Magnetic Resonance Spectroscopy Markers of Disease Progression in Multiple Sclerosis

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**Importance**  Predicting disease evolution is becoming essential for optimizing treatment decision making in multiple sclerosis (MS). Multiple sclerosis pathologic damage typically includes demyelination, neuro-axonal loss, and astrogliosis.

**Objective**  To evaluate the potential of magnetic resonance markers of central nervous system injury to predict brain-volume loss and clinical disability in multiple sclerosis.

**Design, Setting, and Participants**  Participants were selected from the Multiple Sclerosis Center at the University of California–San Francisco. The preliminary dataset included 59 patients with MS and 43 healthy control individuals. The confirmatory dataset included 220 patients from an independent, large genotype-phenotype research project.

**Main Outcomes and Measures**  Baseline N-acetylaspartate (NAA) level, myo-inositol (mI) in normal-appearing white and gray matter, myelin water fraction in normal-appearing white matter, markers of axonal damage, astrogliosis, and demyelination were evaluated as predictors in a preliminary data set. Potential predictors were subsequently tested for replication in a confirmatory data set. Clinical scores and percentage of brain-volume change were obtained annually over 4 years as outcomes. Predictors of outcomes were assessed using linear models, linear mixed-effects models, and logistic regression.

**Results**  N-acetylaspartate and mI both had statistically significant effects on brain volume, prompting the use of the mI:NAA ratio in normal-appearing white matter as a predictor. The ratio was a predictor of brain-volume change in both cohorts (annual slope in the percentage of brain-volume change/unit of increase in the ratio: −1.68; 95% CI, −3.05 to −0.30; P = .02 in the preliminary study cohort and −1.08; 95% CI, −1.95 to −0.20; P = .02 in the confirmatory study cohort). Furthermore, the mI:NAA ratio predicted clinical disability (Multiple Sclerosis Functional Composite evolution: −0.52 points annually, P < .001; Multiple Sclerosis Functional Composite sustained progression: odds ratio, 2.76/SD increase in the ratio; 95% CI, 1.32 to 6.47; P = .01) in the preliminary data set and predicted Multiple Sclerosis Functional Composite evolution (−0.23 points annually, P = .01), Expanded Disability Status Scale evolution (0.57 points annually, P = .04), and Expanded Disability Status Scale sustained progression (odds ratio, 1.46; 95% CI, 1.10 to 1.94; P = .009) in the confirmatory data set. Myelin water fraction did not show predictive value.

**Conclusions and Relevance**  The mI:NAA ratio in normal-appearing white matter has consistent predictive power on brain atrophy and neurological disability evolution. The combined presence of astrogliosis and axonal damage in white matter has cardinal importance in disease severity.
he mechanisms underlying disease evolution in multiple sclerosis (MS) are not fully known. Current predictors based on clinical or conventional magnetic resonance imaging (MRI) data\(^2\) are known to relate to long-term disability but have limited specificity in characterizing and quantifying the heterogeneous pathological features of MS.\(^3\) The study of myelin destruction and repair, axonal injury, and astrogliosis—major pathological events in MS\(^4,5\)—by means of nonconventional MRI techniques\(^6\) could help in achieving that goal.

Magnetic resonance spectroscopy (MRS) has contributed to understanding the pathogenesis and natural history of MS.\(^7\) Metabolic abnormalities in patients with MS are not restricted to lesion sites but are more diffuse in nature.\(^8,9\) \(N\)-acetylaspartate (NAA) is an amino acid found in neurons and axons and is used as a marker of neuronal/axonal integrity and function.\(^10\) N-acetylaspartate is depleted in patients with MS,\(^11,12\) precedes brain atrophy,\(^13\) and moderately correlates with subsequent development of physical disability.\(^12\) Myo-inositol (mI) originates from intracellular astrocyte stores.\(^15\) It is elevated in patients with MS,\(^16\) reflecting astroglial hypertrophy or hyperplasia, even in early stages of the disease\(^14\) and precedes the decrease of NAA and brain volume.\(^15\) Moreover, a relatively new MRI technique allows the estimation of myelin water content derived from the quantification of short T2 relaxometry component.\(^16\) The measure is specific to myelin content and/or its integrity.\(^17\) The myelin water fraction (MWF) is commonly reduced in normal-appearing white matter (NAWM),\(^18\) which may reflect active or chronic demyelination.

The aim of the present study was to conduct a rigorous analysis of spectroscopy and relaxometry markers of axonal integrity, astrogliosis, and demyelination in vivo with respect to predicting long-term clinical disability and brain-volume loss. After performing an initial analysis in a preliminary group of patients with MS (preliminary data set), results were tested for replication in a larger representative MS group (confirmatory data set).

