

Proton Magnetic Resonance Spectroscopy of the Primary Motor Cortex in Patients With Motor Neuron Disease

Subgroup Analysis and Follow-up Measurements

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Objectives: To determine the motor cortex degeneration in patients with amyotrophic lateral sclerosis (ALS) using proton magnetic resonance spectroscopy, and to prove that proton magnetic resonance spectroscopy is suited to monitor the course of disease with follow-up examinations.

Materials and Methods: We studied 33 patients with ALS whose conditions were diagnosed according to the El Escorial World Federation of Neurology criteria. Nine patients with ALS were followed up for up to 2 years. The control group included 20 healthy volunteers and 4 patients with multifocal motor neuropathy. Proton magnetic resonance spectroscopy determined levels of the brain metabolites *N*-acetylaspartate (NAA), choline, inositol-containing compounds, glutamate/glutamine, and phosphocreatine.

Results: Patients with ALS showed a significant reduction in the NAA-choline ($P < .001$) and NAA-phosphocreatine ($P < .005$) metabolite ratios and significantly elevated choline-phosphocreatine ($P < .005$) ratios compared with controls. Inositol-phosphocreatine ratios were also elevated in case patients, but the increase was less

pronounced ($P < .05$). No differences in glutamate/glutamine-phosphocreatine ratios were detected between case patients and controls. An analysis of subgroups demonstrated less significant differences in NAA-choline metabolite ratios ($P < .05$), even in patients with pure lower motor neuron syndrome (suspected ALS). No changes in metabolite T_1 and T_2 relaxation times were observed. Patients with multifocal motor neuropathy showed normal metabolic ratios. Progressive alterations in affected metabolite ratios could be documented in the follow-up examinations.

Conclusions: Spectroscopic changes in the motor cortices of patients with ALS correspond with a reduction in levels of NAA and an elevation in levels of choline and inositol compounds. Since NAA is exclusively expressed in neurons, the observed decrease of NAA reflects neuronal loss or dysfunction. Inositol and choline are associated with plasma membrane metabolism, so the release of these compounds may be related to membrane disorders.

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AMYOTROPHIC lateral sclerosis (ALS) is a motor neuron disease (MND) characterized by a progressive degeneration of upper (UMNs) and lower motor neurons (LMNs) in the motor cortex, brainstem, and spinal cord. Clinical signs include spasticity and amyotrophic paresis as well as bulbar involvement. The disease typically manifests in patients who are 50 to 60 years of age, and the median survival period is approximately 3 years. Until recently, the extent of motor neuron loss in patients with ALS could not be quantified using conventional neuroradiological or electrophysiologic methods. In addition, there is no biological marker reflecting the rate of disease progression in patients with ALS. Even recent trials that tested the efficacy of therapeutic approaches¹ used rating

scales mainly based on muscle strength or other clinical measures. Therefore, a method for direct quantification of the degenerative process is needed to gain greater insight into motor neuron survival in patients with MND, especially for monitoring therapeutic trials.

*For editorial comment
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Noninvasive proton magnetic resonance spectroscopy (¹H-MRS) offers the opportunity to investigate regional changes in the metabolite composition of different brain regions in vivo. Proton metabolites detectable with ¹H-MRS are *N*-acetylaspartate (NAA), phosphocreatine, choline, inositol-containing compounds, and glutamine/glutamate. In recent stud

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SUBJECTS AND METHODS

STUDY SUBJECTS

Thirty-three patients with a progressive MND (21 men and 12 women; mean [\pm SD] age, 50 ± 13 years) were studied using ^1H -MRS. Motor neuron disease was diagnosed according to El Escorial World Federation of Neurology criteria,¹⁰ requiring a progressive disease with clinical and electrophysiologic evidence for LMN degeneration in at least 2 distinct topographical regions (bulbar, cervical, thoracic, or lumbosacral). Patients were separated into 2 subgroups: those with ALS and clinical signs of UMN involvement ($n = 22$) and those with suspected ALS without clinical evidence of UMN involvement ($n = 11$). According to El Escorial criteria, we accepted as clinical signs of UMN involvement positive extensor plantar response, hypertonic tone, increased or clonic tendon reflexes, and pseudobulbar features. Four patients had a familial form of ALS. The control group included 4 patients (3 men and 1 woman; mean [\pm SD] age, 52 ± 14 years) with multifocal motor neuropathy (MMN) defined by electrophysiologic criteria and elevated anti-Gm1 antibodies, and 20 healthy volunteers (13 men and 7 women; mean [\pm SD] age, 45 ± 14 years) without any known medical history of neurodegenerative disease. Clinical and ^1H -MRS data of all patients and controls are listed in **Table 1**. All participants provided informed consent before entering the study.

