Journal of the Neurological Sciences 373 (2017) 329–334

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Quantitative sensory testing and structural assessment of sensory nerve fibres in amyotrophic lateral sclerosis

Baris Isak [a], Kirsten Pugdahl [a,*], Páll Karlsson [b], Hatice Tankisi [a], Nanna Brix Finnerup [b], Jasna Furtula [a], Birger Johnsen [a], Niels Sunde [c], Johannes Jakobsen [d], Anders Fuglsang-Frederiksen [a]

[a] Department of Clinical Neurophysiology, Aarhus University Hospital, Aarhus, Denmark
[b] Danish Pain Research Centre, Department of Clinical Medicine, Aarhus University, Aarhus, Denmark
[c] Department of Neurosurgery, Aarhus University Hospital, Aarhus, Denmark
[d] Department of Neurology, Aarhus University Hospital, Aarhus, Denmark

ARTICLE INFO

Article history:
Received 16 September 2016
Received in revised form 12 December 2016
Accepted 3 January 2017
Available online 06 January 2017

Keywords:
Amyotrophic lateral sclerosis (ALS)
Quantitative sensory testing (QST)
Skin biopsy
Nerve biopsy
Axonal swelling

ABSTRACT

Objective: In this prospective study, involvement of sensory nerve fibres in ALS patients was assessed using functional and structural measures in the form of quantitative sensory testing (QST) and skin and nerve biopsies.
Methods: Thirty-two ALS patients and 32 healthy subjects were evaluated with a QST battery comprising thresholds of mechanical detection, mechanical pain, vibration detection, cold detection, warm detection, heat pain, and pinprick sensation. Skin biopsies were evaluated in 31 ALS patients by intraepidermal nerve fibre density (IENFD) and axonal swelling ratios, and growth-associated protein 43 (GAP-43) antibody staining. Sural nerve biopsies were evaluated using teased fibre analysis in eight patients.
Results: Mean values for QST parameters and IENFD in ALS patients were within normal range. However, the patients had increased axonal swelling ratios and GAP-43 antibody staining was negative in all patients.
Conclusions: Although QST and IENFD were affected in only a small subset of ALS patients, the axonal swellings observed in all patients indicate that the affection is more frequent, and suggests that IENFD count may not be sufficient. The negative GAP-43 staining suggested an insufficiency of regeneration in small sensory nerve fibres.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is commonly accepted as a neurodegenerative disease of cortico-motor nerves with sensory nerves considered to be intact. However, an increasing number of neurophysiological studies have shown involvement of large and small diameter sensory nerve fibres in ALS [e.g., [1–4]]. Concordantly, pathological studies showed the involvement of myelinated sensory nerves in 70–90% [1,5] and unmyelinated nerves in 79% of ALS patients [6]. In a recent paper, skin biopsies and quantitative sensory testing (QST) showed involvement of thinly-/unmyelinated sensory nerves in 84% (11/13) of ALS patients with spinal onset and in only 9% (1/11) with bulbar onset [7].

Yet, ALS patients usually do not complain of sensory changes and physicians do not detect sensory abnormalities in neurological examination [8]. There can be several reasons for this. Firstly, progressive weakness and disability dominate the clinical picture of ALS and sensory disturbances could easily be ignored by both patients and clinicians. Secondly, just like the re-innervation process in motor nerves [9], collateralization and regeneration of the sensory nerves could accompany neurodegeneration and compensate the loss of nerve fibres to some extent. And lastly, sensory nerves could be more resistant to neurodegeneration.

Re-innervation of C-fibres was shown in an experimental model of peripheral neuropathy using growth-associated protein 43 (GAP-43) staining in skin biopsies [10]. Unlike PGP9.5 (transported through the slow axonal component towards the periphery [11]), GAP-43 indicates a regeneration in C-fibres. Injury to axons stimulates an increased synthesis of GAP-43 in early regeneration [10], using fast axonal transport to the growth cone [12–14]. Similarly, axonal swellings are suggested to be predictors of dysfunctional axonal transport [15] or intraepidermal nerve fibre (IENF) loss [16,17], or potential biomarkers of axonal regeneration in C-fibres [18].

