Cerebrovascular disease is the fourth leading cause of death in people >65 years of age in the United States and a major cause of disability and societal burden. Brain infarcts are an irreversible downstream feature of cerebrovascular disease. Improved understanding of risk factors for brain infarcts, in particular of risk factors that are readily identifiable (eg, by blood testing) and modifiable (eg, with treatment), may contribute to identifying possible targets for therapy and to improving public health in the elderly, the fastest-growing segment of the population.

Clinical Perspective on p 189

Antiphospholipid antibodies (aPLs) are a group of antibodies present in blood that are directed against a key component of cell membranes and include anticardiolipin (aCL) antibodies, among others. Data from the literature suggest that aPLs are increasingly common in aging. Indeed, a large study found that a third of people >80 years of age had aPLs. Moreover, aPLs increase the risk of first ischemic stroke by 2-fold in young to middle-aged adults and are routinely tested for in clinical settings in the evaluation of unexplained stroke, and stroke prevention therapies may be beneficial even in the presence of asymptomatic aPLs. Nonetheless, the relationship of aPLs to cerebrovascular disease in the elderly is less clear. In particular, there are no systematic data available on the association of aPLs with brain pathology. Indeed, only a small number of case reports and case series of select autopsied groups are published, and these report a range of pathologies, including brain infarcts and other vascular processes.

Our overarching hypothesis is that aPLs are associated with pathological brain infarcts and are related to cognitive and motor decline in aging. The design of the Antiphospholipid Antibodies, Brain Infarcts, and Cognitive and Motor Decline in Aging (ABICMA) study has been reported previously in detail. Here, we tested whether aPLs are associated with brain infarcts. We used blood specimens in which we measured aPLs combined with clinical and pathological data from >600 older, community-dwelling people who were followed up longitudinally until death in people >65 years of age in the United States and a major cause of disability and societal burden. Brain infarcts are an irreversible downstream feature of cerebrovascular disease. Improved understanding of risk factors for brain infarcts, in particular of risk factors that are readily identifiable (eg, by blood testing) and modifiable (eg, with treatment), may contribute to identifying possible targets for therapy and to improving public health in the elderly, the fastest-growing segment of the population.

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death and who underwent a brain autopsy and standardized neu-
ropathological evaluation. We tested whether aPLs are related to
the presence of any brain infarcts or to subtypes of infarcts,
including gross and microscopic and cortical and subcortical
infarcts. We also considered various characteristics of aPLs,
including an overall aPL assessment of positivity that takes a
panel of 4 aPL measures into account, individual measures (eg,
aCL antibodies), number of positive aPL assays, and measures at
different time points in serially collected blood specimens (eg,
persistence of aPLs over time). Finally, we explored whether
vessel pathology such as atherosclerosis and arteriolosclerosis
affects the relationship of aPLs to brain infarcts.

Methods

Cohorts
Subjects were enrolled in 1 of 2 ongoing community-based, clinical-
pathological studies of aging, the Rush Memory and Aging Project
and the Religious Orders Study, with high follow-up and autopsy
rates. The studies were designed to have essentially identical methods
for recruitment and a large common core of data and specimen collec-
tion, thus facilitating the combination of data to examine the relation
of risk factors to neurological aspects of aging and brain pathology.
All subjects consented to annual clinical evaluations, blood draws,
and brain donation at time of death. Both studies were approved by
the Rush University Medical Center Institutional Review Board and
are funded by the National Institute on Aging. Detailed methods of
the 2 cohort studies are found elsewhere.11,12

The Rush Memory and Aging Project has been enrolling subjects
since 1997. At the time of the present analyses, 1682 subjects had
undergone a baseline clinical evaluation, which included a blood
draw. Of these, 664 have died. Excluding 10 people who withdrew
from further data collection before death, 532 subjects underwent an
autopsy (81% autopsy rate), and neuropathological data were avail-
able on the first 507. Analyses with pathological outcomes were con-
ducted in 405 of these subjects with available blood specimens from
which to measure the predictor of interest for this study (aPLs).

