Proton Magnetic Resonance Spectroscopy of the Motor Cortex in 70 Patients With Amyotrophic Lateral Sclerosis

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Objective: To evaluate proton magnetic resonance spectroscopy for detection and monitoring of upper motoneuron degeneration in patients with amyotrophic lateral sclerosis.

Methods: Seventy patients with amyotrophic lateral sclerosis according to the El Escorial criteria were compared with 48 healthy control subjects. Single-volume proton magnetic resonance spectroscopy (echo time, 272 milliseconds; repetition time, 2000 milliseconds) was performed in both motor cortices for detection of N-acetylaspartate (NAA), phosphocreatine + creatine ([P]Cr), and choline-containing compounds (Cho) to calculate the metabolite ratios NAA/Cho, NAA/(P)Cr, and Cho/(P)Cr. In addition, absolute metabolite concentrations of NAA, (P)Cr, and Cho were obtained in 30 patients and 15 controls with the unsuppressed water signal used as an internal reference.

Results: Absolute concentrations of NAA (P < .001) and (P)Cr (P < .05) were reduced in motor cortices of patients, whereas Cho concentrations remained unchanged. The NAA/Cho and NAA/(P)Cr ratios were reduced in all El Escorial subgroups (P < .001). The Cho/(P)Cr ratio was elevated in patients with definite amyotrophic lateral sclerosis (P < .05). Metabolite ratio changes corresponded to the lateralization of clinical symptoms and were weakly correlated with disease duration and disease severity. In follow-up observations of 16 patients during a mean (±SD) of 12.1 ± 8.7 months, NAA/Cho dropped by 9.1% (P < .01), and Cho/(P)Cr increased by 7.0% (P < .01). Changes of metabolite ratios were significantly correlated with progression of disease severity.

Conclusions: Measurement of NAA concentrations and NAA/Cho ratios appear to be most suitable for detection of motor cortex degeneration by single-volume proton magnetic resonance spectroscopy. Reduced NAA/Cho ratios correspond to aspects of the clinical presentation and reflect disease progression in follow-up measurements.

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A MYOTROPHIC lateral sclerosis (ALS) is a neurodegenerative disorder characterized by progressive degeneration of motoneurons in the motor cortex, brainstem, and spinal cord.1 Whereas lower motoneuron involvement in ALS is easily detected by electromyography, electrophysiologic examination of central motor pathways and conventional magnetic resonance imaging provide only limited information with respect to upper motoneuron (UMN) degeneration or bulbar involvement.2,3 Proton magnetic resonance spectroscopy (1H-MRS) has been introduced as a method to detect and to quantify subtle neurodegenerative changes not apparent on conventional magnetic resonance imaging. It has been repeatedly shown that 1H-MRS demonstrates metabolite changes in the motor cortex and brainstem of patients with ALS.4-12 Since these changes have been attributed to neuronal loss, 1H-MRS may be used to detect cortical and bulbar neurodegeneration.

Even more important, 1H-MRS findings may serve as surrogate markers for ALS disease progression in therapeutic trials.13 However, most 1H-MRS studies in ALS have been performed in a limited number of patients, and the relationship between spectroscopic findings and the heterogeneous clinical presentation of the disease is far from clear. In addition, little is known about long-term changes in 1H-MRS findings in the course of ALS. To resolve these questions, we conducted a follow-up and extension of our previously published study13 analyzing single-volume 1H-MRS for the detection and monitoring of neuronal degeneration in the motor cortex of patients with ALS. Seventy patients were investigated for up to 2 years and compared with 48 healthy controls. For better interpretation of 1H-MRS findings, metabolite
SUBJECTS AND METHODS

SUBJECTS

Seventy patients with ALS according to the El Escorial criteria were recruited from the Department of Neurology at the University Hospital of Bonn, Bonn, Germany (mean ±SD age, 55.4 ± 12.7 years; 39 men and 31 women). No patient had a history of any other neurologic disease. At the time of their first 1H-MRS examination, 15 patients were classified as having suspected ALS, 18 patients as possible ALS, 10 patients as probable ALS, and 27 patients as definite ALS. In 16 patients, follow-up investigations were performed within 4 to 24 months (3 with suspected ALS, 3 with possible ALS, 4 with probable ALS, and 6 with definite ALS). Mean duration of the observation period was 12.1 ± 8.7 months. All but 2 of these patients received riluzole treatment during the whole observation period. Forty-eight healthy volunteers (aged 51.8 ± 17.6 years; 31 men and 17 women) without history of any neurologic disease served as control subjects. All patients and control subjects gave informed consent.