MAGNETIC RESONANCE EXAMINATIONS

Magnetic resonance investigations were performed on 1.5-T whole body magnetic resonance systems (Gyrosan S15/ACS II and Gyrosan ACS-NT, Philips Medical Systems, Best, the Netherlands) using a head coil suited for magnetic resonance imaging and ^1H -MRS. Coronal T_2 -weighted turbo spin echo sequences (repetition time [TR], 2700 milliseconds; echo time [TE], 120 milliseconds), transaxial T_1 -weighted gradient echo sequences (TR, 400 milliseconds; TE, 7 milliseconds; 100° flip angle), and sagittal T_1 -weighted spin echo sequences (TR, 500 milliseconds; TE, 15 milliseconds) were obtained for image-guided localization of the spectroscopic volume of interest (VOI). A cubic VOI of $40 \times 30 \times 25$ mm was placed anterior to the central sulcus in the motor cortex and subjacent white matter (**Figure 1**). Proton magnetic resonance spectra were acquired with PRESS (Point Resolved Spectroscopy)

volume selection¹¹ and water suppression performed using 2 frequency-modulated 180° inversion prepulses.^{12,13} With a TR of 2000 milliseconds, TEs of 272 and 30 milliseconds, and 128 signal averages, the acquisition of each spectrum took approximately 4 minutes. Analysis of the spectra was performed with the spectroscopy software package supplied by the manufacturer of the magnetic resonance system (Philips Medical Systems). Relative metabolite concentrations of NAA, choline, inositol-containing compounds, glutamine/glutamate, and phosphocreatine were determined by fitting the resonance line shape of each metabolite peak to a Lorentz curve before ascertaining the peak integral. For all patients with ALS and MMN, ^1H -MRS of the motor cortex in both hemispheres was performed, whereas for healthy control subjects, either ^1H -MRS was performed on the dominant hemisphere or data were acquired from VOI measurements in both hemispheres. Proton T_2 relaxation times for NAA, choline, and phosphocreatine were determined in 9 patients with ALS compared with 5 healthy volunteers. T_2 values were obtained from a monoexponential fit to the peak areas of NAA, choline, and phosphocreatine in a series of 5 spectra with increasing echo delays (TE: 50, 136, 272, 400, and 600 milliseconds). With a TR of 3000 milliseconds and 32 signal averages, the measurement of a complete spectrum series took approximately 8 minutes. Additionally, in 4 patients and 3 volunteers, T_1 values of NAA, choline, and phosphocreatine were determined from a series of 5 inversion recovery spectra with increasing inversion delays (time of inversion: 180, 500, 1000, 1500, and 2500 milliseconds), a TR of 3500 milliseconds, and 32 signal averages (total measurement time, 10 minutes). For 9 patients with ALS, subsequent investigations were performed within 4 to 22 months. Five patients were examined twice, and 4 of these 5 were examined 3 times.

STATISTICAL ANALYSIS

Analysis of variance of metabolite ratios was performed with a 1-way analysis of variance procedure. Means of NAA:choline, NAA:phosphocreatine, choline:phosphocreatine, inositol:phosphocreatine, and glutamine:glutamate:phosphocreatine ratios were compared with a Scheffé post hoc range test. Metabolite T_2 values of patients and controls were measured using a Mann-Whitney test for independent variables. Correlations between age, disease duration, and MRS ratios were determined using Pearson product moment correlation.

ies, ^1H -MRS has been applied to neurodegenerative disorders such as Alzheimer disease and human immunodeficiency virus dementia, providing evidence of altered patterns of metabolites in the cerebral cortex.²⁻⁴ These studies show a reduction in the NAA:creatinine metabolic ratio in primarily affected areas of the brain. Since NAA is confined to neurons and phosphocreatine is distributed relatively evenly through all cells, these findings have been explained by a reduction in levels of neuronal NAA during the degenerative process.

