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ORIGINAL ARTICLE

Ultrasonic detection of vagus, accessory, and phrenic nerve atrophy in amyotrophic lateral sclerosis: Relation to impairment and mortality

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Abstract

Background and purpose: In amyotrophic lateral sclerosis (ALS), phrenic nerve (PN) atrophy has been found, whereas there is controversy regarding vagus nerve (VN) atrophy. Here, we aimed to find out whether PN atrophy is related to respiratory function and 12-month survival. Moreover, we investigated the relevance of VN and spinal accessory nerve (AN) atrophy in ALS.

Methods: This prospective observational monocentric study included 80 adult participants (40 ALS patients, 40 age- and sex-matched controls). The cross-sectional area (CSA) of bilateral cervical VN, AN, and PN was measured on high-resolution ultrasonography. Clinical assessments included the Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised (ALSFRS-R), the Non-Motor Symptoms Questionnaire, and handheld spirometry of forced vital capacity (FVC). One-year survival was documented.

Results: The CSA of each nerve, VN, AN, and PN, was smaller in ALS patients compared to controls. VN atrophy was unrelated to nonmotor symptom scores. PN CSA correlated with the respiratory subscore of the ALSFRS-R (Spearman test, $r=0.59$, $p<0.001$), the supine FVC ($r=0.71$, $p<0.001$), and the relative change of sitting–supine FVC ($r=-0.64$, $p=0.001$). Respiratory impairment was predicted by bilateral mean PN CSA ($p=0.046$, optimum cutoff value of ≤0.37 mm^2, sensitivity = 92%, specificity = 56%) and by the sum of PN and AN CSA ($p=0.036$). The combination of ALSFRS-R score with PN and AN CSA measures predicted 1-year survival with similar accuracy as the combination of ALSFRS-R score and FVC.

Conclusions: Ultrasonography detects degeneration of cranial nerve motor fibers. PN and AN calibers are tightly related to respiratory function and 1-year survival in ALS.

KEYWORDS
accessory nerve, amyotrophic lateral sclerosis, motor neuron disease, nerve ultrasound, phrenic nerve, respiratory function, vagus nerve

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is characterized by chronic degeneration of bulbar and spinal motor neurons, accompanied by atrophy and loss of mainly the large myelinated alpha-motoneuron fibers in nerve roots and peripheral nerves [1, 2]. The resulting decrease of calibers of cervical nerve roots and of peripheral motor nerves in ALS patients as compared to healthy individuals can be detected with high-resolution ultrasound [3–7]. Recently it was demonstrated using ultrasound that also phrenic nerve (PN) calibers are decreased in ALS [8]. However, the functional relevance of PN atrophy in ALS patients is unclear so far. Controversial findings have been reported regarding the presence of vagus nerve (VN) atrophy in ALS [9–11]. Here, we wanted to find out whether PN atrophy is related to respiratory function and 12-month outcome of ALS patients. Moreover, we investigated the degree and clinical correlates of spinal accessory nerve (AN) and VN atrophy in ALS.

METHODS

Study participants

In this prospective observational monocentric cohort study, 80 adult participants were recruited between October 2019 and January 2022 at our department. Inclusion criteria were adult age and full legal capacity. Forty participants had probable ($n=11$) or definite ($n=29$) ALS (disease duration = 18.1 ± 14.5 months, range = 2–66 months) according to the revised El Escorial criteria [12], and another age-matched 40 were control subjects (Table 1). The patients were classified with regard to ALS manifestation phenotype (24 classic, six bulbar, two flail arm, four flail leg, one respiratory, one pure upper motor neuron, two pure lower motor neuron) [13, 14] and predominantly affected body region at the time of study (11 bulbar, 15 cervical, 14 lumbosacral). The control group comprised healthy volunteers, either from our hospital staff ($n=9$) or those who were recruited via press advertisement ($n=17$) and patients with minor stroke ($n=14$) who had no history of diabetes, neuromuscular disorder, neurodegenerative disease, or other relevant chronic disorders. None of the ALS patients had diabetes, severe hypertension, coronary heart disease, other neurodegenerative disorders, or relevant chronic disorders. The study was approved by the ethics committee of the Medical Faculty at Rostock University (identifier: A2017-0018). Written informed consent was obtained from each participant, and the experimental procedures were conducted according to the policies and ethical principles of the Declaration of Helsinki.

TABLE 1 Demographic, clinical, and ultrasound findings in ALS patients and controls.

	ALS patients, $n=40$	Controls, $n=40$	p
Demographics			
Age, years	66.2 ± 10.5	66.0 ± 8.6	0.95[a]
Gender, female/male, n	14/26	19/21	0.36[b]
Height, cm	171.8 ± 9.4	169.3 ± 6.6	0.23[a]
Weight, kg	72.3 ± 13.7	77.0 ± 13.2	0.20[a]
ALS duration, months	18.1 ± 14.5		
Clinical scores			
ALSFRS-R	35.1 ± 8.2	48.0 ± 0	**<0.001**[a]
NMSQ sum score	7.3 ± 4.4	3.6 ± 2.8	**<0.001**[a]
NMSQ autonomic subscore[c]	2.5 ± 2.3	1.1 ± 1.1	**<0.001**[a]
Spirometry			
FVC_n sitting, percent of reference value[c,d]	56.2 ± 30.6	81.4 ± 18.2	**<0.001**[a]
FVC supine, L[e]	2.03 ± 1.15	2.47 ± 0.77	0.16[a]
ΔFVC, percent change[c,e]	26.8 ± 20.6	15.6 ± 14.0	**0.045**[a]
Ultrasonography [CSA], mm^2			
Right vagus nerve	1.43 ± 0.72	1.99 ± 0.62	**<0.001**[a]
Left vagus nerve	1.37 ± 0.64	1.80 ± 0.60	**0.003**[a]
Bilateral vagus nerve, bilateral mean value	1.40 ± 0.59	1.91 ± 0.59	**<0.001**[a]
Right accessory nerve	0.34 ± 0.21	0.66 ± 0.23	**<0.001**[a]
Left accessory nerve	0.38 ± 0.21	0.69 ± 0.33	**<0.001**[a]
Bilateral accessory nerve	0.37 ± 0.19	0.68 ± 0.22	**<0.001**[a]
Right phrenic nerve	0.45 ± 0.25	0.60 ± 0.27	**0.013**[a]
Left phrenic nerve	0.43 ± 0.24	0.55 ± 0.14	**0.009**[a]
Bilateral phrenic nerve	0.44 ± 0.23	0.58 ± 0.18	**0.003**[a]

Note: Values except gender are mean ± SD. Significance of bold values: $p < 0.05$.