Patients underwent physical examination at the time of each 1H-MRS examination. The following information was obtained for further statistical analysis: (1) hemisphere dominance for motor functions as determined by handedness; (2) disease duration in months, defined as the latency between when the patient first noted weakness subsequently attributed to ALS and the 1H-MRS examination; (3) disease severity as assessed by the score of Jablecki et al., which encompasses the evaluation of speech, swallowing, respiration, ambulation, muscle strength, and extremity function (a high score indicates greater impairment); (4) lateralization of clinical symptoms with respect to the severity of extremity pareses (asymmetry of clinical presentation was assumed if there was a minimum of 1 point difference in at least 1 muscle of the muscle strength subscales of the Jablecki et al score); and (5) location of disease onset differentiating bulbar- vs limb-onset ALS.

MAGNETIC RESONANCE EXAMINATION

Seventy patients with ALS and 48 controls were investigated by 1H-MRS for determination of relative metabolite concentrations of N-acetylaspartate (NAA), phosphocreatine + creatine (P)Cr, and choline-containing compounds (Cho) by means of the NAA/Cho, NAA/(P)Cr, and Cho/(P)Cr ratios. In 30 patients and 15 controls, absolute metabolite concentrations of NAA, (P)Cr, and Cho in millimoles per liter of brain tissue were also determined with the unsuppressed spin-echo spectra with echo times of 30, 70, 136, 272, 400, 700, and 1000 milliseconds and 4 signal averages each. Errors caused by variations in the assumed T1 and T2 relaxation times of cerebrospinal fluid and tissue water within the volume of interest were obtained by a biexponential fit to a series of unsuppressed spin-echo spectra with echo times of 30, 70, 136, 272, 400, 700, and 1000 milliseconds and 4 signal averages each. For this purpose, an additional acquisition of 1H-MR spectra was obtained without water suppression, with repetition time and echo time of 3000 and 272 milliseconds, respectively, and averaged over 16 or 32 free induction decays, yielding NAA/water signal ratios. In the post-processing of these unsuppressed spectra, polynomial baseline subtraction removed the broad lorentzian slopes of the water resonance curve underlying the much smaller metabolite peaks. To determine NAA/water concentration ratios by extrapolation to repetition time of infinity and echo time of 0 milliseconds, T2 relaxation times and relative fractions of cerebrospinal fluid and tissue water within the volume of interest were obtained by a biexponential fit to a series of unsuppressed spin-echo spectra with echo times of 30, 70, 136, 272, 400, 700, and 1000 milliseconds and 4 signal averages each. Absolute concentration measurements were performed in both hemispheres of 30 patients with ALS and 5 healthy controls. Measurements in dominant and non-dominant hemispheres yielded consistent results in these 12 control subjects. We therefore assumed that 1H-MRS findings were independent of hemisphere lateralization of motor functions, and measurements in the remaining 36 controls were thus performed only in the dominant hemisphere. Consequently, data for 140 patient and 60 control motor cortices were available.

Metabolite ratios were calculated in both hemispheres of all patients with ALS and 12 healthy controls. Measurements in dominant and non-dominant hemispheres yielded consistent results in these 12 control subjects. We therefore assumed that 1H-MRS findings were independent of hemisphere lateralization of motor functions, and measurements in the remaining 36 controls were thus performed only in the dominant hemisphere. Consequently, data for 140 patient and 60 control motor cortices were available.

STATISTICAL ANALYSIS

Clinical characteristics and metabolite concentrations of patients and controls were compared by means of χ² tests or t tests for unpaired samples. Clinical characteristics and metabolite ratios in different El Escorial subgroups were compared by 1-way analysis of variance (ANOVA) with
ratios and absolute metabolite concentrations were determined.

RESULTS

PATIENT CHARACTERISTICS

The ALS and control groups were well matched for age \( (P=.21) \) and sex \( (P=.65) \) (Table 1). Disease duration of patients with ALS at the initial \(^1\)H-MRS investigation was \( 22.4 \pm 20.3 \) months; disease severity was \( 10.9 \pm 5.3 \) according to the Jablecki et al score.

