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CLINICAL INVESTIGATIVE STUDY

Autonomic dysfunction in amyotrophic lateral sclerosis: A neurophysiological and neurosonology study

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Abstract

Background and Purpose: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder affecting upper and lower motor neurons. Some ALS patients exhibit concomitant nonmotor signs, and thus ALS is considered a multisystem disorder. The aim of this study is to investigate autonomous nervous system involvement in ALS.

Methods: We investigated 21 ALS patients and 28 age-matched controls. ALS patients were assessed for disease severity with the Revised-ALS Functional Rating Scale (ALSFSR) and for the presence of autonomic symptoms with the Composite Autonomic Symptom Score scale. Sympathetic nervous system was evaluated by sympathetic skin response (SSR) and parasympathetic nervous system by ultrasonography of vagus nerve (VN) at the level of the thyroid gland.

Results: SSR latencies were shorter and SSR amplitudes were higher in controls compared to ALS patients. The cross-sectional area (CSA) of the VN was significantly smaller in ALS patients (mean CSA right/left: 1.73±0.62 mm^2/1.47±0.53 mm^2) compared to controls (mean CSA right/left: 2.91±0.79 mm^2/2.30±0.80 mm^2), right: $p < .001$, left: $p < .001$. There was a significant negative correlation between disease duration and CSA of left-VN ($r = -0.493$, $p = .023$). This correlation was attenuated between disease duration and CSA of right-VN ($r = -0.419$, $p = .059$). ALSFSR-R was positively correlated to CSA of right-VN ($p = .006$, $r = 0.590$). CSA of VN did not correlate with bulbar involvement.

Conclusions: This study confirms the presence of autonomic dysfunction in ALS patients and provides evidence of VN atrophy that correlates with disease severity and duration and is independent of bulbar involvement. Degeneration of dorsal nucleus neurons of the VN is hypothesized.

KEYWORDS
ALS, autonomic nervous system, cross-sectional area, sympathetic skin response, vagus nerve

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease affecting upper and lower motor neurons (UMN and LMN). In view of the lack of specific biomarkers, the diagnosis remains principally clinical and supported by electrodiagnostic studies.[1] The hallmark of the disease is the concurrent presence of UMN and LMN features. Patients can present either with bulbar-onset or limb-onset disease that will progressively spread to involve other regions. Less commonly, some patients may present with pure UMN or LMN involvement.[2]

Even though ALS is considered a "pure" motor disease, atypical presentations exist. It is estimated that half of patents with ALS experience cognitive or behavioral impairment,[3] a percentage of whom develop frontotemporal dementia.[4] A subset of ALS patients, defined as ALS-Plus,[5] exhibits other nonmotor signs, extrapyramidal or autonomic, that may be clinical or subclinical, the latter usually revealed on pathological postmortem grounds.[6] Based on the above, ALS can be considered a multisystem degenerative disease.[7–10]

Regarding autonomous nervous system (ANS) involvement, several researchers have investigated heart rate variability (HRV) as a measure of dysautonomia in ALS patients. All studies reported similar findings, that is, decreased HRV, indicative of sympathovagal imbalance,[7,11–13] that could explain cases of circulatory collapse or sudden death among ALS patients.[10,13,14] The use of HRV in exploring ANS dysfunction has the disadvantage of the inability to measure separately sympathetic and parasympathetic contribution.

The aim of this study is to investigate simultaneously subclinical involvement of both parts of ANS, sympathetic and parasympathetic, in patients with ALS. For the purpose of the study, two separate methods have been chosen. Sympathetic skin response (SSR) to investigate sympathetic nervous system (SNS) and cross-sectional area (CSA) of vagus nerve (VN), measured by ultrasonography to investigate parasympathetic nervous system (PSNS). SSR has been previously used in ALS patients, but with inconsistent results.[15–20] CSA of VN has been used to investigate diseases with known dysautonomia, such as Parkinson disease[21–27] and diabetes mellitus.[28] There are currently only two previous studies evaluating CSA of VN in ALS patients. Tawfik reports a single ALS case with smaller CSA of VN bilaterally, compared to reference values.[29] In his work, Tawfik does not attempt to provide any interpretation of this observation and suggests further research. Holzapfel and Naumann investigated VN in 24 ALS patients with bulbar symptoms.[30] Their study demonstrated VN atrophy, and attributed this finding to degeneration of motor neurons of nucleus ambiguous that supplies pharyngeal and laryngeal muscles, thus explaining bulbar symptoms, dysphagia, and dysarthria, in ALS patients.

In view of the former considerations, we sought to investigate in the present case-control study the involvement of SNS and PSNS in ALS, along with the potential correlation of SNS and PSNS dysfunction with indices of disease severity, disease duration, and the presence of bulbar symptoms in ALS patients. To explore the above hypothesis, we investigated the correlation between clinical and electrophysiological or imaging data, assessing sympathetic and parasympathetic involvement, respectively.

