Multiparametric Analysis of Sniff Nasal Inspiratory Pressure Test in Middle Stage Amyotrophic Lateral Sclerosis

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The relaxation rates and contractile properties of inspiratory muscles are altered with inspiratory muscle weakness and fatigue. This fact plays an important role in neuromuscular disorders patients and had never been extensively studied in amyotrophic lateral sclerosis (ALS). In this cross-sectional study, these parameters were investigated non-invasively through nasal inspiratory sniff pressure test (SNIP) in 39 middle stage spinal onset ALS subjects and compared with 39 healthy controls. ALS patients were also divided into three subgroups according to a decline in their percentage of predicted forced vital capacity (FVC%pred) as well as a decline in the ALS functional rating scale score and its respiratory subscore (R-subscore) in order to determine the best parameter linked to early respiratory muscle weakness. When compared with healthy subjects, middle stage ALS subjects exhibited a significantly lower (p < 0.0001) maximum relaxation rate and maximum rate of pressure development (MRPD), as well as a significantly higher (p < 0.0001) τ (τ), contraction time, and half-relaxation time. The results from receiver operating characteristic curves showed that MRPD (AUC 0.735, p < 0.001) and FVC%pred (AUC 0.749, p = 0.009) were the best discriminator parameters between ALS patients with ≤30 and >30 points in the ALS functional rating scale. In addition, 1/2RT (AUC 0.720, p = 0.01), FVC%pred (AUC 0.720, p = 0.01), τ (AUC 0.824, p < 0.0001), and MRPD (AUC 0.721, p = 0.01) were the parameters more sensitive in detecting a fall of three points in the R-subscore. On the other hand, MRPD (AUC 0.781, p < 0.001), τ (AUC 0.794, p = 0.0001), and percentage of predicted of SNIP (AUC 0.769, p = 0.002) were the parameters able to detect a fall in 30% of the FVC%pred in middle stage ALS patients. The contractile properties and relaxation rates of the diaphragm are altered in middle stage spinal onset ALS when compared with healthy subjects. These parameters are able to discriminate between those middle stage ALS subjects with early decline in inspiratory muscle function and those who not.

Keywords: amyotrophic lateral sclerosis, forced vital capacity, inspiratory muscle weakness, relaxation rates, respiratory subscore, sniff nasal inspiratory pressure
INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a rare neurodegenerative disorder characterized by progressive weakness of the skeletal and respiratory muscle (1). The median survival from first symptoms ranges from 2 to 4 years (2), and although respiratory insufficiency can be present in approximately 3% of patients (3, 4), it frequently emerges in the late phase of the disease representing the most frequent cause of death (1).

Global assessment scores, such as the ALS functional rating scale (5) (ALSFRS-R), is a useful and valid parameter in predicting survival in this population (6, 7) and has proved to be related to forced vital capacity (8) (FVC). Since respiratory function and muscle strength are of clinical importance and represent crucial factors influencing survival in ALS (9, 10), the monitoring of these parameters is essential during disease progression. The gold standard measurement of respiratory muscle strength involves the insertion of esophageal and/or gastric balloon catheters through the nose (11). However, the sniff nasal inspiratory pressure (SNIP) has been proposed as a non-invasive alternative method and proved to accurately reflect diaphragm strength (12) and global inspiratory muscle strength (13).

In ALS patients, the already weakened respiratory muscles are easily suitable to fatigue (14) and this fact may play an important role in the development of ventilatory failure (15). It has been demonstrated that the relaxation rate of inspiratory muscles is altered by the development of inspiratory muscle fatigue (16, 17) and that relaxation rates obtained from a maximal sniff accurately reflect those obtained from esophageal pressure (16, 18). Relaxation rates can be described in terms of maximum relaxation rate (MRR), half-relaxation time (1/2RT), and time constant of the pressure decay curve (t, tau) after voluntary contraction of a muscle (16). Furthermore, the contractile properties of the diaphragm [namely, maximum rate of pressure development (MRPD) and contraction time (CT)] are also altered in fatigue and have been used as an index of the motor output of the respiratory center (19) as well as to assess inspiratory muscle function (11, 20, 21).