The Religious Orders Study Core began enrolling subjects in 1994.
There were 1169 subjects who had a baseline clinical evaluation com-
pleted, including a blood draw. Two subjects withdrew from autopsy.
Of the 652 subjects who have died, 608 have come to autopsy (93% 
autopsy rate), and neuropathological data were available on the first
594. Analyses with pathological outcomes were conducted in 202 of
these subjects with blood specimens available for this study.

Clinical Data
A uniform, structured, baseline clinical evaluation was administered
to all subjects and included medical history, physical examination,
cognitive function testing, and blood draw (see below). Annual fol-
low-up evaluations were identical in all essential components except
that only a subset of Religious Orders Study participants underwent
follow-up blood draws. Data on vascular risk factors and diseases,
data on visually inspected medications, and other clinical data of rel-
evance to this study are available elsewhere.11,12

Blood Specimens and aPL Data
Blood was collected by a trained phlebotomist or nurse in tiger-top
serum separator tubes and lavender top tubes (EDTA). Blood was
then centrifuged for 15 minutes within 1 hour (up to 4 hours in all
cases) and either driven that day or shipped on ice within 24 hours
(for long-distance sites) to the Rush Alzheimer’s Disease Center labo-
ratory. There, serum and plasma specimens were processed, includ-
ing being divided into 0.5-mL aliquots, and stored in a −80°C freezer.

Methods describing blood specimen transfer from Rush to the col-
laborating laboratories with aPLs expertise and quantification of aPL
measures of interest to this study are described in detail elsewhere.10

Briefly, aPL data were collected in the first available blood specimen
(baseline), in the last available blood specimen (proximate to death,
second time point) in a subset, and in a specimen derived from a third
time point in between these 2 time points. Repeated measures of aPLs
allowed the determination of persistence of aPL positivity over time
and may have implications for clinical outcomes, as suggested by
the literature and a consensus statement by a panel of aPL experts.13

An aliquot of plasma was used to quantify 3 measures of aPLs: IgG
and IgM isotypes for aCL, β2-glycoprotein 1 (anti-β2 GPI), and anti-
phosphatidyl-serine (aPS). ELISA kits were used according to the
manufacturer’s instructions for aCL and aPS (Corgenics, Inc) and
anti-β2 GPI (INOVA Diagnostics, Inc), and results were interpreted by
researchers blinded to all clinical data. Tests were done in duplicates,
and assays were repeated if the variance was >15%. If either the IgG
or IgM isotype for a particular aPLs was positive, then that aPLs was
determined to be positive. Specifically, aCL IgG was positive if ≥23
IgG international anti-cardiolipin immunoreactivity units, and aCL IgM
was positive if ≥11 IgM international anti-cardiolipin immunoreactivity
units; anti-β2 GPI IgG was positive if ≥20 standardized IgG units, and
anti-β2 GPI IgM was positive if ≥20 standardized IgM units; and aPS
IgG was positive if ≥16 IgG anti-phosphatidyl-serine units, and aPS
IgM was positive if ≥22 IgG anti-phosphatidyl-serine units.

An aliquot of plasma was used to quantify 1 aPL measure: lupus
anticogulants. This measure was obtained with the use of commer-
cially available reagents on citrated plasma, and positivity was deter-
mined with the use of previously established and published methods.14

Neuropathological Data
Systematic neuropathological evaluations of autopsied brains were
conducted by investigators blinded to clinical data (including all aPL
data), as previously published.15 Briefly, uniform gross and histologi-
cal evaluations, focused on common age-related pathologies, includ-
ing cerebrovascular disease, were performed by a neuropathologist.

On gross examination, number, volume (in millimeters squared), and
location (including side of the brain) of all visualized brain infarcts
were recorded, dissected, confirmed on microscopic examination with he-
maoxylin and eosin stain, and classified by age (chronic, subacute, acute).15

Microscopic infarcts were, by definition, not visible to the naked eye and
identified only under microscopy with the use of blocks of midfrontal,
middle temporal, entorhinal, hippocampal, and inferior parietal corti-
ces, as well as anterior cingulate, thalamus, basal ganglia, and midbrain,
which were paraffin embedded, cut, mounted on slides, and stained with
hematoxylin and eosin.16 Location (and side) and age of microinfarcts
were also recorded. For analyses in this study, only chronic infarcts were
considered in primary analyses, and secondary analyses took several
infarct characteristics into account (eg, location as cortical or subcortical).

