The APASS Investigators

Knowledge of well-established risk factors for recurrent stroke only partially predicts the occurrence of new events, which has led to a search for new risk factors. Several potential hematologic markers of hypercoagulability, including antiphospholipid antibodies (aPL) have been associated with vascular thrombo-occlusive events in most but not all case-control studies. In prospective studies, aPL positivity has been associated with initial thrombotic events including stroke, although not consistently. The role of aPL in predicting recurrent ischemic events, particularly recurrent ischemic stroke, is controversial.

Antiphospholipid antibodies, both antiphospholipid antibodies (aCL) and lupus anticoagulant antibodies (LA), have been linked to an immune-mediated coagulopathy and left-sided cardiac valvular lesions. In the absence of prospective, controlled clinical trial data, recommendations have been put forth for the use of long-term, high-intensity anticoagulation in the management of symptomatic patients testing positive for aPL. Because all prior studies investigating the role of aPL and recurrent events have been compromised by small sample sizes, lack of blinded or standardized therapy, and lack of adjudicated end points, we cannot establish risk factors for recurrent stroke only partially predicts the occurrence of new events, which has led to a search for new risk factors. Several potential hematologic markers of hypercoagulability, including antiphospholipid antibodies (aPL) have been associated with vascular thrombo-occlusive events in most but not all case-control studies. In prospective studies, aPL positivity has been associated with initial thrombotic events including stroke, although not consistently. The role of aPL in predicting recurrent ischemic events, particularly recurrent ischemic stroke, is controversial.

Antiphospholipid antibodies, both antiphospholipid antibodies (aCL) and lupus anticoagulant antibodies (LA), have been linked to an immune-mediated coagulopathy and left-sided cardiac valvular lesions. In the absence of prospective, controlled clinical trial data, recommendations have been put forth for the use of long-term, high-intensity anticoagulation in the management of symptomatic patients testing positive for aPL. Because all prior studies investigating the role of aPL and recurrent events have been compromised by small sample sizes, lack of blinded or standardized therapy, and lack of adjudicated end points, we cannot establish risk factors for recurrent stroke only partially predicts the occurrence of new events, which has led to a search for new risk factors. Several potential hematologic markers of hypercoagulability, including antiphospholipid antibodies (aPL) have been associated with vascular thrombo-occlusive events in most but not all case-control studies. In prospective studies, aPL positivity has been associated with initial thrombotic events including stroke, although not consistently. The role of aPL in predicting recurrent ischemic events, particularly recurrent ischemic stroke, is controversial.

Context The presence of antiphospholipid antibodies (aPL) has been associated with vascular occlusive events. However, the role of aPL in predicting ischemic events, particularly recurrent ischemic stroke, is controversial.

Objective To evaluate the effect of baseline aPL positivity (ie, positivity for antiphospholipid antibodies [aCL], lupus anticoagulant antibodies [LA], or both) on subsequent thrombo-occlusive events, including recurrent stroke.

Design, Setting, and Participants The Antiphospholipid Antibodies and Stroke Study (APASS), a prospective cohort study within the Warfarin vs Aspirin Recurrent Stroke Study (WARSS), a randomized double-blind trial (N=2206) conducted at multiple US clinical sites from June 1993 through June 2000 and comparing adjusted-dose warfarin (target international normalized ratio, 1.4-2.8) and aspirin (325 mg/d) for prevention of recurrent stroke or death. APASS participants were 1770 (80%) WARSS participants who consented to enroll in the APASS, with usable baseline blood samples drawn prior to randomization to the WARSS and analyzed for aPL status within 90 days of index stroke by a central independent laboratory. Quality assurance was performed on approximately 10% of samples by a second independent laboratory.

Main Outcome Measure Two-year rate of the composite end point of death from any cause, ischemic stroke, transient ischemic attack, myocardial infarction, deep vein thrombosis, pulmonary embolism, and other systemic thrombo-occlusive events. The primary analysis assessed the outcome associated with aPL positivity within each WARSS treatment group separately, after risk-factor adjustment (since these aPL-positive vs aPL-negative comparisons were not randomized).

Results Of the 1770 APASS patients, 720 (41%) were classified as aPL-positive and 1050 (59%) as aPL-negative. There was no increased risk of thrombo-occlusive event associated with baseline aPL status in patients treated with either warfarin (relative risk [RR], 0.99; 95% confidence interval [CI], 0.75-1.31; P=.94), or aspirin (RR, 0.94; 95% CI, 0.70-1.28; P=.71). The overall event rate was 22.2% among aPL-positive and 21.8% among aPL-negative patients. There was no treatment \times aPL interaction (P=.91). Patients with baseline positivity for both LA and aCL antibodies tended to have a higher event rate (31.7%) than did patients who tested negative for both antibodies (24.0%) (unadjusted RR, 1.36; 95% CI, 0.97-1.92; P=.07). Classification and regression tree analyses did not identify a specific LA test or aCL isotype or titer that was associated with increased risk of thrombo-occlusive event.