Apart from fatigue in healthy subjects (16–18, 22–24), physiological and/or disease-related changes in diaphragm relaxation have not been extensively investigated in ALS patients through the SNIP test. The present work aimed to non-invasively measure the relaxation rates and the contractile properties of the inspiratory muscles in ALS patients through SNIP test (1) in comparison to healthy subjects and (2) in relation to early respiratory symptoms in order to determine the best parameter linked to early respiratory muscle weakness. We hypothesized that these parameters are altered in ALS patients and can be indicators of inspiratory muscle weakness.

MATERIALS AND METHODS

Subjects

This cross-sectional study was conducted according to the World Medical Association Declaration of Helsinki and approved by the Research Ethics Committee under number 1.344.512/2015. All individuals involved in the study signed an informed consent form.

We investigated 39 subjects with ALS (22 males), recruited from the Hospital Universitário Onofre Lopes and diagnosed by a neurologist according to the El Escorial criteria (25) as “Probable or definite,” and 39 healthy controls (19 males). ALS subjects with cardiovascular, pulmonary, or other neurological diseases, as well as with bulbar dysfunction signs or tracheostomy were not included. Those who failed to perform the assessments or refuse to participate in the study were excluded.

Control group included self-reported age-matched healthy subjects with no history of cardiovascular, neurological or pulmonary diseases. Those with FVC and FEV₁ <80% of predicted were excluded.

Spirometry

Spirometry was performed using a Koko Digidoser spirometer (nSpire Health, Longmont, CO, USA) and carried out with the subjects positioned sitting on a chair with feet supported and trunk flexion of 90° according to the ATS/ERS guidelines (11). All values obtained were compared with absolute and percentage of predicted values for the Brazilian population (26).

Respiratory Muscle Strength

Maximum inspiratory and expiratory pressures [maximum inspiratory pressure (MIP) and maximum expiratory pressure (MEP), respectively] and SNIP were measured using a digital manometer (NEPEB-Labcare, Belo Horizonte) with the subjects seated on a chair. MIP was measured starting from residual volume and MEP from total lung capacity, while SNIP was performed starting from functional residual capacity (FRC) (27). Data obtained were compared with previous reference values (28, 29), and the highest value of each test was considered for analysis.

SNIP Curve Analysis

All subjects were asked to perform a short, sharp inspiratory effort through the nostrils with lips closed. Since some sniff parameters can be affected by changes in muscle length and the activity of expiratory muscles could interfere in the analysis, the sniff maneuvers were performed from FRC and a passive relaxation right after reaching the peak of pressure was requested (23, 30). At least 10 maximal sniffs, with an interval of about 30 s in between, were performed by all subjects. The following criteria were used to select those sniffs suitable for analysis: (1) sniff performed from FRC; (2) peak pressure maintained for less than 50 ms; (3) duration of the inspiratory effort less than 500 ms; and (4) sniff pressure waveform with smooth decay curve (16, 31). Figure 1 shows the parameters derived from the SNIP test. From the sniff maneuver trace, CT and 1/2RT were calculated as the time to reach the peak pressure of the sniff and the half-time of the relaxation curve, respectively (32). MRPD, expressed as cmH₂O·ms⁻², was calculated as the negative peak of the first derivative of pressure–time curve (21, 33) while MRR, expressed as milliseconds⁻¹, was defined as the positive peak of the first derivative of pressure–time curve normalized to the sniff peak pressure, in order to make contractions of different intensities comparable (18).
Figure 1 | Representative tracings of the sniff nasal inspiratory pressure (SNIP) test and its parameters. (A) Tracings of SNIP change; peak sniff pressure ($P_{sniff}$); time to reach $P_{sniff}$, contraction time (CT); and half-time of the relaxation curve ($1/2RT$). (B) Derivative signal of sniff pressure ($dP_{sniff}/dT = \text{cmH}_2\text{O}/\text{ms}$); negative peak $dP_{sniff}/dT$, maximum rate of pressure development (MRPD) positive peak $dP_{sniff}/dT$ normalized by $P_{sniff}$, maximum relaxation rate (MRR). (C) Decay portion of the sniff pressure plotted on semilog scale vs. time (ms). Linear black portion indicates a single exponential function with a time constant, $\tau = 1/$slope. cmH$_2$O, centimeters of water.