Abbreviations: ALS, amyotrophic lateral sclerosis; ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised; CSA, cross-sectional area in square millimeters; FVC, forced vital capacity; FVC_n, percent of normal FVC value; NMSQ, Non-Motor Symptoms Questionnaire; ΔFVC, relative percent change in FVC from sitting to supine.

[a]*t*-test, two-sided.

[b]Fisher exact test.

[c]For details, see text.

[d]Assessed in 36 ALS patients and 18 controls.

[e]Assessed in 23 ALS patients and 18 controls.

Clinical assessments

All participants underwent clinical and sonographic investigations on the same day. The severity of ALS-related motor, salivary, and respiratory symptoms was evaluated with the revised version of the Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised (ALSFRS-R) [15]. Nonmotor symptoms were assessed using the German version of the Non-Motor Symptoms Questionnaire (NMSQ) validated for Parkinson disease [16], which is applicable also for motor neuron diseases [17–19]. The sum score of autonomic symptoms on the NMSQ, calculated from items 1, 3, 4, 5, 6, 7, 8, 11, 19, 20, and 28, was used as measure of autonomic symptom severity [20]. The degree of neurogenic respiratory insufficiency was assessed by conventional (sitting) and dynamic (sitting to supine) pulmonary function

testing using a handheld spirometer (pocket spirometer, Buhl type). Participants completed spirometry in the sitting position first, then in the supine position if tolerated. Approximately 10–30 min elapsed between tests to allow for recovery and to minimize fatigue. The relative percent change in forced vital capacity (FVC; given in liters) from sitting to supine position was calculated using the following formula: $\Delta FVC = (FVC\ sitting - FVC\ supine)/(FVC\ sitting) \times 100$ [21, 22]. For quantifying sitting respiratory function, the age- and gender-related normative values of FCV (FVC_n, given in percentages) were calculated using established reference equations [23]. FVC_n values ≤ 70% were regarded as representing respiratory impairment in ALS patients [24]. In all patients, 1-year survival was documented.

High-resolution ultrasound

For high-resolution ultrasound, a high-end ultrasound system (MyLabTwice; Esaote, Genoa, Italy) equipped with a 15.0-MHz transducer (LA435) was applied. Bilateral VN, AN, and PN were scanned in the axial view. All measurements were performed by one of two well-trained sonographers (G.S., U.W.). To capture the VN, the probe was placed at the midcervical level (at the level of thyroid cartilage; Figure 1a,b). To capture the PN and the AN, the probe was placed at the low cervical level (at the level of interscalene groove; Figure 1c,d). For measurements of cross-sectional area, the target nerve was positioned in the center of the image, at a position where it had a nearly elliptic cross-sectional shape. For the axial transection of these nerves, the longest cross-sectional diameter d1 of the nerve and the diameter d2 perpendicular to d1 were measured separately (within the hyperechoic epineural rim of the nerve), each diameter given by the ultrasound system in millimeters with one digit following the decimal point for measures ≥ 1.0 mm and with 2 digits following the decimal point for measures ≤ 0.99 mm, and the elliptic cross-sectional area (CSA; in square millimeters) was calculated offline according to the formula $CSA = d1 \cdot d2 \cdot \pi/4$ [25]. To assess interrater reliability, 47 nerves were independently measured by both investigators, one of whom was blinded to diagnosis. To ensure blinding, the study participants (patients and controls) were