METHODS

Study design

A case-control study was carried out at an outpatient electrodiagnostic service of a tertiary-care referral academic hospital (Second Department of Neurology, National and Kapodistrian University of Athens, "Attikon" University Hospital) in Athens, Greece.

Written informed consent was obtained by all participants. The present study was approved by the local Ethics Committee of our Institution (ΕΒΔ93/07-04-2021) and followed the principles of the Helsinki Declaration and its later amendments.[31]

Study population

All adult patients with a definite diagnosis of ALS were considered eligible for inclusion to the study. Diagnosis of ALS was based on revised El Escorial criteria.[32] Controls were recruited after open invitation to participate in the research and only adults (>18 years) were included in the study. Exclusion criteria for ALS patients comprised the presence of concomitant diabetes mellitus or other type of peripheral neuropathy, and for healthy controls, the presence of diabetes mellitus or history of neuromuscular disorders.

Demographic and clinical data

The following data were collected: patients' demographic data (age, sex, and body mass index [BMI]) and clinical characteristics (duration of the disease, areas involved, and treatment received).

Composite Autonomic Symptom Score (COMPASS 31)

COMPASS 31[33] is a refined and abbreviated instrument to assess and quantify autonomic symptoms severity across multiple autonomic domains. It is a self-administered tool, requiring less than 10 minutes. COMPASS 31 derived from the well-established 169-item Autonomic Symptom Profile and its validated 84-question scoring instrument, the COMPASS. It includes 31 questions in six domains: orthostatic intolerance, vasomotor, secretomotor, gastrointestinal, bladder, and pupillomotor function. Simple questions are scored as 0 points for no and 1 point for yes. Questions regarding the frequency, severity, and time course of a symptom are scored from 0 points to 3 points. Each domain is assigned a weighting factor, so that total score ranges between 0 and 100. A higher COMPASS 31 score indicates more severe autonomic symptoms.

COMPASS 31 proved to be a sensitive and convenient screening tool for evaluating autonomic function in various diseases: Parkinson disease,[34] multiple sclerosis,[35] diabetic neuropathy,[36] small fiber polyneuropathy,[37] neuromyelitis optica spectrum dosirders,[38]

fibromyalgia,[39] and systemic sclerosis.[40] COMPASS 31 has been translated and linguistically validated in Greek. Permission was received to use for academic purposes.

Revised-Amyotrophic Lateral Sclerosis Functional Rating Scale

Revised-Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R)[41] evaluates the functional status of patients with ALS. It measures 12 parameters: speech, salivation, swallowing, handwriting, cutting food and handling utensils, dressing and hygiene, turning in bed and adjusting bed clothes, walking, climbing stairs, dyspnea, orthopnea, and respiratory insufficiency. Each measure takes 0-4 points (worst to normal performance). Total score ranges between 0 and 48. A higher ALSFRS-R score indicates better functionality. ALSFRS-R has close agreement with objective measures, such as muscle strength and respiratory function, and shows good construct validity, strong internal consistency, and test-retest reliability. ALSFRS-R scores correlate significantly with quality of life.

Sympathetic skin response

SSR examination was performed using Dantec Keypoint v5.13. The examination followed the standard protocol.[42] All subjects were instructed to refrain from smoking and to have a light breakfast that did not include alcohol or caffeine. Room temperature was kept at 23-26° C and limb temperature at 32-33° C.

Recordings were made with surface, circular-shaped electrodes. The active electrodes were placed on the left palm and right sole. The reference electrodes were placed on the dorsum of the hand and foot. Electrical stimuli were delivered on the right wrist at 75 mA and 0.1 ms duration, the band pass was 0.5-2000 Hz. Five stimuli were delivered unexpectedly at random intervals of at least 30 seconds. Latency was measured from the onset of the stimulus artifact to the first deflection from baseline in seconds (s). Amplitude was measured peak to peak (negative peak to positive peak) in millivolts (mV). The shortest of the five responses were used for statistical analysis. Measurements included in the analysis were: SSR upper limb latency, SSR upper limb amplitude, SSR lower limb latency, and SSR lower limb amplitude. The electromyographer was blinded to as to the subject's assignment (patient or control group) and to the ultrasonography results.

