Neurometabolite Markers of Cerebral Injury in the Antiphospholipid Antibody Syndrome of Systemic Lupus Erythematosus

Arman Sabet, MD; Wilmer L. Sibbitt, Jr, MD; Christine A. Stidley, PhD; Jeff Danska, PA; William M. Brooks, PhD

Background and Purpose—To determine the neurometabolic patterns of brain injury in systemic lupus erythematosus with antiphospholipid antibody syndrome (SLE-aPLS).

Methods—Forty-nine SLE patients (12 SLE-aPLS) and 23 control subjects were studied using magnetic resonance imaging and spectroscopy. N-Acetylaspartate/creatine (NAA/Cre) and choline/Cre (Cho/Cre) were measured in normal-appearing tissue. IgG and IgM antiphospholipid antibodies (aPL) were measured by enzyme-linked immunosorbent assay.

Results—Stroke, epilepsy, and elevated IgG-aPL were more common in SLE-aPLS patients than in SLE patients (P<0.001). NAA/Cre was lower (P<0.05) and Cho/Cre higher (P<0.001) in SLE-aPLS patients than in SLE patients without aPLS. Regression models showed NAA/Cre was most related to injury seen by imaging (P<0.01), disease duration (P<0.05), and prior neuropsychiatric SLE (NPSLE) (P=0.07). Reduced NAA/Cre was more closely related to IgG-aPL (P<0.01) than the presence of stroke or aPLS. When adjusted for all factors, Cho/Cre was most associated with the presence of aPLS (P<0.05).

Conclusions—SLE and SLE-aPLS are actually a clinical continuum describing brain injury in SLE, with SLE-aPLS being characterized by increased aPL, NPSLE, stroke, epilepsy, and disturbed neurochemistry. An elevated IgG-aPL level is a potent risk factor for brain injury as measured by NAA/Cre in SLE that is independent of stroke and aPLS. However, thrombotic phenomena and the presence of aPL (aPLS) are most closely associated with increased Cho/Cre in SLE. These results suggest that aPLs exacerbate SLE, resulting in increased thrombotic and nonthrombotic brain injuries. Spectroscopy detects brain injury in SLE and may permit better understanding of the neurological consequences of SLE and SLE-aPLS. (Stroke. 1998;29:2254-2260.)

Key Words: antiphospholipid syndrome ■ brain injuries ■ lupus ■ magnetic resonance ■ neurochemistry ■ spectroscopy

Systemic lupus erythematosus (SLE) can be complicated by the antiphospholipid syndrome (aPLS), which is characterized by antiphospholipid antibodies (aPL) and specific thromboembolic phenomena, including pulmonary emboli, recurrent miscarriage, thrombocytopenia, and arterial or venous thrombi. SLE-aPLS is a particularly debilitating form of neuropsychiatric SLE (NPSLE) that is characterized by focal neurological deficit, epilepsy, recurrent stroke, and multi-infarct dementia. The pathology and clinical evolution of cerebral infarction in SLE-aPLS are assumed to be that of stroke of other causes. Using proton magnetic resonance spectroscopy (1H-MRS), we previously determined that NPSLE is characterized by frequent neurochemical abnormalities including the loss of the neuronal marker N-acetylaspartate (NAA), consistent with neuronal loss, and increased choline-containing compounds (Cho), lipids, and macromolecules, suggesting membrane breakdown. In the present study, we examined the association of aPLS and aPL with neurochemical disturbance in patients with SLE.