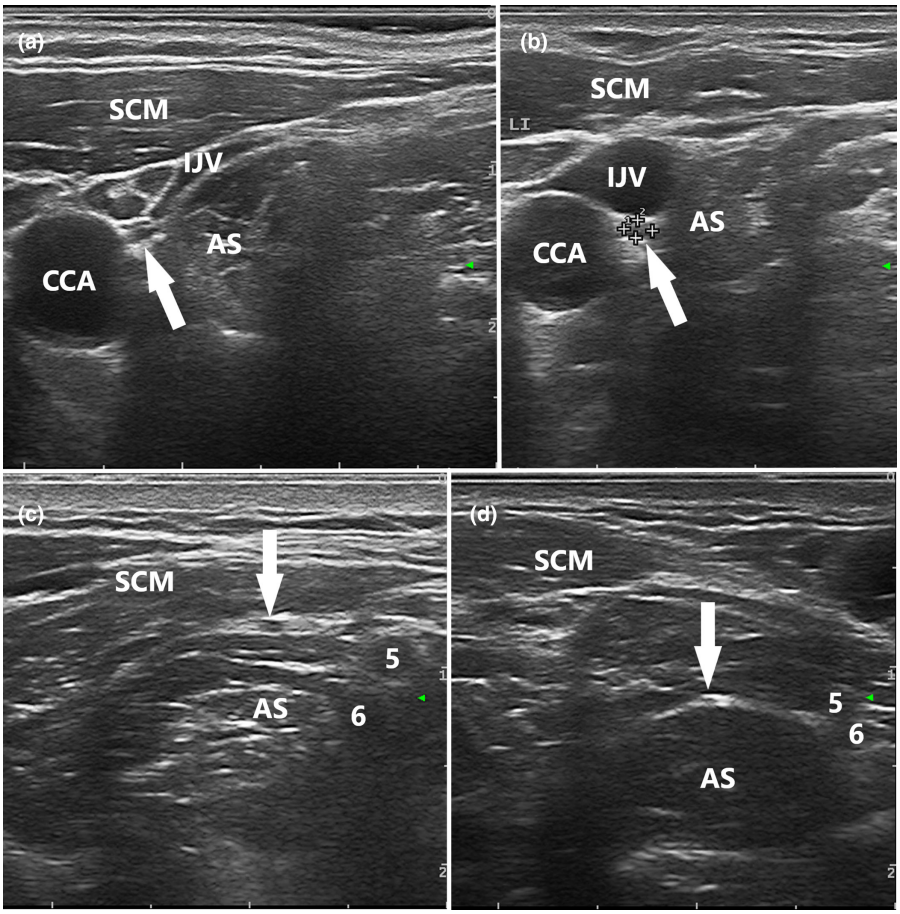

FIGURE 1 Axial sonograms of lateral neck region at midcervical level. AS denotes anterior scalene muscle; CCA, common carotid artery; IJV, internal jugular vein; LI, left-sided insonation of neck; SCM, sternocleidomastoid muscle; 5, fifth cervical nerve root; 6, sixth cervical nerve root. (a) Sonogram of a healthy control subject. Arrow indicates axial transection of vagus nerve. (b) Sonogram of a patient with amyotrophic lateral sclerosis (ALS). Arrow indicates axial transection of vagus nerve. Measurement marks denote longest and shortest diameter of vagus nerve used for calculation of elliptic cross-sectional area. (c) Sonogram of a healthy control subject. Arrow indicates axial transection of phrenic nerve. (d) Sonogram of a patient with ALS. Arrow indicates axial transection of phrenic nerve. Note the marked atrophy of phrenic nerve as compared to normal phrenic nerve shown in panel c.

placed in random order on the investigation chair before the second investigator entered the room, and patients were not allowed to talk with the investigator. High interrater agreement was demonstrated (Pearson correlation, $r=0.88$, $p<0.001$; Bland–Altman plot for mean difference = 0: $p<0.001$).

Statistical analyses

Normally distributed variables were compared with the two-sided *t*-test, and categorical variables with Fisher exact test. The Spearman correlation test was used to compare nerve CSA measures with demographic and clinical parameters. Because eight different parameters (age, ALS duration, ALSFRS-R sum score, ALSFRS-R respiratory subscore, NMSQ sum score, NMSQ autonomic subscore, FVC_n, ΔFVC) were tested in the correlation analyses, a Bonferroni correction was applied, with $p<0.006$ indicating significance. Receiver operating characteristic (ROC) curves were plotted to assess the value of the combination of clinical and sonographic findings for the prediction of respiratory impairment and of 1-year survival in ALS patients. Optimum cutoff values were estimated by calculation of the Youden Index. Statistical analyses were performed with SPSS Statistics software (version 28; IBM, Armonk, NY).

RESULTS

Clinical findings

Findings on clinical assessments and spirometry are summarized in Table 1. Four (10%) ALS patients were not able to perform spirometry at all due to severe bulbar impairment and/or insufficient expiratory force, and 13 (32.5%) patients could not perform spirometry in supine position.

Vagus nerve

CSA of VN was significantly smaller in ALS patients compared to controls (Table 1, Figure 2). CSA of right and left VN were highly correlated considering all study participants (Spearman test, $r=0.62$, $p<0.001$) or ALS patients only ($r=0.50$, $p=0.001$; Figure 3a). In controls, VN CSA (bilateral mean value) did not correlate with age, NMSQ sum score, NMSQ autonomic subscore, or FVC_n (each, $p>0.29$). In patients, VN CSA did not correlate with age, ALS duration, ALS severity assessed on the ALSFRS-R, NMSQ sum score, or FVC_n (each, $p>0.3$). Larger VN CSA correlated, by trend, with higher NMSQ autonomic subscore ($r=0.3$, $p=0.035$).

Accessory nerve

CSA of AN was significantly smaller in ALS patients compared to controls (Table 1, Figure 2). CSA of right and left AN were highly correlated considering all study participants (Spearman test, $r=0.58$, $p<0.001$) or ALS patients only ($r=0.63$, $p<0.001$; Figure 3b). AN CSA correlated with VN CSA ($r=0.49$, $p<0.001$). In controls, AN CSA (bilateral mean value) did not correlate with age or FVC_n (each, $p>0.08$). In patients, AN CSA did not correlate with age, ALS duration, ALS severity assessed on the ALSFRS-R, NMSQ sum score, NMSQ autonomic subscore, FVC_n, or ΔFVC (each, $p>0.08$). AN CSA correlated, by trend, with the respiratory subscore of the ALSFRS-R (Spearman test, $r=0.38$, $p=0.016$), but not with the bulbar subscore ($p=0.29$). Considering all study participants, AN CSA correlated