Vessel pathology data were also systematically collected, includ-
ing pathology in both large (atherosclerosis) and small (arterioloscle-
rosis) vessels. The presence of atherosclerosis in the circle of Willis
was assessed, and severity was graded on a scale from 0 (no athero-
sclerosis) to 6 (severe atherosclerosis with all visualized large arteries
affected or 1 artery completely occluded).17 On histological examina-
tion, the presence of arteriolosclerosis was documented on hematoxy-
lin and eosin–stained sections of the anterior basal ganglia (caudate,
putamen, globus pallidus, and internal capsule), and severity was
graded from 0 (none) to 7 (vessel occlusion). For the purpose of this
study, severity of vessel pathology was grouped into 4 levels (accord-
ing to distribution of data): not present, mild, moderate, and severe.

Analytic Approach

First, descriptive analyses were conducted that included analyses of
cohort characteristics, graphical display of aPLs, and intercorre-
lations among aPLs (Spearman correlations). We created a primary
assessment of overall aPL positivity at baseline based on positivity
in any of the serum and plasma aPL measures at baseline. We also
created other summary variables of aPL data, including an assess-
ment of overall aPL positivity at proximate to death (similar to overall
aPL positivity at baseline but using aPL data proximate to death) and
of persistence of aPL positivity (defined by positivity in any of the
aPL measures at ≥2 time points). Comparison of subjects with and
without overall aPLs positivity at baseline was done with the use of \( \chi^2 \) for categorical variables and \( t \) tests for continuous variables.

All subsequent analyses were adjusted for age and sex. In the primary analysis of the study, we examined the association of overall aPLs positivity at baseline with the presence of any brain infarct (chronic infarct[s], whether macroscopic or microscopic, in any location) using a logistic regression. We then performed similar analyses but for the separate outcomes of gross and microscopic infarcts and for cortical and subcortical infarcts. Additional analyses considered individual aPL positivity, number of aPLs positive at baseline, aPL positivity proximate to death, and persistence of aPL positivity over time. Finally, separate analyses examined whether associations of aPLs with brain infarcts differed by severity of vessel pathology (atherosclerosis and arteriolosclerosis).

Significance level was set at 0.05, and \( P \) values from 2-tailed tests were used. Analyses were programmed with SAS software, version 9.3, of the SAS system for Linux (SAS Institute Inc).17

Results

Subjects

There were 607 subjects with neuropathological data available in whom aPLs were measured at least once, at baseline, who were included in analyses. Demographic, clinical, and neuropathological characteristics of these subjects are shown in Table 1. Overall, subjects were old (mean age at death, 89 years; SD=6.4) and mostly women (two thirds of total group). Vascular conditions (including stroke), risk factors (including hypertension and diabetes mellitus), and medication use for them were very common. Neuropathological data showed that half of the subjects (296 of 607, 49%) had a chronic infarct of any type (gross or microscopic, any location). There were 118 subjects (19%) with gross infarcts without microinfarcts, 74 (12%) with microinfarcts without gross infarcts, and 104 (17%) with both gross infarcts and microinfarcts.

A total of 142 subjects (23%) were positive on the overall aPL assessment at baseline. Most subjects had only 1 positive measure (n=77), followed by 2 positive measures (n=38) and then 3 measures (n=23), and few (n=4) had \( \geq 4 \) positive measures. Comparisons among subjects with and without overall aPLs positivity at baseline showed that both groups were comparable in terms of age, sex, education, and most clinical characteristics (other than myocardial infarction, which was more common among the aPL group), as well as in all pathological characteristics (infarct and vessel pathology; Table 1). Three quarters of subjects (463 of 607, 76%) had aPL measures collected over time: 322 (53%) subjects had aPL data collected over 3 time points, and 141 (23%) subjects had aPL data collected over 2 time points. The median time interval from the baseline aPL measure to death was 4.6 years (lower quartile, 2.6 years; upper quartile, 6.9 years; range, 0.1–15.6 years) and from the last aPL measure to death was 0.8 year (lower quartile, 0.5 year; upper quartile, 1.4 years; range, 0.02–7.2 years). Among subjects with a third measure of aPLs (intermediate time point, n=322), the time interval from the baseline aPL measure to the intermediate measure was 2.1 years (lower quartile, 1.1 years; upper quartile, 3.0 years; range, 0.39–7.0 years).

