

Small-fibre neuropathy related to bulbar and spinal-onset in patients with ALS

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Abstract We aimed at seeking more precise diagnostic information on the sensory nervous system involvement described in patients with amyotrophic lateral sclerosis (ALS). We investigated large myelinated nerve fibres with nerve conduction study and small-nerve fibres with Quantitative Sensory Testing (QST) (assessing thermal-pain perceptive thresholds) and skin biopsy (assessing intraepidermal nerve fibre density) in 24 consecutive patients with ALS, 11 with bulbar-onset and 13 with spinal-onset. In 23 of the 24 patients, regardless of ALS onset, nerve conduction study invariably showed large myelinated fibre sparing. In patients with bulbar-onset ALS, QST found normal thermal-pain perceptive thresholds and skin biopsy disclosed normal intraepidermal nerve fibre density. Conversely, in patients with spinal-onset, thermal-pain thresholds were abnormal and distal intraepidermal nerve fibre density was reduced. Sensory nervous system involvement in ALS differs according to disease onset. Patients with spinal-onset but not those with bulbar-onset ALS have concomitant distal small-fibre neuropathy. Neurologists should therefore seek this ALS-related non-motor feature to improve its diagnosis and treatment.

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Introduction

Although the relentlessly progressing and devastating disease amyotrophic lateral sclerosis (ALS) primarily affects motor neurons [1, 2], convincing observations imply concomitant sensory nervous system involvement [3, 4].

A minimally invasive and reliable tool for investigating sensory nervous system is skin biopsy to assess intraepidermal nerve fibres, mainly thermal-pain unmyelinated C-fibre terminals [5]. The most commonly used markers for epidermal nerve fibres are antibodies against protein gene product 9.5 (PGP 9.5).

An early skin biopsy study showed that patients with ALS have distally distributed intraepidermal nerve fibre loss, thus postulating an ALS-related small-fibre neuropathy [6]. The investigators, however, gave no information on differentiating between bulbar-onset and spinal-onset disease, nor did they use a widely accepted diagnostic test such as Quantitative Sensory Testing (QST) to verify whether the intraepidermal nerve fibre loss leads to clinically evident thermal-pain sensory disturbances. Having more precise information on intraepidermal nerve fibre damage might prompt specific diagnostic testing to assess small-fibre neuropathy in patients with ALS.

In this clinical and skin biopsy study, we aimed at verifying whether ALS is associated with small-fibre neuropathy, and, if so, whether this non-motor feature depends on the ALS onset. To do so, we enrolled 24 consecutive patients with ALS attending a specialized motor neuron disease clinic, distinguished those with bulbar-onset and spinal-onset ALS and tested thermal-pain sensory

disturbances and intraepidermal nerve fibre density using QST and skin biopsy.

Methods

Over the 6 months from April 2014 to October 2014, 27 patients were screened for this study at the ALS Centre, Sapienza University, Rome. All patients were diagnosed by two experienced neurologists (MI, GC) as having clinically definite ALS or clinically probable—laboratory-supported ALS (according to the revised El Escorial criteria) [7]. We collected the Medical Research Council (MRC) score for muscle strength applied to 15 upper and lower limb muscles, and the Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R), a validated measure of functional impairment in ALS [8].

Exclusion criteria were cognitive disturbances, neurological diseases other than ALS and all conditions potentially affecting peripheral nerves such as diabetes, impaired glucose tolerance, vitamin deficits, kidney failure, thyroid diseases, alcohol abuse and previous oncologic diseases. Three patients were excluded (one for cognitive disturbances and two for vitamin B deficiency). Of the 27 screened patients, 24 were enrolled: 11 patients with bulbar-onset ALS (7 men, 4 women, age 52–80 years) and 13 patients with spinal-onset ALS (9 men, 4 women, 42–78 years).

After enrolment, all patients underwent precise sensory profiling using bedside tools. We investigated touch with a piece of cotton wool, vibration with a tuning fork (128 Hz) and pinprick sensation with a wooden cocktail stick. In the clinical interview, we sought information on dysautonomic symptoms such as orthostatic hypotension (by enquiring about dizziness, light-headedness, nausea, headache and blurred vision when standing up or stretching), genitourinary disturbances (incontinence, erectile dysfunction) and gastrointestinal problems (constipation, faecal incontinence, diarrhoea). In patients complaining of pain, we used the widely agreed DN4 questionnaire for identifying neuropathic pain; DN4 is a validated questionnaire, with a sensitivity and specificity of about 80 % in distinguishing neuropathic pain [9].