The time constant ($\tau$), was also calculated. When the natural logarithm of pressure is plotted as a function of time, the lower 50–70% of the pressure decay follows a straight line (18, 34) (Figure 1C), indicating that the pressure follows a monoexponential decay with a time constant $\tau$ ($\tau = 1/$slope). For the measurement of $\tau$ to be accepted, the correlation coefficient of the individual regression line (ln P vs. time) had to be $\geq 0.96$ (35).

Sniff nasal inspiratory pressure curve analysis was performed by custom software developed in MATLAB (The MathWorksInc, Natick, MA, USA).

Functionality and Stage of the Disease
Functionality was measured using the ALSFRS-R (maximum 48 points), validated for the Brazilian population (5), as well as its respiratory subscore (R-subscore) alone (36) (maximum 12 points). In addition, the stage of the disease was determined according to disease progression proposed by Roche et al. (37).

Statistical Analysis
To statistical analysis, data from ALS subjects were divided into three subgroups, defined by the degree of decline of the (1) respiratory function (2, 38, 39) ($\leq$70 and $>70$ of FVC$_{pred}$), (2) ALSFRS-R total score ($\leq$30 and $>30$ points), and (3) R-subscore ($\leq$9 and $>9$ points) (40, 41).

Data are expressed as median [25–75th percentile] unless otherwise stated. Normality and distribution of data were performed using Shapiro–Wilk test. Data between ALS and healthy subjects (intergroup analysis) were studied using the unpaired
$t$-test or Mann–Whitney test for parametric and non-parametric data, respectively. One-way ANOVA or Kruskal–Wallis test was applied to compare subgroup with control group data and, in the event of statistical significance; Bonferroni’s or Dunn’s *post hoc* test was applied, respectively, to identify differences between groups.

To avoid type II error, the power of the study was calculated as well as effect sizes for all data. For parametric data, effect sizes were calculated using Cohen’s $d$ for intergroup analysis and Cohen’s $f$ for subgroup analysis (42). For non-parametric data, Cohen’s $d$ was calculated for intergroup analysis according to Fritz et al. (43) and $\chi^2$ for subgroup analysis according to Tomczak and Tomczak (44) (see Supplementary Material).

Receiver operating characteristic (ROC) curves were calculated for SNIP parameters between middle stage ALS and healthy subjects, as well as between subgroups. The area under the curve (AUC) and its 95% confidence interval were calculated for SNIP parameters between middle stage ALS and healthy subjects, as well as between subgroups. The area under the curve (AUC) and its 95% confidence interval were calculated for intergroup analysis according to Tomczak and Tomczak (44) (see Supplementary Material).

Inferential data analysis was performed using GraphPad Prism® software version 6.01. The power of the study and effect sizes were analyzed using G*Power software, version 3.1.9.2 (Kiel, Germany), and ROC curves were analyzed using MedCalc (Ostend, Belgium) version 14.8.1. For all statistical analysis, a $p$-value of $<0.05$ (two-sided) was considered as statistically significant.

**RESULTS**

Data related to diagnosis criteria, region of onset, clinical phenotype as well as the presence of familial ALS and cognitive impairment of all ALS included in the study are shown in Table S1 in Supplementary Material. Anthropometric, spirometric, respiratory muscle strength, and functionality data are shown in Table 1. ALS subjects were characterized by significant lower spirometric and respiratory muscle strength values. All ALS subjects were classified as middle stage. The mean ALSFRS-R score was 32.5 ± 8.8 (67.7 ± 18.3%), and the mean R-subscore was 10 ± 2 (83.3 ± 16.6) (see Table S2 in Supplementary Material).

All parameters extracted from the sniff curve were significantly different between ALS and healthy subjects. A significantly lower MRR ($p < 0.0001$, Cohen’s $d = 0.44$) and MRPD ($p < 0.0001$, Cohen’s $d = 0.71$) were found in ALS subjects, as well as a higher CT ($p < 0.0001$, Cohen’s $d = 1.21$), 1/2RT ($p < 0.0001$, Cohen’s $d = 0.42$), and $\tau$ ($p < 0.0001$, Cohen’s $d = 0.64$) (Figure 2).