Subjects and Methods

Subjects

The purpose was to determine the relationship between neurochemical markers of brain injury in patients with SLE and the presence or absence of aPLS and aPL. We determined SLE-aPLS by evaluating the presence of elevated aPL and specific thrombotic phenomena associated with aPLS. We then compared the cerebral metabolites of normal control subjects, SLE patients without aPLS, and patients with SLE-aPLS. SLE patients were consecutively recruited to the

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2254
study from our clinics or on hospital admission for more serious conditions over a period of 15 months. The final cohort reflected the typical SLE patients in our university-based clinics. However, 9 potential subjects were too ill to participate in this study. Thus, our general SLE population may have had even more severe systemic and brain diseases than is represented in the reported study cohort. SLE patients (43 women, 6 men) were compared with normal control subjects (13 women, 10 men) who had no history of systemic disease or head trauma, from the local community. Of the patients, 31 had participated in previous MRS studies, although issues of aPLS had not been addressed. SLE patients fulfilled the American College of Rheumatology criteria for SLE. Global SLE activity was assessed by a history of stroke, neuropathy, movement disorder, transverse myelitis, seizure, meningitis, dementia, delirium, major cognitive decline, atypical psychosis, or major affective disorder. This study was approved by the Institutional Review Board. All subjects gave informed consent.

SLE-aPLs was defined as the presence of both SLE and aPLs as causing the stroke. SLE patients (32 women) without aPLs were identified. The aPLs were IgM and IgG. Control samples from 543 normal blood donors were used to standardize the assay. IgM- and IgG-aPL were reported in MPL and GPL units. Normal ranges (±2 SD) for IgG are 5 to 20 GPL and 0 to 10 MPL for IgM. Based on this large control population, normal activity. Control samples from 543 normal blood donors were used to standardize the assay. IgM- and IgG-aPL were reported in MPL and GPL (standardized aPL units based on reactivity to the standard antigen), respectively. Based on this large control population, normal ranges (±2 SD) for IgG are 5 to 20 GPL and 0 to 10 MPL for IgM. aPL testing was performed once on each individual according to the study design. Twelve patients with SLE-aPLs (11 women) and 37 SLE patients (32 women) without aPLs were identified.

**Immunologic Testing**

All SLE patients were then tested for IgM and IgG-aPL, using a standardized antigen in an enzyme-linked immunosorbent assay (ELISA). Immulon I microtiter ELISA plates (Dynatech) were coated with 30 μL (45 μg/mL) of a standardized phospholipid antigen (Louisville aPL Diagnostics, Inc). The plates were blocked for nonselective binding with a 1% gelatin solution. Serum samples were diluted 1:100 in a 10% fetal bovine serum; 200 μL of each sample was placed in duplicate wells and incubated for 2 hours. The plates were then washed with a phosphate-buffered saline solution with 0.05% Tween and 10% fetal bovine serum and developed with a 1:1000 dilution of goat anti-human IgG or IgM conjugated to horseradish peroxidase and 2,2'-azino-di-(3-ethylbenzthiazoline) sulfonate. The optical density at 405 nm was read on a micro-ELISA reader (Dynatech). Previously established negative and positive controls were used as standards to correct for plate-to-plate variability. Control samples from 543 normal blood donors were used to standardize the assay. IgM- and IgG-aPL were reported in MPL and GPL (standardized aPL units based on reactivity to the standard antigen), respectively. Based on this large control population, normal ranges (±2 SD) for IgG were 5 to 20 GPL and 0 to 10 MPL for IgM. aPL testing was performed once on each individual according to the study design. Twelve patients with SLE-aPLs (11 women) and 37 SLE patients (32 women) without aPLs were identified.

**Magnetic Resonance Examination**

MR data were acquired with a 1.5-T clinical scanner (GE Medical Systems). Critically ill or uncooperative SLE patients (n=35, 7 with...
aPLs) and 10 control subjects were studied using single-column short-echo spectroscopic imaging (SI) (TE = 19 ms, TR = 2700 ms; procedure time, 24 minutes), which produced 8 (10 mm)³ voxels in deep occipitoparietal white matter (WM). 8 SLE patients who were medically stable and cooperative (n = 5) were studied using multislice SI (TE = 19 ms, TR = 2300 ms; procedure time, 60 minutes), which produced a 32 × 32 spectroscopic grid of voxels across the field of view. 9–21 Sagittal T1-weighted images (TE = 16 ms, TR = 600 ms) were used to select the location of the spectroscopic data. Three slice locations aligned parallel to the anterior-posterior commissure were chosen for the long TE acquisitions. Oblique-axial T2-weighted MR images (TE = 30/100 ms, TR = 2800 ms; field of view = 200 mm, 15-mm slice thickness, 2.5-mm gap) coinciding with the locations of the spectroscopic images were obtained.

