Serum N-acetylaspartate Level in Amyotrophic Lateral Sclerosis

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Background: N-acetylaspartate (NAA) level is a biomarker of functional integrity and vitality in neurons. In vivo multisection proton (1H)–magnetic resonance spectroscopy studies indicate that NAA level decreases in specific cortical brain areas of patients with amyotrophic lateral sclerosis (ALS).

Objective: To study NAA level in serum samples as a possible biomarker of ALS.

Design: Serum NAA assay by liquid chromatography–mass spectrometry in a case-control series.

Setting: Department of Neurological and Psychiatric Sciences, Policlinico, University of Bari, Bari, Italy.

Patients: One hundred twelve consecutive patients with ALS and 51 age- and sex-matched healthy control subjects.

Main Outcome Measures: General estimating equations tested associations between serum NAA level and clinical variables in patients with ALS.

Results: Serum NAA level was significantly higher in ALS cases than in controls. Multivariate logistic regression analysis showed a direct association between serum NAA level and the presence of ALS. After stratifying serum NAA level based on the median value (0.171 mmol/L), the age- and sex-adjusted odds ratio for ALS was 19.97 (95% confidence interval, 7.18-55.55) (P < .001). N-acetylaspartate level did not differ across ALS clinical phenotypes. Riluzole treatment did not affect NAA level. A significant correlation was found between serum NAA level and ALS progression rate.

Conclusions: High serum NAA level was found in patients with ALS, which may relate to greater excretion of NAA into the blood circulation following increased release of this metabolite from damaged neurons. The correlation between serum NAA level and disease progression rate suggests that it may be a useful biomarker of ALS.

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Amyotrophic lateral sclerosis (ALS) is a chronic progressive neurodegenerative disorder that causes rapid loss of motor neurons in the brain and spinal cord. Diagnosis of ALS is based on clinical grounds, and as in other neurodegenerative disorders, no diagnostic or prognostic biomarkers are available with satisfactory specificity and sensitivity. N-acetylaspartate (NAA) is a free amino acid synthesized predominantly in neuronal mitochondria by the enzyme aspartate-N-acetyltransferase. One of the most abundant molecules in the central nervous system, the exclusive localization of NAA in neurons suggests that it may be a good biomarker of neuronal viability. Daily turnover of NAA is regulated through an intercompartmental cycle involving extracellular fluids among neurons, oligodendrocytes, and astrocytes. Evidence suggests a continuous NAA efflux from neurons to blood circulation, and in physiological conditions, low serum NAA level relates to its rapid glomerular filtration in the kidneys. A slightly decreased NAA level in the brain is a normal part of aging, particularly in older men. In contrast, pathological decreases have been observed in the brain of patients with Alzheimer disease, Parkinson disease, and multiple sclerosis (MS) by in vivo proton (1H)–magnetic resonance spectroscopy or by postmortem histopathological evidence. Low NAA level was found in cortical brain regions of patients with ALS particularly those with bulbar onset or upper motor neuron impairment. To date, few studies have performed NAA assay in biological fluid samples. A high NAA level has been reported in cerebrospinal fluid (CSF) of patients with ALS and recently in CSF and serum of patients with MS suggesting enhanced efflux of NAA in biological fluids secondary to neuronal impairment.
The objectives of the present study were 2-fold: (1) to determine whether serum NAA level differs between patients with ALS and healthy control subjects and (2) to examine what associations exist between serum NAA level and ALS clinical phenotypes.

**STUDY POPULATIONS**

The study analyzed 112 patients with ALS and 51 age- and sex-matched healthy control subjects. Patients with ALS were consecutively recruited among outpatients attending the ALS multidisciplinary center of the Department of Neurological and Psychiatric Sciences, Policlinico, University of Bari, Bari, Italy. For the control group, 11 healthy individuals (age range, 40-59 years) were consecutively recruited among blood donors attending the blood bank service, University of Bari, and the remaining 40 healthy individuals (age range, 61-82 years) were consecutively recruited among patients attending the Center for Brain Aging and Memory, Department of Geriatrics, University of Bari. For the latter group, any systemic disease or central nervous system involvement was preliminarily excluded by clinical evaluation and by hematological and radiological investigations (brain computed tomography or magnetic resonance imaging) and then by a standardized battery of neuropsychological tests.

For the ALS group, diagnosis was made according to Airlie House criteria. All patients had sporadic ALS. No patients with familial ALS were included in the study. Based on the site of symptom onset, patients were classified as having (1) bulbar ALS when the onset of symptoms was in the bulbar region or (2) spinal ALS when the onset of symptoms was in cervical, thoracic, or lumbar regions. Patients were also classified based on the presence of predominantly upper motor neuron signs or lower motor neuron signs, considering the extent of upper and lower involvement and the number of affected regions at the time of evaluation. The motor and functional status of patients with ALS was assessed using the Medical Research Council Scale and the Revised ALS Functional Rating Scale (ALSFRS-R). Disease progression rate was calculated as follows: 

\[ \text{Disease progression rate} = \frac{[48 - \text{ALSFRS-R Score at Evaluation}]}{\text{Disease Duration From Symptom Onset to Evaluation}} \]

All individuals gave their informed consent to participate in the study, which was approved by the institutional review board of the University of Bari.

