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## Agreement between Diaphragm Ultrasound and Pulmonary Function Testing in the Evaluation of Diaphragm Function in Patients with ALS

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**Methods:** Twenty nine spinal ALS and thirteen healthy controls were studied in standing up and lying 30° supine position. All subjects performed PFTs and DU, to assess of diaphragmatic excursion, delta-thickness between end inspiration and end expiration ( $\Delta T$ ), and the thickening fraction (TF).

**Keywords:** *amyotrophic lateral sclerosis; diaphragm ultrasound; pulmonary function testing.*

**GJMR-F Classification:** LCC: RC346



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# Agreement between Diaphragm Ultrasound and Pulmonary Function Testing in the Evaluation of Diaphragm Function in Patients with ALS

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**Results:** The standing position,  $\Delta T$  correlates with VC ( $r=0.58p=0.001$ ) and FVC ( $r=0.61 p<0.001$ ); in lying 30° supine position,  $\Delta T$  correlates with VC( $r=0.59 p=0.001$ ) and FVC ( $r=0.62 p<0.001$ ). In standing up position, TF correlates with VC ( $r=0.55 p=0.003$ ) and FVC ( $r=0.53 p=0.005$ ); in lying 30° supine position, TF correlates with VC ( $r=0.55 p=0.003$ ) and FVC ( $r=0.51 p=0.007$ ). The following correlations were also observed: the standing position, diaphragmatic excursion correlated with VC( $r=0.55 p=0.007$ ) and FVC ( $r =0.65 p<0.001$ ). In lying 30° supine position, diaphragmatic excursion correlated with VC( $r=0.59 p=0.003$ ); and FVC( $r=0.63 p=0.001$ ).

**Conclusion:** DU adequately assesses the diaphragmatic impairment in ALS patients. Thickening and excursion appear as complementary indices in the evaluation of diaphragm motility.

**Keywords:** amyotrophic lateral sclerosis; diaphragm ultrasound; pulmonary function testing.

## I. INTRODUCTION

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease characterized by loss of both upper and lower motor neurons with progressive muscle weakness. In the late stage of the disease, respiratory muscle weakness ensues, leading to hypercapnic respiratory failure, representing the most common cause of death in these patients. The condition has no definitive treatment, and its progression can be

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only partly modified by pharmacological intervention; when respiratory muscle involvement occurs, the only approach that has demonstrated an improvement in survival time and quality of life is the use of non-invasive ventilation (NIV). Early recognition of diaphragm dysfunction and respiratory muscle weakness is crucial to define the optimal timing for starting NIV [1–3]. The European Federation of Neurological Societies (EFNS) guidelines recommend initiating NIV when at least one respiratory clinical symptom plus forced vital capacity (FVC) $<80\%$  of the predicted value[4]. However, the PFTs, which represent the most common and validated parameter to assess the respiratory volumes and the need for NIV, are challenging to perform in this subset of patients, because they require cooperation with the examiner and coordination during the maneuvers. On the other, diaphragmatic ultrasound (DU) is a non-invasive, radiation-free technique to assess the diaphragm function regarding of inspiratory displacement and thickening[5]. Hence, DU could provide clinically relevant information to detect patients at risk of diaphragmatic dysfunction for whom starting the NIV is indicated. Limited literature is available [6–8] on the correlation between the data obtained at DU with those obtained at PFTs in the evaluation of diaphragmatic dysfunction in ALS patients. These studies, however, did not take in to consideration the changes in lung volumes induced by changes in body position observed in ALS patients. The aims of our study in ALS patients were: 1) confirm the utility of DU in assessing diaphragmatic function, and 2) evaluate the correlation between DU and PFTs measurements in both standing and supine position.

## II. METHODS

### a) Study design and cohort

A case-control single-centre study conducted in Rome at Umberto I Hospital. During a nine months run-in period, from May 2021 to January 2022, twenty-nine ALS patients with spinal onset, and thirteen healthy controls were enrolled. The inclusion criteria were: (1) diagnosis of ALS according to El Escorial-Revised Diagnostic Criteria[9]; (2) age  $> 18$  years old; (3) ability and collaboration to perform respiratory tests. The exclusion criteria were (1) ALS with bulbar onset,

because unable to correctly perform PFTs; (2) tracheostomy; (3) ventilatory support at the time of the enrolment (4) pregnancy; (5) breastfeeding (6) actual or previous lung diseases. Healthy controls were selected among age-comparable volunteers, with no biological relationship to patients. This study was conducted per the Declaration of Helsinki and Good Clinical Practice guidelines. The local ethics committee approved the study protocol 0897/2021, and written informed consent was obtained from all the subjects in the study.