**Data Analysis**

Images were scored (0 = normal, 1 = mild, 2 = moderate, 3 = severe) for MRI abnormalities common to SLE (eg, cortical atrophy, ventricular dilation, diffuse WM abnormalities, periventricular WM abnormalities, infarct, small focal WM lesions) as described previously. 22 An injury index was defined as the sum of the individual abnormality scores. Infarcts were defined as focal irreversible high intensity lesions at least 1 mL in volume (10 mm)³ on T2-weighted images. Lesions smaller than 1 mL were categorized as small focal lesions.

SI data sets were processed using cosine filtering in k-space, exponential apodization (3 Hz), zero filling to 1024 time-domain points, and Fourier transformation. Residual water signals were removed by high-pass time-domain convolution filtering. Initially, spectra were selected from normal-appearing occipitoparietal WM in all patients, avoiding voxels that were hyperintense on T2-weighted images. In SLE patients with stroke, further data were obtained from infarcts defined as focal regions of high intensity at least 1 mL in volume on T2-weighted images. A total of 21 lesions from 8 individuals with gross cerebral infarct were studied. Lesions that did not fill a spectroscopic voxel completely were not considered strokes and were not analyzed. Uninvolved areas in the contralateral hemisphere of the individuals with stroke were used to compare infarcts with normal-appearing tissues. Spectra were integrated to determine the area for NAA (1.9 to 2.1 ppm), creatine (Cre; 2.9 to 3.1 ppm), and Cho (3.1 to 3.3 ppm) and the ratios, NAA/Cre and Cho/Cre, were

**TABLE 2. Clinical Characteristics of the Study Populations**

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (n = 23)</th>
<th>SLE Patients Without aPLS (n = 37)</th>
<th>SLE-aPLS Patients (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y¹</td>
<td>33.5 ± 7.1</td>
<td>34.8 ± 13.7</td>
<td>44.0 ± 7.2 *</td>
</tr>
<tr>
<td>Sex, % male², a</td>
<td>43.5 *</td>
<td>13.7</td>
<td>8.3</td>
</tr>
<tr>
<td>Disease duration, y³</td>
<td>N/A</td>
<td>7.2 ± 5.0 †</td>
<td>10.6 ± 5.8 †</td>
</tr>
<tr>
<td>SLEDAI, % low³, a</td>
<td>N/A</td>
<td>32.4</td>
<td>33.3</td>
</tr>
<tr>
<td>Prior NPSLE, %³, a</td>
<td>N/A</td>
<td>73.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Epilepsy, %³, a</td>
<td>N/A</td>
<td>5.4 *</td>
<td>33.3 *</td>
</tr>
<tr>
<td>Infarct, %³, a</td>
<td>N/A</td>
<td>0 ‡</td>
<td>66.7 ‡</td>
</tr>
<tr>
<td>Neurological symptoms, %³</td>
<td>N/A</td>
<td>23.3 ‡</td>
<td>100.0 ‡</td>
</tr>
<tr>
<td>Injury Index³, a</td>
<td>N/A</td>
<td>4.8 ± 4.0 †</td>
<td>10.3 ± 4.4 †</td>
</tr>
<tr>
<td>Anticardiolipin IgG, GPL³</td>
<td>N/A</td>
<td>21.5 ± 8.2 †</td>
<td>48.2 ± 11.3 †</td>
</tr>
<tr>
<td>Anticardiolipin IgM, MPL³</td>
<td>N/A</td>
<td>9.8 ± 7.2</td>
<td>14.7 ± 11.7</td>
</tr>
</tbody>
</table>

N/A indicates not applicable. Data are mean ± SD or %. ¹ANOVA with Fisher’s least significant difference groupings. ²Fisher’s exact test. ³Wilcoxon rank sum test. †Significantly different from other groups at P < 0.05. ‡Significantly different from other groups at P < 0.001.