### Methods

#### Preliminary Data Set

**Study Population**

Fifty-nine patients with MS and 43 control participants were included in a case-control longitudinal study. The MS cases, fulfilling 2001 McDonald criteria,\(^19\) were prospectively selected from the University of California–San Francisco Multiple Sclerosis Center. At baseline (assessment of predictors), only the use of interferon-\(\beta\) and copolymer-1 treatment for MS was allowed. The mean (SD) study follow-up time was 3.5 (1.2) years and 80% of the MS cases (47 of 59) completed 4 years of the study. All participants gave written informed consent to enter the study, which was approved by the University of California–San Francisco ethics committee. Demographic and clinical data are available in Table 1.

**Predictors**

Predictors were derived from a 3-dimensional short-echo proton MRS imaging (3D 3\(^1\)HMRSI) sequence and a multislice multi-echo T2 relaxometry sequence using a single 3-T GE Signa scanner (GE Healthcare) with an 8-channel phased-array coil. Spectroscopic signals were acquired from a supratentorial point-resolved spectroscopy box covering 4 slices centered over the corpus callosum,\(^20\) using a conventional phase encoding, with a repetition time (TR) and echo time (TE) of 1000 and 40 milliseconds, respectively. Metabolite (NAA and mI) contributions within each voxel were estimated by adjusting short echo time signals to a model function created from a prior

### Table 1. Patients’ Baseline Demographic and Clinical Characteristics From the Preliminary and Confirmatory Studies

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Preliminary</th>
<th>Control Participants (n = 43)</th>
<th>All Patients With MS (n = 220)</th>
<th>CIS (n = 33)</th>
<th>RRMS (n = 164)</th>
<th>SPMS (n = 17)</th>
<th>PPMS (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, No. (%)</td>
<td>42 (71.2) 27 (62.8)</td>
<td>156 (70.9) 20 (60.6)</td>
<td>122 (74.4) 11 (64.7)</td>
<td>3 (50)</td>
<td>43.2 (9.4) 38.9 (10.1)</td>
<td>42.4 (9.5) 40.7 (9.6)</td>
<td>41.8 (9.2) 40.6 (9.2)</td>
</tr>
<tr>
<td>Disease duration, y</td>
<td>10.3 (9.7) NA</td>
<td>9.7 (8.2) 3.5 (3.2)</td>
<td>10.2 (8.0) 18.2 (8.7)</td>
<td>8.1 (4.9)</td>
<td>14.6 (6.6) 12 (36.4)</td>
<td>118 (72.0) 13 (76.5)</td>
<td>3 (50.0)</td>
</tr>
<tr>
<td>EDSS score, median (range)</td>
<td>1.5 (0-4.5) NA</td>
<td>2.0 (0-7.0) 1.5 (0-4.0)</td>
<td>2.0 (0-6.0) 5.5 (2.5-7.0)</td>
<td>4.0 (3.0-5.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSFC z score</td>
<td>0.19 (0.5) NA</td>
<td>0.12 (0.67) 0.43 (0.5)</td>
<td>0.17 (0.53) 0.86 (1.2)</td>
<td>0.71 (1.02)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nBPV, cm(^3)</td>
<td>1603.2 (79.5) 1651.4 (66.1)</td>
<td>1599.7 (89.9) 1638.4 (57.2)</td>
<td>1601.1 (89.3) 1507.6 (88.7)</td>
<td>1607.9 (96.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nWMV, cm(^3)</td>
<td>610.4 (38.9) 630.2 (36.9)</td>
<td>606.8 (48.2) 630.4 (38.8)</td>
<td>604.1 (47.7) 575.6 (46.9)</td>
<td>639.2 (47.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nGMV, cm(^3)</td>
<td>987.1 (65.5) 1021.2 (54.1)</td>
<td>987.3 (80.3) 1006.0 (46.7)</td>
<td>991.9 (83.4) 915.1 (65.9)</td>
<td>965.7 (82.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nLV, cm(^3)</td>
<td>5.70 (6.99) NA</td>
<td>5.54 (10.9) 1.96 (2.32)</td>
<td>5.2 (5.8) 16.9 (33.1)</td>
<td>1.3 (2.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CIS, clinically isolated syndrome; DMT, disease-modifying therapy; EDSS, Expanded Disability Status Scale; MS, multiple sclerosis; MSFC, Multiple Sclerosis Functional Composite; NA, not applicable; nBPV, normalized brain parenchymal volume; nGMV, normalized gray matter volume; nLV, normalized lesion volume; nWMV, normalized white matter volume; RRMS, relapsing-remitting MS; PPMS, primary progressive MS; SPMS, secondary progressive MS.
knowledge basis set of metabolite signals.\textsuperscript{21} The percentage gray matter (GM) and WM content within each spectroscopic voxel was calculated\textsuperscript{22} and for patients, the voxels containing lesions on the inversion recovery–spoiled gradient echo sequence were removed from the analysis. Moreover, the spectroscopic voxels were included in a linear fit only if their concentration estimates had estimated Cramer-Rao bounds within threshold values (10% for NAA and 30% for mI) (eAppendix 1 in Supplement).