Cross-sectional area of vagus nerve

The ultrasound study was performed using Philips CX50 Ultrasound System with a linear transducer at 12 Hz. Patients and controls were scanned by the same operator. The operator was blinded to the patient and to the control group and to the results of the SSR study. All participants were in the supine position with the sonographer sitting behind them. The settings of the ultrasound system were individually optimized for each participant with respect to gain, depth, and focus. The VN was visualized bilaterally at the level of the thyroid gland in axial view inside the carotid sheath. The carotid artery and internal jugular vein were used as the anatomical landmarks and they were identified through their anechoic appearance and Doppler signal.[43] Further details regarding CSA of VN measurements by our group have been previously published.[44,45] In most cases, the VN was located laterally to the common carotid artery and dorsally to the internal jugular vein within the carotid sheath appearing as a small-rounded hypoechoic or honeycomb structure. Whenever necessary, color Doppler was used to prevent misinterpreting a small vessel within this hypervascularized region for being the VN. Before measuring the CSA, the operator made sure that the pressure of the transducer was reduced, until the lumen of the internal jugular vein was clearly inflated to avoid compression of the VN. Measurements were conducted using the trace function of the ultrasound system by outlining the contour of the nerve within the (hyper)echoic epineural rim (in mm^2). The mean of two CSA values was used for statistical analyses. CSA of both VNs was included in the analysis. As described by Pelz et al.,[43] this protocol of VN CSA measurement showed good intra- and inter-rater agreement and good agreement between different ultrasound systems.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Science (SPSS Inc., version 24.0 for Windows; IBM, Armonk, NY, USA). Descriptive statistics are given as the mean and standard deviation (SD), frequency, and percentage. Normality of data distribution was investigated with Kolmogorov-Smirnov test. Statistical comparisons between different groups were performed using the chi-square test (or exact test) for binary outcomes, and Student's *t*-test or Mann-Whitney U-test for continuous variables as appropriate. Correlations between variables were tested using Pearson or Spearman correlation coefficients (*r*) as appropriate. A two-tailed *p*-value of less than .05 was considered significant.

RESULTS

Demographic characteristics

A total of 28 healthy subjects (10 women and 18 men) and 21 patients (5 women and 16 men) were included (Table 1). The mean age of controls was 57 years, ranging between 46 and 76 years; the mean age of ALS patients was 61 years, ranging between 41 and 86 years. The two

TABLE 1 Demographic and clinical characteristics of the study population

	ALS ($n = 21$)	Controls ($n = 28$)	
Characteristic			p
Sex (females %)	5 (24)	10 (36)	.533
Age (years)	61±11.075	57±8.94	.133
BMI	26.32±3.41	27.3±4.57	.424
COMPASS 31	11.61± 11.97	–	
ALSFRS-R	35.10±9.85	–	
Disease duration (months)	17.1±16.98	–	
LMN involvement, *n* (%)	20 (95)	–	
UMN involvement, *n* (%)	20 (90)	–	
Bulbar involvement, *n* (%)	13 (62)	–	
Treatment, *n* (%)	11 (52)	–	

Note: All the data represent mean±standard deviation unless otherwise indicated.
Abbreviations: ALS, amyotrophic lateral sclerosis; ALSFRS-R, Revised-Amyotrophic Lateral Sclerosis Functional Rating Scale; BMI, body mass index; COMPASS31, Composite Autonomic Symptom Score; LMN, lower motor neuron; *n*, number of subjects; UMN, upper motor neuron.

groups did not differ in age ($p = .133$) and sex ($p = .533$). Mean BMI was 27.30 kg/m^2, with a range of 19.80-40.00 kg/m^2 for controls, and 26.32 kg/m^2, with a range of 21.00-32.10 kg/m^2 for ALS patients. BMI did not differ between groups ($p = .620$).

Clinical characteristics

The mean duration of the disease was 17.1 months, ranging between 4 and 84 months. Eleven patients, 52% of all cases, were under medication with riluzole. The functional status of the patients was assessed with the use of the ALSFRS-R scale. Bulbar symptoms were present in 13 patients (62%). Mean ALSFRS-R score (±SD) was 35.10 (±9.85), ranging between 14 and 46. Autonomous symptoms were assessed with the use of COMPASS 31 questionnaire. Mean COMPASS 31 score (±SD) was 11.61 (±11.97), ranging between 0 and 50 (Table 1).

SSR measurements

SSR was elicited in all controls but it was absent in three patients (14%). Mean latency for the upper limb in the control group was 1.29±0.21 seconds and 1.52 ±0.14 seconds in the ALS group. Mean latency for the lower limb in the control group was 1.81±0.31 seconds and 1.92 ±0.51 seconds in the ALS group. Mean latencies for upper and lower limbs were significantly shorter in controls compared to patients ($p<.001$ and $p = .024$). Mean amplitudes for the upper limb in the control group were 2.10±1.54 mV and 1.16±0.51 mV in the ALS group. Mean amplitudes for the lower limb in the control group were 1.02±0.69 mV and 0.76±0.58 mV in the ALS group. Mean amplitudes were higher in controls compared to patients, but only in the upper limb, this difference reached statistical significance (Table 2 and Figure 1).

