

Quantitative objective markers for upper and lower motor neuron dysfunction in ALS

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ABSTRACT Objective: To investigate the value of objective biomarkers for upper (UMN) and lower (LMN) motor neuron involvement in ALS. **Methods:** We prospectively studied 64 patients with ALS and its subsets using clinical measures, proton MR spectroscopic imaging (^1H MRSI), diffusion tensor imaging, transcranial magnetic stimulation, and the motor unit number estimation (MUNE) at baseline and every 3 months for 15 months and compared them with control subjects. **Results:** ^1H MRSI measures of the primary motor cortex *N*-acetyl-aspartate (NAA) concentration were markedly reduced in ALS ($p = 0.009$) and all UMN syndromes combined (ALS, familial ALS [fALS], and primary lateral sclerosis; $p = 0.03$) vs control values. Central motor conduction time to the tibialis anterior was prolonged in ALS ($p < 0.0005$) and combined UMN syndromes ($p = 0.001$). MUNE was lower in ALS ($p < 0.0005$) and all LMN syndromes combined (ALS, fALS, and progressive muscular atrophy; $p = 0.001$) vs controls. All objective markers correlated well with the ALS Functional Rating Scale–Revised, finger and foot tapping, and strength testing, suggesting these markers related to disease activity. Regarding changes over time, MUNE changed rapidly, whereas neuroimaging markers changed more slowly and did not significantly differ from baseline. **Conclusions:** ^1H MR spectroscopic imaging measures of the primary motor cortex *N*-acetyl-aspartate (NAA) concentration and ratio of NAA to creatine, central motor conduction time to the tibialis anterior, and motor unit number estimation significantly differed between ALS, its subsets, and control subjects, suggesting they have potential to provide insight into the pathobiology of these disorders. **NEUROLOGY 2007;68:1402-1410**

The search for quantitative objective markers that reliably assess upper (UMN) and lower (LMN) motor neuron dysfunction in ALS and its subsets has been an area of intense research.^{1,2} As abnormalities in the putative neuronal marker *N*-acetyl-aspartate (NAA) by proton MR spectroscopy (^1H MRS) were initially demonstrated in ALS,³ its usefulness as a biomarker has been explored extensively.⁴⁻¹⁷ Although most studies found the ratio of NAA to total creatine (NAA/tCr) was significantly reduced in the motor cortex or brainstem of patients with ALS vs healthy subjects, the diagnostic value of this measure in the clinical assessment of ALS and UMN disease is uncertain and remains to be fully established.^{1,2,18} Because MR diffusion tensor imaging (MRDTI) reveals the structural integrity of neuronal fibers, it has potential for investigating UMN fiber tract integrity in ALS.¹⁹⁻²² In addition to these novel neuroimaging techniques, transcranial magnetic stimulation (TMS), which evaluates the neurophysiologic integrity of the UMN tracts, may be useful for determining UMN dysfunction.^{16,23-26} Whereas UMN function has remained difficult to quantify by objective testing,^{27,28} the motor unit number estimation (MUNE) has emerged as a sensitive and reliable tool for quantifying the change in the number of functioning motor units in ALS.²⁹⁻³¹ Overall, the diagnostic reliability and sensitivity to change over time remain to be established for these techniques. Therefore, we conducted a comprehensive multidisciplinary study to investigate the value of potential markers of UMN and LMN dysfunction in relation to well-established clinical measures used in ALS, at baseline and during disease progression.

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METHODS Study subjects. All patients were recruited and evaluated for eligibility at the Eleanor and Lou Gehrig MDA/ALS Research Center of Columbia University. All patients provided informed consent, and this human research study protocol was approved by the Institutional Review Board of the Columbia University Medical Center. A trained neurologist conducted the examinations for the diagnosis of ALS, which was made on the basis of the well-established El Escorial criteria.³² We enrolled any patients who gave consent and had a diagnosis of suspected sporadic or familial ALS or its subsets, including primary lateral sclerosis (PLS) and progressive muscular atrophy (PMA), as defined below. This study was considered exploratory, and thus we recruited all eligible patients with the goal of substantially increasing the number enrolled as compared with our previous single-voxel ¹H MRS study of ALS and PLS.⁹ Patients with a current or past history of neurologic disease other than ALS and its subsets were excluded, but those enrolled in other clinical trials were not excluded. Baseline and identical follow-up clinical evaluations and diagnostic marker measurement studies were conducted every 3 months for up to 15 months.