aPL Data

At baseline, most correlations were weak, although a few moderately strong correlations were noted among several aPL isotypes (Table 2). In particular, aCL IgM was strongly correlated with aPS IgM (\( r_s=0.87 \)) and moderately correlated with anti-\( \beta_2 \)GPI IgM (\( r_s=0.53 \)), and moderate correlations were noted between aCL IgG and aPS IgG (\( r_s=0.60 \)) and between aPS IgM and anti-\( \beta_2 \)GPI IgM (\( r_s=0.55 \)), as shown in Table 2. Because most correlations, when present, were generally weak, we created an assessment of overall aPL positivity that takes all 7 aPL measures into account to increase our power to detect possible associations with the outcome of interest (brain infarcts).

Data on positivity on the overall aPL assessment and on individual aPL measures at baseline and proximate to death are presented in Table 3. Of note, aCL IgM was the most common aPL (observed in up to a fifth of subjects), whereas lupus anticoagulant was the least (only 1% of subjects). Of subjects with aPL positivity at baseline who had follow-up data, 98 (77%) remained persistently positive (eg, had positivity on 1 or 2 of any follow-up measure). Whereas aPLs were positive in 23% of subjects at baseline, 30% had aPL positivity proximate to death. Figure 1 shows the number of subjects with and without overall aPL positivity at baseline by overall aPL positivity proximate to death. Figure 2 shows the proportion of subjects with and without specific individual aPL positivity (and isotype) at baseline by individual aPL positivity proximate to death.

Relation of aPLs to the Presence and Subtypes of Brain Infarcts

In the primary logistic regression model adjusted for age and sex, we did not find that overall aPL positivity at baseline was related to the presence of brain infarcts among older people (Table 4). In addition, the small odds ratio (OR) suggests that the effect of aPLs on brain infarcts, even if present, is small. In separate models, we did not find relationships with either gross infarcts or microinfarcts. Furthermore, no relationships were found with cortical or subcortical infarcts (Table 4).

To consider different characteristics of infarcts simultaneously, we created 4 additional models, 1 for each of the outcomes of gross cortical, gross subcortical, microcortical, and microsubcortical infarcts. These analyses did not show associations with either gross cortical infarcts or gross subcortical infarcts separately (both \( P>0.1 \)) or with microinfarcts, whether cortical or subcortical (both \( P>0.5 \)).

Analyses by aPL Characteristics

We next considered whether positivity in an individual aPL may be related to infarcts. Given that aCL was the most common individual aPL among our subjects (present in 20%), we performed a logistic regression to test whether aCL at baseline was related to brain infarcts. For this analysis of an individual aPL, we used a conservative approach by creating an aCL variable defined by positivity in either IgG or IgM isotypes with all other aPLs being negative (n=55, 9%), and the reference group included subjects who had negative values on all 7 aPLs measures. The association of aCL with the presence of brain infarcts was not statistically significant (OR=1.73; 95% confidence interval, 0.96–3.11; \( P=0.067 \)). An association was found with gross infarcts (OR=2.35; 95% confidence interval, 1.31–4.21; \( P=0.004 \)) but not for microinfarcts (\( P>0.9 \)) or for cortical (\( P>0.4 \)) or subcortical (\( P=0.07 \)) infarcts. Further analyses for gross infarct outcomes found an association with gross subcortical infarcts specifically (OR=2.17; 95%
confidence interval, 1.20–3.92; \( P = 0.010 \) but not with gross cortical infarcts (\( P > 0.8 \)). We conducted additional analyses of individual aPLs to examine whether using other stringent criteria for aPL positivity would yield different results. We explored the possibility of obtaining an empirical cutoff for aPL positivity by evaluating the receiver-operating characteristic curves. For example, we fitted a logistic regression model with the presence of gross infarcts as the binary outcome and the continuous aCL IgG measure as the predictor. The result showed that higher levels of aCL IgG were associated with

