

SUPPLEMENTARY MATERIAL

Antiphospholipid Antibodies: Cognitive and Motor Decline, Neuroimaging and Neuropathology

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Methods

Participants

Participants from one of two ongoing, prospective, community-based, clinical-pathologic cohort studies of aging were included in this study (www.radc.rush.edu). This research was approved by the Rush University Institutional Review Board. All participants signed an informed consent and an anatomical gift act to donate their brain at time of death.

The Religious Orders Study (ROS) began enrolling Catholic clergy men and women in 1994 from across the United States.¹⁵ Of 1417 people enrolled at the time of analyses starting in June of 2018, 149 had aPL data and two or more clinical evaluations from which to derive change in cognitive function. There were 814 deaths over the years of the study, of which 752 came to autopsy, and among those, 202 had aPL and neuropathologic data collection complete for inclusion in this study. There were separate groups analyzed with aPL data available in ROS (and similarly in MAP). Specifically, one group included participants with two or more cognitive evaluations, so that we could examine the relation of baseline aPL to change in cognitive and motor function (primary analyses). Another group included participants who died and had neuropathologic data. This group is not a subset of the living persons with longitudinal cognitive data. Because aPL data were initially collected among deceased persons, we have a

higher number of persons with aPL data who died. The Rush Memory and Aging Project (MAP) began in 1997, and of 1972 lay-persons in the Chicagoland area enrolled, 807 had aPL data and at least two clinical evaluations.¹⁵ There were 959 who died, of which 790 came to autopsy, and data from 497 were available to be included in analyses. Because there were more persons with frozen serum from the MAP available for this study, there were more persons with aPL data in the MAP cohort than in ROS. Together, data from both cohorts resulted in a total of 956 persons included in analyses of change in brain function (e.g., cognitive decline), and 699 in analyses of postmortem neuropathology. The cohort studies have essentially identical recruitment methods and a large overlap of data and biospecimen collection (including for antemortem serum and postmortem brain biospecimens), allowing for pooling of data to increase statistical power in the examination of risk factors for cognitive and motor decline in aging.¹⁵ Both cohort studies have unusually high follow-up and autopsy rates, above 85%.¹⁵

Cognitive and motor data

Participants underwent baseline and annual clinical evaluations, as previously described elsewhere.¹⁵ Evaluations included a medical history, physical examination with emphasis on the neurological and motor examination, and neuropsychological testing. Annual follow-up assessments were essentially identical to the baseline evaluation. The follow-up rates for the two ongoing cohort studies are in the range of 85% to 95%.

Neuropsychological testing was conducted using a standardized battery of individual tests of different cognitive abilities, as published before.¹⁶ A total of 17 tests were summarized into composite measures of five cognitive domains and an overall score of global cognition (based on all tests). Measures included composite scores on perceptual speed (two tests), working memory

(three tests), episodic memory (seven tests), semantic memory (three tests), and visuospatial abilities (two tests).¹⁶ A neuropsychologist reviewed all data, blinded to data from prior years. Motor testing included a modified version of the Unified Parkinson's Disease Rating Scale (UPDRS), which is more applicable to persons with milder parkinsonian signs and without a clinical diagnosis of PD, as published.¹⁷ An overall score was derived (range 0-100), with higher numbers indicating more parkinsonian signs. Four summary measures of parkinsonian signs sub-scores included parkinsonian gait disturbance and bradykinesia (both as continuous variables), as well as tremor and rigidity (dichotomous variables: present vs. not). Continuous variables were not normally distributed and were square root transformed for analyses. Additional performance-based motor data were collected as part of the uniform structured clinical evaluations, and included the use of testing devices (e.g., dynamometers). A composite global motor score, based on ten motor performances, was constructed to summarize these performances, as described in detail elsewhere.¹⁸ A lower score indicates worse motor function.