Conclusions The presence of aPL (either LA or aCL) among patients with ischemic stroke does not predict either increased risk for subsequent vascular occlusive events over 2 years or a differential response to aspirin or warfarin therapy. Routine screening for aPL in patients with ischemic stroke does not appear warranted.

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sought to clarify the significance of aPL, specifically both LA and aCL, assayed at the time of an initial ischemic stroke, in predicting subsequent thrombo-occlusive events. We undertook a prospective study, the APASS (Antiphospholipid Antibodies and Stroke Study), within a large, randomized, double-blind clinical trial comparing warfarin and aspirin for the prevention of recurrent ischemic stroke or death.22

METHODS
The primary goal of the APASS was to compare the effect of aPL positivity on subsequent thrombo-occlusive events among patients in the Warfarin Aspirin Recurrent Stroke Study (WARSS)23 in each treatment group separately, through 2 prespecified primary null hypotheses: (1) within the warfarin treatment group, there is no difference between aPL-positive patients and aPL-negative patients in risk of subsequent thrombo-occlusive events, and (2) within the aspirin treatment group, there is no difference between aPL-positive patients and aPL-negative patients in risk of subsequent thrombo-occlusive events. A secondary null hypothesis was that aPL-positive patients in the aspirin group and aPL-positive patients in the warfarin group would not have any differences in the rate of subsequent thrombo-occlusive events, including death. Other secondary aims included determining rates of thrombo-occlusive events based on type of aPL positivity, aPL titers, and stroke subtype.

Selection of Patients
Participants in the APASS were recruited from the WARSS, a randomized, double-blind, multicenter clinical trial that compared aspirin (325 mg/d) and adjusted-dose warfarin (target international normalized ratio [INR], 1.4-2.8) for the prevention of recurrent ischemic stroke or death after a previous ischemic stroke.22,23 The APASS began its recruitment 12 months after the initiation of the WARSS. The WARSS assessed patients for 2 years or to a primary end point. Details of the WARSS methodology have been previously published,22-24 as have the main results.21 The APASS was a prospective study with hypotheses that were specified prior to study initiation and was administered independently of the WARSS.22 Forty-four of the 48 WARSS sites participated in the APASS (see list at end of article). Patients were eligible for the APASS if (1) they were enrolled in the WARSS, (2) their site had institutional review board approval for APASS aPL testing before their baseline aPL blood draw, (3) they provided written informed consent prior to venipuncture for aPL determination, and (4) their aPL status based on blood samples obtained at baseline (ie, prior to randomization and treatment in the WARSS) was successfully determined within 90 days of enrollment in the WARSS (Figure 1). Briefly, patients were eligible for the WARSS if they were aged 30 to 85 years, deemed safe for warfarin therapy, had experienced an operation or attributed to a high-grade carotid stenosis for which surgery was planned, or stroke due to a potentially likely cardiac source of embolism (eg, atrial fibrillation). All aspects of eligibility were confirmed via the APASS data management center at Columbia University, New York, NY, prior to enrollment.

aPL Assays
Prior to the initiation of randomization, a training videocassette recording, produced at the APASS coordinating laboratory in San Antonio, Tex, was mailed to each clinical site. This provided standardized, detailed instructions for the drawing and processing of the aPL samples, as well as for shipping the samples to the coordinating laboratory. Special emphasis was placed on techniques to obtain platelet-poor plasma (defined as <5000 residual platelets per cubic milliliter of plasma after processing) for LA testing. After centrifugation (beginning between 30 minutes and 2 hours after blood draw) at room temperature for 15 minutes at 3000g, serum was carefully divided into 1-mL aliquots and plasma was filtered through a 0.22-micron syringe filter to
remove any residual platelets. Residual platelet counts performed on 10 identically processed plasma samples ranged from none detected to 4 platelets per cubic milliliter of plasma, far less than the published criteria for platelet-poor plasma. Specimens were immediately stored at a minimum of −20°C and were shipped monthly via overnight courier on dry ice to the APASS coordinating laboratory in San Antonio. Assays were kept frozen and then thawed for monthly testing.

The LA tests included a sensitive activated partial thromboplastin time (aPTT) (Diagnostica Stago Inc, Parsippany, NJ), the dilute Russell viper venom test (dRVVT) (American Diagnostics Inc, Pendleton, Ind) and a hexagonal phase confirmatory test, the StaClot LA (Diagnostica Stago Inc). Several tests for LA were selected because the literature suggests that a significant number of true-positive LA plasma samples may be missed if only a single test is used.\textsuperscript{25} If times in the aPTT and dRVVT tests were prolonged, mixing studies (1:1) were performed for both tests and a StaClot LA test was performed. For study purposes, an LA test result was called positive if the results of either the aPTT or dRVVT mixing studies were positive or if the results of the StaClot LA tests were positive.

