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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AMYOTROPHIC 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.6-13 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.16 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 study15 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 criteria17 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.,14 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 in the volume of interest.

In 30 patients and 15 controls, absolute metabolite concentrations of NAA, [P]Cr, and Cho were determined from spin-echo spectra in the volume of interest. Measurements in dominant and nondominant hemispheres were calculated by means of the NAA/Cho, NAA/[P]Cr, and Cho/[P]Cr ratios. In 16 patients undergoing follow-up 1H-MRS investigations, 8 were examined twice, 7 were examined 3 times, and 1 was examined 4 times (total of 25 follow-ups) to determine relative metabolite concentrations. The 1H-MRS examiners (W.B. and F.T.) were not aware of clinical data.

Details of the 1H-MRS protocol for determination of relative metabolite concentrations are given elsewhere.13 In brief, investigations were performed on a 1.5-T whole-body magnetic resonance systems (Gyrosan S15/ACS II and Gyroscan ACS-NT; Philips Medical Systems, Best, the Netherlands). A spectroscopic volume of interest (40×30×25 mm) was placed anterior to the central sulcus in the motor cortex and subjacent white matter including the primary motor cortex within Brodmann areas 4 and 6 (Figure 1). The 1H-MR spectra were acquired with a repetition time of 2000 milliseconds, echo time of 272 milliseconds, and 128 signal averages. Relative metabolite concentrations for NAA, (P)Cr, and Cho were determined by lorentzian curve fitting of the corresponding resonance in the frequency spectra.

From these data, the metabolite ratios NAA/Cho, NAA/[P]Cr, and (P)Cr/Cho were determined.

Metabolite ratios were calculated in both hemispheres of all patients with ALS and 12 healthy controls. Measurements in dominant and nondominant 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.

Absolute concentrations of NAA, (P)Cr, and Cho were determined from spin-echo spectra in the volume of interest described above, with the brain water signal used as an internal reference. 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 postprocessing 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 ∞ 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. Errors caused by variations in the assumed T1 values of mixed gray-white matter (800 milliseconds) and cerebrospinal fluid (3000 milliseconds) were minimized by a long repetition time of 6000 milliseconds. Metabolite T1 and T2 values were not measured on an individual basis, but were taken from our comparative analysis in healthy volunteers and patients with ALS published previously.13 Metabolite concentrations were expressed as millimoles per liter of brain tissue by a mixed gray-white matter water content of 72%, corresponding to 40 mol/L.

Metabolite concentration measurements were performed in both hemispheres of 30 patients with ALS and 5 healthy controls. Measurements in dominant and nondominant hemispheres yielded consistent results in these 5 controls. We therefore assumed that 1H-MRS findings were independent of hemisphere lateralization of motor functions, and measurements in the remaining 10 controls were thus performed only in the dominant hemisphere. Consequently, data from 60 ALS and 20 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 test 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.
Table 1. Group Characteristics

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No. of Subjects</th>
<th>Sex, No. M:F</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Age, y</td>
</tr>
<tr>
<td>Controls</td>
<td>48</td>
<td>31:17</td>
<td>51.8 (17.6)</td>
</tr>
<tr>
<td>ALS</td>
<td>70</td>
<td>39:31</td>
<td>55.4 (12.7)</td>
</tr>
<tr>
<td>Control vs ALS†</td>
<td></td>
<td></td>
<td>1.2 (21)</td>
</tr>
<tr>
<td>ALS classification</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suspected</td>
<td>15</td>
<td>12:3</td>
<td>56.4 (10.9)</td>
</tr>
<tr>
<td>Possible</td>
<td>18</td>
<td>9:9</td>
<td>59.7 (11.8)</td>
</tr>
<tr>
<td>Probable</td>
<td>10</td>
<td>6:4</td>
<td>50.3 (16.8)</td>
</tr>
<tr>
<td>Definite</td>
<td>27</td>
<td>14:13</td>
<td>53.6 (12.6)</td>
</tr>
<tr>
<td>Control vs El Escorial‡</td>
<td></td>
<td></td>
<td>1.1 (P = .35)</td>
</tr>
</tbody>
</table>

* ALS indicates amyotrophic lateral sclerosis; NA, not applicable.
† Values are t values.
‡ Statistics for group comparison for sex are given in the “Patient Characteristics” subsection of the “Results” section.
§ Values are F ratios (analysis of variance).
||Analysis only within El Escorial groups.

Table 2. Metabolite Concentration at First Proton Magnetic Resonance Spectroscopy

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No.†</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NAA, mmol/L</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>13.9 (0.9)</td>
</tr>
<tr>
<td>ALS</td>
<td>60</td>
<td>12.3 (1.3)</td>
</tr>
<tr>
<td>Control vs ALS‡</td>
<td></td>
<td>4.6 (P &lt; .001)</td>
</tr>
</tbody>
</table>

* NAA indicates N-acetylaspartate; (P)Cr, phosphocreatine + creatine; Cho, choline-containing compounds; and ALS, amyotrophic lateral sclerosis.
† Numbers of evaluated motor cortex voxels.
‡ Values are t values.