#### b) *Measurements*

Demographic and anthropometric variables were collected in all patients at the time of enrolment.

#### c) *Pulmonary Function Testing (PFTs)*

Lung function evaluation followed the American Thoracic Society (ATS) guidelines [10,11]. The best of at least three measurements was taken to assure reproducibility. The assessment of Vital Capacity (VC) and Forced Vital Capacity (FVC), Forced Expiratory Volume during the first second (FEV<sub>1</sub>) was performed standing upright and lying in a 30° supine position, using the automated equipment microQuark (Cosmed).

#### d) *Diaphragm ultrasound (DU)*

DU evaluates diaphragm thickening and excursion [5,7,12]. It was assessed by the same observer during the run-in period using Biosound Esaote - AU5 Harmonic EPI, according to Sarwal et al. [13]:

1. Diaphragm thickness was evaluated both at FRC (Te) and at TLC (Ti) by using a B-Mode 7.5-10 Hertz linear probe. Te is the thickness at FRC, and Ti is the thickness at TLC. This technique measures, in a lying 30° supine position, the diaphragmatic thickness in the zone of apposition to the rib cage between the middle and posterior axillary line. These body and probe positions are utilized to detect the thicker part of the muscle and to minimize possible measurement errors [5,14–16].

The diaphragmatic thickening is then calculated by using two different equations:

$$\Delta \text{ Thickness } (\Delta T) = T_i - T_e$$

and

$$\text{Thickening Fraction (TF)} = [(T_i - T_e) / T_e] \times 100$$

2. The real-time diaphragmatic excursion of the right hemidiaphragm was evaluated using a right anterior subxiphoid approach in a lying 30° supine position during maximal inspiratory effort by M-Mode 3.5 Hertz convex probe [5,15].

#### e) *Statistical analysis*

The data are presented for two groups, ALS patients and controls (Table 1). Categorical variables are presented as a number of patients and percentage and

compared between groups with a chi-square test. Numerical data are presented as mean ± standard deviation (SD) or median [interquartile range (IQR)], as appropriate. Shapiro-Wilk test was used to test the normal distribution of numerical data. Student's t-test or Wilcoxon test was used to compare numerical values between the two groups, as appropriate. The correlation between respiratory function tests and diaphragmatic ultrasonographic indices was performed using the Pearson test. A value of  $p \leq 0.05$  was considered statistically significant. Receiver operating characteristic (ROC) curves were constructed to evaluate the performance of ultrasound-derived indices in predicting  $FVC < 80\%$ , one of the criteria for starting NIV. The optimal cut-point was determined as the maximal value of sensitivity plus specificity. The statistical analysis was performed with R statistical software version 4.1.

### III. RESULTS

Patient characteristics, PFTs as well as ultrasound-derived indices are shown in Table 1. No significant differences in age, sex, and BMI were observed in the two groups.

Table 1: Demographic, PFTs and ultrasonography evaluation of the study population

	Spinal ALS n=29	Controls n=13	p value
Male/female n (%)	20/9 (68/32)	6/7 (46/54)	0.06
Age (year)	60 [54-72]	46 [37-68]	0.06 <sup>§</sup>
BMI (Kg/m <sup>2</sup> )	24.2 ± 5.2	26.3 ± 4.3	p=0.16 <sup>#</sup>
FVC standing up (%)	67.5 ± 23.4	110.7 ± 12	< 0.001 <sup>#</sup>
FVC lying 30° supine position (%)	59.4 ± 27.3	106.9 ± 13	< 0.001 <sup>#</sup>
VC standing up(%)	71 [54-89]	108 [105-121]	< 0.001 <sup>§</sup>
VC lying 30° supine position (%)	64 [41-73.5]	109 [99-113]	< 0.001 <sup>§</sup>
ΔFVC (%)	10[5-26.7]	4 [2-5]	< 0.001 <sup>§</sup>
ΔVC (%)	12 [4-26]	4 [1-7]	p=0.02 <sup>§</sup>
Diaphragmatic excursion during deep breath (mm)	52.3[32.3-62]	62.3 [54.6-70.9]	p=0.04 <sup>§</sup>
TF%	44.5 [34.5-79]	67 [53-105]	p=0.05 <sup>§</sup>
ΔT	0.8 [0.6-1]	1.3 [1.1-1.9]	< 0.001 <sup>§</sup>
Ti (mm)	2.3 [2-3.2]	3.3 [2.7-4.6]	p=0.01 <sup>§</sup>
Te (mm)	1.5 [1.3-2]	1.7 [1.6-2.2]	p=0.05 <sup>§</sup>