The aCL tests were performed on samples using a commercial enzyme-linked immunosorbent assay technique (Corgenix Inc, Denver, Colo) according to the manufacturer’s instructions. For the baseline samples, a polyvalent screening that detected aCL IgG, IgM, and IgA was performed first. All samples that tested positive for polyvalent testing were subsequently isotyped for aCL IgG, IgM, and IgA immunoreactivity. Cutoff values for positive results were IgG >21 µg aCL per deciliter of serum; IgM >12 µg IgM aCL per deciliter; and IgA >23 µg IgA aCL per deciliter.

Patients were classified as aPL-positive if they tested negative for both aCL and LA at baseline. As part of the study protocol, approximately 10% of the samples (262 aCL, 228 LA), randomly chosen, were also assayed yearly by the APASS quality assurance laboratory in Muncie, Ind. For all assays of aCL and LA, there were no significant differences in mean values (by t test) between the 2 laboratories (Dr Brey in San Antonio and Dr Triplett in Muncie). All intraclass correlation coefficients were greater than 0.8 (P<.001).

**Participant Follow-up**

Following baseline aPL assays, patients were followed up for 24 months postrandomization or until a primary end point occurred. Participants underwent detailed baseline histories and examinations and were evaluated in person by the local principal investigator every 4 months thereafter and with monthly telephone contact in between. Detailed information was obtained on all possible new thrombo-occlusive events and study medication adverse events. Results of the aPL testing by the APASS coordinating laboratory were not made available to the local sites. Clinical aPL testing performed by the local sites was monitored (85% of all charts); such testing was found to be infrequent (12.6% of patients) and did not interfere with blinding for therapy after randomization.

**Definitions of End Points**

The primary end point was the occurrence of death from any cause, or any thrombo-occlusive event (ie, ischemic stroke, myocardial infarction, transient ischemic attack, deep venous thrombosis, pulmonary embolism, systemic visceral arterial embolism, or peripheral arterial embolism). Thrombo-occlusive event was defined conservatively to include all-cause rather than vascular death. This has 3 advantages. It avoids the problem that some deaths are unclassifiable as vascular or nonvascular because of missing data, ambiguity, etc; it provides consistency with the WARSS; and it avoids the problem of censoring. (If thrombo-occlusive event was defined using vascular death, then non-vascular deaths without prior recurrent ischemic stroke, myocardial infarction, transient ischemic attack, deep venous thrombosis, pulmonary embolism, systemic visceral arterial embolism, or peripheral arterial embolism would be censored observations. It is conceivable that death could be accelerated by some undetected vascular process in a number of these cases.)

The local site investigator, who was blinded to treatment assignment, made the initial determination of an end point on the basis of information obtained from the patient, medical records, and review of diagnostic studies.

**Adjudication of End Points**

Information concerning end points was sent to the WARSS-APASS data management center for central, independent adjudication. Prior to adjudication, all information related to patient identifiers, treatment assignment, and aPL status was masked. Ischemic stroke and transient ischemic attack were adjudicated by the WARSS adjudicators, and myocardial infarction was adjudicated by a cardiologist at Columbia University. Data for all other thrombo-occlusive events were sent to independent adjudicators expert in vascular surgery or pulmonary and critical care medicine, depending on the type of event. All adjudicators were blinded to treatment assignment.

**Statistical Analysis**

The statistical analysis was intent-to-treat, with a 2-sided, Bonferroni-corrected α level of .025 for each test reflecting the separate analyses within the warfarin and aspirin treatment groups, and end point imputation analogous to WARSS\textsuperscript{23} for cases lost to follow-up. A Cox proportional hazards model was used to determine the relative risks (RRs) and associated confidence intervals (CIs). Reported event rates are actuarialized estimates from the Kaplan-Meier curves that adjust for censoring. Similar analyses were performed for the secondary hypothesis. For these analyses, a multivariate proportional hazards model was used to ad-
just for variables that were imbalanced between aPL-positive and aPL-negative participants (P=.20) and were associated with a thrombo-occlusive event (P=.20) in either the aPL-positive or aPL-negative group.