There have been a few reports on proton magnetic resonance spectroscopic imaging⁵ and ^1H -MRS⁶⁻⁸ showing reduced NAA:phosphocreatine ratios in different brain

regions of patients with ALS/MND, most prominently in the primary motor cortex. Most recently, a decrease in the absolute concentrations of NAA in the motor cortex of patients with MND and clinical signs of UMN involvement has been demonstrated using ^1H -MRS with a water signal as an internal standard.⁹ This study failed to show any changes in the concentrations of choline and phosphocreatine, but revealed a reduction of NAA in patients with UMN signs. Furthermore, to our knowledge no investigations have analyzed inositol and/or glutamine:glutamate metabolite ratios in patients with MND.

In this study, we analyzed the relative resonance intensities of NAA, phosphocreatine, choline, inositol, and glutamine:glutamate in the primary motor cortex

Table 1. Clinical and MRS Data for Patients With ALS and Controls*

	Age, y	Metabolite Ratios				
		NAA/Cho	NAA/PCr	Cho/PCr	Ins/PCr	Glx/PCr
Suspected ALS ₁ (n = 11)	53 ± 15	2.34 ± 0.24†	2.77 ± 0.29	1.20 ± 0.18	1.05 ± 0.21	1.44 ± 0.76
ALS ₂ (n = 22)	47 ± 13	2.06 ± 0.28§	2.64 ± 0.31‡	1.29 ± 0.15‡	1.25 ± 0.37†	1.50 ± 0.39
Controls (n = 24)	45 ± 14	2.48 ± 0.19	2.86 ± 0.29	1.16 ± 0.11	1.05 ± 0.25	1.59 ± 0.39
MMN (n = 4)	52 ± 14	2.45 ± 0.19	2.80 ± 0.29	1.15 ± 0.11	0.95 ± 0.25	1.76 ± 0.39
Controls (n = 20)	43 ± 14	2.50 ± 0.18	2.89 ± 0.29	1.16 ± 0.12	1.08 ± 0.27	1.52 ± 0.37

*Data are given as mean ± SD. MRS indicates magnetic resonance spectroscopy; ALS, amyotrophic lateral sclerosis; NAA, N-acetylaspartate; Cho, choline-containing compounds; PCr, phosphocreatine; Ins, inositol; Glx, glutamine/glutamate; MMN, multifocal motor neuropathy; ALS₁, without clinical signs for upper motor neuron (UMN) involvement; and ALS₂ with clinical signs for UMN involvement.

†P < .05.

‡P < .005.

§P < .001.

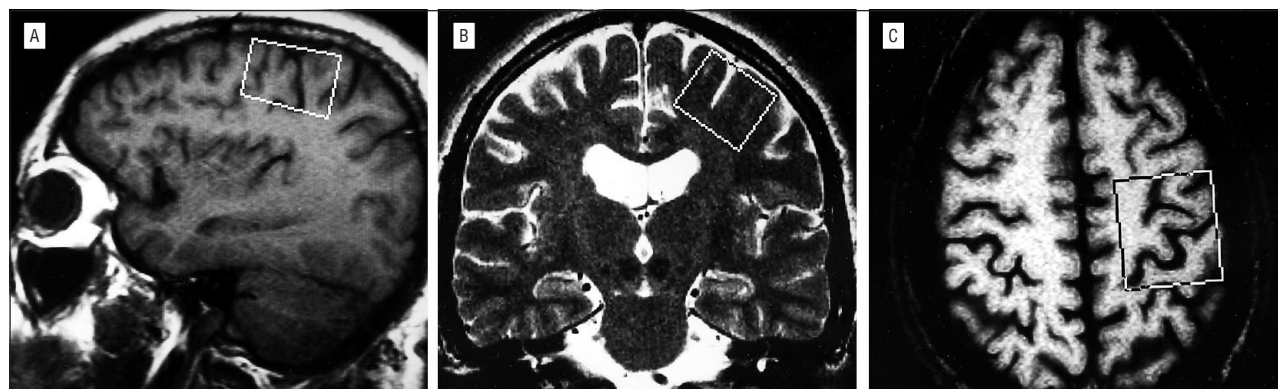


Figure 1. Sagittal (A), coronal (B), and transaxial (C) magnetic resonance images demonstrating the localization of the volume of interest. Proton magnetic resonance spectra were acquired in a cubic volume of interest of 40 × 30 × 25 mm placed anterior to the central sulcus in the motor cortex and subjacent to the white matter.