Table 3. Metabolite Ratios at First Proton Magnetic Resonance Spectroscopy

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No.†</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NAA/Cho</td>
</tr>
<tr>
<td>Control</td>
<td>60</td>
<td>2.45 (0.21)</td>
</tr>
<tr>
<td>ALS classification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suspected</td>
<td>30</td>
<td>2.17 (0.20)</td>
</tr>
<tr>
<td>Possible</td>
<td>36</td>
<td>2.20 (0.28)</td>
</tr>
<tr>
<td>Probable</td>
<td>20</td>
<td>2.12 (0.31)</td>
</tr>
<tr>
<td>Definite</td>
<td>54</td>
<td>2.05 (0.24)</td>
</tr>
<tr>
<td>Control vs El Escorial‡</td>
<td></td>
<td>19.1 (P &lt; .001)</td>
</tr>
</tbody>
</table>

* NAA indicates N-acetylaspartate; Cho, choline-containing compounds; (P)Cr, phosphocreatine + creatine; and ALS, amyotrophic lateral sclerosis.
† Numbers of evaluated motor cortex voxels.
‡ Values are F ratios (analysis of variance).

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 = .07) or the Cho/(P)Cr ratio (P = .29).

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 = −0.16; P = .03; Figure 3) as well as elevated Cho/(P)Cr ratios (r = 0.18; 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...
as well as elevated Cho/(P)Cr ratios \( (r = 0.20; P = .04-08) \). 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 \(^1\)H-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) \( (\text{Table 4}) \). In individual patients, different patterns of NAA/Cho ratio changes emerged with respect to onset and slope of NAA/Cho deterioration \( (\text{Figure 4}) \). 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 \(^1\)H-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 \(^1\)H-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 \( (r = 0.55; P < .001; \text{Figure 5}) \). 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 \( (P = .07) \).

<table>
<thead>
<tr>
<th>Table 4. Change of Metabolite During Follow-up*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean (SD)</strong></td>
</tr>
<tr>
<td>NAA/Cho</td>
</tr>
<tr>
<td><strong>First investigation</strong></td>
</tr>
<tr>
<td><strong>Last investigation</strong></td>
</tr>
<tr>
<td><strong>First vs last‡</strong></td>
</tr>
<tr>
<td><strong>Disease progress§</strong></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.

(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 \(^1\)H-MRS studies in ALS have demonstrated that either NAA concentrations \(^6,9\) or NAA/(P)Cr, \(^7,11,12\) NAA/Cho, \(^10,11\) and NAA/(P)Cr + Cho \(^14\) ratios are reduced in the motor cortex of patients with ALS. Since NAA is present only in neurons, \(^20\) 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 \(^1\)H-MRS truly reflects neurodegeneration in ALS. There is a substantial debate on the interpretation of data obtained by different \(^1\)H-MRS protocols. By theory, absolute quantification of NAA appears to be more attractive than relative quantification of metabolite ratios, since data are ro-

**COMMENT**

This study is an expansion of our previous work \(^15\) on motor cortex \(^1\)H-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.
bust against changes of metabolites serving as denominators. However, because the clinical application of quantitative 
$^1$H-MRS is time consuming and still suffers from technical
limitations, measurements of relative metabolite con-
centrations are still in common use. In this context, the
NAA/(P)Cr ratio was considered the most valuable surro-
gate 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
$^1$H-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 de-
crease 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 spec-
troscopic investigations of the ALS motor cortex.6,9 Al-
ternatively, 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. How-
ever, a parallel decrease of NAA and (P)Cr concentra-
tions without changes of Cho concentrations has also been
observed in the cortex of patients with Alzheimer dis-
ease, suggesting that cortical (P)Cr reduction might ind-
deed be found in various neurodegenerative disor-
ders.21 The (P)Cr peak detected by $^1$H-MRS consists of
signals derived from creatine and phosphocreatine lo-
cated in astrocytes, oligodendrocytes, and neurons.22
Therefore, changes in (P)Cr concentrations detected by
$^1$H-MRS are not specific for neuronal damage and do not
show whether the equilibrium between creatine and phos-
phocreatine is altered or whether the concentrations of
both compounds are decreased to the same degree. There-
fore, decreased (P)Cr concentrations might be nonspe-
cifically linked to neuronal death. Alternatively, it might
be speculated that decreased (P)Cr indicates a dis-
turbed cellular energy buffering and transport system in
degenerating UMNs. This would be consistent with ac-
tual hypotheses that adenosine triphosphate depletion and
oxidative damage might play a role in the pathogenesis
of ALS.23 In particular, a recent experimental study re-
ported that orally administered creatine extended sur-
vival 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 $^1$H-MRS for diagnostic pur-
poses, we compared measurements in different ALS sub-
groups 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-