A *post hoc* analysis considering a $p$-value of $<0.01$ and the calculated effect size for $\tau$ between ALS and healthy subjects (Cohen's $d = 0.64$) showed a statistical power ($1 - \beta$) = 0.99 for this study.

**ALSFRS-R, R-Subscore, and FVC%pred Subgroups**

As shown in Table 2, all subgroups of ALS subjects presented a lower FVC%pred, SNIP%pred, MRR, and MRPD and a higher CT, 1/2RT, and $\tau$ when compared with healthy subjects. However, subjects with functional capacity ≤30 (13 subjects) exhibited significantly lower values of FVC%pred when compared to ALS subjects with >30 points; and those with ≤9 (14 subjects) presented a significantly lower FVC%pred as well as higher 1/2RT and $\tau$ values when compared to those with >9 points. On the other hand, when ALS subjects were classified according to FVC%pred, those with <70% exhibited significantly higher $\tau$ and lower SNIP%pred values when compared to ALS with values >70%.

**ROC Analysis**

Since SNIP%pred is one of the respiratory prognostic markers mostly considered in ALS (38, 46), this parameter was also included in the ROC analysis. As shown in Table 3, all sniff parameters were significantly able to discriminate between ALS and healthy. Of these, MRPD was the parameter with the highest AUC. When dividing the ALS subjects between those with ALSFRS-R score ≤30 and >30, only the MRPD and FVC%pred were statistically significant (Table 4). However, when taking into account the subdivision between those ALS with R-subscore ≤9 and >9 points, MRPD, 1/2RT, $\tau$, and FVC%pred showed to be statistically significant (Table 5). On the other hand, MRPD, $\tau$, and SNIP%pred parameters were statistically significant when subjects were classified according to FVC%pred classification (Table 6; Figure 3).

**DISCUSSION**

The main findings of this study are that (1) the sniff test provides parameters, apart from its peak pressure, able to discriminate...
between healthy and middle stage ALS subjects, and that (2) some of these parameters, namely $\tau$, MRPD, and 1/2RT, are more sensitive in detecting impaired inspiratory muscle function in ALS than the peak pressure itself.

According to Kyroussis et al. (22), measurements of relaxation rates obtained from nasal sniffs accurately reflect those from esophageal pressure curves and can be used as an index of the onset and recovery of respiratory muscle fatigue. Moreover, measurements of nasal sniffs are simple, tolerated, and minimally invasive and can provide a quantitative response index to fatigue and therapeutic interventions in neuromuscular disease patients (47, 48). In our study, all parameters derived from the

**FIGURE 2** Data are shown as median [25-75th percentile]. Comparisons between the parameters obtained from the sniff nasal inspiratory pressure (SNIP) curve [maximum relaxation rate (MRR), maximum rate of pressure development (MRPD), contraction time, half-relaxation time (1/2RT), and tau ($\tau$)] and percentage of predicted of the SNIP test (SNIP%pred) between amyotrophic lateral sclerosis (ALS) and healthy subjects. cmH$_2$O: centimeters of water; +, mean for parametric analysis.
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Table 2: Relaxation rates and contraction properties of the diaphragm extracted from the SNIP curve of healthy and ALS subgroup subjects.

| Parameter | Healthy | ALS | ES | P-value |
|-----------|---------|-----|----|---------|
| MRR (ms)  | 0.0067 [0.0056 - 0.0078] | 0.0054 [0.0049 - 0.0062] | -0.19 | <0.001 |
| MRPD (cmH2O·ms) | 0.0065 [0.0061 - 0.0069] | 0.0071 [0.0063 - 0.0074] | 0.05 | 0.0078 |
| τ (ms)    | 50.4 [42.3 - 58.8] | 78.6 [57.0 - 121.1] | 0.19 | <0.001 |
| RT (ms)   | 142 [116 - 168] | 174 [138 - 204] | 0.25 | <0.001 |
| FVC (%)   | 97.5 [91.5 - 107.5] | 68.3 [54.3 - 82.4] | 0.44 | <0.001 |

Values are shown as median [25th–75th percentile].