Myelin water fractions from NAWM were extracted from a 16-slice T2 prep spiral sequence (TR = 2000 milliseconds; TEs = 7, 17, 28, 38, 49, 60, 70, 92, 124, 177, 220, and 294 milliseconds; in-plane resolution of 2 × 2 mm\textsuperscript{2}; 16-5-mm-thick slices; number of excitations = 6) that acquires nonlinearly spaced 12-echo data sets.\textsuperscript{23} Lesion and WM masks were regressed to the MWF map derived from the 12-echo data, which was fit to a distribution of T2 values using a nonnegative least square algorithm. The MWF maps (defined as ratio between peak area for T2 component <50 milliseconds and total water) were created to yield the percentage content within each voxel. The MWF median value was calculated from the NAWM mask for each patient at baseline and used as a predictor.

Confirmatory Data Set

Study Population
An independent group of patients with MS from the University of California–San Francisco Multiple Sclerosis Center were prospectively recruited from a large genotype-phenotype research project and included here to confirm the results obtained in the preliminary study. A total of 220 patients with MS meeting the 2005 revised McDonald criteria\textsuperscript{24} were included, with a mean (SD) follow-up time of 3.6 (0.9) years. The use of MS therapies was permitted. Eighty-eight percent of the patients (193 of 220) completed 3 years and 68% (150 of 220) completed 4 years of follow up. Demographic and clinical data are provided in Table 1.

Predictors
Four predictors from the preliminary data set analysis were retained (MWF was not used). N-acetylaspartate and mI from NAWM and GM were derived from a 2-dimensional TE-averaged spectroscopic imaging technique (TE-Averaged-CSI).\textsuperscript{25} The spatial data were acquired with a nominal in-plane spatial resolution of 1.2 × 1.0 cm with a volume selection box placed in the supratentorial brain, covering a single 1.5-cm-thick slice, just above the corpus callosum body (TR = 1 second; 64 TE steps starting at 35 milliseconds with a TE increment of 2.5 milliseconds). The resulting 8-coil combination data were TE averaged. Then NAA and mI values were quantified using the LCModel software in millimoles per liter and corrected for T1 and T2 metabolite relaxation times.\textsuperscript{22} After obtaining the percentage GM and WM content within each voxel the same way as for the preliminary data set, pure GM and NAWM metabolite concentrations were extrapolated\textsuperscript{26} by modeling the metabolite concentrations as a linear function of WM content. The same estimated Cramer-Rao lower bound threshold as for the preliminary data set was used.

Study Outcome Measures for Preliminary and Confirmatory Data Sets

All outcome metrics were collected similarly for both data sets. Brain atrophy progression for each pair of scans (baseline-year 1, year 1-year 2, etc) over the observation period was measured by estimated brain-volume change using SIENA (Image Analysis Group). Clinical outcomes were measured longitudinally using the Expanded Disability Status Scale (EDSS) and the Multiple Sclerosis Functional Composite (MSFC) scores over the observation period.

Brain-Volume Changes

Structural MRIs for both studies were acquired on the same 3-T GE scanner. The main MRI outcome was the slope in the percentage brain-volume change (PBVC) over the study period based on annual brain images acquired using a 3-dimensional inversion recovery–spoiled gradient echo (TR = 7 milliseconds; TE = 2 milliseconds; inversion time = 400 milliseconds; 15° flip angle; matrix: 256 × 256 × 180; field of view: 240 × 240 × 180 mm\textsuperscript{3}; 180 1-mm slices).

Clinical Measures

Annual neurological evaluations included standardized MS clinical metrics (EDSS and MSFC). All examiners were blinded to radiological predictors and outcomes. Patients with a baseline EDSS score of 5.5 or less were defined to have a sustained progression in their EDSS if an increase of 1.0 or more points was observed for at least 2 consecutive measures (sustained EDSS progression for 12 months); for patients with a baseline EDSS score of 6.0 or more, progression was defined as an increase of 0.5 or more points over 2 consecutive measures. Multiple Sclerosis Functional Composite standardized scores ($z$ scores) were derived by the methods previously described by the National Multiple Sclerosis Society’s Clinical Assessment Task Force\textsuperscript{26} and calculated from a reference population published previously.\textsuperscript{27} Sustained progression in MSFC $z$ score was defined as having a score that worsened by 20% or more from the baseline value over 2 consecutive points. The clinical outcomes of the study were the longitudinal change in EDSS score and MSFC $z$ scores and the binary summary–sustained EDSS score or MSFC progression over 12 months.