TABLE 2 Comparison of sympathetic skin response measurements and cross-sectional area values of the vagus nerve between groups

	ALS ($n = 21$)	Controls ($n = 28$)	
Characteristic			p
SSR PALM LAT (seconds)	1.52±0.14	1.29±0.21	<.001
SSR PALM AMP (mV)	1.16±0.51	2.10±1.54	.024
SSR PLANT LAT (seconds)	1.92±0.51	1.81±0.31	.024
SSR PLANT AMP (mV)	0.76±0.58	1.02 ±0.69	.212
CSA VN R (mm^2)	1.73±0.62	2.91±0.79	<.001
CSA VN L (mm^2)	1.47±0.53	2.30±0.80	<.001

Note: All the data represent mean±standard deviation unless otherwise indicated.
Abbreviations: AMP, amplitude; CSA, cross-sectional area; L, left; LAT, latency; mV, millivolts; *n*, number of subjects; PALM, palmar; PLANT, plantar; R, right; SSR, sympathetic skin response; VN, vagus nerve.

TABLE 3 Correlations between the cross-sectional area of the right and left vagus nerve

Variable	Spearman's correlation (r)	p
CSA VN R versus CSA VN L (ALS)	0.439	.047
CSA VN R versus CSA VN L (Controls)	0.559	.003
CSA VN R versus CSA VN L (ALL)	0.698	<.001

Note: Right VN is significantly larger than left VN in both patients and controls.
Abbreviations: ALL, refers to ALS patients and controls; ALS, amyotrophic lateral sclerosis; CSA, cross-sectional area; L, left; R, right; VN, vagus nerve.

CSA VN

For the control group, mean CSA of the right VN was 2.91±0.79 mm^2 and of the left VN was 2.30±0.80 mm^2. For the patient group, mean CSA of the right VN was 1.73±0.62 mm^2 and of the left VN was 1.47±0.53 mm^2. CSAs of both right and left VN were significantly smaller in patients compared to controls ($p = .001$) (Table 2). We also documented significantly higher values of the right VN compared to the left VN in both groups (Table 3, Figures 2 and 3).

Correlations between SSR measurements and clinical characteristics

There was no correlation between SSR measurements and clinical characteristics: duration of the disease, ALSFRS-R score, and COMPASS 31 score (Table 4).