In all patients the diagnostic, objective sensory nervous system assessment included a standard nerve conduction study (NCS) to assess large myelinated A β -fibre, QST and skin biopsy to assess small-nerve fibres. Sensory nerve conduction was studied with surface recording electrodes placed according to the international standards. Variables assessed were sensory nerve action potentials and conduction velocities recorded from sural, ulnar and superficial radial nerves. Methods used and normative ranges adhered

to those recommended by experts of the International Federation of Clinical Neurophysiology [10].

For the QST, we used a thermode (ATS, PATHWAY, Medoc, Israel). The computer-driven PATHWAY system contains a metal contact plate (contact area 30 × 30 mm) equipped with an external Peltier element that cools and heats the plate to target levels. Temperature was ramped from a baseline of 32 °C to target at a rate of 1 °C/s. Quantitative sensory variables were tested on the right and left foot. We investigated thermal detection thresholds for the perception of cold (cold detection threshold) and warmth (warm detection threshold), thermal-pain thresholds for cold (cold pain threshold) and heat stimuli (heat pain threshold). In the QST assessment, we used the procedures and normative ranges indicated by the German Research Network on Neuropathic Pain [11].

Skin biopsies were taken from the thigh, 20 cm below the anterior iliac spine and from the distal leg, 10 cm above the lateral malleolus, within the sural nerve territory on the right side. Biopsies were taken after local anaesthesia using a 3 mm disposable punch under a sterile technique. Three sections randomly chosen from each biopsy were immunoassayed with PGP 9.5 antibodies using the free-floating protocol for bright-field immunohistochemistry. To assess the intraepidermal nerve fibre density, we used the recommendations and normative ranges recommended by the European Federation of the Neurological Societies [12].

As primary outcome variables for investigating the sensory nervous system, we selected sural nerve action potential amplitudes (the most reliable item for investigating distal-symmetric peripheral neuropathy) [13], the intraepidermal nerve fibre density at the proximal thigh and distal leg, and the cold and warm detection thresholds and heat and cold pain thresholds.

The collected variables were compared with normative reference data, and considered abnormal when they exceeded the normative reference ranges. The sural nerve sensory action potential was compared with normative ranges established in our laboratory (60 subjects, age and gender matched with patients with ALS). Conversely, the QST and skin biopsy variables were compared with the widely agreed, published normative ranges, distinguished for age and gender [11, 12] (these reference studies provide reliable normative ranges for the warm and cold detection thresholds and the distal intraepidermal nerve fibre density). We also assessed differences in each QST and skin biopsy variables between patient with bulbar-onset and spinal-onset disease.

The local institutional review board approved the research, and patients gave their informed consent.

Statistical analysis

Because data had a Gaussian distribution (D'Agostino and Pearson omnibus normality test), we used the unpaired *t* test to compare differences between patients with bulbar-onset and spinal-onset disease, and the paired *t* test to compare intra-individual differences in intraepidermal nerve fibre density at the thigh and distal leg. *P* values <0.05 were considered to indicate significance. All data are reported as mean ± SD.

Results

No differences were found in demographic data for age or duration of disease between the 11 patients with bulbar-onset and 13 patients with spinal-onset disease (Table 1).

Clinical examination showed that six patients with spinal-onset disease had mild sensory disturbances (-paraesthesia and hypoesthesia distributed in the distal legs); two complained of neuropathic pain affecting the feet, as assessed by the DN4.

Sural nerve sensory action potential amplitudes came within the reference ranges in all 24 patients, except one, without significant differences according to disease onset.

While QST in patients with bulbar-onset disease showed warm and cold detection thresholds within the reference ranges (except for one patient aged 80), in 11 of the 13 patients with spinal-onset it identified reduced cold detection thresholds and increased warm detection thresholds, when compared with the reference ranges [11, 12].

Thermal-pain thresholds differed significantly between patients with bulbar-onset and spinal-onset ALS (*P* < 0.01, by unpaired *t* test).