The analysis addresses potential confounding, given that the aPL-positive vs aPL-negative comparison within each WARSS treatment group is not randomized. The candidate variables tested as confounders in the multivariate analyses included age, sex, race (white vs nonwhite), marital status, education (college vs no college), insurance status (Medicaid vs non-Medicaid), history of diabetes, systolic blood pressure, history of cardiac disease, history of stroke, cigarette smoking (ever), alcohol consumption (moderate vs low or high), sedentary lifestyle, Glasgow Outcome Scale score less than 5 points, and Barthel Index less than 95. A treatment × aPL interaction term was added to this model to test the secondary null hypothesis that aPL-positive patients in the aspirin group and aPL-positive patients in the warfarin group would not have any differences in the rate of subsequent thrombo-occlusive events, including death. An interaction was considered significant if the computed P value was .10 or less.

The original prospective APASS power calculation assumed an aPL positivity rate of 20%, recruitment of 80% of the WARSS cohort (approximately 1000 in each treatment group), an α level of .025, 2-sided tests, and that the aPL-negative patients in each treatment group would experience the 2-year event proportion expected for the WARSS primary end point of ischemic stroke or death (16% for aspirin, 11.2% for warfarin). This provided 80% power to detect absolute differences between aPL-positive and aPL-negative patients of 10.2% in the warfarin group and 11.4% in the aspirin group. In the APASS cohort actually recruited, both the aPL positivity rates and the overall event rates were greater than expected. The final sample provided 87% power to detect a 10.2% difference in the warfarin group, and 96% power to detect an 11.4% difference in the aspirin group.

Classification and regression tree analysis (CART 4.0, Salford Systems, San Diego, Calif)26 was performed to investigate the significance of specific aCL titers and isotypes in predicting death or thrombo-occlusive event. The CART analysis constructed decision trees for outcome classification (death or thrombo-occlusive event vs no death or thrombo-occlusive event) based on algorithms that determine optimal cutpoints for each individual assay. The analytic technique is nonparametric and can select the set of variables that are jointly most important in determining outcome from among a large set of candidates. Prediction accuracy is assessed by cross-validation among randomly chosen subsets of the data.

RESULTS

The APASS enrolled 1770 patients from June 1994 through June 1998. Follow-up continued through June 30, 2000. Figure 1 accounts for the APASS enrollment status of all 2206 WARSS participants. Of the patients randomized in the WARSS, 89% were eligible for the APASS. Of those, more than 90% were enrolled in the APASS. Patients enrolled in APASS were more often women and less often cigarette smokers compared with those not enrolled in the APASS, with all other sociodemographic factors not significantly different (Table 1).

Patients were compared for baseline variables within each treatment group per the primary, prespecified hypotheses of interest. Within the warfarin group (n=881), no baseline sociodemographic or risk factor variables were both imbalanced between the aPL-positive and the aPL-negative patients and associated with outcome. Within the aspirin group (n=889), history of cardiac disease (28.3% vs 21.4%, P=.02), history of stroke (19.9% vs 14.7%, P=.06), sedentary lifestyle (47.5% vs 40.3%, P=.04), and age (63.4% [SD, 11.5] years vs 62.2 [SD, 11.3] years, P=.12) differed between aPL-positive and aPL-negative patients and were associated with out-

### Table 1. Generalizability of APASS to WARSS Sociodemographic and Clinical Factors

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>WARSS Patients, No. (%)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>in APASS (n=1770)</td>
<td>Not in APASS (n=436)</td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>62.5 (11.3)</td>
<td>62.2 (11.4)</td>
</tr>
<tr>
<td>Women</td>
<td>746 (42)</td>
<td>151 (35)</td>
</tr>
<tr>
<td>White (vs nonwhite)</td>
<td>1014 (57)</td>
<td>239 (65)</td>
</tr>
<tr>
<td>Smoking (ever)</td>
<td>1155 (66)</td>
<td>309 (71)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1203 (69)</td>
<td>296 (68)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>560 (32)</td>
<td>145 (33)</td>
</tr>
<tr>
<td>Cardiac disease</td>
<td>411 (24)</td>
<td>93 (22)</td>
</tr>
</tbody>
</table>

Abbreviations: APASS, Antiphospholipid Antibodies and Stroke Study; WARSS, Warfarin vs Aspirin Recurrent Stroke Study.

### Table 2. Comparison of Sociodemographic and Clinical Factors by aPL Status (Combined Treatment Groups)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>aPL+ Patients, No. (%)</th>
<th>aPL− Patients, No. (%)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=720)</td>
<td>(n=1050)</td>
<td></td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>63.1 (11.4)</td>
<td>62.2 (11.2)</td>
<td>.11</td>
</tr>
<tr>
<td>Women</td>
<td>275 (38)</td>
<td>471 (45)</td>
<td>.005</td>
</tr>
<tr>
<td>White (vs nonwhite)</td>
<td>393 (65)</td>
<td>621 (69)</td>
<td>.06</td>
</tr>
<tr>
<td>Smoking (ever)</td>
<td>497 (69)</td>
<td>658 (63)</td>
<td>.005</td>
</tr>
<tr>
<td>Hypertension</td>
<td>486 (69)</td>
<td>717 (69)</td>
<td>.07</td>
</tr>
<tr>
<td>Diabetes</td>
<td>227 (32)</td>
<td>333 (32)</td>
<td>.91</td>
</tr>
<tr>
<td>Cardiac disease</td>
<td>187 (26)</td>
<td>224 (21)</td>
<td>.02</td>
</tr>
</tbody>
</table>