ALS: Amyotrophic Lateral Sclerosis; BMI: Body Mass Index; FRC: Functional Residual Capacity; FVC: Forced Vital Capacity; PCF: Peak Cough Flow; SD: Standard Deviation; TF: Thickening Fraction; TLC: Total Lung Capacity; VC: Vital Capacity; Te: thickness at FRC; ΔT: Δ Thickness; Ti: Thickness at TLC.

\*p ≤ 0.05 (**in bold**) indicates significant differences

<sup>#</sup>T test expressed as mean ± SD (standard deviation)

<sup>§</sup>Wilcoxon test expressed as median [IQR]

As expected, PFTs and DU values were significantly different between the two groups, the most striking differences being ΔT, VC and FVC in a lying 30°supine and standing up position and the changes in FVC and VC when moving from standing to the supine position (i.e., three-fold greater in ALS patients).

a) Correlation between ΔT and PFTs in both ALS patients and controls

As shown in Fig.1, in ALS, ΔT significantly correlates with VC and FVC, in standing up (r=0.58

p=0.001; r=0.61 p<0.001) and lying 30° supine position (r=0.59 p=0.001; r=0.62 p<0.001), respectively. A weak correlation was observed in controls between ΔT with VC and FVC, in standing up and lying 30° supine position.

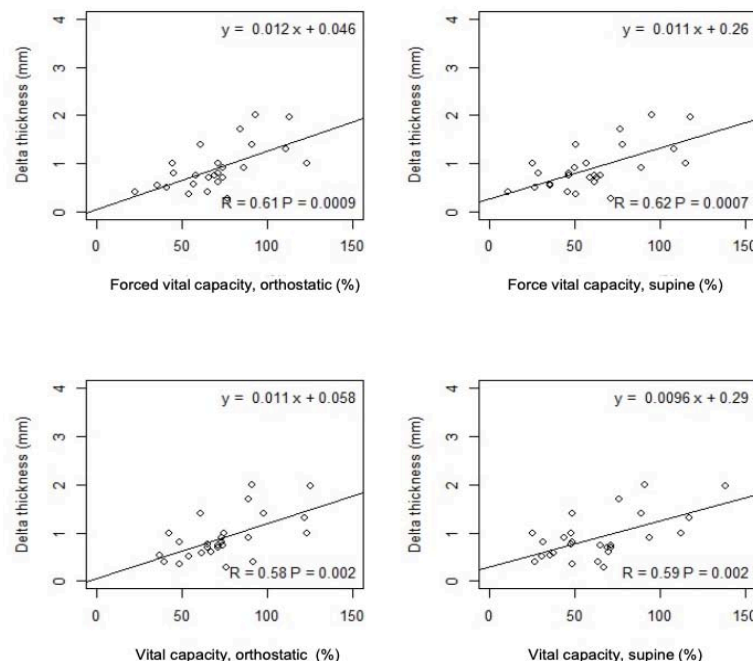


Fig.1: Correlation between ΔT and PFTs

b) Correlation between TF and PFTs in both ALS patients and controls

As shown in Fig. 2, in ALS patients but not in controls, TF significantly correlates with VC and with

FVC in standing up ( $r=0.55$   $p=0.003$ ;  $r=0.53$   $p=0.005$ ) and lying 30° supine position ( $r=0.55$   $p=0.003$ ;  $r=0.51$   $p=0.007$ ), respectively.