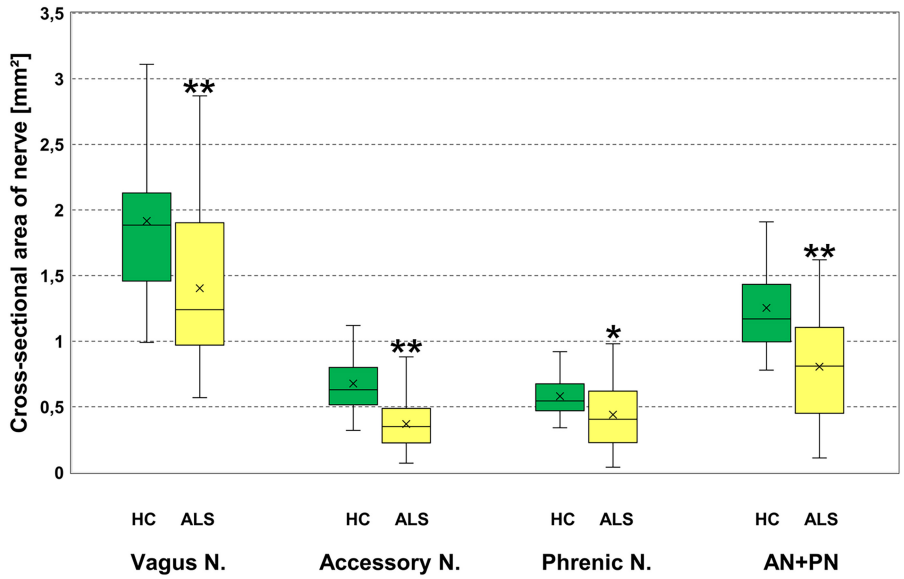

FIGURE 2 Box-and-whisker diagram showing the cross-sectional area of vagus nerve (individual mean of bilateral measures), accessory nerve (AN), and phrenic nerve (PN) in amyotrophic lateral sclerosis patients (ALS) and age-matched healthy controls (HC). Boxes denote median and interquartile ranges; crosses denote mean values. Note that all three nerves are atrophic in ALS patients. *$p<0.005$, **$p<0.001$. N., nerve.

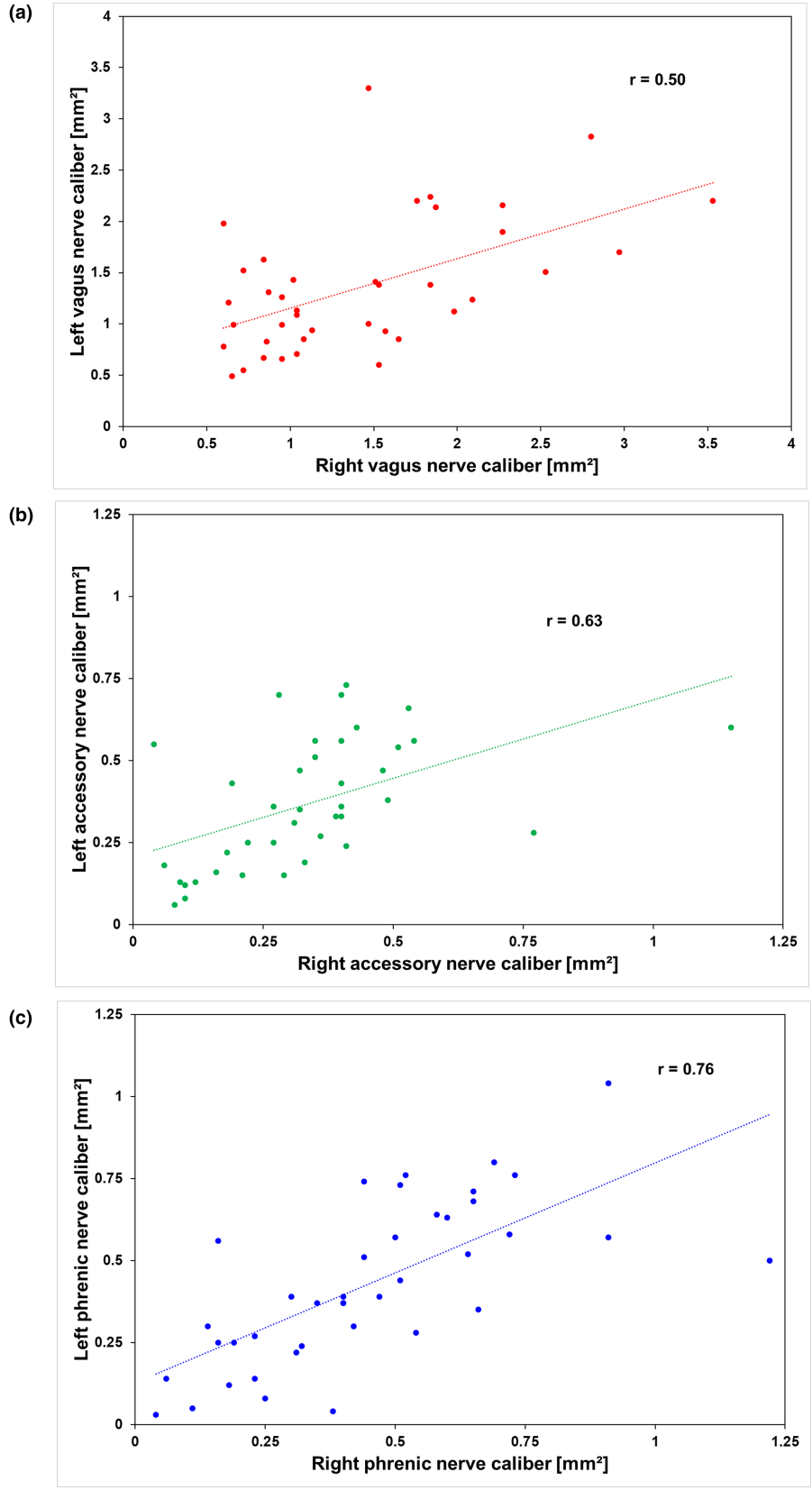

FIGURE 3 Diagram showing the correlation between right and left nerve calibers (cross-sectional area at midcervical level) in amyotrophic lateral sclerosis patients. (a) Vagus nerve; note right-sided dominance especially if atrophy is less advanced, and rather symmetrical caliber reduction. (b) Accessory nerve; note symmetrical caliber reduction. (c) Phrenic nerve; note symmetrical caliber reduction.

with FVC_n ($n=54$, $r=0.44$, $p<0.001$). AN CSA did not predict respiratory impairment ($FVC_n \leq 70\%$) in ALS patients (ROC curve analysis, $p=0.19$).