Clinically healthy control subjects with a normal neurologic examination and no history of a major neurologic or neuropsychiatric disorder were solicited from the general public through advertisement or referral. Each control subject underwent only the neuroimaging studies (MRSI, MRDTI), as normal values for MUNE and TMS had been previously established in prior studies at the Columbia University EMG Laboratory and Clinical Motor Physiology Laboratory.

Neuromuscular assessment. All patients were examined at baseline and on follow-up visits by a neurologist and clinical evaluator who had participated in previous multicenter clinical trials in ALS. Each examination consisted of testing for muscle stretch reflexes, pathologic reflexes, muscle tone, finger- and foot-tapping speed,³³ full manual muscle testing on both sides in a total of 36 skeletal muscles,³⁴ manual grip power measurements using a Jammer hydraulic hand-held dynamometer (Preston Company, Jackson, MI), finger-pinch strength testing using a pinch dynamometer,³⁵ and forced vital capacity (FVC) using a Puritan-Bennett spirometer (Pleasanton, CA) containing a microprocessor. For FVC, the best of three trials was recorded at each exam, and capacity was expressed as percent predicted FVC (%FVC).^{35,36} The ALS Functional Rating Scale-Revised (ALSFRRS-R), which is the most widely used and extensively validated global scale³⁷⁻³⁹ for assessing motor function in ALS, was administered by a clinical evaluator at each visit. The entire clinical evaluation lasted 45 to 60 minutes.

Neuroimaging studies. Structural MRI and ¹H MRSI. All neuroimaging studies were conducted on a 1.5 T GE MRI system, using a standard quadrature head coil. Following a sagittal T1-weighted localizer imaging series, a four-section (15 mm thick, 3.5-mm gap) T1-weighted axial oblique MRSI localizer imaging series was acquired at the same slice locations and with the same slice thickness and angulation as would be used in the subsequent ¹H MRSI scan. Next, the multislice ¹H MRSI scan was performed⁴⁰ to record the spectroscopic data from four interleaved 15-mm brain slices in 29 minutes, with the following acquisition parameters: echo time/repetition (TE/TR) = 280/2,300 milliseconds; field of view = 240 mm; 32 × 32 phase-encoding steps with circularly sampled *k* space; and 256 time domain points. The resulting nominal MRSI voxel size was 7.5 × 7.5 × 15 mm.

The recorded raw MRSI data were transferred to an off-line

Sun Microsystems (Mountain View, CA) workstation for post-processing by two study investigators blinded to the diagnosis, who used MRS data processing and analysis software of their own design. For all analyses, a single experienced neuroradiologist, who was blinded to the identity of patients and their diagnoses, was responsible for selecting the MRSI voxels of interest in the left and right precentral gyrus (primary motor cortex) for analysis, using a registered grid overlay on the matching T1-weighted MRSI localizer images (figure E-1 on the *Neurology* Web site [www.neurology.org]). The voxels were selected on the basis of whether the cortex of the precentral gyrus or the subcortical U-fibers of the precentral gyrus intersected the center of the voxel. In each region of interest, the area under the peaks of the major brain metabolites (NAA, tCr, total choline [tCho]) was obtained using a frequency-domain nonlinear least-squares fitting routine. Each metabolite level was expressed as both the peak area ratio relative to tCr and the absolute or molar concentration (mmol), which was derived from the metabolite peak area using the phantom replacement method.⁴¹

MRDTI. Without changing hardware or moving the subjects, an MRDTI scan was performed immediately following the ¹H MRSI scan, using a single-shot, multislice, diffusion-weighted, echo planar, diffusion tensor imaging pulse sequence,⁴² with a 128 × 128 image matrix, a slice thickness of 5 mm, and a TE/TR ratio of 100/6,000 milliseconds. Thirty axial slices covering the entire brain were acquired in a total imaging time of 6.4 minutes, with a total of 32 images per slice: 26 diffusion-weighted images and 6 images without diffusion weighting. The maximum diffusion-weighting factor per axis was 840 s/mm². The recorded MRDTI images were transferred to a workstation for tensor components analysis. Maps of the average diffusion constant (D_{av} = diffusion trace/3), and the average diffusion anisotropy (fractional anisotropy, relative anisotropy, and ultimate anisotropy) was calculated.⁴² These MRDTI parameters were analyzed for the posterior limb of the internal capsule bilaterally. The total scan time for all the neuroimaging studies, including the structural MRI, MRSI, and MRDTI scans, was approximately 60 minutes.