regions of a large number of patients with MND to detect the brain metabolite ratios that best reflect the neuronal degeneration of the motor cortex. Patients without clinical signs of UMN involvement were included in these investigations to find possible biological markers for subclinical motor cortex degeneration. To prove the potential of this quantitative method of following the course of the neurodegenerative process in the motor cortex, follow-up examinations were performed for 2 years.

RESULTS

Proton magnetic resonance spectra were obtained from 66 motor regions of 33 patients with MND. The results were compared with data obtained from 4 patients with MMN and 20 healthy controls. **Figure 2** shows a set of ¹H-MR spectra acquired with TEs of 272 and 30 milliseconds comparing exemplary results from a control and a patient with ALS. Table 1 shows NAA-choline, NAA-phosphocreatine, choline-phosphocreatine, inositol-phosphocreatine, and glutamine/glutamate-phosphocreatine metabolic ratios in patients with ALS compared with patients with MMN and healthy controls.

Patients with MMN had normal values for all metabolic ratios compared with healthy controls. In comparison with the control group, patients with MND and UMN

involvement showed a significant reduction in the mean NAA-choline and NAA-phosphocreatine ratios ($P < .001$ and $P < .005$, respectively), as well as a significant elevation in choline-phosphocreatine ($P < .005$) and inositol-phosphocreatine ratios ($P < .05$). In patients with a clinical diagnosis of a pure LMN syndrome, there was a significant reduction in the NAA-choline ratio ($P < .05$), while their NAA-phosphocreatine, choline-phosphocreatine, and inositol-phosphocreatine ratios did not differ from those of the control group. N-acetylaspartate–choline, NAA-phosphocreatine, and choline-phosphocreatine ratios calculated from spectra with short TEs times revealed similar alterations compared with controls. The significance of the changes was less pronounced because of higher SDs (data not shown). Differences in glutamine/glutamate–phosphocreatine ratios were not observed between the MND group and the controls. Correlation analysis revealed no significant correlation between age or disease duration and any metabolite ratio for patients with ALS and healthy controls.

Measurements of the T₂ relaxation time in 9 patients with MND failed to show any significant changes compared with 5 healthy controls. Preliminary results also indicated no difference in the T₁ determination between patients with MND and controls (**Table 2**).

Sequential studies in 9 patients with ALS (5 with and 4 without UMN involvement) were performed. Except

