



Clinical Study

Glial fibrillary acidic protein as a marker of astrocytic activation in the cerebrospinal fluid of patients with amyotrophic lateral sclerosis



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ABSTRACT

Glial fibrillary acidic protein (GFAP) has been shown to be increased in the cerebrospinal fluid (CSF) of patients suffering from neurological diseases involving the activation of astrocytes, but has not been studied in amyotrophic lateral sclerosis (ALS) patients to our knowledge. CSF samples of patients with definite ALS and of those with other neurological diseases were evaluated for their GFAP concentrations. CSF-GFAP concentrations of patients with ALS were significantly elevated by 53% compared to patients with other neurologic diseases. GFAP might serve as a biomarker in ALS. Our findings support the concept that astrocytes play a role in ALS pathogenesis.

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1. Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease [1,2] characterized by selective, rapid degeneration of motor neurons [3,4]. Time to diagnosis is an average of 16–18 months from symptom onset. Since the diagnosis is purely clinical and no biomarkers exist, patients should undergo a thorough workup to exclude other diseases that may mimic ALS. At the time of writing, cerebrospinal fluid (CSF) analysis of ALS patients has not revealed a specific pattern helpful in diagnosis. Thus diagnosis can be firmly established only at an advanced stage. The pathophysiology of the sporadic disease remains elusive [5–9]. Though the major abnormality in ALS is the progressive and selective death of motor neurons, in recent years glial cells have been shown to have a pathogenic role [10,11] and *post mortem* studies of ALS patients have revealed astroglial changes in the spinal cord and motor cortex [12–14]. However, astrocytic markers have not been investigated in the CSF of ALS patients to our knowledge. As astrocytes may play a role in the pathophysiology of disease, and their activation involves the upregulation of glial fibrillary acidic protein (GFAP), CSF GFAP may serve as a marker for astrocytic injury in neurologic disease [15]. In experimental, as well as *post mortem* studies of ALS patients, increased tissue levels of GFAP have been

detected [16,17]. Studies on the clinical usefulness of a CSF biomarker of astrocytic damage and its relation to motor neuron loss in ALS are lacking [18,19]. We therefore examined, in a prospective manner, the CSF concentration of GFAP in ALS.

2. Patients and methods

Patients were recruited from the Department of Neurology, Rabin Medical Center, a tertiary medical facility in Israel, over a period of 3 years (June 2010 to September 2014). The El Escorial diagnostic criteria [20,21] were used to classify patients with ALS and we included only patients with definite ALS. The institutional Ethics Committee approved the study, and all patients gave informed consent to participate. All patients suspected of suffering from ALS underwent a full diagnostic workup including peripheral electrodiagnostic and electromyographic evaluation, exclusion of underlying malignant neoplastic diseases, brain and cervical spinal cord imaging, and an analysis of the CSF. We identified 14 patients with definite ALS (mean age $64.6 \pm$ standard deviation [SD] 8.0 years, range 51–76 years; 12 males, two females) and 14 randomly chosen, non-matched patients (mean age $42.2 \pm$ SD 18.6 years, range 19–73 years; 10 males, four females; Table 1) undergoing lumbar puncture (LP) for other reasons than ALS, including pseudotumor cerebri, multiple sclerosis, acute confusional state, headache, peripheral neuropathy, and seizures.

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Table 1
Demographics and results of the CSF analysis in ALS and control patients

	ALS	Control
Patients, n	14	14
Age, years	64.6 ± 8.0 [51–76]	42.2 ± 18.6 [19–73]
Male/female	12/2	10/4
CSF		
Leukocytes (n/dl)	1.9 ± 4.4 [0–17]	6.6 ± 9.6 [0–32]
Protein (mg/dl)	44.0 ± 18.4 [22.3–83.0]	46.4 ± 25.2 [17.5–96.0]
CPK (mg/dl)	275.2 ± 166.6 [95–532]	127 ± 95 [50–364]
Symptoms prior to LP (months)	9.3 ± 5.1 [2–18]	–
Reason for LP in control:	–	Demyelination (3) Confusional state (2) Headache (2) Neuropathy (3) Pseudotumor cerebri (2) Seizure (2)

Data are presented as mean ± standard deviation [range] unless otherwise indicated.

ALS = amyotrophic lateral sclerosis, CPK = creatine phosphokinase, CSF = cerebrospinal fluid, LP = lumbar puncture.