No skin biopsy procedures resulted in adverse effects. Skin biopsy showed in all 11 patients with bulbar-onset ALS, except one (the patient aged 80 with abnormal thermal-pain thresholds), spared intraepidermal nerve fibre density when compared with the normative ranges [12]. Conversely, in 11 of the 13 patients with spinal-onset, skin biopsy showed a significantly reduced intraepidermal nerve fibre density at the distal leg, when compared with the normative ranges [12]. In two patients with spinal-onset disease, QST showed normal findings as well as spared intraepidermal nerve fibre density. In patients with spinal-onset disease, skin biopsy also showed sparing of the intraepidermal nerve fibre density proximal–distal gradient, i.e. the intraepidermal nerve fibre density was significantly higher at the proximal thigh than at the distal leg (*P* = 0.002, by paired *t* test). The intraepidermal nerve fibre density at the distal leg was higher in patients with bulbar-onset than in patients with spinal-onset ALS (*P* = 0.008, unpaired *t* test) (Fig. 1). Conversely, no onset-related statistical difference was found for this variable at the proximal thigh.

Discussion

In this study, conducted in patients with ALS attending our specialized clinic for motor neuron disease, and distinguished according to bulbar-onset or spinal-onset, we show

Table 1 Clinical and diagnostic test findings (mean ± SD)

	Bulbar-onset ALS	Spinal-onset ALS	<i>P</i>
Age (years)	68.4 ± 10.2	61.5 ± 9.9	0.1
Gender (F:M)	7:4	9:4	
Disease duration (months)	22.9 ± 9.9	28.6 ± 12.5	0.2
Strength MRC	126.9 ± 23.7	87.7 ± 47.4	0.02
ALS FRS	33 ± 10.7	30.2 ± 9.9	0.5
Sural SNAP (μV)	11.3 ± 4.0	10.6 ± 2.7	0.7
Sural NCV (m/s)	50.4 ± 3.4	51.3 ± 4.2	0.6
CDT (°C)	26.5 ± 3.7	19.9 ± 3.4	0.0001
WDT (°C)	38.6 ± 0.9	48.4 ± 2.8	0.0001
CPT (°C)	8.5 ± 1.6	4.9 ± 4.2	0.01
HPT (°C)	45.6 ± 1.8	48.6 ± 1.5	0.0002
Proximal IENF density (/mm)	5.4 ± 2.3	3.8 ± 2.2	0.09
Distal IENF density (/mm)	4.1 ± 2.1	2.0 ± 1.4	0.008

MRC medical research council (score based on MRC applied to 15 muscular groups of upper and lower limbs), *SNAP* sensory nerve action potential, *NCV* nerve conduction velocity, *CDT* cold detection threshold, *WDT* warm detection threshold, *CPT* cold pain threshold, *HPT* heat pain threshold (Sural SNAPs and QST variables are referred to the right side, the same side of the skin biopsy), *IENF* intraepidermal nerve fibre

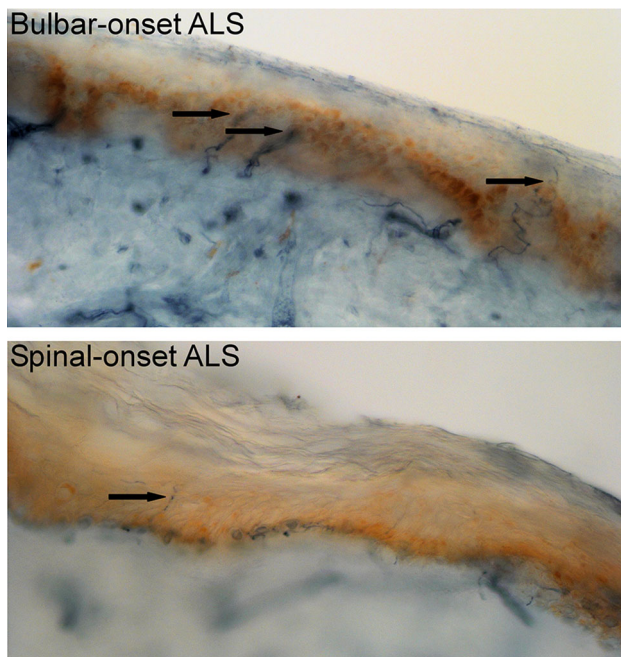


Fig. 1 Skin biopsy representative findings. Bright-field images ($\times 40$ magnification) showing intraepidermal nerve fibre density at the distal leg in patients with bulbar-onset and spinal-onset amyotrophic lateral sclerosis (ALS). Arrows indicates nerve fibres