**TABLE 3. Comparison of Uncorrected Neurometabolites at TE = 19 ms and 270 ms and the Corrected Neurometabolites of the Combined Group**

<table>
<thead>
<tr>
<th>Metabolite Ratio</th>
<th>Control Subjects</th>
<th>SLE Patients Without aPLS</th>
<th>SLE-aPLS Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA/Cre(19 ms)</td>
<td>2.26 ± 0.12 (10)†</td>
<td>1.88 ± 0.24 (28)</td>
<td>1.77 ± 0.16 (7)</td>
</tr>
<tr>
<td>NAA/Cre(270 ms)</td>
<td>2.33 ± 0.23 (13)†</td>
<td>1.96 ± 0.22 (9)</td>
<td>1.78 ± 0.04 (5)†</td>
</tr>
<tr>
<td>NAA/Cre(270 ms)</td>
<td>2.26 ± 0.17 (23)†</td>
<td>1.90 ± 0.23 (37) *</td>
<td>1.76 ± 0.13 (12) *</td>
</tr>
<tr>
<td>Cho/Cre(19 ms)</td>
<td>0.82 ± 0.05 (10)</td>
<td>0.80 ± 0.13 (28)</td>
<td>1.25 ± 0.43 (7)†</td>
</tr>
<tr>
<td>Cho/Cre(270 ms)</td>
<td>1.17 ± 0.27 (13)</td>
<td>1.22 ± 0.07 (9)</td>
<td>1.55 ± 0.34 (5)†</td>
</tr>
<tr>
<td>Cho/Cre(320 ms)</td>
<td>0.82 ± 0.09 (23)</td>
<td>0.82 ± 0.12 (37)</td>
<td>1.17 ± 0.39 (12)†</td>
</tr>
</tbody>
</table>

Data are mean ± SD; values in parentheses indicate subject numbers in each cell; statistical significance determined using ANOVA with Fisher’s least significant difference groupings. *Significantly different from other groups at P < 0.05. †Significantly different from other groups at P < 0.001.
calculated. In normal-appearing tissues, the ratios from 5 adjacent voxels in each anatomic region were averaged to obtain values for each metabolite in each individual.

Data were analyzed individually for the TE=19 ms and TE=270 ms cohorts. The statistical observations among normal control subjects, SLE patients, and patients with SLE-aPLS in the 2 data sets were similar, although each cohort was composed of different individuals. To determine whether the trends in the independent cohorts represented true differences between patient subgroups, the 2 data sets were combined to provide greater statistical power, especially to determine unique characteristics of the SLE-aPLS subgroup (a total of 12 patients). Data from the long TE acquisitions were normalized to data at TE=19 ms using correction factors obtained from control data. Correction factors for individual metabolites were derived by dividing the mean metabolite ratio (i.e., NAA/Cre or Cho/Cre) acquired at short TE by the mean value acquired at long TE. Thus, the corrections were made as follows: NAA/Cre\(^{19\text{ms}}\) = 0.97\times \text{NAA/Cre}\(^{270\text{ms}}\), Cho/Cre\(^{19\text{ms}}\) = 0.70\times \text{Cho/Cre}\(^{270\text{ms}}\). These pooled data provided values from 23 control subjects and 49 SLE patients normalized to TE=19 ms.

Previous evaluation of SI reproducibility has shown the mean coefficient of variation to be 3.2% for NAA/Cre and 6.6% for Cho/Cre. The mean coefficient of variation for analysis reproducibility for NAA/Cre was 3.5% and for Cre/Cho was 4.4%.^9

### Statistical Evaluation

Summary statistics were obtained for all variables. Plots of continuous variables were examined for distributional shape and for outliers. Comparisons of continuous variables from 2 populations were made using the 2-sample \(t\) test and the nonparametric analogue, the Wilcoxon rank sum test. Because results from both tests were similar, the Wilcoxon test results are presented. When 3 populations were compared, ANOVA was used with Fisher’s least significant difference test to assess differences between individual groups. Comparisons of categorical data from 2 or more populations were made using Fisher’s exact test.