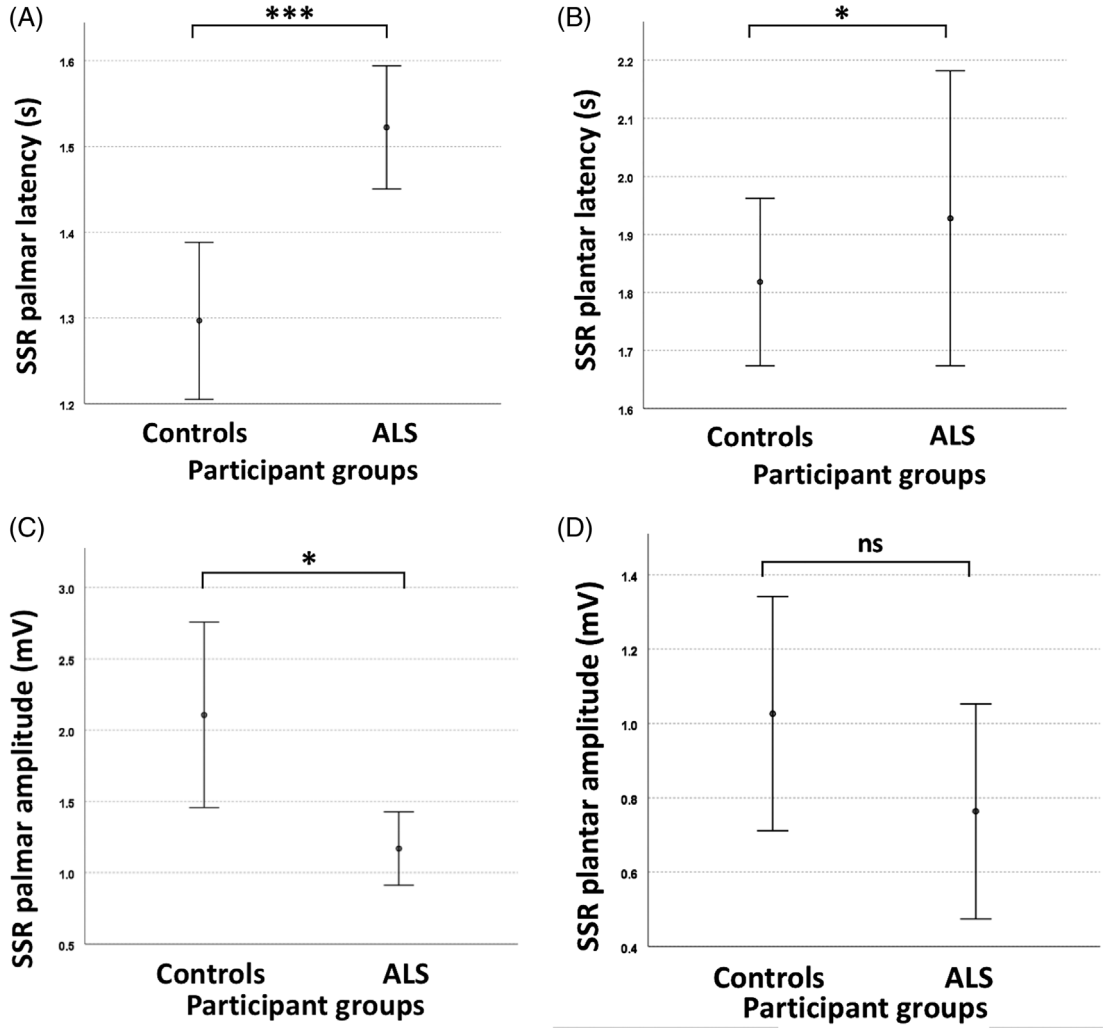

FIGURE 1 Comparison of sympathetic skin response latencies and amplitudes between patients and controls. Mean latencies were significantly shorter in controls compared to patients. Mean amplitudes were higher in controls compared to patients, but only in the upper limb, this difference reached statistical significance. One asterisk (*) indicates statistically significant differences at $p < .05$, three asterisks (***) indicate statistically significant differences at $p < .001$. Error bars represent two standard errors. Abbreviations: ALS, amyotrophic lateral sclerosis; mV, millivolts; ns, nonsignificant; SSR, sympathetic skin response; s, seconds

Correlations between CSA measurements and clinical characteristics

There was a significant negative correlation between disease duration and CSA of left VN ($r = -0.493$, $p = .023$), and a trend for statistical significance between CSA of right VN ($r = -0.419$, $p = .059$). ALSFSR-R was positively correlated to CSA of right VN ($r = 0.590$, $p = .006$). No other significant correlations were observed (Table 4).

Correlations between SSR-CSA measurements

No correlation was observed in any group (Table 5).

Correlations between clinical characteristics

ALSFRS-R was significantly negatively correlated to the presence of bulbar symptoms ($r = -0.475$, $p = .034$) and marginally to disease duration ($r = -0.390$, $p = .089$) and the presence of UMN signs ($r = -0.380$, $p = .099$). No other correlation was observed between clinical characteristics, disease duration, presence of autonomic symptoms, and functional status.

DISCUSSION

The aim of this study was to investigate ANS dysfunction in ALS patients and to distinguish SNS and PSNS involvement. The results of

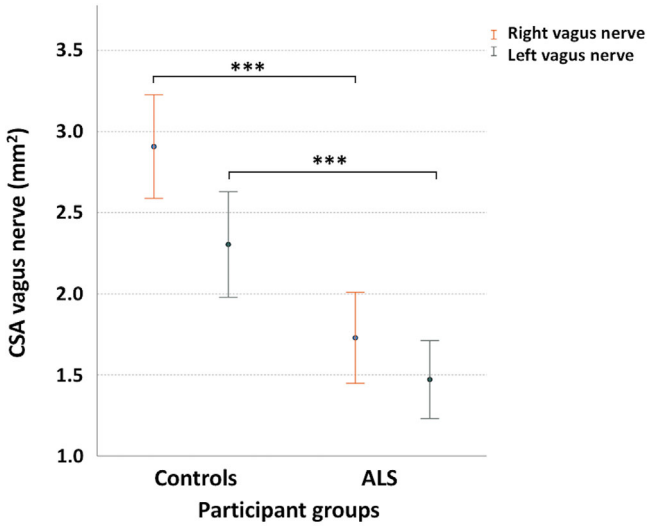

FIGURE 2 The right vagus nerve is significantly larger than the left vagus, in both controls and patients. Three asterisks (***) indicate statistically significant differences at $p < .001$. Error bars represent two standard errors. Abbreviations: ALS, amyotrophic lateral sclerosis; CSA, cross-sectional area

TABLE 4 Correlations between sympathetic skin response measurements, cross-sectional area of the vagus nerve, and clinical characteristics

Variable	Spearman's correlation (*r*)	*p*
SSR PALM AMP versus DURATION	0.101	.663
SSR PLANT AMP versus DURATION	−0.17	.941
SSR PALM AMP versus ALSFRS-R	0.336	.148
SSR PLANT AMP versus ALSFRS-R	0.124	.602
SSR PALM AMP versus COMPASS 31	−0.329	.183
SSR PLANT AMP versus COMPASS 31	−0.172	.495
SSR PALM LAT versus DURATION	−0.316	.163
SSR PLANT LAT versus DURATION	−0.188	.413
SSR PALM LAT versus ALSFRS-R	0.250	.288
SSR PLANT LAT versus ALSFRS-R	−0.009	.968
SSR PALM LAT versus COMPASS 31	−0.344	.162
SSR PLANT LAT versus COMPASS 31	−0.351	.153
CSA VN R versus DURATION	−0.419	.059
CSA VN L versus DURATION	−0.493	.023
CSA VN R versus ALSFRS-R	0.590	.006
CSA VN L versus ALSFRS-R	0.176	.458
CSA VN R versus COMPASS 31	−0.179	.476
CSA VN L versus COMPASS 31	0.174	.489