Neurophysiologic studies. TMS. TMS stimulation was performed using a cap stimulator at C_z (Cadwell MES-10, Cadwell Inc., Kennewick, WA) to obtain total conduction latencies. Electrical spinal stimulation was performed at the C7 and L1 spinal levels using a high-voltage stimulator (Digitimer, Ltd., Hertfordshire, UK) to obtain peripheral conduction latencies. Central motor conduction time was calculated by subtracting the minimal peripheral latency from the minimal total evoked response latency, as previously described.^{16,43} Motor responses were recorded from the right and left abductor digiti minimi and tibialis anterior muscles. The normative TMS data were obtained from an independent group of 33 healthy control subjects previously tested at the Columbia University Motor Physiology Laboratory. The mean central motor conduction time to the abductor digiti minimi in the control group was 7.8 ± 1.5 (SD) milliseconds; the time to the anterior tibialis muscle was 13.5 ± 1.6 milliseconds.

MUNE. A previously described multiple-point stimulation MUNE method was performed²⁹ in the thenar eminence of the strongest hand, along with concurrent thenar strength measures. If strength was equal in both hands, the right hand was tested. Carpal tunnel syndrome was excluded first by standard

Table 1 Patient demographic and clinical features

Diagnosis	n	Age (range), y	Gender, M:F	Duration (range) from symptom onset to baseline, mo	Duration (range) from diagnosis to baseline, mo	ALSFRS-R score (range)	%FVC (range)
ALS	43	52.6 ± 10.9 (31.6–82.1)	31:12	30.0 ± 39.6 (4.8–255.6)	6.2 ± 5.0 (1.0–18.0)	36.4 ± 7.8 (4–47)	86.7 ± 17.6 (50–128)
PMA	9	65.9 ± 11.9 (44.5–82.5)	9:0	26.4 ± 12.0 (13.2–49.2)	5.1 ± 1.5 (2.0–7.0)	37.9 ± 6.1 (27–45)	78.2 ± 23.3 (49–117)
PLS	6	55.3 ± 9.1 (41.5–62.0)	2:4	80.4 ± 72.0 (10.3–193.2)*	7.5 ± 7.4 (2.0–22.0)	41.8 ± 3.7 (37–46)	101.2 ± 6.9 (91–110)
fALS	6	61.3 ± 17.3 (44.9–86.5)	0:6	46.8 ± 34.8 (13.2–85.2)	5.3 ± 6.3 (1.0–18.0)	37.2 ± 6.9 (31–48)	75.5 ± 6.9 (43–109)
Control subjects	29	55.8 ± 9.0 (36.5–75.0)	10:19	—	—	—	—

All values = mean ± SD.

* When patients enrolled, the duration of disease from onset to baseline was less than 4 years, but the patients were followed more than 4 years to satisfy the criteria for PLS.⁴⁶

PMA = progressive muscular atrophy; PLS = primary lateral sclerosis; fALS = familial ALS; ALSFRS-R = ALS Functional Rating Scale-Revised; FVC = forced vital capacity.

electrophysiologic techniques. The normative MUNE data used in this study were obtained from an independent group of 20 healthy control subjects tested previously at the Columbia University EMG Laboratory.

Data analysis. All statistical analyses were performed with the SPSS 13.0 software (SPSS Inc., Chicago, IL). At the conclusion of the study, a final clinical diagnosis was made for each patient on the basis of the accumulated clinical data. Patients with ALS had a combination of UMN and LMN signs (including EMG findings) and were considered to have ALS regardless of whether the diagnosis was definite, probable, laboratory-supported probable, or possible ALS as defined by the El Escorial criteria.³² PMA was diagnosed if pure LMN disease was present without any clinical UMN signs. Primary lateral sclerosis (PLS) was diagnosed if pure UMN involvement was present without any LMN signs, including negative electromyography (EMG) findings for at least 4 years from symptom onset.⁴⁴ fALS was diagnosed in five patients with *SOD1* mutations (four with a Leu144Phe mutation and one with Leu113Thr) and one patient with non-*SOD1*-linked fALS. All patients with fALS had both UMN and LMN involvement with a clear family history of ALS in first-degree relatives. As, by definition, all patients with ALS, PLS, or fALS had clinical UMN signs, these patients were combined as a UMN syndrome group for analysis when appropriate and are referred to as the UMN syndrome group.