When patients with ALS were analyzed according to El Escorial subgroups, there were no significant differences between patients and controls with respect to age \( (P=.35) \) and sex \( (P=.41) \) (Table 1). There were no significant differences of disease duration among different El Escorial subgroups. In contrast, disease severity was significantly different, with most severe clinical presentation in definite ALS and less severe presentation in possible ALS (ANOVA: disease duration, \( P=.12 \); disease severity, \( P<.001 \)).

GROUP COMPARISON OF \(^1\)H-MRS MEASUREMENTS

Group comparison of patients and control subjects showed a significant reduction in NAA concentrations \( (P<.001) \) and \( (P)Cr \) concentrations \( (P=.04) \), whereas Cho concentrations remained unchanged \( (P=.85) \) (Table 2).

When ALS subgroups according to El Escorial criteria were compared with healthy control subjects, NAA/Cho and NAA/(P)Cr ratios were significantly reduced in all El Escorial groups (ANOVA: \( P<.001 \)), whereas the Cho/(P)Cr ratio was significantly elevated only in the subgroup with definite ALS (ANOVA: \( P=.03 \)) (Table 3). Group differences were not influenced by the covariates age, sex, or hemisphere dominance of motor functions.

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 \( 40 \times 30 \times 25 \) mm placed anterior to the central sulcus and subjacent to the white matter.
Receiver operating characteristic curves illustrated that the NAA/(P)Cho ratio and the NAA concentration had a greater accuracy in the detection of UMN abnormality than the NAA/(P)Cr ratio (Figure 2).

CLINICAL FINDINGS AND 1H-MRS MEASUREMENTS

In patients with asymmetric clinical presentation (n = 48), NAA/Cho ratios were significantly lower in the motor cortex contralateral to the clinically predominantly affected side (P = .04). There were no such side differences for the NAA/(P)Cr ratio (P = .96; NAA/(P)Cr, P = .92; Cho/(P)Cr, P = .99). No significant differences were observed between patients with bulbar onset (n = 24) vs limb onset (NAA/Cho, P = .96; NAA/(P)Cr, P = .91; Cho/(P)Cr, P = .82).

There was a weak, but significant, correlation between disease duration and reduced NAA/Cho ratios (r = −.16; P = .03; Figure 3) as well as elevated Cho/(P)Cr ratios (r = .018; P = .02). No significant correlation was detected between disease duration and NAA/(P)Cr ratios (P = .73).

There was a weak, but significant, correlation between disease severity and reduced NAA/Cho ratios...
(r = −0.24; P = .001; Figure 3) as well as elevated Cho/(P)Cr ratios (r = 0.20; P = .04-0.08). No significant correlation was detected between disease severity and NAA/(P)Cr ratios (P = .28).

FOLLOW-UP MEASUREMENTS

In patients with ALS undergoing follow-up 1H-MRS measurements (n = 16), NAA/Cho ratio decreased by 9% (P = .003) and NAA/(P)Cr ratio by 2% (P = .49), whereas Cho/(P)Cr ratio increased by 7% (P = .006) between the first and the most recent measurement (mean observation period, 12.1 ± 8.7 months) (Table 4). In individual patients, different patterns of NAA/Cho ratio changes emerged with respect to onset and slope of NAA/Cho deterioration (Figure 4). In late disease stages, no further decrease in NAA/Cho ratios was observed at values of about 1.7. Of 3 patients without clinical signs of UMN involvement at first 1H-MRS measurement, 2 exhibited a marked decrease of NAA/Cho and presented clinical signs of UMN dysfunction at follow-up investigations, whereas 1 had stable 1H-MRS measurements and no clinical UMN involvement during a period of 6 months.

There was a significant correlation between the decline of the NAA/Cho ratio and the progress of disease severity as indicated by an increase in the Jablecki et al score (r = 0.55; P < .001; Figure 5). Moreover, there was a significant correlation between the progress of disease severity and an increase of the Cho/(P)Cr ratio (r = 0.52; P = .001), whereas the NAA/(P)Cr change over time did not correlate with disease progress (P = .07).

This study is an expansion of our previous work on motor cortex 1H-MRS in ALS. We confirmed our previous results of reduced NAA/Cho and NAA/(P)Cr ratios. In addition, we observed reduced absolute NAA and (P)Cr concentrations by quantitative measurements and found that NAA/(P)Cr ratios as well as NAA concentrations have a greater accuracy in the detection of UMN abnormality than NAA/(P)Cr ratios.