<table>
<thead>
<tr>
<th>Table 1. Demographic, Clinical, and Neuropathological Characteristics of Subjects by aPL Status at Baseline*</th>
</tr>
</thead>
<tbody>
<tr>
<td>aPL Positivity on Any Measure</td>
</tr>
<tr>
<td>--------------------------------</td>
</tr>
<tr>
<td>Subjects, n</td>
</tr>
<tr>
<td>Demographic</td>
</tr>
<tr>
<td>Age at baseline (SD), y</td>
</tr>
<tr>
<td>Female, n (%)</td>
</tr>
<tr>
<td>Education (SD), y</td>
</tr>
<tr>
<td>Clinical†</td>
</tr>
<tr>
<td>Vascular conditions, n (%)</td>
</tr>
<tr>
<td>Stroke</td>
</tr>
<tr>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>Claudication</td>
</tr>
<tr>
<td>Vascular risk factors, n (%)</td>
</tr>
<tr>
<td>Hypertension</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>Smoking (current, former)</td>
</tr>
<tr>
<td>Vital signs</td>
</tr>
<tr>
<td>Systolic blood pressure, average (SD), mm Hg</td>
</tr>
<tr>
<td>Diastolic blood pressure, average (SD), mm Hg</td>
</tr>
<tr>
<td>Body mass index at baseline (SD), kg/m²</td>
</tr>
<tr>
<td>Medications, n (%)</td>
</tr>
<tr>
<td>Aspirin</td>
</tr>
<tr>
<td>Antihypertensive</td>
</tr>
<tr>
<td>Statin</td>
</tr>
<tr>
<td>Amiodarone</td>
</tr>
<tr>
<td>Cardioglycoside</td>
</tr>
<tr>
<td>Insulin</td>
</tr>
<tr>
<td>Oral hypoglycemic</td>
</tr>
<tr>
<td>Laboratory measures</td>
</tr>
<tr>
<td>Total cholesterol at baseline (SD), mg/dL</td>
</tr>
<tr>
<td>Neuropathological, n (%)</td>
</tr>
<tr>
<td>Brain infarcts (all chronic)</td>
</tr>
<tr>
<td>Any infarct present</td>
</tr>
<tr>
<td>Gross infarct present</td>
</tr>
<tr>
<td>Cortical</td>
</tr>
<tr>
<td>Subcortical</td>
</tr>
<tr>
<td>Microinfarct present</td>
</tr>
<tr>
<td>Cortical</td>
</tr>
<tr>
<td>Subcortical</td>
</tr>
<tr>
<td>Vessel pathology‡</td>
</tr>
<tr>
<td>Atherosclerosis</td>
</tr>
<tr>
<td>Arteriosclerosis</td>
</tr>
</tbody>
</table>

aPL indicates antiphospholipid antibody.

*Mean (SD) at time of first aPL measure unless otherwise specified; aPL positivity is defined as positivity on any of the 7 blood measures at baseline.

†At any time point during (or averaged across) study unless otherwise specified.

‡Grade of moderate to severe.
Vessel Pathology

Previously published studies have shown that aPLs increase the risk of first clinical stroke. Because cerebral vessel pathology may affect the relationship of aPLs with brain infarcts, we explored whether the severity of vessel pathology affected our results. First, we examined the effect of arteriolosclerosis on the relationship of aPLs with brain infarcts. Using similar models but replacing large-vessel pathology with small-vessel pathology, we did not find relationships of aPLs with infarcts controlling for arteriolosclerosis (all $P>0.25$) or evidence of mediation by arteriolosclerosis (all $P>0.24$ for interaction terms).