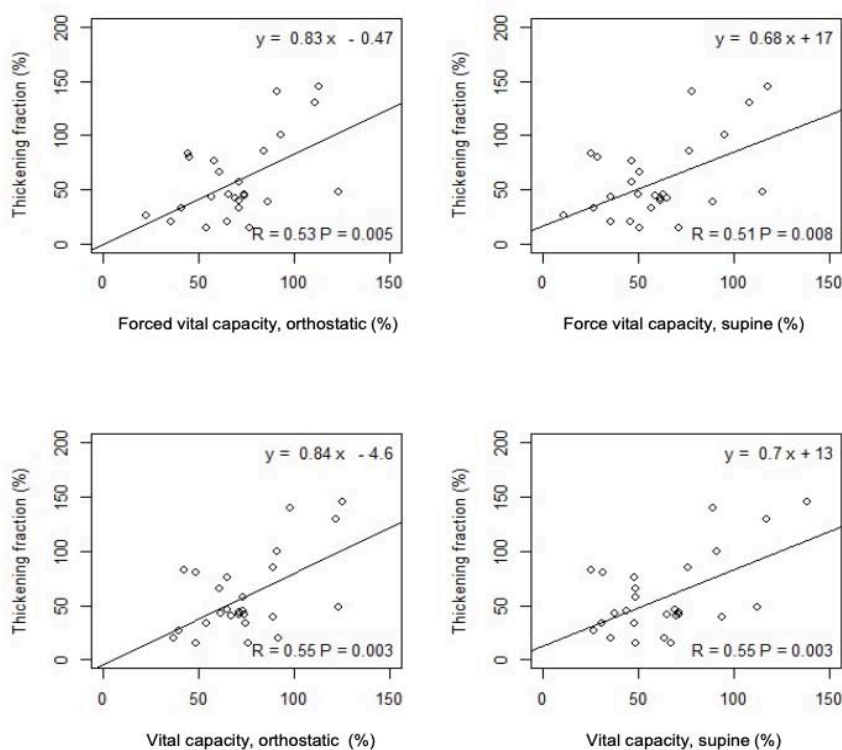


Fig. 2: Correlation between TF and PFTs

c) Correlation between diaphragmatic excursion and PFTs in both ALS patients and controls

As shown in n Fig. 3, in ALS patients but not in controls, DE significantly correlates with VC and FVC in standing up ( $r=0.55$   $p=0.007$ ;  $r=0.65$   $p<0.001$ ) and

lying 30° supine position ( $r=0.59$   $p=0.003$ ;  $r=0.63$   $p=0.001$ ), respectively. Moreover, in ALS patients, DE negatively correlated with the change in VC ( $r = -0.54$   $p = 0.01$ ) and FVC from standing up to lying 30° supine position ( $r = -0.50$   $p = 0.02$ ).

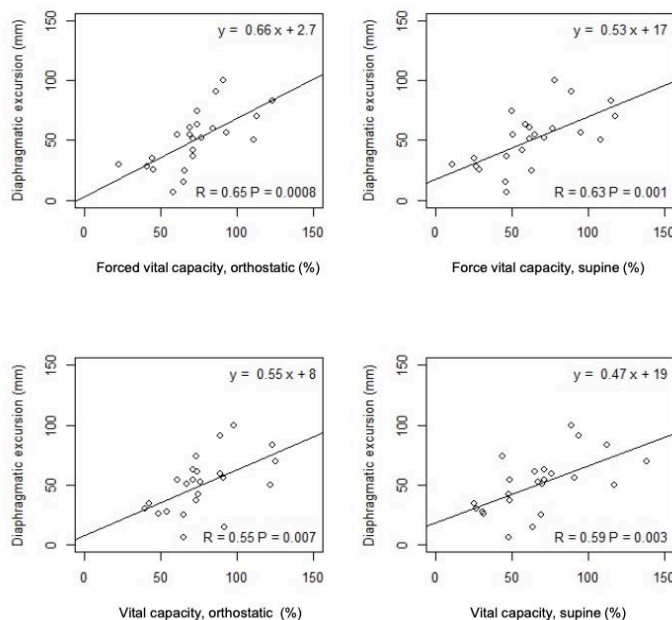


Fig. 3: Correlation between DE and PFTs

d) *ROC curve analysis: predictive value of DU indices for NIV timing initiation*

In our cohort, 23 out of 29 patients had a standing up FVC < 80% of predicted. As shown in Fig.4, in those patients, the AUC for  $\Delta T$ , TF and,

diaphragmatic excursion were 0.94 [0.87-1], 0.87 [0.69-1], 0.88 [0.72-1] respectively. The optimal cut-point found was 0.9 mm for  $\Delta T$ , 85% for TF, and 56 mm for diaphragmatic excursion.