Linear regression models were used to explore the relationships of the predictor variables (aPLS, stroke, IgG-aPL, and IgM-aPL) to the outcome variables (NAA/Cre and Cho/Cr), aPLS was coded so that the coefficient for aPLS gives the change in mean NAA/Cre or Cho/Cr for the group with aPLS relative to the group without aPLS. Similarly, the coefficient for stroke gives the change in mean NAA/Cre or Cho/Cr for the group with stroke relative to the group without. Because of associations among the main predictor variables (aPLS, stroke, IgG-aPL, and IgM-aPL), these variables were assessed individually and with the other variables in the model. The association between aPLS and the outcome variables NAA/Cre and Cho/Cho was the primary focus of this study, so most models presented here include aPLS. However, to determine the effect of aPLS that is independent of stroke, IgG-aPL, and IgM-aPL, further models with subsets or all of these predictor variables are presented. Finally, to adjust for potential confounders (such as age, duration, SLEDAI, a history of prior NPSLE episodes, and injury index) were added to the predictor variables. SLEDAI was categorized as high (\(\geq 10\)) versus low (\(< 10\)). Because of the association between injury index and IgG-aPL, the independent effect of IgG-aPL was assessed by comparing the model with all of the predictor variables to the model with all of the predictor variables except injury index. To assess possible nonlinear effects and to reduce the effects of outliers, the continuous predictor variables IgG-aPL and IgM-aPL were also categorized. Because the results were similar to those from models with continuous variables, which are more informative, results for the models with categorical variables are not presented.

Two subjects had high Cho/Cre values. Thus, Cho/Cre was categorized as high (\(\geq 0.9\)) and low (\(< 0.9\)). Logistic regression models were developed to determine variables that predict high Cho/Cre. The same variables that were important in the linear regression analysis were the important predictors in the logistic regression models. However, because of the influence of outliers in the estimates of the coefficients for the linear regression models, the results for the logistic regression models are presented in terms of odds ratios.

Although statistical significance was ascertained using \(p=0.05\), some results that have probability values between 0.05 and 0.10 are discussed, because of an indication of an effect. Analyses were conducted using SAS software (SAS Institute).

### Table 4. Analysis of Linear Regression Results for NAA/Cre

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1*</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
<th>Model 5</th>
<th>Model 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>aPLS</td>
<td>-0.133† (0.06)‡</td>
<td>0.113 (0.30)</td>
<td>0.092 (0.48)</td>
<td>0.123 (0.34)</td>
<td>0.055 (0.65)</td>
<td></td>
</tr>
<tr>
<td>Stroke</td>
<td>0.035 (0.79)</td>
<td>0.011 (0.93)</td>
<td>0.098 (0.43)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG-aPL</td>
<td>-0.007 (&lt;0.01)</td>
<td>-0.009 (&lt;0.01)</td>
<td>-0.009 (&lt;0.01)</td>
<td>-0.008 (0.02)</td>
<td>-0.004 (0.21)</td>
<td></td>
</tr>
<tr>
<td>IgM-aPL</td>
<td>0.001 (0.83)</td>
<td>0.001 (0.80)</td>
<td>-0.001 (0.84)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>-0.001 (0.64)</td>
<td>0.000 (0.99)</td>
<td>0.008 (0.21)</td>
<td>0.013 (0.04)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration, y</td>
<td>0.008 (0.90)</td>
<td>-0.009 (0.88)</td>
<td>0.193 (0.02)</td>
<td>0.146 (0.07)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLEDAI</td>
<td>0.008 (0.00)</td>
<td>0.009 (0.00)</td>
<td>0.025 (&lt;0.01)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prior NPSLE</td>
<td>-0.193 (0.02)</td>
<td>0.146 (0.07)</td>
<td>0.025 (&lt;0.01)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Presence of coefficient estimates indicates variables used in model.