When multiple measurements were obtained from the corresponding right and left sides of the body, the mean value for the right and left sides combined was calculated for each subject and used in analysis. Manual muscle testing was expressed as the mean strength, that is, the total scores divided by the number of muscles tested. A one-way analysis of variance (ANOVA) was used to detect potential differences between all disease categories at baseline. When ANOVA tests indicated the groups differed significantly at the 0.05 level of significance, Student *t* tests were used for specific analyses between the disease groups. Because of the exploratory nature of the study, no further adjustments for multiple comparisons were applied. Pearson product-moment correlation coefficient was used to analyze the correlation between continuous clinical variables and objective markers at baseline. The change over time in patients with ALS was analyzed by using generalized estimation equations to test for a linear trend in the percentage change

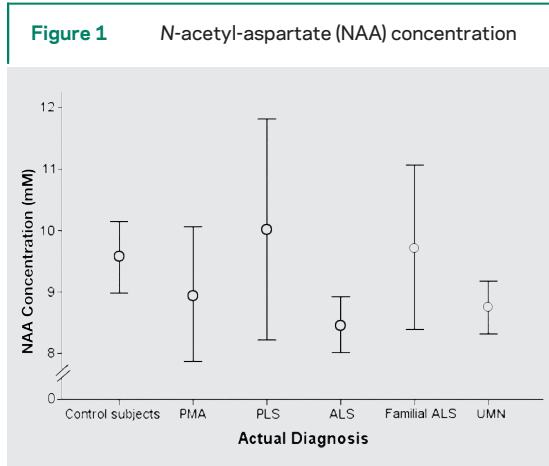
with time. For this we treated each patient as a cluster with repeated measurement at different visits and used an exchangeable working correlation matrix.^{45,46} In the ALS group (*n* = 43), 13 patients dropped out by the time of follow-up examination. These patients were compared by a *t* test with those who had at least one follow-up visit (*n* = 30) for differences in demographic variables and functional impairment (ALSFRS-R and %FVC). Patients with PMA, PLS, or fALS were not analyzed over time because the number of patients with sufficient follow-up assessments in these groups was small, and the changes were expected to occur slowly, unlike in ALS. Potential correlations over time between clinical measures and the quantitative markers were analyzed using Pearson product-moment correlation coefficient.

All data are expressed as the means ± 1 SD, or with a two-sided 95% CI. The significance level for all tests was set at the 0.05 level.

RESULTS Patient demographics and clinical features.

In total, we studied 64 patients and 29 recruited control subjects. Table 1 summarizes the diagnostic categories and clinical features. ANOVA among the groups showed significant differences in age and gender. Subsequent *t* tests revealed that mean age did not differ between control subjects and patients (*p* = 0.11) except for those with PMA, who were older than the control subjects (*p* = 0.02). The control group had more women than the patient group (*p* = 0.005). Mean disease duration was 34.8 ± 42.0 (SD) months (range 4.8 to 256 months) from symptom onset to the baseline study, indicating that substantial disease progression may have occurred prior to obtaining baseline measurements for some patients. Clinical features showed several differences among the disease categories; for example, patients with PLS had a significantly higher ALSFRS-R score and %FVC than patients with ALS or PMA.

The NAA concentration (mM) at the primary cortex in ALS was reduced by 11%, which differed from controls ($p = 0.009$); that of all UMN diseases combined (ALS, primary lateral sclerosis [PLS], and familial ALS [fALS]) also significantly differed from control subjects. No differences were found in progressive muscular atrophy (PMA), PLS, or fALS. The graph shows means and 95% CI.



Baseline analyses. ^1H MRSI. Both the NAA concentration and NAA/tCr ratio significantly differed among groups by ANOVA, and post-hoc tests showed that the mean NAA concentration in the precentral gyrus was 11% lower in ALS ($p = 0.009$; mean, 8.47 mM; 95% CI, 8.02 to 8.92) compared with control subjects (mean, 9.57 mM; 95% CI, 9.02 to 10.12) (figure 1). When ALS, PLS, and fALS were combined as a UMN syndrome group, the NAA concentration for the primary motor cortex was reduced by 8.4% compared with the control group ($p = 0.03$).