**ANALYSIS OF NAA LEVEL**

N-acetylaspartate level was measured in serum samples from cases and controls using liquid chromatography–mass spectrometry according to the method described by Ruggieri et al. The NAA level was expressed in millimoles per liter. N-acetylaspartate for use as an internal standard was obtained commercially (Sigma; St Louis, Missouri). Analysis was performed on a C18 column in a high-performance liquid chromatogra-
 Phy system (Agilent 1100 Series; Agilent Technologies, Palo Alto, California). An isocratic gradient was used, and liquid chromatography–mass spectrometry was performed on a system (Finnigan Mat Spectra System P4000; Agilent Technologies, Palo Alto, California) connected to an ion trap mass spectrometer (Thermo Quest LCQ Duo; Agilent Technologies). The range of 5.7 to 57 μmol/L was explored, with a detection limit of 0.1 μmol/L. The intraday and interday (10 times for both) coefficients of variation were 2.1% and 6.2%, respectively. Readers (M.R. and E.C.) were blinded to all clinical information.

### STATISTICAL ANALYSIS

Clinical and paraclinical variables were expressed as means (SDs) and as medians (ranges). Nonparametric statistical tests were used because of the nonnormal distribution of most variables. Comparisons between groups were performed using the Mann–Whitney and Kruskal–Wallis tests, followed by pairwise post hoc analysis corrected for multiple comparison (Dunn post hoc correction). Correlations between variables were tested by Spearman rank correlation and by multiple linear regression analyses. Finally, we studied the association between serum NAA level and ALS case or control status using a multivariate logistic regression model. We used NAA level in the model as a categorical variable, stratifying serum NAA level based on the median value. P < .05 was considered statistically significant. All statistical analyses were performed using commercially available software (SPSS version 8.0; SPSS Inc, Cary, North Carolina).

### RESULTS

**Table 1** gives the clinical and demographic characteristics of cases and controls, who did not differ in age or sex distributions. Based on revised El Escorial criteria for diagnosis,24 fifty-nine patients had definite ALS, 22 had probable ALS, 8 had probable ALS with laboratory evidence, and 23 had possible ALS. Eighty-seven patients (77.7%) had spinal onset of disease. Upper motor neuron signs were predominant in 39 patients (34.8%) at the time of evaluation. The mean disease duration from symptom onset to evaluation was 40.2 (42.7) months. The mean diagnostic delay (interval between symptom onset and diagnosis) was 18.7 (19.7) months. The mean disease progression rate was 0.73 (0.79) per month. Sixty-five patients (58.0%) were receiving riluzole therapy.

Serum NAA level was significantly higher in ALS cases (mean, 0.184 [0.027] mmol/L; median, 0.185 [0.110-0.260] mmol/L than in controls (mean, 0.086 [0.062] mmol/L; median, 0.060 [0.025-0.300] mmol/L) (P < .001, Mann–Whitney test) (Figure). Using ALS status as a dependent variable in a logistic regression model, high serum NAA level was strongly associated with the presence of ALS. After stratifying serum NAA level based on the median value (0.171 mmol/L), the unadjusted odds ratio for ALS was 15.20 (95% confidence interval, 5.94-38.85; P < .001); the results did not change after adjustment for sex and age (Table 2). In controls, NAA level was significantly higher in men (mean, 0.110 [0.068] mmol/L; median, 0.090 [0.029-0.297] mmol/L) than in women (mean, 0.046 [0.016] mmol/L; median, 0.049 [0.025-0.088] mmol/L) (P = .001, Mann–Whitney test). In cases, there was no difference in NAA level by sex (mean, 0.188 [0.027] mmol/L; median, 0.188 [0.147-0.256] mmol/L in men; and mean, 0.179 [0.027] mmol/L; median, 0.178 [0.110-0.234] mmol/L in women). Furthermore, a negative correlation between serum NAA level and current age was found in controls (r = −0.64; P < .001, Spearman rank correlation) but not in cases. The N-acetylaspartate level did not differ across ALS clinical phenotypes. Riluzole treatment did not affect NAA level. However, subgroups of cases showed significantly higher serum NAA level than controls (Table 3).

In cases, a significant correlation was found between serum NAA level and ALS progression rate (r = 0.3; P = .01, Spearman rank correlation). This was confirmed by multiple linear regression analysis (r² = 0.18, P = .01). No correlation was found between NAA level and site of symptom onset, Medical Research Council Scale score, ALSFRS-R score, diagnostic delay, or riluzole treatment duration.

### COMMENT

In a previous study,21 a high NAA level was observed in the CSF of patients with ASL by high-performance liquid chromatography. To date, our study is the first to demonstrate that a serum NAA level is significantly higher in patients with ALS compared with healthy controls. High NAA level in CSF and in serum is consistent with decreased NAA noted in the motor cortex by in vivo 1H-magnetic resonance spectroscopy studies.24-17

Although our data are preliminary, possible mechanisms underlying the observed increased serum NAA level should be analyzed. In normal conditions, the first route for NAA clearance is its transfer from neurons to oligodendrocytes, where the enzyme aspartoacylase cleaves the acetate moiety for use in fatty acid and steroid synthesis.31 In pathological conditions, such as ALS, damaged neurons may increase the release of NAA in the extracellular space. From here, NAA is then preferentially...
regression rate, which considers the ALSFRS-R score and diagnostic delay. A possible explanation is that ALSFRS-R score and diagnostic delay are, by themselves, less reliable clinical measures because they are affected by several factors.29,37 Disease progression rate, which considers the ALSFRS-R score and diagnostic delay, is independent of possible confounding factors, such as age and sex, and could be an expression of the neurodegenerative process involving abnormal neuronal loss.

In conclusion, the serum NAA level may be considered a biomarker of disease progression in patients with ALS. The results of this study are preliminary and need to be confirmed in a prospective study.

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