†For indicator variables, coefficients give the estimate of the difference in the mean NAA/Cre for those with aPLS versus those without (variable aPLS), with stroke versus without (Stroke), with high SLEDAI (\(>10\)) versus low SLEDAI (SLEDAI), and for prior NPSLE versus no prior NPSLE (Prior NPSLE).

‡For continuous variables, IgG-aPL, IgM-aPL, Age, Duration, and Injury Index, coefficients give the estimate of the mean change in NAA/Cre for a 1-unit increase in the predictor variables.

†Probability from test of significance of coefficient.
TABLE 5. Analysis of Linear Regression Results for Cho/Cre

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1*</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
<th>Model 5</th>
<th>Model 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>aPLS</td>
<td>18.1† (&lt;0.01)‡</td>
<td>58.7 (0.01)</td>
<td>95.2 (0.04)</td>
<td>90.6 (0.05)</td>
<td>90.0 (0.05)</td>
<td>90.0 (0.95)</td>
</tr>
<tr>
<td>Stroke</td>
<td>1.51 (0.84)</td>
<td>4.37 (0.55)</td>
<td>4.40 (0.56)</td>
<td>4.40 (0.56)</td>
<td>4.40 (0.56)</td>
<td>4.40 (0.56)</td>
</tr>
<tr>
<td>IgG-aPL</td>
<td>1.06 (0.02)</td>
<td>0.96 (0.34)</td>
<td>0.96 (0.42)</td>
<td>0.97 (0.56)</td>
<td>0.97 (0.59)</td>
<td>0.97 (0.59)</td>
</tr>
<tr>
<td>IgM-aPL</td>
<td>0.92 (0.16)</td>
<td>0.92 (0.20)</td>
<td>0.92 (0.20)</td>
<td>0.92 (0.20)</td>
<td>0.92 (0.20)</td>
<td>0.92 (0.20)</td>
</tr>
<tr>
<td>Age, y</td>
<td>1.01 (0.81)</td>
<td>1.01 (0.81)</td>
<td>1.01 (0.81)</td>
<td>1.01 (0.81)</td>
<td>1.01 (0.81)</td>
<td>1.01 (0.81)</td>
</tr>
<tr>
<td>Duration, y</td>
<td>0.83 (0.12)</td>
<td>0.83 (0.13)</td>
<td>0.83 (0.13)</td>
<td>0.83 (0.13)</td>
<td>0.83 (0.13)</td>
<td>0.83 (0.13)</td>
</tr>
<tr>
<td>SLEDAI</td>
<td>0.75 (0.74)</td>
<td>0.75 (0.74)</td>
<td>0.75 (0.74)</td>
<td>0.75 (0.74)</td>
<td>0.75 (0.74)</td>
<td>0.75 (0.74)</td>
</tr>
<tr>
<td>Prior NPSLE</td>
<td>1.63 (0.67)</td>
<td>1.63 (0.67)</td>
<td>1.63 (0.67)</td>
<td>1.63 (0.67)</td>
<td>1.63 (0.67)</td>
<td>1.63 (0.67)</td>
</tr>
<tr>
<td>Injury Index</td>
<td>1.00 (0.99)</td>
<td>1.00 (0.99)</td>
<td>1.00 (0.99)</td>
<td>1.00 (0.99)</td>
<td>1.00 (0.99)</td>
<td>1.00 (0.99)</td>
</tr>
</tbody>
</table>

*Presence of coefficient estimates indicates variables used in model.
†Odds ratio from the logistic regression model. Coefficients give the odds ratio for high Cho/Cre for those with aPLS relative to those without (variable aPLS), with stroke versus without (Stroke), high SLEDAI (≥10) versus low SLEDAI (SLEDAI), and prior NPSLE versus no prior NPSLE (Prior NPSLE). For the continuous variables IgG-aPL, IgM-aPL, Age, Duration, and Injury Index, coefficients give the odds ratio for high Cho/Cre for a 1-unit increase in the predictor variables.
‡Probability from test of significance of odds ratio.