Analysis of the NAA/tCr ratio in the primary motor cortex revealed greater differences between the UMN disease groups and the control group at baseline (figure 2). The ratios for the PLS and ALS groups were 20% ($p = 0.001$) and 24% ($p < 0.0005$) lower than those for the control group. The mean NAA/tCr ratio for the combined UMN syndrome group was lower compared with the control group ($p = 0.007$), whereas a more modest difference was found between the controls and patients with PMA ($p = 0.03$).

DTI. The diffusion anisotropy value measured for the posterior limb of the internal capsule differed significantly among groups. Further analyses showed that the diffusion anisotropy value was lower in patients with fALS than control subjects ($p < 0.002$); otherwise groups did not differ at baseline.

TMS. One-way ANOVA revealed significant differences among all groups. Central motor conduction time at the tibialis anterior muscle was prolonged in patients with ALS (mean, 24.0 milliseconds; 95% CI, 19.7 to 28.3 milliseconds; $p < 0.0005$) and in those with fALS (23 milliseconds; 95% CI, 17.7 to 28.3 milliseconds; $p = 0.01$) as compared with the control data from our normal human subject database (mean, 13.5 milliseconds; 95% CI, 12.0 to 15.0). Central conduction time remained prolonged when the UMN syndromes were ana-

lyzed as a group ($p < 0.0005$) (figure E-2). In contrast, in ALS the central motor conduction time at the abductor digiti minimi did not significantly differ from the control group. At our center, we already use TMS as an adjunct diagnostic test and have found central conduction time to be abnormal and to indicate UMN involvement when it is longer than the value of the mean plus 2 SD based on the normal values used in this study. In the current study, the detection rate based on this method was 78% when conduction time was abnormal in any limb tested in all patients. However, when values from the right and left sides of the body were averaged, the rate declined to 55%.

MUNE. Analysis among groups showed substantial differences in MUNE ($p = 0.0005$). The mean MUNE value for the established normal subject data were 267 (95% CI, 234 to 280). The mean values for the disease groups were markedly lower vs controls: PMA = 29 (95% CI, 13 to 45; $p < 0.0005$), ALS = 76 (95% CI 54 to 98; $p < 0.0005$), fALS = 80 (95% CI, 20 to 110; $p < 0.0005$), and PLS = 174 (95% CI, 116 to 229; $p = 0.01$) (figure E-3). When all syndromes with LMN signs (ALS, PMA, and fALS) were combined, the mean MUNE value also differed from controls ($p = 0.001$). For PLS, the mean MUNE value was greater than that for ALS ($p = 0.004$) and PMA ($p < 0.0005$).

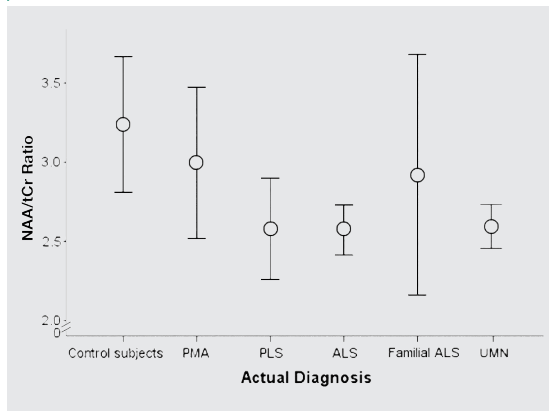
Correlation analyses at baseline. Correlations among clinical measures. All clinical measures except %FVC correlated highly with each other (Pearson $r > 0.39$, $p < 0.001$). The %FVC correlated well only with the ALSFRS-R score ($r = 0.40$, $p = 0.001$) and manual muscle testing ($r = 0.40$, $p = 0.001$).

Correlations between quantitative markers and clinical measures. Objective markers generally correlated with clinical measures, suggesting that these markers did detect clinically meaningful changes. The NAA concentration in all patients correlated well with average finger-tapping speed ($r = 0.38$, $p = 0.001$), average grip strength ($r = 0.45$, $p < 0.0005$), and average pinch strength ($r = 0.41$, $p < 0.0005$), but less strongly with the ALSFRS-R score ($r = 0.30$; $p = 0.03$). The NAA/tCr ratio modestly correlated with finger tapping, foot tapping, and average pinch strength. Central motor conduction time to the hand and leg muscles correlated with finger tapping ($r = 0.28$; $p = 0.03$) and foot tapping ($r = 0.33$; $p = 0.01$). MUNE correlated well with %FVC ($r = 0.32$; $p = 0.01$), manual muscle testing ($r = 0.52$; $p < 0.0005$), grip strength ($r = 0.34$; $p = 0.007$), and pinch strength ($r = 0.49$; $p < 0.0005$).