Imaging Covariates

Experienced neuroradiologists created T1-lesion masks using semi-automated thresholding and manual editing methods from the inversion recovery–spoiled gradient echo images. Subsequent brain segmentation and normalization were performed using SIENAX (Image Analysis Group), which was fully automated once the T1-lesion mask had been used to avoid pixel misclassifications. The final normalized brain parenchymal volume (nBPV) and normalized lesion volume (nLV) metrics were used as covariates in statistical modeling.

Statistical Analysis

All statistical analyses were performed using R (http://www.r-project.org/). Cross-sectional comparisons of metabolites and MWF between patients with MS and control participants were performed using Wilcoxon rank-sum tests. Linear mixed-
effects models were used to longitudinally model all outcomes (disability scores and brain-volume changes). The linear mixed-effects models were fitted using restricted maximum likelihood with a random intercept only. In contrast to the specifica- tion in the study by Frost et al., we only modeled PBVC measurements between subsequent pairs of time points (individual patient changes range, −1.5 to 4.5). Forty-eight percent (24 of 50) of the patients presented 12-month disability score progression. No predictors had a statistically significant influence on EDSS score evolution. However, mI:NAA ratio in NAWM was a significant predictor of change in disability score point annually for each increase in 1 mM; 95% CI, −0.08 to −0.009; \( P = .02 \). An EDSS score decline (Table 3).

## Prediction of Brain Atrophy Evolution

Overall, the mean (SD) PBVC from baseline to year 4 was −1.63% (1.1%). In the single-predictor analyses, we did not find any statistically significant associations between metabolite levels or MWF and PBVC evolution (Table 3). However, in a multiple-predictor analysis that included mI, NAA, and their interaction as predictors, there was a statistically significant positive interaction between mI and NAA in NAWM. This positive interaction indicates that NAA levels may modify, in this case reducing, the influence of mI on volume loss (estimated interaction of +0.018 annualized change in slope of PBVC for each simultaneous unit increase in NAA and mI; 95% CI, 0.006-0.031; \( P = .003 \)). A statistically significant interaction in the same direction as for NAWM was also observed in GM (+0.008 annualized change in slope; 95% CI, 0.003-0.014; \( P = .003 \)). These statistically significant interactions between mI and NAA along with biological plausibility (increased gliosis and reduced axonal integrity) prompted consideration of the mI:NAA ratio in NAWM as a predictor. Higher baseline mI:NAA ratio in NAWM predicted increased longitudinal brain-volume loss. Specifically, for each unit of increase in the mI:NAA ratio in NAWM, we estimated a corresponding annual slope of PBVC of −1.68 (95% CI, −3.05 to −0.30; \( P = .02 \); Table 3).

## Prediction of Change in Disability

The median EDSS score increase over the study was only 0.5 points (individual patient changes range, −1.5 to 4.5). Forty-eight percent (24 of 50) of the patients presented 12-month EDSS score sustained progression and 20% (10 of 49) showed MSFC score progression. No predictors had a statistically significant influence on EDSS score evolution. However, mI (<0.043 MSFC score point annually for each increase in 1 mM; 95% CI, −0.08 to −0.009; \( P = .02 \)) and mI:NAA ratio (<0.522 MSFC score point annually; 95% CI, −0.83 to −0.23; \( P < .001 \)) in NAWM were statistically significant predictors of longitudinal MSFC score decline (Table 3). Moreover, the mI:NAA ratio in NAWM was a significant predictor of MSFC score sustained progression over 12 months (estimated odds ratio [OR]/SD increase in the ratio, 2.76; 95% CI, 1.32–6.47; \( P = .01 \)) but not of EDSS score sustained progression (OR, 1.04; 95% CI, 0.60–1.79; \( P = .87 \)).

### Results

#### Preliminary Study

**Predictors**

All metabolites and MWF measures are reflected in Table 2. Statistically significant differences were found between patients with MS and healthy control participants for all predators except for NAA concentration levels in GM. The mI:NAA ratio in NAWM provided the largest percentage difference (31%; \( P < .001 \)).