In this study, we investigated the functional and structural involvement of sensory fibres in ALS using QST, sural nerve biopsy, and skin biopsy. Sural nerve biopsies were obtained in a subset of patients to investigate the structural changes in myelinated sensory fibres.

* Corresponding author at: Department of Clinical Neurophysiology, Aarhus University Hospital, Nørrebrogade 44, Building 10, 8000 Aarhus C, Denmark.
E-mail addresses: barisisak@gmail.com (B. Isak), pugdahl@aarhus.rm.dk (K. Pugdahl), pall@clin.au.dk (P. Karlsson), hatitank@rm.dk (H. Tankisi), finnerup@clin.au.dk (N.B. Finnerup), furtula@gmail.com (J. Furtula), birgjohn@rm.dk (B. Johnsen), Niels.Sunde@aarhus.rm.dk (N. Sunde), johannes.klitgaard.jakobsen@regionh.dk (J. Jakobsen), anders.fuglsang@aarhus.rm.dk (A. Fuglsang-Frederiksen).

http://dx.doi.org/10.1016/j.jns.2017.01.005
0022-510X/

2. Materials and methods

The study was carried out at the Department of Clinical Neurophysiology, Aarhus University Hospital, Denmark and the Danish Pain Research Centre, Aarhus, Denmark. Approval was given by the local Ethical Committee of the Central Denmark Region and by the Danish Data Protection Agency, and all participants signed an informed consent document at inclusion.

2.1. Patients and controls

Thirty-two prospectively collected ALS patients (9 females, 23 males) aged 40–80 years (mean age 61.6 ± 10.0) and 32 controls (15 females, 17 males), aged 43–79 (mean age 60.3.1 ± 10.9), were enrolled in the study. The patients were recruited from the Department of Neurology, Aarhus University Hospital and Department of Neurology, Vejle Hospital, Denmark. The controls consisted of healthy volunteers recruited from employees at Aarhus University Hospital, the patients' relatives, and from flyers and spreads in local newspapers.

Six (18.8%) patients had bulbar onset and 26 (81.2%) had spinal onset. Mean disease duration prior to neurophysiological testing was 32.9 ± 19.2 months, and mean revised ALS-functional rating scale (ALSFRS-R) [19] score was 36.1 ± 10.1 (range: 5–47).

The ALS diagnosis was given using clinical evidence of upper motor neuron degeneration, and clinical and electromyographic evidence of progressive lower motor degeneration in at least one of four regions (brain stem, cervical, thoracic, or lumbosacral) according to the revised El-Escorial criteria [20]. Eleven patients were categorized as definite (34.4%), 8 as probable (25%), 11 as probable-lab supported (34.4%), and 2 as possible (6.3%). All patients showed progression during a follow-up period of 6 months or more.

Exclusion criteria were: (1) radiological or clinical evidence of radiculopathy, plexopathy, or entrapment neuropathy, (2) evidence indicating neurological diseases other than ALS; including ALS-mimic disorders [21], atypical motor neuron syndromes as ALS variants showing sensory involvement (e.g., Kennedy's disease) as determined by information from history (relatively long duration of symptoms, impotence), clinical findings (gynecomastia), and neurological examination (lack of evidence of UMN involvement) [22], (3) conditions affecting peripheral nerves such as diabetes, impaired glucose tolerance, vitamin deficits, kidney failure, thyroid diseases, alcohol abuse, and previous oncologic diseases, (4) cerebellar or extrapyramidal signs, (5) pregnancy, (6) patients who could not tolerate neurophysiologic procedures due to severe cachexia or/and respiratory insufficiency, and (7) dementia, as QST requires full cooperation of the subjects.

2.2. Quantitative sensory testing

QST was performed on the foot dorsum in all patients and controls. Mechanical detection threshold (MDT), mechanical pain threshold (MPT), vibration detection threshold (VDT), warm detection threshold (WDT), cold detection threshold (CDT), heat pain threshold (HPT), and pinprick sensation were used to assess the function of large and small diameter sensory nerve fibres. All patients and controls were examined by the same research nurse in a quiet room, with the subjects lying on a couch in a supine relaxed position. The weakest side was selected in the ALS patients and the right side in controls (unless an entrapment neuropathy was referred or detected). All data were analysed by N.B.F.