Laboratory measures

The clinical evaluations included a blood draw, from which aPL and other serum measures were quantified, blinded to all clinical data. Briefly, a panel of three serum aPL measures, each with IgG and IgM titers (six measures in all), was collected from the first time point at which a frozen serum specimen was available for the study.¹³ In a subset of about half of participants, a serum specimen from a second or third time (each about two years apart) was also used to collect longitudinal data on aPL. Commercial kits were used for anticardiolipin antibodies (aCL) and antiphosphatidyl-serine antibodies (aPS) (Corgenics, Inc.), and Antibodies to β_2 -glycoprotein I (anti- β_2 GPI) (INOVA Diagnostics, Inc.), according to manufacturer's instructions. All tests are run in duplicate and if the variance was >15%, assays were repeated. For analyses, we created a

summary variable of overall aPL positivity (yes vs no), if any of the aPL assays were positive according to the manufacturer's threshold. For secondary analyses, aPL positivity in a particular category of aPL (aCL, aPS, and anti- β_2 GPI) was determined, and based on whether IgG and/or IgM was positive for that type.

Serum markers of inflammation and blood brain barrier (BBB) permeability were also collected in the same first sample available for the aPL measure, in a subset of about half of participants. Commercial kits were used for C-reactive protein (C-Reactive Protein Ultrasensitive ELISA, Calbiotech), and for matrix metalloproteinase 9 and matrix metalloproteinase tissue inhibitor (R&D Systems, Inc.). For analyses, we created a summary variable ratio of matrix metalloproteinase (MMP_r), by dividing matrix metalloproteinase 9 by matrix metalloproteinase tissue inhibitor, in keeping with published research suggesting that a ratio may be more informative than individual measures.¹⁹

Neuroimaging data

All brain magnetic resonance imaging (MRI) data were collected blinded to clinical and other data, including laboratory measures. A 1.5 Tesla General Electric (Waukesha, WI) MRI scanner was used to collect 3D T₁-weighted and 2D T₂-weighted FLAIR data, using previously described methods and parameters.²⁰ White matter hyperintensities were automatically segmented using a support vector machine classifier considering both 3D T₁-weighted and T₂-weighted FLAIR information (WMLS, SBIA, University of Pennsylvania, PA).²¹ The total volume of white matter hyperintensities was measured for each participant and then divided by the corresponding intracranial volume (ICV) measured with Freesurfer on 3D T₁-weighted images. MRI data collected at the nearest time interval after the time of the blood draw from which serum aPL were derived were used in this study. For analyses, given the skewed distribution of the total

white matter hyperintensity volumes across participants, the values were logarithmically transformed.

Lacunar infarcts larger than 3 mm along the anterior-posterior or left-right axes and larger than 3mm along the superior-inferior axis were manually segmented based on the T₁-weighted and T₂-weighted FLAIR data in a subset of 330 participants. Inter-rater and intra-rater reliability of the manual infarct segmentation were assessed by means of the intraclass correlation (ICC) based on segmentations of two expert raters on 7 participants, and based on segmentations of one rater on the same 10 participants, respectively. For analyses, we created a dichotomous variable for the presence of one or more infarcts with volume of 3³ mm³ or more.

Neuropathologic data

Detailed and systematic neuropathologic examinations were conducted in deceased and autopsied participants over the course of the study.¹⁵ Documentation of common age-related neuropathologies was collected, including for cerebrovascular disease. All postmortem evaluations were conducted blinded to clinical and other data, as previously published.¹⁵ All brain infarcts identified on gross (macroscopic) examination were classified by number, volume (in mm²) and location, and then confirmed on microscopic examination, and classified by age (chronic, subacute, acute). Microinfarcts were also documented, and defined as infarcts not visible to the naked eye and identified only under microscopy. Location and age were recorded. For analyses, only chronic infarcts were considered, and all infarct variables were categorized into two levels: no infarct (reference group) vs one or more infarcts. We also considered infarct size (gross- and micro-infarcts) and location (cortical and subcortical). In addition, we used data on cerebral vessel disease for large vessels assessed on gross examination (atherosclerosis), and small vessels based on histologic examination (arteriolosclerosis), as

reported elsewhere.²² For analyses, we categorized level of severity of vessel pathology into none, mild, moderate, and severe.

Statistical analysis

Descriptive analyses were performed. All subsequent analyses were adjusted for age, sex, and education. To test the primary hypothesis that overall aPL positivity at baseline is related to change in the primary outcome variables of cognitive and motor function, we conducted two linear mixed effect models, one with the global cognitive function score as the outcome and another with the global parkinsonian signs score as the outcome. Models included terms for overall aPL positivity (level effect), time from baseline, and the interaction between the two variables (overall aPL positivity \times time: change effect). In secondary analyses, we conducted similar analyses but replacing the outcome each time with one of the five cognitive domains (perceptual speed, working memory, episodic memory, semantic memory, and visuospatial abilities), and then with the motor function measures including the parkinsonian signs sub-scores (gait disturbance, bradykinesia) and the global motor score measure. We used logistic mixed effect models for additional parkinsonian sign scores for the presence/absence of tremor and, separately, for the presence/absence of rigidity as outcomes.