### Table 2. Summary of Imaging Parameters Used as Predictors in the Preliminary Study

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Mean (SD)</th>
<th>Patients With MS vs Control Participants, %</th>
<th>( P ) Value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA in NAWM, mM/L</td>
<td>7.72 (1.42)</td>
<td>8.08 (1.19)</td>
<td>( \downarrow ) 8.4 ( .01 )</td>
</tr>
<tr>
<td>NAA in GM, mM/L</td>
<td>9.44 (2.0)</td>
<td>9.18 (1.54)</td>
<td>( \uparrow ) 2.8 ( .49 )</td>
</tr>
<tr>
<td>mI in NAWM, mM/L</td>
<td>2.57 (0.52)</td>
<td>2.22 (0.45)</td>
<td>( \uparrow ) 15.8 ( .001 )</td>
</tr>
<tr>
<td>mI in GM, mM/L</td>
<td>4.03 (0.95)</td>
<td>3.39 (0.78)</td>
<td>( \uparrow ) 18.9 ( .001 )</td>
</tr>
<tr>
<td>mI:NAA ratio in NAWM</td>
<td>0.34 (0.04)</td>
<td>0.26 (0.06)</td>
<td>( \uparrow ) 30.8 ( &lt;.01 )</td>
</tr>
<tr>
<td>mI:NAA ratio in GM</td>
<td>0.44 (0.1)</td>
<td>0.37 (0.08)</td>
<td>( \uparrow ) 18.9 ( .004 )</td>
</tr>
<tr>
<td>MWF, %</td>
<td>10.52 (1.12)</td>
<td>11.18 (0.92)</td>
<td>( \downarrow ) 5.9 ( .04 )</td>
</tr>
</tbody>
</table>

Abbreviations: GM, gray matter; mI, myo-inositol; MS, multiple sclerosis; MWF, myelin water fraction; NAA, N-acetylaspartate; NAWM, normal-appearing white matter; ▼, increase relative to control participants; ▲, decrease relative to control participants.<sup>a</sup> For healthy control participants, metabolite concentrations are extracted from WM and GM regions.<sup>b</sup> \( P \) values are derived from the Wilcoxon rank-sum test.
When the additional covariates (ie, disease duration, nBPV, nLV, and treatment status) were added into the models, the pattern of results was unchanged. Myelin water fraction did not show any predictive value on the evolution of brain atrophy or disability.

**Confirmaitory Study**

**Predictors**

Imaging parameters used in the confirmatory study are summarized in Table 4. Our main goal in this part of the study was to confirm our preliminary findings and prioritize the assessment of the ml:NAA ratio in NAWM.

**Prediction of Brain Atrophy Evolution**

The overall mean (SD) PBVC from baseline to year 4 was −2.02% (1.15%). Similar to the preliminary study, higher ml:NAA ratio in NAWM predicted larger brain-volume loss (−1.08 annual slope; 95% CI, −1.95 to −0.20; P = .02) (Table 5).

**Prediction of Change in Disability**

The median EDSS score change was 0.0 points (range, −3.0 to 3.0) at the end of the study. For each unit increase of the ml:NAA ratio in NAWM, there was an estimated corresponding annual mean EDSS score increase (0.57 points annually; 95% CI, 0.015−1.13; P = .04) over the subsequent 4 years. Longitudinal

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### Table 3. Effect of Imaging Predictors on Disease Progression (Annual Slopes) From the Preliminary Study’s 59 Patientsa

<table>
<thead>
<tr>
<th>Predictor</th>
<th>PBVC (%/y)</th>
<th>P Value</th>
<th>EDSS Score (Points/y)</th>
<th>P Value</th>
<th>MSFC Z Score (Points/y)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA in NAWM</td>
<td>0.03 (−0.03 to 0.09)</td>
<td>.34</td>
<td>−0.007 (−0.06 to 0.05)</td>
<td>.81</td>
<td>−0.0004 (−0.014 to 0.014)</td>
<td>.95</td>
</tr>
<tr>
<td>NAA in GM</td>
<td>0.03 (−0.02 to 0.08)</td>
<td>.19</td>
<td>−0.01 (−0.06 to 0.03)</td>
<td>.58</td>
<td>−0.007 (−0.02 to 0.005)</td>
<td>.24</td>
</tr>
<tr>
<td>ml in NAWM</td>
<td>−0.1 (−0.14 to 0.03)</td>
<td>.22</td>
<td>0.008 (−0.14 to 0.15)</td>
<td>.91</td>
<td>−0.04 (−0.08 to −0.009)b</td>
<td>.02b</td>
</tr>
<tr>
<td>ml in GM</td>
<td>−0.004 (−0.09 to 0.09)</td>
<td>.94</td>
<td>−0.02 (−0.11 to 0.07)</td>
<td>.70</td>
<td>−0.02 (−0.04 to 0.004)</td>
<td>.11</td>
</tr>
<tr>
<td>ml:NAA ratio in NAWM</td>
<td>−1.68 (−3.05 to −0.30)b</td>
<td>.02b</td>
<td>0.29 (−0.93 to 1.52)</td>
<td>.64</td>
<td>−0.52 (−0.82 to −0.23)b</td>
<td>&lt;.001b</td>
</tr>
<tr>
<td>ml:NAA ratio in GM</td>
<td>−0.78 (−1.69 to 0.14)</td>
<td>.09</td>
<td>−0.02 (−0.81 to 0.78)</td>
<td>.97</td>
<td>−0.06 (−0.27 to 0.14)</td>
<td>.55</td>
</tr>
<tr>
<td>MWF in NAWM</td>
<td>−0.03 (−0.1 to 0.03)</td>
<td>.33</td>
<td>−0.02 (−0.1 to 0.05)</td>
<td>.48</td>
<td>0.003 (−0.02 to 0.02)</td>
<td>.78</td>
</tr>
</tbody>
</table>