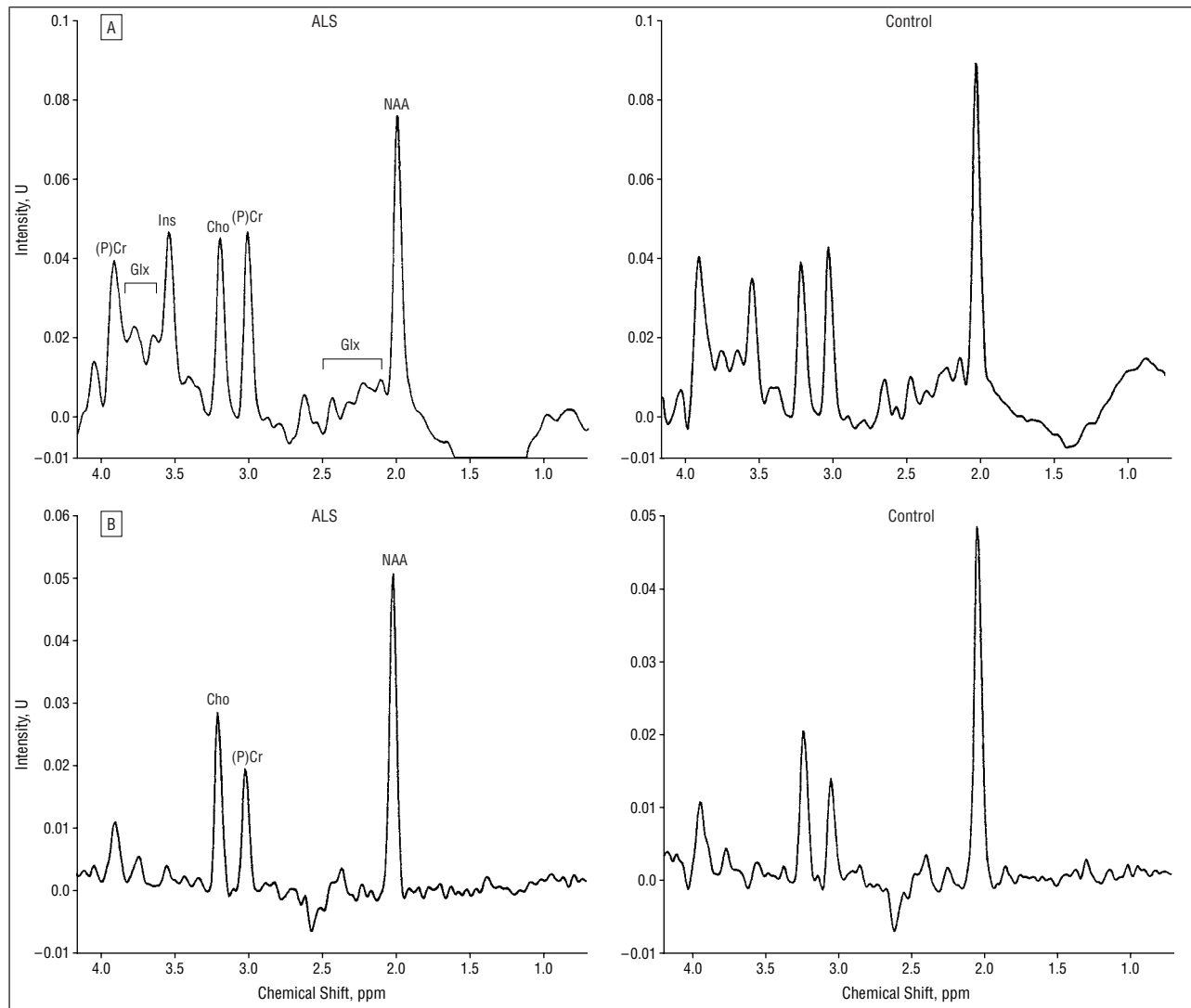


Figure 2. Comparison of localized proton magnetic resonance spectra from a patient with amyotrophic lateral sclerosis (ALS) and a healthy volunteer. A, Short echo time (TE) spectrum (repetition time [TR], 200 milliseconds; TE, 30 milliseconds). B, Long TE spectrum (TR, 2000 milliseconds; TE, 272 milliseconds) with 128 signal averages for each spectrum. Cho indicates choline-containing compounds; (P)Cr, phosphocreatine; Ins, inositol-containing compounds; Glx, glutamine/glutamate; and NAA, N-acetylaspartate.

for 1 patient with suspected ALS, these patients showed a continued reduction in the NAA-choline ratio in follow-up examinations (**Figure 3**), accompanied by smaller changes in the choline-phosphocreatine, inositol-phosphocreatine, and NAA-phosphocreatine ratios. As shown in Figure 3, the time course of the decrease in the NAA-choline ratio varied between patients. The highest changes in metabolite ratios were observed in patients with normal data in the first examination; 1 of these patients was initially diagnosed as having a pure LMN syndrome, but then developed clinical signs of LMN disease. Four patients were examined a third time, and 3 of these 4 showed a further progression of the metabolite pathologic pattern, while in 1 case the NAA-choline ratio stabilized at a low level.

COMMENT

To assess metabolic changes in brain metabolism in patients with MND, the motor cortex of patients with ALS

was studied using ^1H -MRS. Proton spectra with both short and long TEs were analyzed. According to previous findings,⁵⁻⁸ the spectra from the primary motor cortex of patients with ALS revealed significantly lower NAA-choline and NAA-phosphocreatine ratios compared with healthy controls. Additionally, we found significantly elevated choline-phosphocreatine and inositol-phosphocreatine ratios in patients with ALS, while glutamate/glutamine-phosphocreatine ratios were unchanged.