Table 3. aPL Positivity at Baseline and Proximate to Death

<table>
<thead>
<tr>
<th>Subjects, n (%)</th>
<th>Baseline</th>
<th>Proximate to death, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall aPL positivity</td>
<td>142 (23.4)</td>
<td>138 (29.8)</td>
</tr>
<tr>
<td>aCL</td>
<td>117 (19.3)</td>
<td>115 (24.4)</td>
</tr>
<tr>
<td>IgG</td>
<td>27 (4.5)</td>
<td>26 (7.8)</td>
</tr>
<tr>
<td>IgM</td>
<td>93 (15.3)</td>
<td>87 (18.8)</td>
</tr>
<tr>
<td>Anti-β2GPI</td>
<td>43 (7.1)</td>
<td>33 (7.1)</td>
</tr>
<tr>
<td>IgG</td>
<td>10 (1.7)</td>
<td>10 (2.2)</td>
</tr>
<tr>
<td>IgM</td>
<td>33 (5.4)</td>
<td>23 (5.0)</td>
</tr>
<tr>
<td>aPS</td>
<td>67 (11.0)</td>
<td>59 (12.7)</td>
</tr>
<tr>
<td>IgG</td>
<td>23 (3.8)</td>
<td>21 (4.5)</td>
</tr>
<tr>
<td>IgM</td>
<td>49 (8.1)</td>
<td>40 (8.6)</td>
</tr>
<tr>
<td>LA</td>
<td>6 (1.1)</td>
<td>6 (1.4)</td>
</tr>
</tbody>
</table>

aCL indicates anticardiolipin antibody; anti-β2GPI, antibody to β2-glycoprotein I; aPL, antiphospholipid antibody; aPS, anti–phosphatidyl-serine antibodies; and LA: lupus anticoagulant.

Table 2. Spearman Correlation Coefficients Among 7 aPL Measures at Baseline

<table>
<thead>
<tr>
<th></th>
<th>aCL IgG</th>
<th>aCL IgM</th>
<th>Anti-β2GPI IgG</th>
<th>Anti-β2GPI IgM</th>
<th>aPS IgG</th>
<th>aPS IgM</th>
<th>LA</th>
</tr>
</thead>
<tbody>
<tr>
<td>aCL IgG</td>
<td>1</td>
<td>0.20*</td>
<td>0.13*</td>
<td>0.17*</td>
<td>0.60*</td>
<td>0.17*</td>
<td>−0.02</td>
</tr>
<tr>
<td>aCL IgM</td>
<td>…</td>
<td>1</td>
<td>−0.01</td>
<td>0.53*</td>
<td>0.07</td>
<td>0.87*</td>
<td>0.14*</td>
</tr>
<tr>
<td>Anti-β2GPI IgG</td>
<td>…</td>
<td>…</td>
<td>1</td>
<td>0.21*</td>
<td>0.13*</td>
<td>0.03</td>
<td>−0.10*</td>
</tr>
<tr>
<td>Anti-β2GPI IgM</td>
<td>…</td>
<td>…</td>
<td>…</td>
<td>1</td>
<td>0.04</td>
<td>0.55*</td>
<td>0.08*</td>
</tr>
<tr>
<td>aPS IgG</td>
<td>…</td>
<td>…</td>
<td>…</td>
<td>…</td>
<td>1</td>
<td>0.12*</td>
<td>−0.02</td>
</tr>
</tbody>
</table>
| aPS IgM  | …       | …       | …              | …             | 1       | …       | "1"

* $P<0.05$.
people. Thus, our data add to the scientific knowledge by assessing a panel of 4 aPLs (aCL, anti-β2GPI, aPS, and lupus anticoagulant) and by showing the aCL was the most common aPL in the elderly and lupus anticoagulant was the least common. Nonetheless, although aPLs are common in aging, their clinical-pathological significance is less well understood.

Published literature shows that aPLs increase the risk of first ischemic stroke in young to middle-aged adults, most notably, but not exclusively, in the context of specific disease states such as the aPL syndrome and systemic lupus erythematosus.4,21 Our study is not well positioned to identify the aPL syndrome,13 and this syndrome is likely to be uncommon among this group of community-based people who volunteered for participation in research and were relatively healthy, with an average age at death of nearly 90 years. The relation of the aPL syndrome to brain infarcts remains to be studied. However, our study is uniquely positioned to examine the association of aPLs with pathologically proven brain infarcts, a field of research with a paucity of data. Several case reports with pathology in people of any age describe aPL syndrome associated with widespread (multorgan) microvascular thrombotic injury and in the brain specifically with brain infarcts or hemorrhages and no evidence of inflammation.6,9,22,23 Few case series are available, almost all with <10 subjects with aPL and brain pathology data available. These series describe thrombo-occlusive disease in the brain, small but also some large infarcts, and no inflammation or vasculitis.7,24–27 One larger hospital-based autopsy series of 156 consecutive subjects with and without aPLs showed that aPLs were common and associated with thrombi but not vasculitis.8