Abbreviations: ALSFRS-R, Revised-Amyotrophic Lateral Sclerosis Functional Rating Scale; AMP, amplitude; COMPASS31, Composite Autonomic Symptom Score; CSA, cross-sectional area; LAT, latency; L, left; PALM, palmar; PLANT, plantar; R, right; SSR, sympathetic skin response; VN, vagus nerve.

the present work support the existing knowledge of ANS involvement in ALS patients, and further demonstrate the implication of both sympathetic and parasympathetic ANS branches.

In the present study, SSR was not elicited in 14% of patients but was present in all controls. SSR amplitudes were significantly smaller and SSR latencies were significantly longer in the upper limbs in ALS patients compared to controls. The finding of longer SSR latencies in lower limbs in ALS patients, although it reached statistical significance, should be interpreted with caution, due to the noted overlap of confidence intervals. SSR measurements did not correlate with any clinical characteristic nor with CSA measurements. These findings are similar to those of previous studies, in which SSR was not elicited in 18-40% of ALS patients.[15,17,18,20] Additionally, in these studies, SSR latencies were prolonged and amplitudes were smaller in ALS patients compared to controls. Moreover, in all of these studies, SSR abnormalities did not correlate with disease severity and disease duration. SSR impairment typically occurs earlier than clinical manifestations of ANS dysfunction. A recent review of SSR findings in 120 ALS patients[16] reached the same conclusion, supporting the idea that the disease is more disseminated than its definition describes. ALS is a multisystem disease affecting principally but not exclusively motor neurons, even though involvement of other systems may not always be clinically apparent. SSR abnormalities are mainly attributed to loss of neurons in the intermediolateral nucleus between the dorsal and ventral horns of

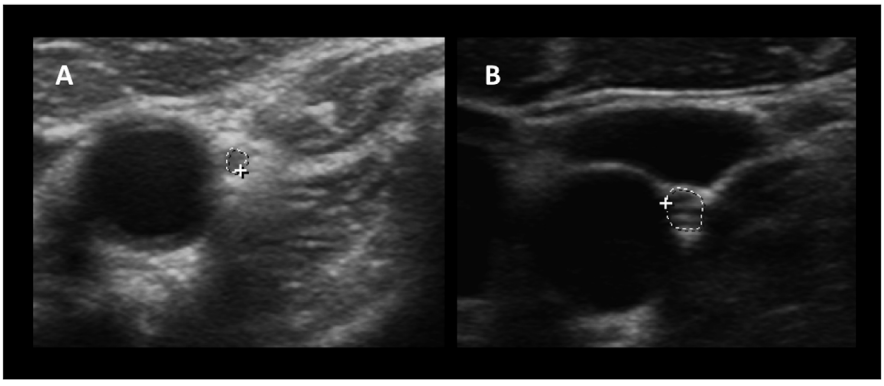

FIGURE 3 Ultrasound images of vagus nerve in a patient with amyotrophic lateral sclerosis (ALS) and a healthy control. (A) Cross-sectional area of the right vagus nerve in an ALS patient (0.011 cm^2); (B) cross-sectional area of right vagus nerve in a healthy individual (0.04 cm^2)

TABLE 5 Correlations between sympathetic skin response measurements and cross-sectional area of the vagus nerve

Variable	Spearman's correlation (*r*)	*p*
SSR UL LAT versus CSA VN R	−0.001	.998
SSR UL AMP versus CSA VN R	−0.109	.666
SSR LL LAT versus CSA VN R	−0.47	.854
SSR LL AMP versus CSA VN R	−0.365	.136
SSR UL LAT versus CSA VN L	0.83	.743
SSR UL AMP versus CSA VN L	−0.239	.340
SSR LL LAT versus CSA VN L	0.196	.436
SSR LL AMP versus CSA VN L	−0.308	.213

Abbreviations: AMP, amplitude; CSA, cross-sectional area; L, left; LAT, latency; LL, lower limb; R, right; SSR, sympathetic skin response; UL, upper limb; VN, vagus nerve.

the spinal cord, and this notion is further supported by histological findings.[46,47]

The most prominent finding of our study was the smaller CSA of both the right and left VNs in ALS patients compared to controls. Mean values of VN in controls are similar to those reported previously[43,48] and confirm the characteristic discrepancy between the bigger right VN compared to a smaller left VN.[21,22,28,43,44,49] This finding is unique among all other symmetrical peripheral nerves and could be explained by the fact that the right VN has a longer course in the abdomen innervating gastrointestinal viscera down to the colon, whereas the left VN is limited to the anterior gastric plexus.[43]

In the present study, CSA of VN was not correlated with the severity of autonomic symptoms or with the clinical presentation of the disease (involvement of UMN, LMN, or bulbar area). Additionally, the findings of significant correlation of CSA of the left VN with disease duration and the right VN with disease severity should be interpreted with caution, as type I errors could account for the noted discrepancies across sides (ie, nonsignificant associations of right and left VN CSA with disease duration and severity, respectively).