For spectra with long TEs, the signal intensity depends on both the local metabolite concentrations and the T_2 relaxation times. Our T_2 relaxation time measurements suggest that the T_2 values in the primary motor cortex of patients with ALS were unchanged. The TR of 2000 milliseconds used in this study is longer than the measured T_1 values. Therefore, T_1 alterations can have only minor effects on the metabolite ratios. Furthermore, our T_1 determinations do not support changes in the T_1 relaxation times in patients with ALS. Therefore,

Table 2. T₂ and T₁ Relaxation Times of Metabolites in Patients With ALS and Controls*

Metabolite	Patients With ALS (n = 9)	Controls (n = 5)
T₂ Relaxation Time, Mean ± SD Milliseconds		
Cho	297 ± 23†	307 ± 21
PCr	210 ± 21†	206 ± 17
NAA	315 ± 31†	317 ± 32
T₁ Relaxation Time, Milliseconds		
Cho	1230	1440
PCr	1280	1250
NAA	1420	1460

*ALS indicates amyotrophic lateral sclerosis; Cho, choline-containing compounds; PCr, phosphocreatine; and NAA, N-acetylaspartate.

†P > .05, using the Mann-Whitney test for independent variables.

the observed alterations have to be interpreted as changes in brain metabolite concentrations.

The decrease in the NAA-choline and NAA-creatine ratios in patients with neurodegenerative disorders is commonly explained by reduced NAA concentrations.²⁻⁸ Since NAA is exclusively expressed in neurons (as has been demonstrated by immunohistochemical studies¹⁴), reduced NAA levels suggest neuronal loss or dysfunction. Lower NAA-creatine ratios in the motor cortex of patients with ALS have been reported by other research groups using single-volume MRS and magnetic resonance spectroscopic imaging.⁵⁻⁸ Most recently, the decrease in the absolute concentrations of NAA in the motor cortex of patients with ALS has been demonstrated using ¹H-MRS with a tissue water signal as an internal standard.⁹

With respect to the marked decrease in the NAA-choline ratio associated with an increase in the choline-phosphocreatine ratio, we believe that the decrease in the NAA-choline ratio is also related to an increase in choline-containing compounds. Increasing choline-phosphocreatine ratios have also been reported in ¹H-MRS studies of Alzheimer disease and lesion analysis in multiple sclerosis.^{2,3,15,16} The choline signal detected by ¹H-MRS originates predominantly from glycerophosphocholine and phosphocholine, whereas free choline is only a minor constituent.¹⁷ Glycerophosphocholine and phosphocholine are choline phosphoglycerides, which are constituents of membrane lipids and myelin lipids. A release of choline phosphoglycerides following membrane damage or breakdown may contribute to a stronger choline signal detected by MRS. Another explanation for an increase in choline concentration could be an increase in cell membrane material from glial proliferation.¹⁸⁻²⁰ The observed elevation in levels of inositol compounds has also been reported in association with Alzheimer disease.^{21,22} This corroborates the hypothesis of increased membrane turnover in the primary motor cortex of patients with ALS, which possibly reflects the release of inositol phospholipids in the plasma membrane. Since we detected no alterations in glutamate/glutamine-phosphocreatine ratios, this study failed to provide further evidence for glutamate excitotoxic effects in patients with ALS.²³