Correlations among quantitative markers. In general, all objective biomarkers correlated well within the same assessment: Central motor conduction

Analysis of the NAA/tCr in the primary motor cortex revealed a pattern similar to that seen for the NAA concentration, but the differences were larger: In ALS, values were 24% lower than in control subjects ($p < 0.0005$), in primary lateral sclerosis (PLS), the ratio was 20% lower ($p = 0.01$), and in the combined upper motor neuron (UMN) syndrome group, it also was lower compared with the control group ($p = 0.007$). The graph shows means and 95% CI.

Figure 2 Analysis of the N-acetyl-aspartate/total creatine ratio (NAA/tCr) in the primary motor cortex



time at the abductor digiti minimi and anterior tibialis were strongly correlated ($r = 0.94$, $p < 0.0005$), and the MRDTI diffusion constant correlated well with fractional anisotropy ($r = 0.60$, $p < 0.0005$); the NAA/tCr ratio and NAA concentration were modestly correlated ($r = 0.26$, $p = 0.02$). High correlations were also observed on right-to-right and left-to-left comparisons within the same marker (data not shown).

Analysis of changes over time. This analysis was performed in all patients with ALS who had at least one follow-up visit ($n = 30$). The mean number of visits was three, and the mean time from baseline to the final assessment was 9.2 months (range, 6 to 15 months). The 13 patients who did not return for any follow-up examination were compared at baseline with those who did return to determine if those who dropped out had a more aggressive disease. There were no significant differences in age, gender, or duration of the disease at enrollment. However, those who dropped out had a lower ALSRFS-R score (32 ± 10) than those who returned (38 ± 6 ; $p = 0.02$) as well as a reduced %FVC (75 ± 16 vs $92 \pm 17\%$; $p = 0.007$).

Clinical measures and objective markers. Generalized estimation equations analysis showed that most clinical measures changed significantly over time (table 2). In contrast, the imaging markers (NAA, NAA/tCr, MRDTI diffusion coefficient, and fractional anisotropy) did not change significantly. Of the neurophysiologic measures, central motor conduction time at TA and MUNE changed significantly over time.

Patient follow-up. Of the 64 enrolled patients, 22 had died by the time this report was prepared. Three patients diagnosed with ALS underwent postmortem examination. All three had biomarker evidence of abnormal UMN and LMN dysfunction (a value

Table 2 Linear trend for percent change per month in patients with ALS*

Study	Mean change (SE)	p Value
Clinical studies		
ALSFRS-R	-1.07 (0.27)	0.001
%FVC	-1.04 (0.29)	0.001
Finger tap	-1.68 (0.86)	0.052
Foot tap	-1.90 (0.65)	0.003
Mean MMT	-1.18 (0.31)	0.001
Manual grip	-2.31 (0.59)	0.001
Pinch strength	-0.93 (0.83)	0.26
Imaging studies		
NAA/Cr (motor cortex)	-0.44 (0.24)	0.06
NAA (motor cortex)	-0.10 (0.34)	0.79
DTI diffusion constant	-0.07 (0.06)	0.22
DTI fractional anisotropy	0.10 (0.08)	0.22
Neurophysiologic studies		
CMCT ADM	0.05 (0.56)	0.93
CMCT TA	1.56 (0.62)	0.01
MUNE	-2.35 (0.89)	0.008

All patients were evaluated about every 3 months. Of the 43 patients with a diagnosis of sporadic ALS, 30 had at least one follow-up study, and the average duration of follow-up was 9.2 months. Only these 30 patients were analyzed here. Generalized estimation equations were used to test for linear trends. ALSFRS-R = ALS Functional Rating Scale-Revised; FVC = forced vital capacity (% normal); MMT = manual muscle testing; NAA/Cr = N-acetyl-aspartate/creatinine ratio; DTI = diffusion tensor imaging; CMCT = central motor conduction time; ADM = adductor digiti minimi; TA = tibialis anterior; MUNE = motor unit number estimate.

was considered abnormal when the marker value exceeded the mean of the control ± 2 SD for that particular marker). Autopsy study confirmed the diagnosis of ALS and indicated histopathologic evidence of both UMN and LMN involvement.