Results

Table 1 summarizes the clinical characteristics, imaging, and aPL testing results of the SLE-aPLS patients. Age, disease duration, SLE disease activity, and sex of the patient groups were similar. Figure 1 shows typical imaging findings in an SLE-aPLS patient, including cerebral atrophy, diffuse WM abnormalities, punctate focal lesions, and multiple gross focal infarcts. Current neurological symptoms, stroke, and epilepsy were more common in SLE-aPLS patients than in SLE patients (Table 2). Infarcts were observed only in SLE-aPLS patients (P<0.001). IgG-aPL levels were markedly increased, while IgM-aPL levels were more common in SLE-aPLS patients than in SLE patients (Table 2). Infarcts were observed only in SLE-aPLS patients (P<0.001). IgG-aPL levels were markedly increased in patients with SLE-aPLS (P<0.001), although IgM-aPL levels were similar between the 2 groups (Table 2). Although women were more highly represented in the SLE groups, the control group revealed no sex-related difference in metabolite ratios (P=0.21).

Table 3 shows the mean values for the 19-ms and 270-ms TE cohorts. Both data sets show decreased NAA/Cre in SLE and SLE-aPLS patients and increased Cho/Cre in SLE-aPLS patients. To confirm the trends noted in the individual data sets, the data were combined as described above (Table 3). NAA/Cre was significantly decreased in SLE and SLE-aPLS patients relative to normal control subjects (P<0.001; Figure 2). Moreover, in SLE-aPLS patients a significant (P<0.05) reduction in NAA/Cre was demonstrated relative to SLE without aPLS.

To address potential correlations among the factors discussed above, we used linear regression models to control for the effects of previous NPSLE, injury index, age, IgG-aPL, IgM-aPL, SLE duration, and SLE activity. For each metabolite ratio, a series of models was examined, beginning with a univariate model of only aPLS and ending with a model that adjusted for all of the potential predictor variables. The univariate model for NAA/Cre indicates that aPLS is associated with decreased NAA/Cre (Table 4, model 1). However, the effect of aPLS is not independent of IgG-aPL (model 3), a variable that is highly associated with NAA/Cre regardless of whether aPLS is included (models 2 and 3). After further adjustment for stroke and IgM-aPL, IgG-aPL is still an important predictor of NAA/Cre (model 4). Similarly, after adjustment for previous NPSLE, age, SLE duration, and SLE activity, but not injury index (because of its high correlation with IgG-aPL), IgG-aPL is associated with NAA/Cre (model 5). Finally, to consider the effect of IgG-aPL adjusted for MRI-visible abnormalities, injury index was added, resulting in an erosion of the significance of IgG-aPL (model 6). Thus, for NAA/Cre, the important predictors are injury index or IgG-aPL, disease duration, and, possibly, prior NPSLE episodes.

Similar linear regression models were examined to assess the association between aPLS and Cho/Cre. However, the estimates of the linear regression coefficients differed significantly if 2 extreme values were excluded. Thus, Cho was categorized into high Cho (≥0.9) and low Cho (<0.9), and logistic regression models were developed. We present only the results from the logistic regression modeling, although the same predictor variables were important in both models. In the univariate models, aPLS and IgG-aPL are associated with increased Cho/Cre (Table 5, models 1 and 2). The effect of aPLS remains significant when adjusted for either IgG-aPL or stroke, but the estimate of the effect increases (models 3 and 4). Even after adjustment for the potential confounders (age, duration, SLEDAI, prior NPSLE, and injury index), the effect of aPLS remains significant (models 5 and 6). One limitation of estimating the stroke and aPLS effects is the high correlation between these 2 variables: most aPLS patients had stroke (n=8), whereas only 4 aPLS patients did not, and no patients had stroke without aPLS. Thus, it is difficult to separate the aPLS effect from the stroke effect.