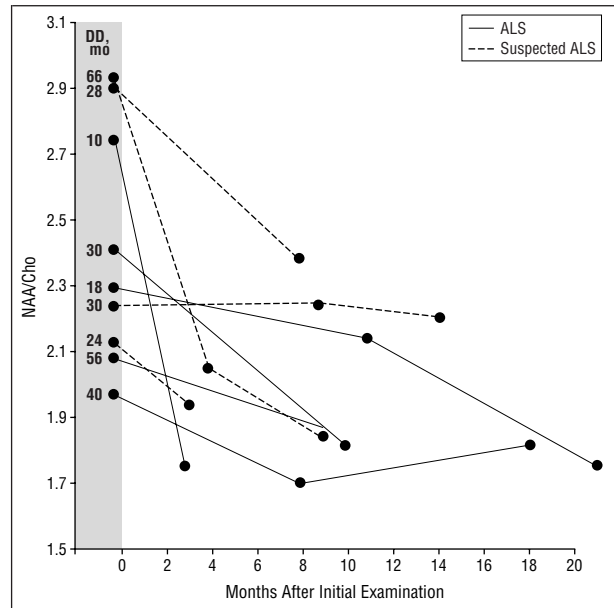


Figure 3. Proton magnetic resonance spectroscopy of the primary motor cortex in patients with amyotrophic lateral sclerosis (ALS) demonstrating decreases in N-acetylaspartate-choline (NAA/Cho) ratios in follow-up investigations. DD indicates disease duration before initial spectroscopic investigation.

Even patients with MND without any clinical signs of UMN involvement demonstrated significant differences in NAA-choline metabolite ratios compared with the control group, while a significant decrease in the NAA-phosphocreatine ratio and elevations in the choline-phosphocreatine and inositol-phosphocreatine metabolite ratios could not be detected. Because a decrease in NAA levels and an elevation in choline levels equally contribute to a reduction in the NAA-choline ratio, this finding might be explained by nonsignificant changes in the concentrations of these 2 metabolites.

As defined in the El Escorial criteria, the subgroup of patients with suspected ALS consisted of those without any clinical signs of UMN involvement at the time of the first spectroscopic examination. As a result, the subgroup of patients with suspected ALS was heterogeneous, including those who developed clinical UMN involvement as their disease progressed and those with pure LMN disease. Moreover, some patients with MND present with motor cortex degeneration only on neuropathologic examination. We think that the range of metabolic results from normal to strongly pathologic in the patients with suspected ALS reflects the heterogeneity of the pathologic characteristics of the motor cortex. Thus, we assume that for some patients diagnosed as having an LMN syndrome early in the disease, and even for patients without clinical signs of UMN, pathologic spectroscopic data could indicate a biological marker for motor cortex degeneration. Supporting this hypothesis, a progressive decrease in the NAA-choline ratio was observed in the follow-up ¹H-MRS examinations of all patients with pure LMN syndromes. One of these patients presented with clinical UMN involvement 1 year after normal ¹H-MRS examination findings.

During follow-up examinations of the patient group, trends toward decreasing NAA-choline, NAA-creatine, and progressively increasing choline-creatine ratios were observed over a period of 4 to 22 months. These findings may reflect the wide clinical variety of the presentation and progression of ALS, which always leads to a severe degeneration of motor neurons in the spinal cord, brainstem, and motor cortex. Correlation analysis revealed no significant linear interrelations between disease duration (defined by the onset of paresis) and any proton metabolite ratio. This is probably due to the fact that the patients with short disease duration showed inhomogeneous spectroscopic results. There are cases with seriously pathological results as well as subjects with almost normal metabolite ratios. In the later stages of the disease, all patients with ALS presented with pathologic metabolite ratios. Therefore, we think that ¹H-MRS could be used either to evaluate the different pathologic features of patients with MND, especially during disease onset, or to detect interindividual differences in the progression of the disease. Proton magnetic resonance spectroscopic examinations of the motor cortex might help to differentiate distinct clinical entities of MND and to monitor pharmacological effects in therapeutic trials, providing a quantitative biological marker for motor neuron degeneration. Further serial studies focusing on the deterioration of metabolite ratios and the correlation between ¹H-MRS findings and clinical and electrophysiologic characteristics of UMN involvement, ie, central motor conduction time, are warranted.

CONCLUSIONS

Alterations in metabolic ratios suggest reduced levels of NAA and elevated levels of choline and inositol compounds, reflecting neuronal loss or dysfunction associated with an increased membrane turnover in the upper motor neurons of patients with ALS. Therefore, the NAA-choline ratio could be considered the most sensitive marker for neuronal degeneration in patients with MND, possibly detecting subclinical UMN involvement. Furthermore, ¹H-MRS provides a quantitative method for obtaining more information about the progression of MND and for monitoring therapeutic trials.

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