DISCUSSION ¹H MRSI showed a significant reduction in the putative neuronal marker NAA in the primary motor cortex in ALS and UMN syndromes. TMS measures of central motor conduction time were markedly prolonged to the tibialis anterior muscle in ALS and UMN syndromes. Variability was large in all the markers for patients with PLS, and thus changes in PLS did not differ significantly from controls. Although the sample size for PLS was small, other reasons for this variability include the possibility that patients with atypical ALS were part of this group. These findings suggest that PLS may, in fact, be quite heterogeneous and warrant further study.⁴⁴ Values for MUNE, an LMN marker, were significantly decreased in all disease groups. MUNE was the objective marker that more consistently correlated with clinical findings at baseline. Al-

though we found that none of the novel technologies immediately discriminated patients from normal subjects for diagnostic purposes at this point, the differences we did find suggest that the markers have the great potential to provide insight into the biology of motor neuron disease pathophysiology and to be clinically useful in the future.

In a previous single-voxel ^1H MRS study, we reported significant differences in NAA/tCr ratios between patients with ALS and control subjects.⁹ However, because a relatively large voxel size (8 cm^3) was used, we postulated that partial volume averaging of extramotor and motor cortex tissue within this large voxel might have contributed to the relatively small magnitude of the observed NAA/tCr differences. Therefore, in the current study, we used the multivoxel MRS approach, which provides a 10-fold higher spatial resolution (a voxel size of 0.83 cm^3) to assess whether the increased spatial resolution and reduced motor cortex voxel tissue heterogeneity would permit more robust and reliable detection of NAA abnormalities in the primary motor cortex. However, in our hands multivoxel MRS (i.e., MRSI) did not perform markedly better than single-voxel MRS, suggesting that single-voxel MRS, which is substantially simpler to perform, requires a relatively short scan time, and is available on virtually every clinical MR system with MRS capability, might be the more viable technique for clinical evaluation of these disorders.

Previous investigations of potential MR spectroscopic markers for ALS, most of which had reported significant decreases similar to those reported here, varied widely in methodology and the measures selected for analysis. Moreover, among studies using the single-voxel approach, the selected region of interest varied. Most often it was the motor cortex, but the frontal white matter,⁶ pons,^{5,8} and medulla¹⁰ also have been investigated. Use of the multivoxel MRS technique has been limited to two studies by another group^{15,17} and the current study. The metabolites selected for analysis have also varied. Most studies examined the ratio of NAA to tCr,^{3,5-7,9-11,16} NAA to tCho,¹³ or NAA to tCr + tCho^{15,17}; only a few studies reported absolute NAA concentrations.^{4,8,11,14} The effects of short-term treatment with antiglutamate agents were investigated but yielded inconsistent results between studies.^{7,8} Only a few investigations examined temporal change in MRI markers, and study designs consisted of a relatively short follow-up period of no more than 18 weeks,¹¹ with assessment at variable observation points.¹³ One study assessed patients every 3 months up to 12 months,¹⁷ a design similar to ours. In general, most previous studies of MRS markers in ALS were

largely exploratory and primarily aimed at establishing the best experimental conditions and variables for measuring and reporting differences in NAA concentrations in various regions of interest.

We previously reviewed² neuroimaging technology in ALS using an evidence-based medicine approach and concluded that the technology for quantitative neuroimaging marker detection and analysis is rapidly evolving. Although nearly all previous studies were conducted on 1.5 T MR systems, clinical MR scanners equipped with high-field magnets ($3.0+\text{ T}$), which may offer higher sensitivity and reliability for measurement of regional concentrations of the major brain metabolites in motor neuron disease, have recently become commercially available.⁴⁷ However, it should be stressed that using uniform and consistent study designs and analyses in future MRS investigations of neuroimaging markers is most likely to reduce variability and permit more meaningful comparisons between studies.^{2,18}

In the current study, MRDTI-derived measures of the diffusion coefficient and fractional anisotropy (two parameters that are presumed to detect pyramidal tract abnormalities)¹⁹⁻²² did not show significant changes for the posterior limb of the internal capsule except in fALS. The pyramidal tracts extend from the centrum semiovale, via the posterior limb, to the cerebral peduncle, so analysis of these areas might potentially reveal diagnostic information on MRDTI.