Phrenic nerve

CSA of PN was significantly smaller in ALS patients compared to controls (Table 1, Figures 1 and 2). CSA of right and left PN were highly correlated considering all study participants (Spearman test, $r=0.63$, $p<0.001$) or ALS patients only ($r=0.76$, $p<0.001$; Figure 3c). PN CSA correlated with AN CSA ($r=0.57$, $p<0.001$) rather than VN CSA ($r=0.24$, $p=0.028$). In controls, PN CSA (bilateral mean value) did not correlate with age, NMSQ sum score, or FVC_n (each, $p>0.2$). In patients, PN CSA did not correlate with age, ALS duration, or NMSQ sum score (each, $p>0.16$). PN CSA correlated with the ALSFRS-R sum score ($r=0.45$, $p=0.003$), the respiratory subscore of the ALSFRS-R ($r=0.59$, $p<0.001$), and ΔFVC ($r=-0.62$, $p=0.001$, $n=23$; Figure 4a), but not the bulbar subscore of the ALSFRS-R ($p=0.082$). PN CSA correlated with supine FVC ($r=0.71$, $p<0.001$, $n=23$; Figure 4b) and, by trend, with FVC_n in ALS patients ($r=0.37$, $p=0.027$, $n=36$). Considering all study participants, PN CSA correlated with FVC_n ($r=0.44$, $p<0.001$, $n=54$), as did the sum of PN and AN CSA ($r=0.51$, $p<0.001$; Supplemental Figure). Respiratory impairment ($FVC_n \leq 70\%$) was predicted in patients by PN CSA $\leq 0.37\,mm^2$ (ROC curve analysis, area under the curve [AUC] $=0.70$, 95% confidence interval [CI] $=0.52$–0.89, $p=0.046$, sensitivity $=92\%$, specificity $=56\%$). Prediction specificity of respiratory impairment was improved by the sum of PN and AN CSA, with an optimum cutoff value of

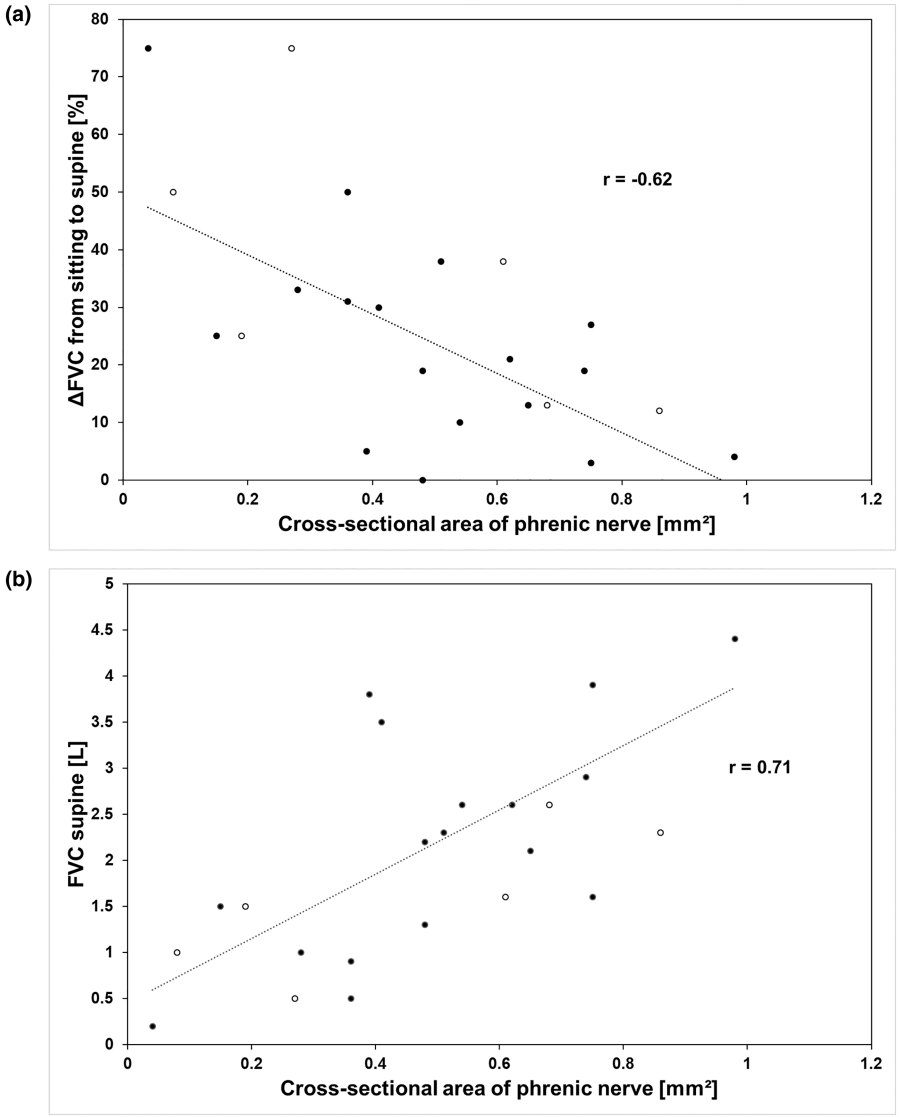

FIGURE 4 Diagram showing the relationship between phrenic nerve caliber (individual mean of bilateral measures) in amyotrophic lateral sclerosis patients and handheld spirometer measures of forced vital capacity (FVC). Black points indicate patients who survived for >1 year; white points indicate patients who died within 1 year. (a) Relative percent change of FVC (ΔFVC) from sitting to supine increases with smaller phrenic nerve caliber. (b) Supine FVC, which largely depends on diaphragm function, lowers with smaller phrenic nerve caliber.