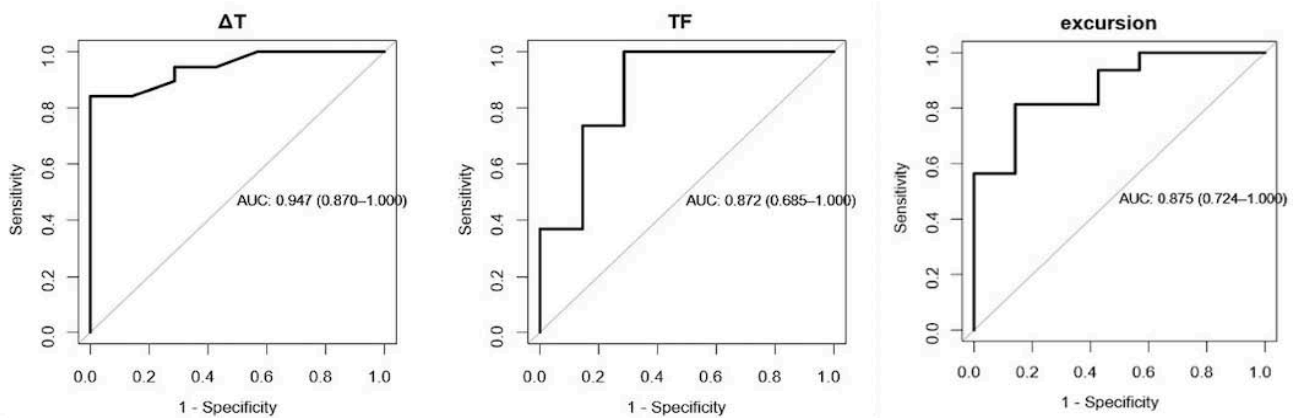


Fig. 4: ROC curve analysis: predictive value of DU indices for NIV timing initiation

#### IV. DISCUSSION

Our study has demonstrated that VC and FVC measurements, in standing up and 30° lying position, correlate with ultrasonographic diaphragmatic indices for assessing diaphragmatic dysfunction in patients with ALS and, perhaps more importantly, we found that DU indices predict with good accuracy the timing for NIV initiation. Of notice, neuromuscular diseases lead to an increased elastic load, increased alveolar surface tension and stiffness of the thoracic wall causing a reduction in maximal inspiratory strength and diaphragmatic fatigue. The best way to assess the mechanical properties of the diaphragm and its ability to generate pressure is the transdiaphragmatic pressure measured by magnetic phrenic nerve stimulation with simultaneous measurements of oesophageal and gastric pressures[17]. Because of invasiveness,  $\Delta T$  measurement at DU has been proposed as an alternative non-invasive approach evaluating of diaphragmatic contractile reserve[18,19]. In fact, in line with Fantini et al. [8], we postulate that  $\Delta T$  might express the maximal transdiaphragmatic pressure. Specifically,  $\Delta T$  cannot measure directly the pressure generated by the diaphragm shortening, but can assess how the pressure generated by a maximum inspiratory effort can short the diaphragm itself. Although not significant, maybe because of the small sample, this trend of correlation is also preserved in controls. Otherwise, in our cohort of recently diagnosed ALS patients, although TF correlates well with lung volumes in both positions, we cannot consider it an index of early diaphragmatic fatigue[16]. TF may differ based on the presentation of the disease: in acute or subacute ALS, the diaphragm may have a preserved thickness with reduced excursion