To the best of our knowledge, our study is the first prospective cohort study to examine the relation of aPLs to pathologically proven brain infarcts. In this community-based sample of older individuals, we found that aPLs were generally not associated with brain infarcts. Findings were consistent across different characteristics of infarcts (gross and microscopic, cortical and subcortical) and of aPLs (number, timing of measure, persistence over time). Perhaps one exception was the finding of a possible association of aCL with gross infarcts (nonstatistically significant association), in particular gross subcortical infarcts (2-fold increased odds of infarcts). Given the number of analyses conducted in this study, this finding may be chance. In any case, the result needs to be further explored and replicated in another large cohort, and clinical implications of associations, if indeed present, need elucidation. Given the challenges of conducting large, clinical-pathological studies with a high autopsy rate, other study designs, including with neuroimaging, will be useful in examining the association of aCL with large subcortical infarcts and of aPLs with clinical stroke. Furthermore, although we studied brain infarcts observed on postmortem neuropathological examination among older deceased individuals, other study designs, for example, in vivo neuroimaging of people of differing age groups, including younger people, would be better poised to determine a sequence of events (eg, whether aPLs occurred before the infarct) and to clarify the relation of aPLs to infarct in differing age groups (eg, young versus old adults). Several reasons may explain why the literature has shown a relation of aPLs with stroke in young to middle-aged adults, but our study of older adults did not find a relation with the key pathological feature of stroke, brain infarct. These explanations include that stroke and brain infarct are not equivalent outcomes (clinical stroke remains a challenging diagnosis and is more
likely to be misdiagnosed compared with the pathological diagnosis of infarct), the pathogenicity of aPLs may differ by age (eg, aPLs may be more pathogenic in younger adulthood but more common and nonspecific in aging), and others. Further study needs to clarify why aPLs appear to increase the risk of stroke in the younger but not older adult age groups.

Several possible explanations may be considered for the overall null finding of our study. On the one hand, there may truly be no association of aPLs with brain infarcts in the older segment of the population. Indeed, several risk factors, including vascular risk factors, have differing biological effects on the brain at different time points in the life span (eg, effect of blood pressure on cognition). It may be that aPLs accumulate over the life span but are nonspecific markers of aging and are not associated with poor health conditions in older people but perhaps are only more benign markers of immune senescence or other as-yet undefined biological mechanisms. Furthermore, aPLs may have deleterious effects on brain function (such as cognitive and motor impairment) but exert their effects via nonvascular mechanisms, as recently reviewed elsewhere.28 On the other hand, our study may have erroneously missed an association of aPLs with brain infarcts. There may be several reasons for this. First, this is an observational study, and the study design cannot test whether aPLs cause brain infarcts. However, this limitation is inherent to many studies, including clinical-pathological ones. Whereas prospective neuroimaging studies are likely to shed light on the question of aPLs and risk of brain infarcts, reliability of infarct identification on brain scans is lower than on pathological evaluation. Second, other vascular pathologies may play a role in the association of aPLs with brain infarcts. We considered this possibility and conducted analyses controlling for atherosclerosis and arteriolosclerosis, as well as analyses testing for interactions of aPLs with vessel diseases, to explicitly examine whether the severity of vessel disease affected the results. Although this does not seem to be the case, other pathological processes may play a role, and we will examine some of these (eg, annexins) in future studies.29 Third, another possible explanation for the null finding is that our primary measure of aPLs was not well constructed and did not capture the important characteristic(s) that may be related to infarcts (eg, perhaps a specific molecular domain of the aPLs or a ratio of 2 aPLs isotypes is important). Although our overall aPLs assessment is indeed a rather crude dichotomous measure at a single time point at baseline, we considered new variables (eg, not based on manufacturer’s cutoffs but using higher titers, using all data as continuous measures) and conducted analyses taking other aPL characteristics into account (number of aPLs, timing of measure proximate to death, persistence of aPLs over time). Fourth, the null finding may reflect insufficient power to detect a weak association. If our data had suggested an association of aPLs with infarcts that was clinically important (even if not statistically significant), there may be value in examining the question in more subjects, and we considered increasing the sample size of the study to address this possibility. The study was designed to have 80% power to detect a clinically relevant effect of aPLs on infarcts, quantified by an OR of ≥1.6.10 In the main finding presented here, we found an OR of 1.08, corresponding to an effect of aPLs on infarcts, small enough to be of little clinical relevance. With such a small effect size, we do not recommend that the question be studied in a larger number of subjects. Taken as a whole, our data suggest that, without a specific indication, aPLs need not be tested for in the clinical setting of unexplained strokes in older people.