2.1. Serum, CSF examination, and enzyme-linked immunosorbent assay testing for GFAP

Serum samples were obtained from all patients and evaluated for complete blood count, electrolytes, creatine kinase and liver enzymes. CSF samples were sent for routine testing (cell count and differentiation, protein, glucose and chloride evaluation, gram-stain, bacterial culture, and microscopic cytology). Two sample vials of CSF (3 ml) were immediately frozen and stored at -80°C for later testing. All CSF samples were quantified for GFAP concentration using a commercially available sandwich enzyme-linked immunosorbent assay (GFAP, #A05188, Bertin Pharm, Montigny le Bretonneux, France), and assays were performed according to the manufacturer's protocols. In short, CSF samples (100 μL) were applied to anti-GFAP antibody-precoated 96-well plates and incubated for 2 hours. After washing three times with phosphate buffer saline, biotin-labeled monoclonal antihuman GFAP antibody was added and incubated for 1 hour. After washing, streptavidin-horseradish peroxidase tracer was applied for 1 hour and then washed three times. Finally, hydrogen peroxide/tetramethylbenzidine substrate was added, and after 10 minutes, the reaction was stopped with sulfuric acid solution. We measured the absorbance at wavelength 450 nm. The detection limit of GFAP in this test is 0.04 ng/mL. All CSF samples were tested twice independently with separate test kits from the same manufacturer.

2.2. Statistical analysis

Findings were evaluated using analysis of variance to determine pairwise comparisons amongst multiple data sets with a significance level of 0.05. Statistical analysis was carried out using GraphPad Prism-5 software (GraphPad, La Jolla, CA, USA). Either Student's t-test to compare two groups or the Mann-Whitney U test was applied.

3. Results

3.1. Concentrations of GFAP in the CSF are elevated in ALS patients

Comparing the mean GFAP concentration in ALS patients with that of patients with other neurological diseases, a 53% elevation in GFAP was detected. Mean GFAP levels were $1.43 \pm \text{SD } 0.89$ ng/ml in ALS patients and 0.94 ± 0.62 ng/ml in the control group ($n = 14$ each; $p = 0.049$; Fig. 1A). Distribution of GFAP concentrations across the ALS and control patients was wide and did not enable a clear cut-off value with high enough specificity to identify ALS solely by the CSF GFAP concentration (Fig. 1B). No differences in CSF GFAP concentrations between patients with bulbar signs, with monoparetic disease, paraparesis or quadriplegia were detected. Likewise, on comparison of CSF GFAP concentrations in patients with fasciculations with patients with mainly spastic upper motor neuron signs, no significant differences were detected.

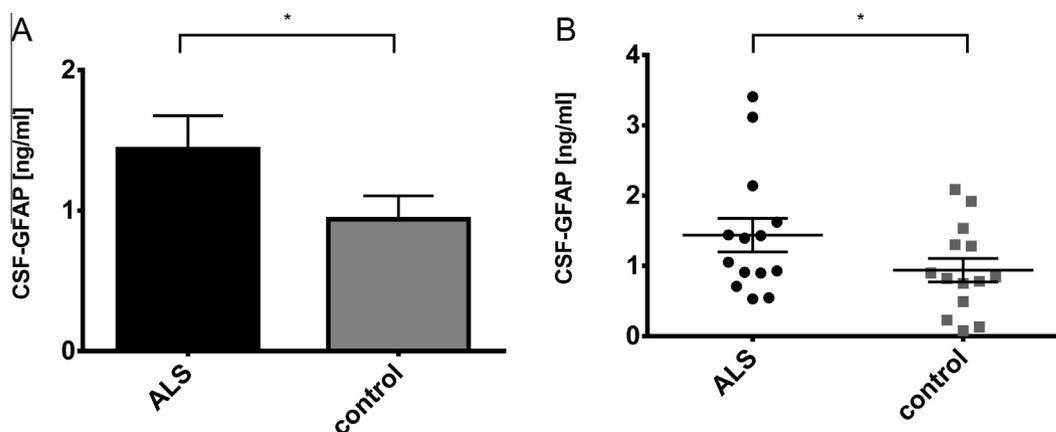


Fig. 1. Cerebrospinal fluid glial fibrillary acidic protein (CSF-GFAP) concentrations in patients with amyotrophic lateral sclerosis (ALS) and other neurological diseases. (A) Mean GFAP levels (ng/ml) in ALS and control individuals (asterisk indicating statistical significance; $p = 0.049$). (B) Individual patient and control subject CSF-GFAP concentrations.