Most 1H-MRS studies in ALS have demonstrated that either NAA concentrations or NAA/(P)Cr, NAA/Cho, and NAA/(P)Cr + Cho ratios are reduced in the motor cortex of patients with ALS. Since NAA is present only in neurons, it has been concluded that these metabolite changes indicate loss or dysfunction of UMN. The present observation that metabolite changes are more severe on the hemisphere contralateral to the clinically predominantly affected extremities further supports that 1H-MRS truly reflects neurodegeneration in ALS. There is a substantial debate on the interpretation of data obtained by different 1H-MRS protocols. By theory, absolute quantification of NAA appears to be more attractive than relative quantification of metabolite ratios, since data are ro-

Table 4. Change of Metabolite During Follow-up*

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD)</th>
<th>NAA/Cho</th>
<th>NAA/(P)Cr</th>
<th>Cho/(P)Cr</th>
</tr>
</thead>
<tbody>
<tr>
<td>First investigation</td>
<td>2.21 (0.29)</td>
<td>2.70 (0.24)</td>
<td>1.23 (0.15)</td>
<td></td>
</tr>
<tr>
<td>Last investigation</td>
<td>2.02 (0.21)</td>
<td>2.65 (0.22)</td>
<td>1.32 (0.10)</td>
<td></td>
</tr>
<tr>
<td>First vs last‡</td>
<td>3.0 (P = .003)</td>
<td>1.3 (P = .49)</td>
<td>2.2 (P = .006)</td>
<td></td>
</tr>
<tr>
<td>Disease progression§</td>
<td>−0.55 (P &lt; .001)</td>
<td>−0.26 (P = .07)</td>
<td>0.52 (P &lt; .001)</td>
<td></td>
</tr>
</tbody>
</table>

* NAA indicates N-acetylaspartate; Cho, choline-containing compounds; (P)Cr, phosphocreatine + creatine; and ellipses, not applicable.
† Numbers of evaluated motor cortex voxels.
‡ Values are t values.
§ Values are Pearson correlation coefficients.
bust against changes of metabolites serving as denominators. However, because the clinical application of quantitative 1H-MRS is time consuming and still suffers from technical limitations, measurements of relative metabolite concentrations are still in common use. In this context, the NAA/(P)Cr ratio was considered the most valuable surrogate marker, since the (P)Cr peak was thought to remain unaffected by the neurodegenerative disease process.7,12 However, we and others observed a stronger decrease of the NAA/Cho than the NAA/(P)Cr ratio, suggesting that either Cho levels are increased or (P)Cr levels are reduced in the ALS motor cortex.11,15 In the present study, we used 1H-MRS with absolute quantification to interpret the changes in metabolite ratios. We found that (P)Cr concentrations were indeed reduced, whereas Cho concentrations were unchanged in the ALS motor cortex. We conclude that NAA/Cho appears to be more valuable than NAA/(P)Cr to characterize motor cortex degeneration in ALS, since the latter metabolite ratio is confounded by a parallel decrease of (P)Cr levels, leading to a decrease in sensitivity and specificity for identification of UMN degeneration.

The mechanisms leading to a decrease in (P)Cr concentrations in cortical areas of patients with ALS are unclear. Possibly as a result of the high variability of quantitative (P)Cr measurements, reduction of (P)Cr concentrations has not been observed in 2 smaller spectroscopic investigations of the ALS motor cortex.6,9 Alternatively, reduction of (P)Cr might be due to the larger spectroscopic volume of interest used in the present study, including subcortical apart from cortical regions. However, a parallel decrease of NAA and (P)Cr concentrations without changes of Cho concentrations has also been observed in the cortex of patients with Alzheimer disease, suggesting that cortical (P)Cr reduction might indeed be found in various neurodegenerative disorders.21 The (P)Cr peak detected by 1H-MRS consists of signals derived from creatine and phosphocreatine located in astrocytes, oligodendrocytes, and neurons.22 Therefore, changes in (P)Cr concentrations detected by 1H-MRS are not specific for neuronal damage and do not show whether the equilibrium between creatine and phosphocreatine is altered or whether the concentrations of both compounds are decreased to the same degree. Therefore, decreased (P)Cr concentrations might be nonspecifically linked to neuronal death. Alternatively, it might be speculated that decreased (P)Cr indicates a disturbed cellular energy buffering and transport system in degenerating UMNs. This would be consistent with actual hypotheses that adenosine triphosphate depletion and oxidative damage might play a role in the pathogenesis of ALS.23 In particular, a recent experimental study reported that orally administered creatine extended survival of transgenic ALS mice and had a neuroprotective effect because of a reduction of oxidative damage in spinal and cortical motoneurons.24