1.28 mm^2 (AUC = 0.72, 95% CI = 0.53–0.90, $p = 0.036$, sensitivity = 42%, specificity = 100%).

Findings in ALS subtypes

CSAs of VN, AN, and PN did not differ between the three subgroups of predominantly affected body regions at the time of study (each, $p > 0.05$). However, comparing all patients with present bulbar symptoms ($n = 21$) and those without ($n = 19$), CSA of PN was smaller in patients with bulbar symptoms (0.38 ± 0.20 vs. 0.50 ± 0.24 mm^2; one-sided *t*-test, $p = 0.045$), as was, by trend, the sum of PN and AN CSA (0.71 ± 0.35 vs. 0.91 ± 0.39 mm^2; $p = 0.052$). VN CSA was unrelated to presence of bulbar symptoms ($p = 0.33$). VN CSA was lower in the patients with pure lower motor neuron phenotype (0.70 ± 0.18 mm^2) compared to those with classical phenotype (1.54 ± 0.56 mm^2; two-sided *t*-test, $p = 0.014$). Both PN CSA and the sum of PN and AN CSA were larger in patients with flail leg phenotype (0.64 ± 0.09 mm^2, 1.10 ± 0.19 mm^2) compared to those with classical phenotype (0.43 ± 0.26 mm^2, $p = 0.007$ for PN; 0.78 ± 0.41 mm^2, $p = 0.037$ for PN + AN). Other phenotypes were unrelated to nerve CSAs.

Prediction of 1-year mortality

Sixteen (40%) of 40 ALS patients died during the 12-month follow-up period (Table 2). The causes of death were respiratory failure as a consequence of weakness of respiratory muscles ($n = 7$, 44%), terminal cachexia/feeding refusal ($n = 5$, 31%), pneumonia ($n = 1$, 6%), sudden death from presumed cardiovascular cause ($n = 1$, 6%), or unknown ($n = 2$, 13%). One-year mortality was predicted by combining ALSFRS-R and PN CSA (AUC = 0.70, 95% CI = 0.53–0.88, $p = 0.034$), or by combining ALSFRS-R and sum of PN and AN CSA (AUC = 0.72, 95% CI = 0.55–0.90, $p = 0.019$; Figure 5a) but not by a single clinical or sonographic feature (ALSFRS-R, VN CSA, AN CSA, PN CSA, AN + PN CSA) or other combinations of these features. The optimum cutoff value of the product of ALSFRS-R score and PN CSA (in square millimeters) for discriminating 1-year survivors from nonsurvivors was 8.2 (sensitivity = 83%, specificity = 56%). The optimum cutoff value of the product of ALSFRS-R score and AN + PN CSA (in square millimeters) for discriminating 1-year survivors from nonsurvivors was 16.8 (sensitivity = 87%, specificity = 62%).

Considering the 36 ALS patients in whom FVC_n could be assessed, 1-year mortality was not predicted by a single clinical or sonographic feature (age, weight, ALSFRS-R, FVC, VN CSA, AN CSA, or PN CSA; each, $p > 0.05$). However, 1-year mortality was predicted by combining ALSFRS-R and FVC_n (AUC = 0.71, 95% CI = 0.53–0.89, $p = 0.040$) or by combining ALSFRS-R, FVC_n, and PN CSA (AUC = 0.72, 95% CI = 0.53–0.91, $p = 0.034$; Figure 5b). The optimum cutoff value of the product of ALSFRS-R score and FVC_n for discriminating 1-year survivors from nonsurvivors was 1680 (sensitivity = 74%, specificity = 61%). The optimum cutoff value of the product of ALSFRS-R score, FVC_n, and PN CSA (in square millimeters) for discriminating 1-year survivors from nonsurvivors was 285.0 (sensitivity = 91%, specificity = 54%).

TABLE 2 Demographic, clinical, and ultrasound findings in 1-year survivors and nonsurvivors.

	Survivors, $n = 24$	Nonsurvivors, $n = 16$	*p*
Demographics			
Age, years	63.8 ± 12.2	69.7 ± 6.2	0.052[a]
Gender, female/male, *n*	9/15	5/11	0.75[b]
Height, cm	172.0 ± 8.8	171.6 ± 10.5	0.90[a]
Weight, kg	71.3 ± 14.6	73.9 ± 12.6	0.54[a]
ALS duration, months	18.8 ± 15.7	17.0 ± 12.7	0.71[a]
Clinical scores			
ALSFRS-R	37.3 ± 7.1	31.8 ± 8.9	**0.047**[a]
NMSQ sum score	6.6 ± 4.4	8.4 ± 4.2	0.21[a]
NMSQ autonomic subscore[c]	1.9 ± 2.1	3.4 ± 2.4	0.054[a]
Spirometry			
FVC_n sitting, percent of reference value[c,d]	64.8 ± 28.5	41.1 ± 29.1	**0.026**[a]
FVC supine, L[e]	2.19 ± 1.24	1.58 ± 0.78	0.19[a]
ΔFVC, percent change[c,e]	23.7 ± 19.0	35.5 ± 24.3	0.32[a]
Ultrasonography [CSA], mm^2			
Right vagus nerve	1.33 ± 0.67	1.57 ± 0.79	0.34[a]
Left vagus nerve	1.21 ± 0.44	1.61 ± 0.81	0.09[a]
Bilateral vagus nerve, bilateral mean value	1.28 ± 0.49	1.59 ± 0.70	0.14[a]
Right accessory nerve	0.37 ± 0.20	0.28 ± 0.21	0.20[a]
Left accessory nerve	0.41 ± 0.20	0.34 ± 0.22	0.27[a]
Bilateral accessory nerve	0.40 ± 0.19	0.31 ± 0.19	0.14[a]
Right phrenic nerve	0.49 ± 0.22	0.38 ± 0.29	0.20[a]
Left phrenic nerve	0.47 ± 0.25	0.36 ± 0.23	0.14[a]
Bilateral phrenic nerve	0.48 ± 0.21	0.37 ± 0.24	0.14[a]

Note: Values except gender are mean ± SD. Significance of bold values: $p < 0.05$.