Abbreviations: APASS, Antiphospholipid Antibodies and Stroke Study; aPL, antiphospholipid antibody.
come. Table 2 provides a comparison of sociodemographic and clinical factors by baseline aPL status for both treatment groups combined.

Baseline aPL Immunoreactivity

There were 720 (41%) participants who demonstrated aPL positivity for either aCL or LA, and 120 (6.7%) who tested positive for both. There were 362 (20.5%) aCL-positive/LA-negative participants and 238 (13.4%) who tested aCL-negative/LA-positive. One thousand fifty (59%) participants were aPL-negative. Of the 926 patients who tested positive on the initial aCL screening test, the isotype distribution and titer were 330 (35.6%) IgG-positive (190 low, 136 moderate, 4 high-positive); 88 (9.5%) IgM-positive (58 low, 19 moderate, 11 high-positive); and 169 (18.3%) IgA-positive (134 low, 30 moderate, 5 high-positive). There were 105 occasions when patients tested positive for more than 1 isotype. Antiphospholipid antibody status was not significantly associated with age, race, educational level, physical activity level, hypertension, diabetes mellitus, cigarette smoking, remote history of cerebral ischemia or other thrombotic events, or index stroke subtype, but was significantly associated with patients’ sex. Men were significantly more often positive for aPL than were women (43% vs 37%, respectively; odds ratio, 1.32; 95% CI, 1.08-1.60; P = .005).

aPL Immunoreactivity and Thrombo-occlusive Events

The distribution of thrombo-occlusive event outcomes by baseline aPL status is shown in Table 3. After risk-factor adjustment (Cox model), there was no observed increase in the risk of death or thrombo-occlusive event associated with aPL positivity in either the warfarin treatment group (26.2% from Kaplan-Meier curve at 2 years for aPL positivity vs 26.2% for aPL negativity; RR, 0.99; 95% CI, 0.75-1.31; P = .94) or the aspirin treatment group (22.2% for aPL positivity vs 21.8% for aPL negativity; RR, 0.94; 95% CI, 0.70-1.28; P = .71) as shown in Table 4. There was no treatment × aPL status interaction (P = .91).

Table 3. APASS End Points, by aPL Status

<table>
<thead>
<tr>
<th>Primary End Point</th>
<th>aPL+ (n = 720)</th>
<th>aPL− (n = 1050)</th>
<th>Proportion With Event at 2 Years*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death from any cause</td>
<td>29 (3.6)</td>
<td>35 (3.3)</td>
<td>0.99 (0.76-1.28)</td>
</tr>
<tr>
<td>Recurrent ischemic stroke</td>
<td>81 (11.3)</td>
<td>112 (10.7)</td>
<td>1.03 (0.77-1.37)</td>
</tr>
<tr>
<td>Transient ischemic attack</td>
<td>42 (4.0)</td>
<td>4 (0.4)</td>
<td>1.01 (0.83-1.22)</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>15 (2.1)</td>
<td>25 (2.4)</td>
<td></td>
</tr>
<tr>
<td>Symptomatic pulmonary embolism</td>
<td>1 (0.1)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Symptomatic deep vein thrombosis</td>
<td>6 (0.8)</td>
<td>8 (0.8)</td>
<td></td>
</tr>
<tr>
<td>Symptomatic peripheral/arterial thromboembolism</td>
<td>1 (0.1)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Symptomatic systemic/visceral arterial thromboembolism</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: APASS, Antiphospholipid Antibodies and Stroke Study; aPL, antiphospholipid antibody. *aPL+ indicates positivity for anticardiolipin antibodies (aCL) and/or lupus anticoagulant antibodies (LA); aPL− indicates negativity for both aCL and LA.