* Mean and 95% CIs of longitudinal effect of metabolite predictors on annual change on the outcome for each predictor. Based on single-predictor models analysis (longitudinal mixed-effects models). The PBVC results are described in terms of annualized percentage (%) change, whereas EDSS score and MSFC z score are given as annualized change in points score. The results are estimates from linear mixed-effects models.

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### Table 4. Imaging Parameters From the Confirmatory Study

<table>
<thead>
<tr>
<th>Predictor</th>
<th>All MS (n = 220)</th>
<th>CIS (n = 33)</th>
<th>RRMS (n = 164)</th>
<th>SPMS (n = 17)</th>
<th>PPMS (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA in NAWM, mM/L</td>
<td>9.8 (1.35)</td>
<td>10.1 (1.22)</td>
<td>9.84 (1.36)</td>
<td>9.42 (1.24)</td>
<td>9.03 (1.44)</td>
</tr>
<tr>
<td>NAA in GM, mM/L</td>
<td>8.76 (1.8)</td>
<td>8.93 (1.46)</td>
<td>8.79 (1.66)</td>
<td>8.07 (1.30)</td>
<td>8.53 (1.93)</td>
</tr>
<tr>
<td>ml in NAWM, mM/L</td>
<td>3.92 (0.69)</td>
<td>3.93 (0.59)</td>
<td>3.90 (0.72)</td>
<td>3.93 (0.64)</td>
<td>3.92 (0.69)</td>
</tr>
<tr>
<td>ml in GM, mM/L</td>
<td>4.74 (1.05)</td>
<td>4.99 (1.45)</td>
<td>4.70 (0.99)</td>
<td>4.52 (0.72)</td>
<td>5.00 (0.99)</td>
</tr>
<tr>
<td>ml:NAA ratio in NAWM</td>
<td>0.40 (0.07)</td>
<td>0.39 (0.06)</td>
<td>0.40 (0.06)</td>
<td>0.42 (0.07)</td>
<td>0.47 (0.1)</td>
</tr>
<tr>
<td>ml:NAA ratio in GM</td>
<td>0.54 (0.09)</td>
<td>0.56 (0.12)</td>
<td>0.54 (0.08)</td>
<td>0.57 (0.11)</td>
<td>0.60 (0.08)</td>
</tr>
</tbody>
</table>

* All patients with MS and MS subtypes are included.

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### Table 5. Confirmatory Effect of the ml:NAA Ratio in NAWM Tested as Predictor of Disease Progression (Annual Slopes)

<table>
<thead>
<tr>
<th>Predictor</th>
<th>PBVC (%/y)</th>
<th>P Value</th>
<th>EDSS Score (Points/y)</th>
<th>P Value</th>
<th>MSFC Z Score (Points/y)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ml:NAA ratio in NAWM</td>
<td>−1.08 (−1.95 to −0.20)b</td>
<td>.02b</td>
<td>0.57 (0.015 to 1.13)b</td>
<td>.04b</td>
<td>−0.23 (−0.41 to −0.05)b</td>
<td>.01b</td>
</tr>
</tbody>
</table>

* Mean and 95% CIs of longitudinal effect of metabolite predictors on annual change on the outcome for each predictor. Based on single-predictor models analysis (longitudinal mixed-effects models). The PBVC results are described in terms of annualized percentage (%) change, whereas EDSS score and MSFC z score are given as annualized change in points score. Results are estimates from linear mixed-effects models.