due to prominent neuropathic damage[20]; on the contrary, with a more chronic course of the disease, the diaphragm becomes thin and atrophic with poor inspiratory thickening and may even become paradoxically thinner with inspiration[21]. Indeed, in our cohort, we found a reduced baseline diaphragm thickness ( $T_e$ ) compared to the control and, TF can vary greatly depending on the value of thickening at FRC. With this perspective, we can speculate that the role of TF is different between acute/subacute ALS, with early TF reduction and normal  $T_e$  value, and chronic ALS, with a similar reduction in  $T_i$  and  $T_e$  values until late stage, maintaining a normal TF value. According to our results, also Fantini et al. [16] show that TF is an index of late stage of diaphragmatic dysfunction, that is not useful for detecting early diaphragmatic dysfunction. Moreover, no significant correlation was found between TF and lung volumes in controls, because of the stability of lung volumes in both positions measured. We also have shown that diaphragmatic excursion measured during maximal inspiratory effort significantly correlates positively with all lung volumes and negatively in position changing. At the beginning of the disease, the diaphragmatic dysfunction expressed as muscle fatigue and reduced excursion, could be compensated by accessories respiratory muscle. In this way, if there is a diaphragmatic decreased capacity to generate force and reduced excursion, this is not translated into a reduction in respiratory function tests. When the disease progress in patients with ALS, the respiratory system elastic load is much higher than the muscle strength, leading to atelectasis, reduced lung elasticity, and, finally increased elastic load. Whith this scenario, there is a definitive patient deterioration with generalized muscle weakness and, finally, reduction of lung volumes. Based

on this finding, we could speculate that DU evaluation by transabdominal approach is a feasible and reproducible technique and integrate the transthoracic approach, better reflecting a direct and dynamic evaluation of this muscle, supporting physicians in an early subclinical diagnosis of diaphragmatic dysfunction in ALS patients. According to our results, Aliberti et al.[5] evaluated diaphragmatic excursion performed bedside confirming our data. Moreover, in ALS patients, the excursion is negatively correlated with the change in VC and FVC from standing up to lying 30° supine position showing, confirming that more is the change between the two positions, less is the diaphragmatic excursion. In this study, we also investigated ROC analysis of all the DU indices ( $\Delta T$ , TF, Excursion) to identify the cut-off point to predict FVC lower than 80% of the predicted value, which is an indication for mechanical ventilation, following the current consensus guidelines[4,10]. Interestingly, when the  $\Delta T$  is lower than 0.9 mm and the diaphragmatic excursion during a deep breath is lower than 56 mm predicts diaphragmatic dysfunction in patients who are not able or cooperative to perform respiratory functional tests, and could guide clinicians in the decision to start NIV. An important limitation of our study is the absence of a comparison between the DU measurement and the gold standard method used to assess its contractility, transdiaphragmatic pressure measured by phrenic nerve stimulation, because it was invasive and not easy to perform in routine clinical practice. Another considerable limitation is the small sample size, there was not easy to recruit vulnerable patients because of their physical and emotional frailty. Another limitation was the lack of inter and intra-variability analysis of the DU performed. Finally, the absence of solid literature references about our indices needs further research to confirm and corroborate the feasibility of our findings.

## V. CONCLUSION

In summary, the relevance of finding out an index of early diaphragmatic dysfunction is mandatory in patients with neuromuscular diseases who cannot perform traditional lung function tests. If TF is affected by the baseline value of diaphragm thickness, the DU indices  $\Delta T$  and diaphragmatic excursion are independent of this value and could provide specific information about muscle fatigue and an early diaphragmatic dysfunction before the exhausting muscle phase become clinically evident. This finding thus opens new perspectives on the use of this imaging technique in the early diagnosis and follow-up of diaphragmatic function impairment.

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*Conflict of Interest:* None of the authors have potential conflicts of interest to be disclosed" should appear in the paper.

*Data Availability Statement:* The data that support the findings are available in the Supplementary material.

*Human/Animal Ethics Approval Declaration:* The local ethics committee approved study protocol number 0897/2021 and written informed consent was obtained from all the subjects in the study.

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### Abbreviations

ALS: Amyotrophic Lateral Sclerosis  
 ATS: American Thoracic Society  
 BMI: Body Mass Index  
 DU: diaphragm ultrasound  
 EFNS: European Federation of Neurological Societies  
 FEV<sub>1</sub>: Forced Expiratory Volume during the first second  
 FRC: Forced Residual Capacity  
 FVC: Forced Vital Capacity  
 IQR: Intequartile Range  
 NIV: Non-invasive ventilation  
 PFTs: Pulmonary Function Testing  
 ROC: Receiver operating characteristic  
 SD: Standard Deviation  
 Te: thickening at FRC  
 TF: thickening fraction  
 Ti: thickening at TLC  
 TLC: Total Lung Capacity  
 VC: Vital Capacity  
 $\Delta T$ : delta-thickness  
 $\Delta FVC$ : the change in FVC from standing upright to supine position  
 $\Delta VC$ : the change in VC from standing upright to supine position

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