Table 4. APASS Primary Analysis (n = 1770)

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Patients, No.</th>
<th>Proportion With Event at 2 Years†</th>
<th>Unadjusted RR (95% CI)</th>
<th>P Value‡</th>
<th>Adjusted§ RR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warfarin</td>
<td>361</td>
<td>520</td>
<td>26.15</td>
<td>26.19</td>
<td>0.99 (0.76-1.28)</td>
<td>.93</td>
</tr>
<tr>
<td>Aspirin</td>
<td>359</td>
<td>530</td>
<td>22.18</td>
<td>21.76</td>
<td>1.03 (0.77-1.37)</td>
<td>.85</td>
</tr>
<tr>
<td>Treatment × aPL interaction</td>
<td>720</td>
<td>1050</td>
<td>24.18</td>
<td>23.95</td>
<td>1.01 (0.83-1.22)</td>
<td>.95</td>
</tr>
</tbody>
</table>

Abbreviations: APASS, Antiphospholipid Antibodies and Stroke Study; aPL, antiphospholipid antibody; CI, confidence interval; RR, relative risk. *aPL+ indicates positivity for anticardiolipin antibodies (aCL) and/or lupus anticoagulant antibodies (LA); aPL− indicates negativity for both aCL and LA.
†From Kaplan-Meier curve at 24 months.
‡From Wald statistic.
§Cox model adjusted for history of cardiac disease, history of stroke, exercise status (sedentary vs not sedentary), and age. These covariates were included in the model due to an imbalance (P = .00) across aPL+ and aPL− groups and were associated with an event (P = .20) in either the aPL+ or aPL− groups.
APASS was 1.9, the same as in the WARSS. A time-dependent covariate Cox model of time to thrombo-occlusive event that adjusted for interruption of therapy found no significant difference in INR response between aPL-positive and aPL-negative warfarin-treated patients not on interruption of therapy (P = .91).

Classification and regression tree analyses were performed to identify a specific titer and isotype associated with death or thrombo-occlusive event. For each of the 3 specific aCL isotypes (IgG, IgM, and IgA), there was no cutpoint that was able to demonstrate a positive predictive value greater than 30%; similar results were obtained for the components of LA positivity. No specific titer for aCL isotypes or LA positivity was able to demonstrate more

### Table 5. APASS Primary Event by LA and aCL Combinations Within Treatment Group

<table>
<thead>
<tr>
<th>LA/aCL Status</th>
<th>No.</th>
<th>Proportion With Event at 2 Years*</th>
<th>Unadjusted RR (95% CI)</th>
<th>P Value†</th>
<th>Adjusted‡ RR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Warfarin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA+/aCL+</td>
<td>64</td>
<td>35.94</td>
<td>1.44 (0.93-2.24)</td>
<td>.11</td>
<td>1.47 (0.91-2.36)</td>
<td>.11</td>
</tr>
<tr>
<td>LA+/aCL−</td>
<td>128</td>
<td>21.26</td>
<td>0.78 (0.51-1.17)</td>
<td>.23</td>
<td>0.78 (0.50-1.21)</td>
<td>.27</td>
</tr>
<tr>
<td>LA−/aCL+</td>
<td>169</td>
<td>26.22</td>
<td>0.99 (0.71-1.39)</td>
<td>.96</td>
<td>0.99 (0.69-1.41)</td>
<td>.95</td>
</tr>
<tr>
<td>LA−/aCL−</td>
<td>520</td>
<td>26.19</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
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<tr>
<td>LA × aCL interaction</td>
<td></td>
<td></td>
<td>.06</td>
<td>.07</td>
<td></td>
<td></td>
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<tr>
<td><strong>Aspirin</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA+/aCL+</td>
<td>56</td>
<td>26.79</td>
<td>1.26 (0.74-2.16)</td>
<td>.40</td>
<td>1.28 (0.74-2.22)</td>
<td>.37</td>
</tr>
<tr>
<td>LA+/aCL−</td>
<td>110</td>
<td>18.43</td>
<td>0.82 (0.51-1.32)</td>
<td>.41</td>
<td>0.71 (0.42-1.18)</td>
<td>.19</td>
</tr>
<tr>
<td>LA−/aCL+</td>
<td>193</td>
<td>22.96</td>
<td>1.08 (0.77-1.53)</td>
<td>.64</td>
<td>0.99 (0.69-1.42)</td>
<td>.96</td>
</tr>
<tr>
<td>LA−/aCL−</td>
<td>530</td>
<td>21.76</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>LA × aCL interaction</td>
<td></td>
<td></td>
<td>.36</td>
<td>.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA+/aCL+</td>
<td>120</td>
<td>31.67</td>
<td>1.36 (0.97-1.92)</td>
<td>.07</td>
<td>1.41 (0.99-2.02)</td>
<td>.06</td>
</tr>
<tr>
<td>LA+/aCL−</td>
<td>238</td>
<td>19.94</td>
<td>0.79 (0.58-1.08)</td>
<td>.15</td>
<td>0.75 (0.54-1.05)</td>
<td>.09</td>
</tr>
<tr>
<td>LA−/aCL+</td>
<td>362</td>
<td>24.48</td>
<td>1.04 (0.81-1.32)</td>
<td>.77</td>
<td>1.00 (0.78-1.29)</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>LA−/aCL−</td>
<td>1050</td>
<td>23.95</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>LA × aCL interaction</td>
<td></td>
<td></td>
<td>.04</td>
<td>.02</td>
<td></td>
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</tr>
</tbody>
</table>

Abbreviations: APASS, Antiphospholipid Antibodies and Stroke Study; aCL, anticardiolipin antibody; CI, confidence interval; LA, lupus anticoagulant antibody; RR, relative risk.