To evaluate motor cortex 1H-MRS for diagnostic purposes, we compared measurements in different ALS subgroups according to the El Escorial criteria. We found the most prominent reduction of NAA/Cho in patients with definite ALS, indicating that more widespread clinical involvement is associated with pronounced motor cortex degeneration. However, there was also a substantial decrease in NAA/Cho ratios in patients without an es-

Figure 4. Follow-up data of the ratios of N-acetylaspartate (NAA) to choline-containing compounds (Cho) in 16 patients with amyotrophic lateral sclerosis (first vs last measurement). A, Measurements in the dominant motor cortex. B, Measurements obtained in the nondominant motor cortex. Note that, in individual patients, different patterns of NAA/Cho ratio changes emerged with respect to onset and slope of NAA/Cho deterioration. In late disease stages, no further decrease of NAA/Cho ratios was observed at values of about 1.7.

Figure 5. Scatterplot of the decrease of the ratio of N-acetylaspartate (NAA) to choline-containing compounds (Cho) (motor cortex measurements of both hemispheres) in a total of 25 follow-up investigations of patients with amyotrophic lateral sclerosis vs increase of disease severity. The continuous line represents the correlation graph.
established diagnosis of probable or definite ALS. Although the diagnostic value of ¹H-MRS is limited by the overlap of metabolite ratios in the motor cortex of healthy control subjects and patients with ALS, these findings indicate that the method may increase diagnostic certainty in some patients with missing clinical UMN signs. Because ¹H-MRS is a well-tolerated procedure that can be easily performed along with conventional magnetic resonance imaging, it might add to the spectrum of procedures used in the diagnosis of motoneuron disorders.

Unlike a previous study, this study did not find an association of altered NAA ratios with bulbar vs limb onset of the disease. Therefore, our data do not point to a more severe UMN involvement or different pathologic process in the bulbar group. Because of considerable variation of the metabolite ratios in early disease stages, we found only weak correlations of ¹H-MRS findings with duration and severity of ALS symptoms. These findings reflect a marked interindividual variability of UMN involvement in the course of ALS, as known from pathological examinations. Moreover, they suggest that the extent of motor cortex degeneration is not of critical importance for the overall clinical impairment in patients with ALS. Motor cortex ¹H-MRS findings are correlated with limb function, as previously shown by other investigators.

Apart from diagnostic issues, ¹H-MRS might be used for monitoring of UMN degeneration in patients with ALS. Herein we report ¹H-MRS findings in 16 patients with ALS followed up longitudinally for an average period of 1 year. We found a significant decline in NAA/Cho ratios along with a significant increase in Cho/(P)Cr ratios. By contrast, decrease in NAA/(P)Cr ratios failed to reach significance, indicating that NAA/Cho ratios might be superior not only for diagnostic purposes but for disease monitoring as well. Interestingly, the average 9.1% decline in NAA/Cho observed in this study resembles the annual 9.3% decrease in NAA concentrations found in cortical areas of patients with Alzheimer disease. However, as we did not perform serial ¹H-MRS measurements within predetermined observation intervals, our data on natural course of NAA deterioration in the ALS motor cortex clearly need extension and reproduction. Nevertheless, they might help to determine what observation periods are necessary to detect neuroprotective effects on ¹H-MRS findings in therapeutic trials.

As observed in our previous study, there was considerable interindividual variability for onset and slope of NAA/Cho deterioration, ranging from patients with marked early NAA/Cho reduction to patients without progressive changes of the NAA/Cho ratio up to 2 years after disease onset. Investigations by ¹H-MRS in patients with advanced cases of definite ALS and severe but stable disease demonstrated a deteriorated plateau of NAA/Cho ratios reaching values of about 1.7. In addition, we found a moderate correlation between decline of metabolite alterations with the clinical progression of the disease. These findings suggest that changes in NAA/Cho ratios might be taken as surrogate markers to estimate the activity of the neurodegenerative process in the motor cortex of individual patients with ALS. Therefore, ¹H-MRS appears to be a promising tool for monitoring the active phase of the ALS disease process.