Abbreviations: ALS, amyotrophic lateral sclerosis; ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised; CSA, cross-sectional area in square millimeters; FVC, forced vital capacity; FVC_n, percent of normal FVC value; NMSQ, Non-Motor Symptoms Questionnaire; ΔFVC, relative percent change in FVC from sitting to supine.

[a]*t*-test, two-sided.

[b]Fisher exact test.

[c]For details, see text.

[d]Assessed in 23 survivors and 13 nonsurvivors.

[e]Assessed in 17 survivors and six nonsurvivors.

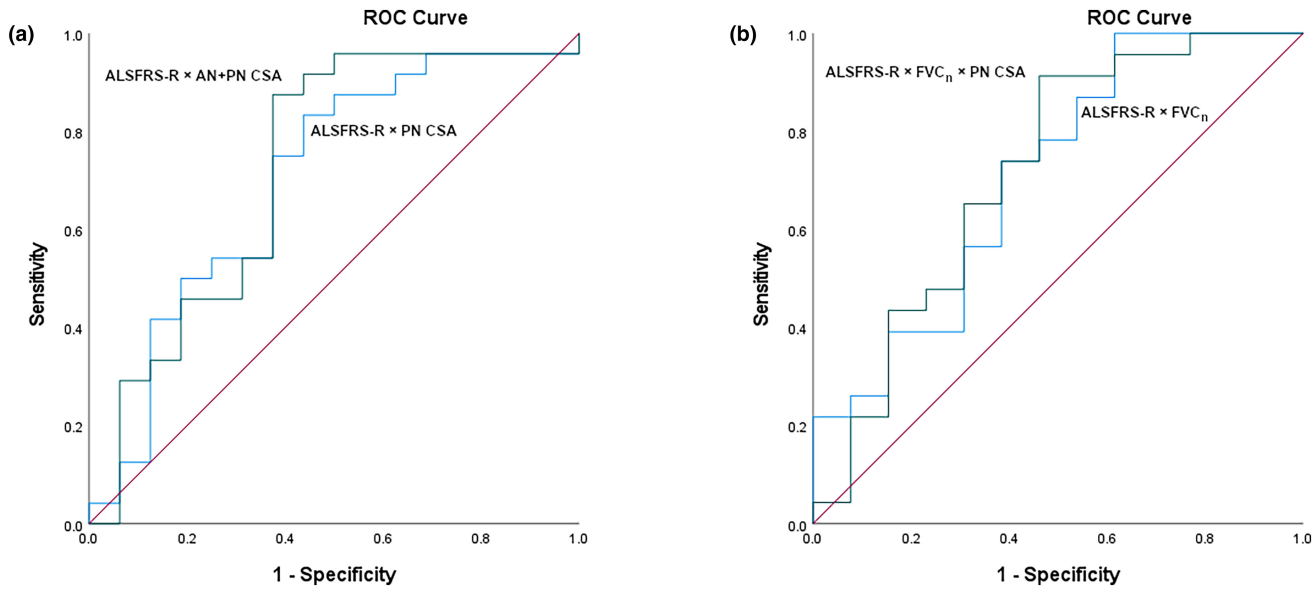

FIGURE 5 Receiver operating characteristic (ROC) curve of prediction of 1-year survival in amyotrophic lateral sclerosis patients. (a) The combination of Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised (ALSFRS-R) score and phrenic nerve caliber (individual mean of bilateral measures) discriminated 1-year survivors from nonsurvivors (area under the curve [AUC] = 0.70, $p = 0.034$); discrimination was slightly improved by combining ALSFRS-R score and sum of phrenic and accessory nerve caliber (AUC = 0.72, $p = 0.019$). (b) The combination of ALSFRS-R score and forced vital capacity (percent of normal value [FVC$_n$]) discriminated 1-year survivors from nonsurvivors (AUC = 0.71, $p = 0.040$); discrimination was slightly improved by combining ALSFRS-R score, FVC$_n$, and phrenic nerve caliber (AUC = 0.72, $p = 0.034$). AN, accessory nerve; CSA, cross-sectional area; PN, phrenic nerve.

The patient who died from presumed cardiovascular cause did not differ from the other decedents with respect to AN, PN, or VN CSA at baseline investigation (each, $p > 0.2$).

DISCUSSION

Data obtained in this study show thinning of VN, AN, and PN in ALS. AN and PN thinning was more pronounced in patients with bulbar symptoms and occurred the least in patients with flail leg phenotype. PN atrophy and, to a lesser degree, AN atrophy are related to decreasing respiratory function in ALS. The combination of ALSFRS-R score with PN and AN CSA measures predicts 1-year survival in ALS patients, and has a similar prediction accuracy as the combination of ALSFRS-R score and FVC$_n$. Decrease of VN calibers in ALS is not associated with higher (vagal) autonomic symptom score, suggesting predominant alpha-motoneuron fiber degeneration in the VN in ALS, and/or inadequate depiction of ALS nonmotor symptoms on the NMSQ.

A potential limitation of the present study could be a bias caused by the first sonographer's awareness of the participants' classification as patient or control. However, we found high interrater agreement of nerve CSA measures between the unblinded and the blinded sonographer. Therefore, we consider a relevant bias unlikely. To minimize the potential influences of comorbidity known to be associated with neuropathy especially of the VN (e.g., diabetes mellitus, severe coronary heart disease, chronic inflammatory neuropathies), we included only patients and controls who had no history and no diagnostic evidence from electrocardiogram or laboratory findings for any of these conditions.