*From Kaplan-Meier curve at 24 months.
†From Wald statistic.
‡Cox model adjusted for history of cardiac disease, history of stroke, exercise status (sedentary vs not sedentary), and age. These covariates were included in the model due to an imbalance (P ≤ .20) across aPL+ and aPL− groups and were associated with an event (P ≤ .20) in either the aPL+ or aPL− groups.

### Figure 2. Kaplan-Meier Analysis of the Time to Thrombo-occlusive Event, by aPL Status of APASS Patients Receiving Warfarin (n=881) or Aspirin (n=889)

For the warfarin group: log-rank z score, 0.68; P = .93; relative hazard ratio, 0.99; 95% confidence interval (CI), 0.76-1.28. For the aspirin group: log-rank z score, −1.28; P = .85; relative hazard ratio, 1.03; 95% CI, 0.77-1.37. APASS indicates Antiphospholipid Antibodies and Stroke Study; aPL, antiphospholipid antibody.
than a 4% increase in positive predictive accuracy above the level expected by chance. Maximum sensitivity was 31% and maximum specificity was 73%. In selected subgroup analyses, aPL positivity was not associated with increased risk in patients younger than 55 years (hazard ratio, 1.02; 95% CI, 0.68-1.52) or with cryptogenic stroke subtype (hazard ratio, 1.14; 95% CI, 0.76-1.70).

**COMMENT**

Immunoreactivity to aPL (ie, presence of either aCL or LA at the time of a first ischemic stroke) did not influence the risk of subsequent thrombo-occlusive events over the subsequent 2 years in this cohort of patients enrolled in the WARSS. Furthermore, warfarin (median INR, 2), alleged to be superior to aspirin in patients with aPL-associated thrombosis, was not associated with fewer thrombo-occlusive events than was treatment with aspirin. Our data from this large, prospectively studied cohort showed that testing for aCL or LA did not confer important knowledge for prognosis or for treatment, a finding that may apply to the ischemic stroke population in general. However, a small subgroup (n=120, or less than 7% of the sample) suggests that patients who test positive for both aCL and LA (ie, a possible higher specificity) may have more thrombo-occlusive events, regardless of type of treatment. Since this was a secondary, prespecified analysis, this finding would require larger studies to confirm it. We also did not find a specific titer or isotype of aCL that was associated with ischemic events over the 2-year follow-up period. While there is variability in aPL assays, we used 2 independent laboratories, both of which have undergone internationally accepted standardization procedures, are internationally recognized for their expertise in this field, and assayed samples after only a single freeze-thaw cycle. Because the majority of aCL immunoreactivity was low-positive, the sample size with higher titers was inadequate to detect a modest titer effect (only 0.2% of 1770 patients with ischemic stroke had high-positive IgG aCL).

Because our study design was double-blind, our data are less likely to be subject to bias compared with data from previous retrospective, unblinded reports. Testing for aPL in patients experiencing their first ischemic stroke may not offer enough value for decisions on therapy or prediction of future ischemic events to warrant routine testing. Even a prior history of thrombo-occlusive events did not predict increased risk conferred by aPL positivity.

The failure to demonstrate a treat-ment effect for warfarin does not appear to be due entirely to a low INR. The median INR after day 28 among warfarin-treated patients enrolled in the APASS was 1.9, the same as in the WARSS. A time-dependent covariate Cox model of time to thrombo-occlusive event that adjusted for interruption of therapy found no significant difference in INR response between aPL-positive and aPL-negative warfarin-treated patients not on interruption of therapy (P=.91). Earlier studies indicating that higher INRs were needed were retrospective, had smaller sample sizes, and were not double-blinded, precluding direct comparison with our data. However, a recent double-blind, randomized clinical trial did not demonstrate lower event rates with INRs of 3 to 4 compared with INRs of 2 to 3. Our failure to demonstrate a better outcome in the warfarin treatment group or any direct link with aPL status along with the study by Crowther et al raises doubts concerning the weight that should be given to earlier recommendations for warfarin therapy. The appropriate use of warfarin in the hypercoagulable states is an understudied area.