To test the association of overall aPL positivity with other secondary outcomes with only one measurement in time (in serum, on neuroimaging, on neuropathology), we used linear regressions for continuous outcomes, logistic regressions for binary outcomes, and ordinal logistic regressions for ordinal level outcomes. The outcomes were CRP values, MMP_r values, MRI white matter hyperintensities values, the presence of MRI infarcts, and the presence of postmortem infarcts on neuropathology (and separately for gross- and micro-infarcts; cortical

and subcortical infarcts), as well as ordinal levels of severity of cerebral vessel disease outcomes (separately for atherosclerosis and arteriolosclerosis).

The core models were validated graphically and analytically and analyses were programmed in SAS v9.4 (SAS Institute Inc.) system for Linux.

Results

Participant characteristics and laboratory measures

The demographic, clinical, neuroimaging, and neuropathologic characteristics of participants are shown in Table 1, for the total group of participants, and separately for those with and without overall aPL positivity at baseline, defined by positivity on any of the assays at the first measurement. One in five participants had aPL positivity at baseline. When comparing participants with and without overall aPL positivity, there was no difference in age ($t(343.72) = -0.57, p=0.568$), sex ($\chi^2(1) = 0.010, p=0.752$), or education ($t(954) = 0.40, p=0.686$). The descriptive data on the panel of the three individual aPL measures, including for IgG and IgM separately for each, is provided in Table 2. Anticardiolipin antibodies were the most frequent single type of aPL, present in 17.3% of participants at baseline. Further, aPLs were measured over time in 463/956 (48.4%) participants (463 with two time points; 324 with three time points with measures about 2 years apart), during an average time interval from first-to-last measurement of 4.10 (SD=2.30) years. Comparing the first measurement to the last, among the 115 participant with overall aPL positivity at the first measure, 82/115 (71.30%) remained positive at the last time point measured, suggesting variability in the aPL over time. A subset of 481/953 participants also had laboratory serum markers of inflammation measured at baseline at

the same time point as the first aPL measurement: CRP was on average lower, and the MMP ratio higher, in participants with aPL positivity compared to those without (Table 1; both $p > 0.123$ for comparisons).

A subset of 413 participants had neuroimaging brain MRI data on white matter hyperintensities conducted after the first aPL measure was collected (within 3.3 (SD=3.1) years after the aPL, on average; range 0-12 years), and there were 330 participants with MRI data on infarcts (Table 1).

Over the course of the study, 699 participants with aPL data died and underwent an autopsy and had neuropathologic data available at the time of these analyses (mean age at death =89.2 (SD =6.4) years). Neuropathologic data showed that cerebrovascular disease was very common, with half (50.7%) of participants having one or more brain infarcts present (chronic, and of any size or location), and about a third of subjects having evidence of moderate-to-severe cerebral vessel diseases (Table 1).

Relation of individual aPL as continuous measures to change in cognitive and motor function

Each of the six individual aPL were measured and data are shown in the Table. We conducted secondary analyses using a series of mixed effects models, all adjusted for age, sex, and education, to examine the relation of each aPL as a continuous variable, to decline in the global cognitive function score and to worsening in the global parkinsonian signs score. Result from these 12 analyses did not show significant relations (data not shown), except for anti- β_2 GPI which was related to a faster decline in global cognition (estimate= -0.002, SE=0.001; $p < 0.001$).

Table. Individual aPL measurements among 956 participants

Variable	Median	Interquartile range		Minimum	Maximum
		Lower quartile	Upper quartile		
Anticardiolipin antibodies (aCL)					
IgG	5.543	3.309	9.52	0.392	85.247
IgM	3.456	1.799	6.834	0.234	66.636
Antibodies to β_2 -glycoprotein I (anti- β_2 GPI)					
IgG	2.412	1.821	3.506	0.502	111.130
IgM	1.956	1.111	3.716	0.001	197.015
Antiphosphatidyl-serine antibodies (aPS)					
IgG	2.549	1.478	4.643	0.104	66.709
IgM	3.712	1.968	7.639	0.188	76.501