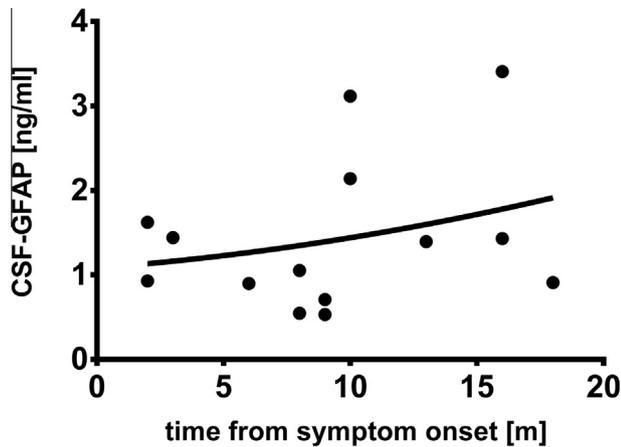


Fig. 2. Correlation of cerebrospinal fluid glial fibrillary acidic protein (CSF-GFAP) concentrations and time (months) from symptom onset in amyotrophic lateral sclerosis. GFAP (ng/ml) concentration in the CSF showed a trend in correlation with disease duration though it was not statistically significant.

3.2. GFAP and disease progression

As ALS progresses, the clinical symptoms spread in a continuous fashion at a local spinal level as well as in the cortical motor strip [4,22,23]. By correlating the concentration of GFAP in the CSF of patients with the time of LP after disease onset, we detected a trend, though non-significant, for increased CSF GFAP levels in temporally advanced disease (Pearson correlation coefficient: $r = 0.27$; $p = 0.17$; Fig. 2).

4. Discussion

In this study we show that GFAP is elevated by 53% in the CSF of patients diagnosed with ALS, compared to those with other neurologic conditions (Fig. 1). This observation did not correlate with disease type or the presence of fasciculations, but tended to increase and be associated with duration of symptoms (Fig. 2). The wide range of distribution for individual patients did not enable us to define a cut-off level to facilitate diagnosis of ALS with adequate specificity. Thus, CSF GFAP currently cannot serve as a biomarker for ALS.

Recently, in a model of specific toxin-induced alpha motor neuron damage, a selective astrogliosis with GFAP activation was observed [24]. Altered glutamate reuptake by astrocytes, leading to neuronal excitatory cell death, has also been suggested as a possible direct link between astrocytes and ALS pathogenesis [25,26]. Furthermore, the focal transplantation of normal astrocytes into the spinal cord of mice carrying the SOD1 mutation has a limited neuroprotective effect [27]. The discovery of an, until now, unidentified neurotoxic soluble factor released by SOD1 mutated astrocytes, strongly insinuates the role of astrocytes in ALS [28,29]. This lethal effect of SOD1 mutated astrocytes on motor neurons has been shown in human embryonic cell-derived motor neurons and can theoretically be limited by astrocytic cell replacement [27,30,31].

GFAP in the CSF is a useful marker to detect astrocytic damage and activation in a relatively convenient and non-invasive way. Compared to tissue biopsies and autopsies, LP can be performed easily, repetitively, and during the acute phase of disease with very low or no risk [32,33]. GFAP elevations in the CSF have been reported in several disorders, including multiple sclerosis [34,35] or acute spinal stroke [36,37]. Unfortunately our findings do not show a similar clear-cut pattern as was found in neuromyelitis optica (NMO), where significantly higher levels of GFAP in CSF of

patients with acute NMO were demonstrated [38–40]. In addition, we cannot exclude the possibility that included patients might suffer from diseases independently raising the level of GFAP in the CSF, as has been shown in NMO and spinal stroke.

The neurologic deficit in ALS is constantly progressing. Even though not reaching statistical significance, our results indicate a trend for a correlation between GFAP CSF levels and duration of disease.

Our findings may also corroborate the involvement of astrocytes in the patho-mechanism of motor neuron loss in ALS.

Conflicts of Interest/Disclosures

Dr. Israel Steiner serves on the Editorial Boards of the Journal of Neurological Sciences, Journal of Neurovirology and Neurology, Neuroimmunology & Neuroinflammation. He is a consultant and member of Data Safety and Monitoring Boards for Actelion and Genentech/Roche. He received honoraria from Teva Pharmaceutical Industries. The other authors declare that they have no financial or other conflicts of interest in relation to this research and its publication.

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