TMS revealed significant differences between control data and patients with ALS or UMN syndromes. At our clinical center, we use TMS as an adjunct test to detect objective signs of UMN involvement. In the current study, TMS was able to detect upper motor prolongation in at least one of four limbs in 78% of all cases (ALS, PLS, fALS, and PMA) and 81% of all cases with UMN involvement. In our previous retrospective study, the ability of TMS to detect abnormal prolongation also was 77%.¹⁶ In the current study, however, TMS detection drops to 55% if the right and left sides are averaged together. Abnormally prolonged central motor conduction time has been reported in 51 to 93% of patients with ALS.^{16,23-25} Detection by TMS could be improved perhaps by combining it with other neurophysiologic methods or by using a more sophisticated TMS peristimulus histogram, which is reported to increase detection of electrophysiologic abnormalities in UMN dysfunction.⁴⁸⁻⁵¹

In contrast to the potential markers for UMN dysfunction, MUNE was useful in detecting LMN dysfunction in patients with clinical LMN signs, confirming its usefulness in LMN disease. Interestingly, a small but statistically significant reduction

in the mean MUNE value was seen in PLS patients when compared with control subjects. However, unlike the ALS groups, MUNE values in the PLS subjects did not significantly decline over time. Although we did not observe any LMN signs in this group, this unexpected finding could occur if very slowly progressive, UMN-onset ALS cases had been included in the PLS group, despite our use of the most stringent diagnostic criteria for clinical classification of patients at entry into the study. MUNE is a highly sensitive technique and documents LMN loss long before clinical weakness or LMN signs appear. Further studies are needed to determine the reliability of current diagnostic criteria in distinguishing PLS from slowly progressive UMN-onset ALS. Finally, this study has clearly confirmed that MUNE is a sensitive and reliable tool for quantitative detection of changes in the LMN over time in patients with ALS.³¹

All the neuroimaging markers changed over time, but these changes did not reach significance (table 2). Because significantly decreased levels of NAA were found at baseline and for all time points in ALS compared with control subjects, that these markers did not change consistently with time may indicate that the decrease due to pathology had already occurred and stabilized at the lower observed levels by the relatively late time of our measurements (see study limitations discussed below), with only very small changes occurring thereafter. Thus, it is possible that significant temporal changes would be observed if studies are conducted relatively early after disease onset, as the slow rate of change in the neuroimaging markers later in the disease process can plausibly be inferred to represent a genuine rate of biologic change in already affected primary motor cortex neurons. Nevertheless, the consensus seems to be^{17,18} that with the current technology, none of the quantitative neuroimaging markers is sufficiently consistent to permit accurate assessment of temporal changes in motor neuron disease. Clinically, change in function may appear faster than biologic change. Electrophysiologic measures, including central motor conduction time at the tibialis anterior and MUNE, may also detect changes that are more likely to directly produce dysfunction than measures of pure cellular changes. Whether quantitative neuroimaging markers can detect abnormal biologic change in UMN disease, especially early during the disease process, warrants further investigation because small changes in neuroimaging data may provide important clues to the pathobiology of UMN involvement in ALS.

We found significant correlations between a number of the objective UMN markers and clinical

measures at baseline, indicating that objective markers can detect clinically meaningful change. In particular, finger tapping and foot tapping, which were highly correlated with NAA, NAA/tCr, and central motor conduction times, are simple, reliable, and routinely used clinical markers for quantitating UMN dysfunction.^{33,52} The NAA concentration also modestly correlated with the ALSFRS-R score, one of the most widely used ALS global scales. In contrast, MUNE highly correlated with muscle strength, grip and pinch strength, and respiratory muscle strength, all of which are primarily influenced by LMN dysfunction. It is essential that quantitative biomarkers be clinically meaningful, particularly because they have potential as natural history markers and even as surrogate markers to supplement clinical findings.^{1,53}

This study has several limitations. The average time from disease onset to the baseline study was 30 months and that from onset to diagnosis was 24 months (table 1); this 24-month duration is longer than the mean of 14 months between onset and diagnosis reported in the ALS CARE database.⁵⁴ Patients referred to our center appeared to be well along in their disease course, which might explain our failure to detect significant temporal changes in the markers. We also had some difficulty in recruiting patients, as most were generally more interested in participating in clinical trials and were generally reluctant to spend the substantial time required for completing the assessments at each visit. In the follow-up studies, patients who had greater impairment as measured by the ALSFRS-R score and reduced pulmonary function dropped out, probably owing in part to difficulty in undergoing prolonged neuroimaging procedures. Therefore, the results of the temporal analysis may reflect the changes in patients who had a more stable disease course.

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