that those with spinal-onset have abnormal thermal-pain thresholds as assessed with QST and reduced distal intraepidermal nerve fibre density as assessed with skin biopsy. These clinical and skin biopsy data extend previous reports indicating that ALS affects the sensory nervous system as well as the motor system. They also provide new information specifying that small-nerve fibre damage differs according to onset; unlike patients with bulbar-onset, patients with spinal-onset ALS have concomitant sensory abnormalities compatible with small-fibre neuropathy [6].

The large myelinated $A\beta$ -fibre sparing NCS documented in this study, regardless of disease onset, contrasts with sural nerve biopsy investigations in patients with ALS showing large myelinated $A\beta$ -fibre abnormalities [14]. Hence, we hypothesize that large myelinated fibre abnormalities might be common, though mild in severity and thus mainly detectable in sural nerve biopsy specimens.

A new finding in this clinical and skin biopsy study is the abnormal thermal-pain thresholds and reduced intraepidermal nerve fibre density at the distal leg, with a spared intraepidermal nerve fibre density ratio between proximal thigh and distal leg in patients with spinal-onset ALS. These findings indicate that patients with spinal-onset ALS have a distally distributed small-fibre neuropathy. The most likely reason why ALS leads to small-nerve fibre damage only in patients with spinal-onset is that sensory system damage follows motor system involvement. Hence,

we cannot exclude the possibility that patients with bulbar-onset disease might have trigeminal intraepidermal nerve fibre loss, nor that these patients, with the possible spread to spinal segments, might manifest a distally distributed small-nerve fibre loss. The concomitant small-fibre distal neuropathy in patients with spinal-onset disease indicates, as others have proposed, that among its non-motor features, such as cognitive and behavioural impairment, ALS also causes sensory nervous system damage [15, 16].

Given that we collected a relatively small sample of patients, all having a similar disease duration, we cannot state whether the severity of the sensory nervous system impairment in ALS depends on the duration of disease; in theory, the longer the disease duration the more severe the sensory nervous system damage.

While QST and skin biopsy showed abnormal thermal-pain thresholds and a reduced intraepidermal nerve fibre density in most patients with spinal-onset ALS (11 of 13), only two had neuropathic pain as assessed with the DN4 questionnaire. Although potentially unexpected, this lack of a direct relationship between thermal-pain system damage and neuropathic pain has been reported in other neurological diseases affecting small-nerve fibres (including familial amyloid polyneuropathy) [17]. This dissociation might depend on the type of thermal-pain fibre damage. We conjecture that pain probably arises from nerve degeneration–regeneration rather than from mere nerve fibre loss [18].

Although our study provides previously unavailable data describing small fibre damage in spinal-onset ALS, it has limitations. First, we collected a relatively small sample of patients. Although the numbers match those in previous published studies [6, 19], they prevented us from reliably analysing correlations between clinical and diagnostic variables. We included only 24 patients for ethical reasons. We were exploring a minor and mostly subclinical feature in this devastating disease, and all our clinical and diagnostic tests are time-consuming and potentially tiring for patients given that skin biopsy is a relatively invasive and uncomfortable procedure. Another limitation is that as a technique for assessing intraepidermal nerve fibre density, we used bright-field immunohistochemistry, rather than the more sensitive immunofluorescence with confocal microscopy with double and triple immunostaining. Admittedly, bright-field immunohistochemistry using the pan-neuronal marker PGP9.5 cannot distinguish the different intraepidermal nerve fibres [20], but it is the only technique that has normative ranges, and thus the only technique that can reliably disclose intraepidermal nerve fibre loss in patients with ALS [12].

Although patients with spinal-onset ALS rarely complain of pain, QST and skin biopsy commonly disclose sensory deficits and intraepidermal nerve fibre loss,

compatible with onset-dependent small-fibre neuropathy [6]. Our findings should prompt neurologists to seek possible ALS-related sensory nervous system damage, thus improving the way they diagnose and cure the non-motor features in this multisystem neurodegenerative disorder.

Conflicts of interest The authors declare that they have no conflict of interest.

Ethical standard All patients gave their informed consent.

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