Despite the scientific challenges in demonstrating a null finding, the study has several important strengths. This is the largest study to date on aPLs and pathological brain infarcts. Neuropathological data were systematically collected in community-dwelling older women and men participating in a clinical-pathological cohort study with a high autopsy rate and included vascular markers of brain infarcts (presence of any infarct and subtypes of infarcts) and vessel disease (large- and small-vessel pathology). Furthermore, aPL data were collected, by investigators blinded to neuropathological and all other data, on 7 serum and plasma measures in up to 3 time points, allowing the examination of individual aPLs, the number of aPL positivity, the timing of measures (baseline versus proximate to death), and the persistence of aPLs.

**Acknowledgments**

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**Disclosures**

None.

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**Table 4.** Relation of Overall aPL Positivity at Baseline to Brain Infarcts (n=607)*

<table>
<thead>
<tr>
<th>Infarct Outcome</th>
<th>Odds Ratio (95% Confidence Interval)</th>
<th>PValue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of any brain infarcts</td>
<td>1.08 (0.74–1.58)</td>
<td>0.19</td>
</tr>
<tr>
<td>Gross infarcts</td>
<td>1.29 (0.88–1.91)</td>
<td>0.20</td>
</tr>
<tr>
<td>Microinfarcts</td>
<td>0.93 (0.61–1.41)</td>
<td>0.72</td>
</tr>
<tr>
<td>Cortical infarcts</td>
<td>0.88 (0.57–1.35)</td>
<td>0.56</td>
</tr>
<tr>
<td>Subcortical infarcts</td>
<td>1.11 (0.74–1.64)</td>
<td>0.62</td>
</tr>
</tbody>
</table>

*aPL indicates antiphospholipid antibody.

*All models adjusted for age and sex.

**Table 5.** Relation of aPL Positivity to Brain Infarcts in Subjects With at Least 2 Measures Over Time (n=463)*

<table>
<thead>
<tr>
<th>Infarct Outcome</th>
<th>Odds Ratio (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of any brain infarcts</td>
<td>0.96 (0.64–1.43)</td>
</tr>
<tr>
<td>Gross infarcts</td>
<td>1.12 (0.74–1.70)</td>
</tr>
<tr>
<td>Microinfarcts</td>
<td>1.11 (0.72–1.71)</td>
</tr>
</tbody>
</table>

*aPL indicates antiphospholipid antibody.

*All models adjusted for age and sex.
REFERENCES


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CLINICAL PERSPECTIVE

Although antiphospholipid antibodies (aPLs) are putative vascular risk factors and are routinely tested for in the clinical context of unexplained stroke, few data are available on the relation of aPLs to pathologically proven infarcts in the human brain. Indeed, only a few small pathological studies to date have suggested that aPLs may be related to brain infarcts. We used blood specimens and clinical and neuropathological data from >600 deceased and autopsied women and men participating in a community-based, longitudinal, clinical-pathological cohort study with a high autopsy rate to test the hypothesis that aPLs are associated with a higher odds of brain infarcts among older individuals (mean age at death, 89 years). In this study, we did not find evidence that aPLs increase the odds of brain infarcts (odds ratio, 1.08; 95% confidence interval, 0.74–1.58; P=0.19). Findings were consistent across different characteristics of brain infarcts (gross and microscopic, cortical and subcortical) and of aPLs (number, timing of measure, persistence of positivity over time). Thus, although aPLs are putative vascular factors that are known to increase risk of stroke in young to middle-aged adults, our findings do not support an association of aPLs with brain infarcts in older age groups. These results have clinical implications and inform medical practice by suggesting that, without a specific indication, aPLs need not be tested for in the clinical setting of unexplained strokes in older patients.
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