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Abbreviations: CIS, clinically isolated syndrome; GM, gray matter; ml, myo-inositol; MSFC, Multiple Sclerosis Functional Composite; MWF, myelin water fraction; NAA, N-acetylaspartate; NAWM, normal-appearing white matter; PBVC, percentage of brain-volume change.

* Mean and 95% CIs of longitudinal effect of metabolite predictors on annual change on the outcome for each predictor. Based on single-predictor models analysis (longitudinal mixed-effects models). The PBVC results are described in terms of annualized percentage (%) change, whereas EDSS score and MSFC z score are given as annualized change in points score. Results are estimates from linear mixed-effects models.

* Statistically significant results at α = 0.05.
changes in MSFC $z$ scores were also predicted by baseline ml:NAA ratio in NAWM (−0.23 points annually; 95% CI, −0.41 to −0.05; $P = .01$) (Table 5).

Twenty-seven percent of patients (55 of 204) experienced 12-month sustained EDSS score progression, and 16% (31 of 191) sustained MSFC $z$ score progression. Twelve-month sustained EDSS score progression was significantly predicted by the ml:NAA ratio in NAWM (OR/SD increase, 1.46; 95% CI, 1.10-1.94; $P = .009$) but not by the ml:NAA ratio in GM (OR, −0.25; 95% CI, −0.64 to 0.15; $P = .22$). However, contrary to the preliminary data set, we did not observe a significant effect of the ml:NAA ratio in NAWM on the 12-month MSFC $z$ score sustained progression (OR, 1.19; 95% CI, 0.84-1.66; $P = .32$).

When the additional covariates were added into the models, the pattern of results on brain atrophy and disability was unchanged.

Lastly, as the confirmatory data set had a larger number of patients, other imaging predictors and potential correlations between variables were explored and presented in eAppendix 2 and the eTable in the Supplement.

Discussion

The main focus of our study was to investigate longitudinally the predictive value of pathologically specific MR metrics on MS disease progression and to replicate our findings using an independent data set. Our observations provided evidence that the relationship between axonal damage and astroglisiosis from MS WM areas is a key element in the development of clinical disability and brain-volume loss in MS. More specifically, we reported that the ml:NAA metabolite ratio in NAWM is a predictor of MS progression.

Patients with MS were first recruited in a preliminary study. Percentage brain-volume change from SIENA$^{30}$ served in this long-term study as the MRI correlate of brain-tissue loss. Our multiple-predictor analyses included baseline ml and NAA from both MS WM and GM areas. The interaction between NAA and ml showed a statistically significant effect on PBVC. This statistical interaction between ml and NAA (reduced NAA and increased ml) along with biological plausibility (reduced axonal integrity and increased gliosis) prompted consideration of the ml:NAA ratio as a predictor. For instance, the observed interaction suggested that a decrease of NAA could accelerate the effect of ml on atrophy. The ml:NAA ratio would represent a practical and simple approach to define and exploit such a relationship. The rationales for the choice of a ratio, rather than any other functional combinations of ml and NAA, were (1) they are convenient for general use in MRS because they require simpler acquisitions and postprocessing compared with absolute metabolite quantification; (2) ratios derived from other metabolites, such as NAA:creatinine, have been widely used in spectroscopy for decades; and (3) ratios are more intuitive for readers less engaged in mathematical background. Indeed, higher baseline ml:NAA ratio in NAWM was a statistically significant predictor of increased longitudinal brain-volume loss, MSFC evolution, and sustained MSFC progression.

Myelin water fraction, a marker of myelin integrity, was not a statistically significant predictor in any statistical models we performed. The lack of sensitivity and robustness (despite good quality fits of all compartment peaks; data not shown) of our technique in detecting myelin injury could explain these findings or, alternatively, it could support the notion that permanent disability in MS may be driven by axonal rather than myelin damage.$^{31,32}$

A large confirmatory study using an independent MS group of patients and a different metabolite quantification method was conducted to replicate the results from the preliminary study. Notably, the ml:NAA ratio in NAWM was again able to predict brain-volume loss, MSFC $z$ score, EDSS score change over time, and 12-month sustained EDSS score progression, overall confirming the main findings of the preliminary results. Of note, WM lesion volume correlated only modestly with ml:NAA ratio in NAWM (Spearman correlation range, 0.22-0.28; eAppendix 2 in the Supplement). Lesion volume’s independent contribution (in addition to the ml:NAA ratio in NAWM) to brain-volume change was also minimal, with no contribution in our data sets to EDSS and MSFC $z$ score evolution. This could suggest an important and independent role of diffuse astrogliaosis and axonal WM injury in MS disease evolution.