Our data would indicate no defined role for aPL in patients evaluated following a noncardioembolic stroke. However, despite our large cohort and essentially negative findings, we cannot definitively state that no such relationship exists for younger patients with stroke who may have other features of the aPL syndrome, including Sneddon syndrome, or with multiple cerebrovascular events. Our subgroup analyses do not suggest that aPL positivity confers increased risk of subsequent events in either young patients or in the cryptogenic stroke subtype.
As our data suggest that immunoreactivity to more than 1 aPL may confer increased risk of thrombo-occlusive events, there still may be differential effects of treatment and outcome using newer aPL markers such as antibodies to β2-glycoprotein I and antibodies to other phospholipids such as phosphatidylserine, which remain untested to the same degree achieved by our APASS study. However, routine aCL testing for aCL and LA in the clinical setting of first ischemic stroke cannot be recommended on the basis of our data because aPL positivity (as we defined it) did not confer increased risk of thrombo-occlusive events, nor did it determine a differential treatment response.

This study definition of LA positivity is slightly different than more recent recommendations, however, at the time the study was undertaken these recommendations were not available. A recent survey of different LA reagents using standardized samples with known LA activity found the overall sensitivity to range from 84% to 99% and the specificity to range from 84% to 99%. The SiaClot and StaClot LA tests were among those tested in this survey.

Our methods for aPL determination were strengthened by the use of a quality assurance laboratory that tested approximately 10% of the study’s sample. That the results for both aCL and LA were highly concordant and correlated adds confidence to our results, given the known variability of aCL assays and the newest guidelines (those issued since our study was conceived and executed) that require at least 2 aPL determinations 6 to 8 weeks apart before considering the presence of the aPL syndrome. As we used only 1 measurement to determine aPL positivity for our primary goal, it is possible that a substantial number of these patients were transiently positive or would not fulfill current guidelines for aPL positivity. This is likely given the relatively higher than expected proportion of patients (4%) who tested aPL positive. We previously studied the acute effect of stroke on aCL immunoreactivity and did not find that aCL immunoreactivity increased over time from stroke in the first week after stroke.

The relatively high prevalence of aPL immunoreactivity suggests that we may be measuring nonspecific immune activation and B cell secretion in response to infection, other inflammatory conditions, medications, or measuring cross-reactivity, consistent with the preponderance of low-positive titers, which have had the most uncertain clinical significance. If some aPL (alone or in combination) truly confer an increased risk of thrombo-occlusive events, better ways are needed to sort out pathogenic aPL prior to making firm treatment recommendations.

Author Contributions: Dr Levine, as principal investigator of the APASS study, had full access to all of the data in this study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Levine, Brey, Tilley, Thompson, Sacco, Mohr.

Acquisition of data: Levine, Brey, Thompson, Sacco, Costigan, Rhine, Trippett, Mohr.

Analysis and interpretation of data: Levine, Brey, Tilley, Thompson, Sacco, Trippett, Sciacca, Murphy, Lu, Levin, Mohr.

Drafting of the manuscript: Levine, Brey, Tilley, Thompson, Sacco, Mohr.

Critical revision of the manuscript for important intellectual content: Levine, Brey, Tilley, Thompson, Mohr.

Statistical expertise: Tilley, Thompson, Murphy, Lu, Sciacca, Levin.

Obtained funding: Levine, Mohr.

Administrative, technical, or material support: Levine, Costigan, Rhine, Murphy, Lu, Sciacca, Mohr.

Study supervision: Levine, Brey, Tilley, Thompson, Mohr.

Authors/Members of the APASS Writing Committee: Steven R. Levine, MD (Department of Neurology, Mt Sinai School of Medicine, New York, NY); Robin L. Brey, MD (Department of Medicine, University of Texas Health Science Center, San Antonio); Barbara C. Tilley, PhD (Department of Biometry and Epidemiology, Medical University of South Carolina, Charleston); J. L. P. Thompson, PhD (Department of Biostatistics, Mailman School of Public Health, Columbia University, New York, NY); Ralph L. Sacco, MD, MS (Department of Neurology, College of Physicians and Surgeons, Columbia University); Robert R. Sciacca, EngScD, MD, MSc, Yimeng Lu, MS, and Teresa M. Costigan, BS (Department of Biostatistics, Mailman School of Public Health, Columbia University); Candri Rhine, BS (Department of Medicine (Neurology), University of Texas Health Science Center, San Antonio); Bruce Levine, PhD (Department of Biostatistics, Mailman School of Public Health, Columbia University); Douglas A. Trippett, MD (Department of Pathology, Memorial Hospital, Muncie, Ind); and J. P. Mohr, MD (Department of Neurology, College of Physicians and Surgeons, Columbia University).


Data Management Center: J. L. P. Thompson, B. Levin.


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ANTIPHOSPHOLIPID ANTIBODIES IN PATIENTS WITH ISCHEMIC STROKE
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