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In patients with SLE (SLE-aPLS), thrombosis and aPL are associated with severe neurological morbidity. Focal neuro-
logical deficits, stroke, epilepsy, recurrent cerebral infarcts, and subclinical ischemic sequelae, especially microemboli and asymptomatic focal brain lesions, are common.\textsuperscript{1,2,3,4,23,24} However, neurocognitive dysfunction has been reported in patients with aPLS even in the absence of obvious focal lesions, indicating substantial microscopic disease.\textsuperscript{25} Migraine, dementia, delusional states, and depression, each of which can be symptoms of NPSLE, have been associated with aPL.\textsuperscript{26–28}

The most prominent resonance in \textsuperscript{1}H-MRS of adult brain is the neuronal marker NAA.\textsuperscript{29} A reduced presence of NAA suggests neuronal injury or death and has been associated with cognitive impairment, indicating an important functional consequence of NAA depletion.\textsuperscript{9,30,31} The current study demonstrates abnormal brain metabolite ratios in SLE and SLE-aPLS patients. The markedly reduced NAA/Cre of large focal lesions in patients with SLE-aPLS is characteristic of infarction, whereas reductions in normal-appearing tissue may indicate extensive microlesions.\textsuperscript{3,6,28} These findings are consistent with previous reports of disturbed neurometabolites in NPSLE.\textsuperscript{7,2} although the metabolic abnormalities observed here are more severe in patients with SLE-aPLS, indicating a different or more extensive injury to brain. The presence of aPLS alone is associated with reduced NAA/Cre (Table 3). However, when modeling included other clinical variables, including IgG-aPL and injury index, the change in NAA/Cre in patients with SLE-aPLS relative to those with SLE was not significant, indicating that the majority of the observed change in NAA/Cre was associated with MRI-visible brain injury, IgG-aPL, or, possibly, disease duration and prior NPSLE (Table 4).

Elevated Cho/Cre was observed in focal lesions and normal-appearing tissues of patients with SLE-aPLS consistent with infarct, the activation of cellular membranes, catabolism of myelin, or inflammation.\textsuperscript{30,32,33} Cho/Cre was increased in normal-appearing tissues even when other clinical factors were included, suggesting exaggerated injury to normal-appearing tissue in patients with SLE-aPLS consistent with widespread microinfarction.\textsuperscript{6,8,28,34} The effect of aPLS remained significant even after adjusting for all clinical factors (Table 5). The increase in Cho/Cre was associated with the presence of aPLS but not stroke, IgG-aPL, or other clinical factors. However, decreased NAA/Cre was not associated with aPLS after adjustment for IgG-aPL, indicating a different or more complicated relationship with IgG-aPL.\textsuperscript{35}

The histological changes of NPSLE and aPLS can be similar. Bland vasculopathy with or without microthrombosis is common in NPSLE and aPLS, but inflammation is rarely seen in aPLS.\textsuperscript{28,36} Perivascular cuffing with inflammatory cells, microinfarcts, cortical atrophy, gross infarcts, hemorrhage, ischemic demyelination, and leukostasis has been observed in SLE.\textsuperscript{28,37–42} Thus, although NPSLE and primary aPLS have certain histological similarities, noninflammatory vasculopathy and thrombosis predominate in aPLS, whereas NPSLE has a more complex and variable pattern.

SLE and SLE-aPLS demonstrate similar patterns of neuronal injury by MRS. Reduced NAA/Cre and elevated Cho/Cre ratios characterize both disorders, consistent with neuronal injury, ischemic demyelination, and postischemic inflammation. These data also demonstrate that IgG-aPL may have a potent independent effect on brain injury, even after correcting for stroke and aPLS. Thus, the disorders may represent a continuum of SLE and IgG-aPL-mediated disease. Future studies are required to determine whether the presence of IgG-aPL in a SLE patient should prompt therapy and whether the observed metabolite abnormalities in normal-appearing tissues are due to microscopic ischemic injury or to cytotoxic extension from adjacent gross infarct. Therapeutic options for the treatment of SLE-aPLS remain controversial, and efficacy for any intervention is extremely difficult to monitor even with widely accepted assays.\textsuperscript{4,40,41,43} Combined MRI/S may provide the means to detect brain injury and monitor therapy.

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References


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Arman Sabet, Wilmer L. Sibbitt, Jr, Christine A. Stidley, Jeff Danska and William M. Brooks

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