CSA of VN has been previously used to investigate parasympathetic involvement in diseases with known ANS involvement. Most studies have previously explored CSA of VN in patients with Parkinson's disease.[21–27] In most of these studies, atrophy of VN was documented, based on smaller CSA, and attributed the observed VN atrophy to degeneration of the dorsal nucleus in the brainstem due to alpha-synuclein aggregates.[24,27] VN atrophy has also been demonstrated in patients with diabetes mellitus[28] and in patients with fibromyalgia.[44] In the latter cases, the findings of VN atrophy were attributed to dying back neuropathy.

Ultrasonography of VN in ALS patients has been evaluated for the first time by Grimm et al.[50] to differentiate ALS patients from patients suffering from multifocal motor neuropathy (MMN). In their study, CSA of VN in all patients did not differ from controls, but patients suffering from MMN showed larger CSA of all examined nerves, the vagus included, compared to ALS patients. According to the authors of this study, VN hypertrophy in MMN patients could be attributed either to parasympathetic nerve involvement or to involvement of motor fibers. It is noteworthy that in the study by Grimm et al., CSA of the VN was measured in the carotid sheath beneath the carotid bifurcation, higher than the common site at the level of thyroid gland. Only at the level of the thyroid gland, where the body of the VN is composed mainly of parasympathetic fibers, can VN atrophy become apparent due to autonomic involvement.

Atrophy of VN has been described previously in patients with exclusively bulbar ALS, evident either clinically or electrophysiologically.[29,30] In these studies, VN atrophy was attributed to the involvement of the nucleus ambiguous in the brainstem. The nucleus ambiguous hosts somatic motor neurons distributed to pharyngeal and laryngeal muscles that are responsible for the functions of swallowing and speech.

In our study, no correlation was found between CSA of VN and clinical involvement of bulbar region. Our results suggest that VN is smaller in ALS patients regardless of bulbar involvement. Interestingly, Holzapfel and Naumann[30] state that "there was also no significant difference in vagus nerve CSA between patients with and without clinical signs of bulbar involvement," a statement that is in agreement with our findings and suggests that involvement of the ANS may occur independently of motor neuron involvement.

Furthermore, in those studies as in ours, CSA of VN was measured at the level of the thyroid gland, as described by Pelz et al.[43] At this level, branches to laryngeal and pharyngeal muscles, derived from nucleus ambiguous, have already left the main trunk of VN[51] with the exception of the recurrent laryngeal nerve, as illustrated in Figure 4. Thus, it is reasonable to assume that CSA of VN at the level of thyroid gland measures predominantly fibers of PSNS, the efferent ones emerging from the dorsal nucleus of the brainstem. Supporting evidence for the above comes from the fact that SOD1-related ALS patients show severe degenerative lesions in the autonomic nuclei, including the dorsal nucleus along with other.[52] The same conclusion is reached by researchers who investigated VN atrophy in Parkinson's disease.[21,22,24] They also assume that VN atrophy is due to PSNS involvement, and this assumption is further supported by histopathological evidence of degeneration of the dorsal nucleus of the VN due to aggregation of the α-synuclein protein that accumulates in Lewy bodies in the cells.[53] Further studies are needed to confirm dorsal nucleus involvement in ALS patients.

ALS is more than a pure motor disease and encompasses degeneration of neurons that belong to other functional systems, such as cognitive, extrapyramidal, and autonomic. In most cases, clinical manifestations of these disorders are not always evident, probably due to the short survival of patients and the overwhelming prevalence of muscle weakness and its consequences. Thus, future studies should evaluate the extent of ANS involvement in different ALS types and at different stages. Moreover, particularly with respect to cardiac autonomic dysfunction, it is highly recommended that ALS patients regularly undergo cardiological evaluation, including prolonged cardiac monitoring, with the aim to detect promptly myocardial involvement and cardiac arrhythmias that may precipitate circulatory collapse and sudden death in ALS patients.