Present measures of PN CSA are lower than values reported earlier for ALS patients and normal population [8]. This can well be explained by the method applied here with separate measuring of the long and short axis diameter and consecutive calculation of the elliptic CSA [25]. If instead a measuring method with tracing of nerve CSA is used, larger values typically result, which would require adaptation of the cutoff values reported in the present study. Our finding that AN and PN atrophy are symmetrical in ALS patients agrees well with the earlier observation of symmetrical loss of motor units in sternocleidomastoid muscle and diaphragm [26]. AN and PN thinning was most pronounced in patients with bulbar symptoms and occurred the least in those with flail leg phenotype, which is in line with the previously noted, albeit weak, association between cervical nerve root atrophy and ALS phenotype [27].

Earlier studies did not assess the relationship between PN or AN CSA and spirometric measures. However, unlike a recent study [8], we found a correlation between PN CSA and the ALSFRS-R sum score as well as the ALSFRS-R respiratory subscore. The latter agrees with previously reported parallel decline of ALSFRS-R and PN stimulation-evoked diaphragm compound muscle action potential [28]. Moreover, we demonstrate a clear association of PN caliber and spirometric measures of respiratory function, especially ΔFVC. Sitting-to-supine fall in vital capacity is a well-established indicator of bilateral diaphragm dysfunction [29]. Our finding that PN, rather than AN, atrophy predicts lowered FVC

(especially supine FVC) is in agreement with the well-documented dominant contribution of diaphragm over other respiratory muscles to the inspiratory volume, which largely determines vital capacity [30]. On the other hand, we found a high correlation between PN and AN calibers, which is in line with earlier reported parallel loss of motor units in the sternocleidomastoid muscle and diaphragm in ALS [26]. Recruitment of extradiaphragmatic muscles, especially the sternocleidomastoid muscle, has been reported as a mechanism for offsetting the effects of increased load on a weak diaphragm [31, 32]. Our findings suggest that assessment of AN CSA in addition to PN CSA improves the indication of respiratory impairment (here, sitting $FVC_n \leq 70\%$). Future studies may assess the relationship between ultrasonic PN and AN calibers and individual chance of improvement on optimized respiratory strength training programs [33].

The causes of death and their relative frequencies in our ALS cohort are in line with earlier reported data [34, 35]. One-year mortality in ALS patients can be predicted by combining ALSFRS-R and FVC, rather than by a single of these criteria [36]. According to our data, a similarly high predictive value can be achieved by combining ALSFRS-R and PN + AN CSA measures. Because it is often easier to obtain ultrasonic than spirometric measures in ALS patients, with failure of portable spirometry devices in some patients as shown in the present and earlier studies [37], nerve ultrasound may be an attractive option for the evaluation of 1-year survival. Sonographic proximal-to-distal median nerve CSA ratio has been reported to improve the identification of fast ALS progressors [38]. Present data underpin the potential value of nerve ultrasound for the prognostic evaluation of ALS patients.

Our findings provide further support to the notion of VN atrophy in ALS [9, 10, 39]. Another study has failed to show VN atrophy in ALS patients [11], which might be due to inclusion of patients with diabetes mellitus, a condition known to be associated with VN atrophy [40]. Interestingly, VN atrophy in ALS patients was unrelated to autonomic dysfunction in the present and an earlier study [11]. In the earlier study, right VN CSA was also unrelated to heart rate variability in ALS patients, other than the control group [11]. Another study that involved patients with a wider range of ALS duration (up to 84 months) demonstrated a negative correlation of VN CSA with ALS duration, and positive correlation with ALSFRS-R score, but no association with autonomic function measures [10]. In contrast, ultrasonic VN atrophy correlated with parasympathetic cardiovascular dysfunction in patients with Parkinson disease [20, 41]. Taken together, these findings support the view that predominantly somatomotor nerve fibers of cranial nerves degenerate in ALS. At midcervical level, the VN contains 15%–20% myelinated fibers, which comprise the motor fibers innervating the laryngeal muscles [42]. Our finding that AN atrophy closely correlates with both VN and PN atrophy, whereas there is less correlation between VN and PN atrophy, is well explained by the craniocaudal order of motor nuclei of VN (medulla oblongata), spinal AN (cervical segments C1–C4), and PN (cervical segments C3–C5) [43–45]. It remains to be elucidated whether VN atrophy in ALS patients is specifically related to dysphonia. The NMSQ was developed originally for the assessment of nonmotor symptoms in Parkinson disease [16, 46], but has also been applied in motor neuron diseases [17–19]. Our results could also indicate that the NMSQ is not perfect in capturing nonmotor symptoms in ALS, because some of its items (e.g., dribbling, swallowing, falling) are likely to reflect motor rather than nonmotor impairment in motor neuron disease. There is, nevertheless, evidence of autonomic dysfunction in ALS [19, 47, 48], including cardiovagal dysfunction [49, 50]. Optimized nonmotor symptom assessment scales may therefore be desirable for ALS patients, especially at mild-to-moderate disease stages.

In conclusion, present data provide further evidence of a detectable degeneration of cranial and cervical motor neurons in ALS, even at disease stages with predominant clinical affection of lower body regions. Further studies are warranted to evaluate the value of combing ALSFRS-R sum score with sonographic measures of PN and AN, and possibly other nerve calibers, in the early prediction of respiratory failure and 1-year survival.

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CONFLICT OF INTEREST STATEMENT

The authors state explicitly that there are no conflicts of interest in connection with this article.

DATA AVAILABILITY STATEMENT

Deidentified participant data will be shared by request from any qualified investigator. Data sharing requests are made in writing through Dr. Walter (uwe.walter@med.uni-rostock.de) and require a formal data sharing agreement with approval from the Rostock University Medical Center. Data sharing agreements must include details on how the data will be stored, who will have access to the data, and intended use of the data, and agreements as to the allocation of intellectual property.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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