Mechanical detection and pain thresholds were tested using Semmes-Weinstein monofilaments (Stoelting, IL, USA) as described by Chaplan et al. [23]. Pinprick sensation was tested using a safety pin. Five pinprick and five dull stimuli were given in random order with the patient's eyes closed. A percentage based on the number of correct identification of stimuli (0/10–10/10) was calculated.

Vibration detection threshold was tested using an electronic vibrameter (Somedic AB, Sweden) as described by Goldberg and Lindblom [24]. The vibration frequency was 120 Hz, the application pressure was kept at ~650 g, and the amplitude was given as peak to peak amplitude. The average of vibration perception threshold and vibration disappearance threshold was taken as the VDT; the average of three consecutive measurements was used as the final VDT.

Thermal detection and pain thresholds were performed using a computer-driven thermo-test (Medoc, TSA, Israel). Starting from a baseline temperature of 32 °C, temperatures were changed at a rate of 1 °C/s (cut-off limits at 50.0 and 0 °C). WDT, CDT, and WPT were recorded using the method of limits. Thresholds were calculated as the average of 3 successive measurements.

In addition, reaction time (RT) to light and sound stimuli was measured with a self-constructed reaction time detector [25]. Subjects were required to let go of a button as soon as the assessor activated sound and light. The reaction times were measured 3 times and the mean of these measures was used as the reaction time.

QST and RT were compared with the normative values obtained from the healthy subjects. QST was defined as abnormal if at least two tested parameters had abnormal findings, i.e. values exceeding ±2 standard deviations from mean of controls.

2.3. Skin biopsy

Skin biopsies from the leg of 31 ALS patients were used to evaluate C-fibre morphology by quantification of intraepidermal nerve fibre density (IENFD) and focal axonal swelling, and by GAP-43 antibody staining.

The skin biopsy was taken approximately 10 cm above the lateral malleolus on the weakest side using a disposable 3-mm punch (Miltex, York, PA, USA). The specimens were fixed in 2% paraformaldehyde-lysine-periodate for 18–24 h, followed by an overnight cryoprotection in 20% glycerol and 0.08 M Sorenson's phosphate-buffered saline. The cryostat specimens were cut into 50-μm sections vertically in relation to the epidermis direction (Microm Cryostat HM 500 OM, Zeiss, Germany) and three sections immuno-stained using standard protocols; sections were exposed to the primary antibody rabbit anti–human PGP-9.5 (1:1000; AbD Serotec, Dusseldorf, Germany) and then biotinylated goat anti-rabbit secondary antibody (1:100; Vector Laboratories, Burlingame, CA).

To obtain IENFD, epidermal length of three sections was measured and the number of nerve fibres crossing the dermal–epidermal junction and passing into the epidermis was counted using a normal light microscope (60x oil immersion lens, Olympus UPlanSApo, NA = 1.35). Quantification of IENFD was done based on the recommendations by the European Federation of the Neurological Societies (EFNS) [26,27], and was blinded to origin and characteristics of the subjects. The age- and gender adjusted reference values published by EFNS was used as normal limits [27].

Axonal swellings were defined as enlargements with a diameter of ≥1.5 μm [17,28,29]. Smaller enlargements were considered to be part of the normal IENF variance and were excluded. Axonal swellings were only counted if they occurred within fibres contributing to IENFD determination [17,28,29]. The axonal swellings were measured using a measurement tool in the stereological software NewCast [28]. Due to the possibility that normal IENFs can terminate in a single swollen enlargement, terminal enlargements of IENFs were excluded from swelling ratio quantification. The axonal swellings were presented as median axonal swelling ratio (i.e., number of axonal swellings/IENFD).

GAP-43 staining of skin biopsies was used to determine axonal regeneration in ALS patients. Three sections from the specimens were fixed, cut into 50-μm sections, and incubated with GAP-43 antibody (Bio-Rad Laboratories, CA, USA) using the same protocol as described above. Additionally, skin biopsies from six randomly selected healthy controls available from another study were stained using the same