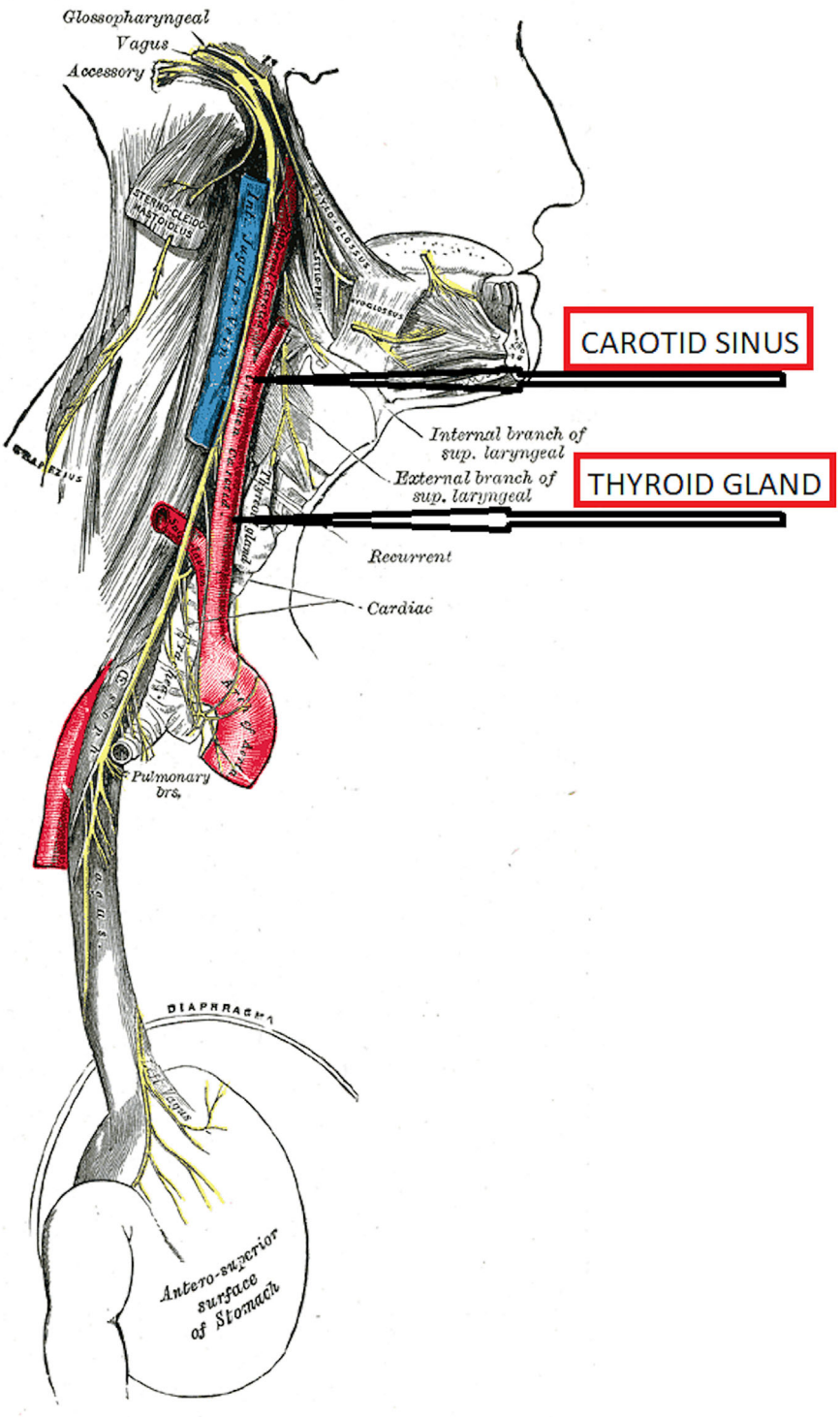

FIGURE 4 Anatomy of the vagus nerve. Adapted from: Henry Gray (1821—1865). Anatomy of the Human Body. 1918. This image is in the public domain. At the level of the thyroid gland, motor fibers to pharyngeal and laryngeal muscles have already left the main trunk of vagus nerve, with the exception of recurrent laryngeal nerve

The present study has certain limitations. The number of patients recruited is moderate. Moreover, the heterogeneity of clinical forms of ALS patients might have affected our results. Thus, larger cohorts of ALS patients, with homogenous clinical features, are warranted to systematically investigate ANS involvement, evidenced by electrophysiological and neuroimaging studies, in association with clinical parameters, such as disease duration and severity.

This study of ANS dysfunction in ALS patients contributes to the limited relevant literature, providing new insights into the implication of both ANS parts in ALS. Sympathetic involvement, as measured by SSR,

has been previously described and attributed to neuronal loss in the intermediolateral nucleus of the spinal cord. We suggest that similarly, neurons of the dorsal nucleus of the VN, which belong to the PSNS, may also degenerate, and that axonal loss may be reflected in the reduced CSA of the VN, as measured at the level of the thyroid gland.

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