Previous smaller studies had found that ml correlated cross-sectionally with clinical disability$^{33,34}$ and that low levels of NAA and high ml in NAWM of clinically isolated syndrome patients were predictors of conversion to clinically definite MS.$^{35}$ Higher ml:NAA ratio levels in patients with MS are likely to reflect the combination of astroglisiosis and axonal damage. From both data sets, ml levels (mainly from NAWM) consistently predicted brain-volume changes and disability. Although causality was not determined in this study, the results could highlight the importance of reactive astrocytes in MS, potentially having a deleterious action on disability and brain atrophy when astroglisiosis increases. However, its deleterious effect can be influenced by axonal status. This reinforces the theory of the dual role of astrocytes in MS: on one side, they may contribute to degeneration and demyelination by promoting inflammation, damage of oligodendrocytes and axons, and glial scarring, but on the other side, they may create a permissive environment for remyelination.$^{36,37}$ Nonetheless, both in vivo metabolite levels taken together seem to be important to predict the outcomes of interest.

Overall, the predictive power of the metabolites in GM was less pronounced than metabolites estimated from WM areas. However, the importance of GM pathology in MS is not questioned by these findings. Estimating metabolites is challenging in the cortical GM especially when using a 2-dimensional spectroscopy single-slice technique such as the one used in the confirmatory study. Additionally, issues related to partial voluming may be at play, this being particularly important for cortical GM owing to its ribbonlike morphological nature. Nonetheless, this does not invalidate the robust predictive value of the ml:NAA ratio derived from NAWM.

Our study had limitations. We did not evaluate the longitudinal variations of our metabolites and of MWF. Therefore, we were unable to characterize the longitudinal progression...
of all predictors considered in the study; obtaining such data would address a different question but could provide additional insight. Nonetheless, a cross-sectional (baseline) predictor derived from a single scan could offer clinicians a practical and convenient tool for patient monitoring. Additionally, all spectroscopy scans were acquired on a single 3-T GE platform and participants were recruited from a single site. Although generalization of the findings and potential population biases could represent limitations, a single-site study design has the advantage of minimizing scanner and site heterogeneity, especially in estimating metabolite concentration levels. Furthermore, the effect sizes of the changes in PBVC and disability measures predicted by the ratio were overall low, preventing the application of this measure for prediction at the individual level. We rather think our results inform about the pathological substrates of disease evolution.

A practical result of this experiment is that a metabolite ratio came up as our most robust predictor. A major advantage for the use of metabolite ratios over individual metabolite concentrations is that they are relatively easy to measure and can be readily obtained from clinical MRI facilities. However, we think that the biological interpretation of the mI:NAA ratio is different than the NAA:creatinine ratio, another metabolite ratio that has been used in MS research. The mI:NAA ratio rather reflects 2 known pathological processes of central nervous system injury instead of using creatinine as a normal reference. As such, its validation in other neurodegenerative disorders may also prove to be promising. Additionally, the mI:NAA ratio could be of significant interest to evaluate MS therapies aiming to achieve neuroprotection.

Conclusions

We concluded that the mI:NAA ratio derived from NAWM in MS is a robust cross-sectional predictor of brain-volume loss and clinical disability over time. Our work demonstrated that the combination of astrogliosis and axonal damage has cardinal importance in the evolution of MS. Further studies should evaluate its potential in clinical settings.

ARTICLE INFORMATION

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Author Contributions: Dr Pelletier had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Llufriu, Cree, Hauser, Pelletier. Acquisition, analysis, or interpretation of data: All authors. Drafting of the manuscript: Llufriu, Pelletier. Critical revision of the manuscript for important intellectual content: Kornak, Ratiney, Oh, Brenneman, Cree, Sampat, Hauser, Nelson, Pelletier. Statistical analysis: Kornak, Pelletier. Obtained funding: Hauser, Pelletier. Administrative, technical, or material support: Llufriu, Ratiney, Oh, Brenneman, Cree, Sampat, Hauser, Nelson, Pelletier. Study supervision: Pelletier.

Conflict of Interest Disclosures: Dr Llufriu has served on scientific advisory boards for Teva, Biogen Idec, and Novartis. Dr Llufriu has also received honoraria for speaking from Merck Serono and Biogen Idec and funding for travel from Teva and Novartis. Drs Oh and Sampat are employees of Synarc Inc. Dr Cree has received personal compensation for consulting to Abbvie, Biogen Idec, EMD Serono, Genzyme/sanofi-aventis, MedImmune, Novartis, and Teva Neurosciences and has received contracted research support from Avarian, Acorda, Hoffman-La Roche, and Novartis. Dr Hauser was a (retired) scientific advisory board member for BioMarin and Receptos. Dr Pelletier has received personal compensation from CNS Imaging Consultant LLC. No other disclosures were reported.

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