The ALSFRS-R is a simple and reliable scale that predicts survival and can be used as the only functional outcome measure in early phase trials (40), while its R-subscore was designed to assess indirectly the respiratory function (36) being also sensitive in detecting early respiratory symptoms of ventilatory insufficiency (57–59). Castrillo-Viguera et al. (41) suggested that a percentage change of at least 20–25% in the slope of decline of the ALSFRS-R scale would represent a clinically meaningful...
### Table 3: Receiver operating characteristic analysis between healthy and ALS subjects.

|                              | Healthy and ALS                                                                 |
|------------------------------|---------------------------------------------------------------------------------|
|                              | AUC (95% CI) | Optimal cutoff (95% CI) | Sensitivity (%) | Specificity (%) | p     |
| MRR (ms⁻¹)                  | 0.755 (0.645 to 0.845) | 0.0073 (0.0068 to 0.0073) | 66.67          | 89.74          | <0.0001 |
| MRPD (cmH₂O ms⁻¹)           | 0.916 (0.830 to 0.967) | −0.420 (−0.540 to −0.398.5) | 74.36          | 97.44          | <0.0001 |
| τ (ms)                      | 0.874 (0.779 to 0.938) | 66 (53.7 to 79.8) | 74.36          | 89.74          | <0.0001 |
| 1/2 RT (ms)                 | 0.743 (0.631 to 0.835) | 154 (120.9 to 164) | 71.79          | 71.79          | <0.0001 |
| CT (ms)                     | 0.795 (0.688 to 0.878) | 215 (202 to 262) | 92.31          | 84.62          | <0.0001 |
| SNIP (%pred)                | 0.936 (0.856 to 0.979) | 81.5 (73.7 to 81.5) | 79.49          | 97.44          | <0.0001 |
| FVC (%pred)                 | 0.911 (0.825 to 0.964) | 81.1 (67.9 to 83.7) | 79.49          | 97.44          | <0.0001 |

AUC, area under curve; CI, confidence interval; MRR, maximum relaxation rate; MRPD, maximum rate of pressure development; τ, tau; 1/2RT, half-relaxation time; CT, contraction time; SNIP, sniff nasal inspiratory pressure; FVC, forced vital capacity; ES, effect size; %pred, percentage of predicted; cmH₂O, centimeter of water; ALS, amyotrophic lateral sclerosis.

### Table 4: Receiver operating characteristic analysis between ALS subjects classified according to a decrease in the ALSFRS-R scale score.

|                              | ALS—ALSFRS-R                                                                 |
|------------------------------|--------------------------------------------------------------------------------|
|                              | AUC (95% CI) | Optimal cutoff (95% CI) | Sensitivity (%) | Specificity (%) | p     |
| MRR (ms⁻¹)                  | 0.533 (0.366 to 0.694) | 0.0053 (0.0031 to 0.0073) | 30.77          | 100            | 0.779 |
| MRPD (cmH₂O·ms⁻¹)           | 0.735 (0.570 to 0.863) | −0.303 (−0.535.7 to −0.232.1) | 76.92          | 73.98          | <0.001 |
| τ (ms)                      | 0.655 (0.486 to 0.800) | 89.08 (48.5 to 147.6) | 69.23          | 61.24          | 0.094 |
| 1/2 RT (ms)                 | 0.506 (0.341 to 0.669) | 160 (106 to 206) | 76.92          | 42.31          | 0.853 |
| CT (ms)                     | 0.648 (0.479 to 0.794) | 250 (198 to 282) | 76.92          | 65.38          | 0.118 |
| SNIP (%pred)                | 0.618 (0.449 to 0.769) | 46.33 (17.4 to 67.0) | 76.92          | 53.85          | 0.194 |
| FVC (%pred)                 | 0.749 (0.584 to 0.873) | 41.7 (25.3 to 106.3) | 53.85          | 92.31          | 0.009 |

ALSFRS-R, amyotrophic lateral sclerosis functional rating scale-revised; AUC, area under curve; CI, confidence interval; MRR, maximum relaxation rate; MRPD, maximum rate of pressure development; τ, tau; 1/2RT, half-relaxation time; CT, contraction time; SNIP, sniff nasal inspiratory pressure; FVC, forced vital capacity; ES, effect size; %pred, percentage of predicted; cmH₂O, centimeter of water; ALS, amyotrophic lateral sclerosis.

### Table 5: Receiver operating characteristic analysis between ALS subjects classified according to a decrease in the respiratory subscore (R-subscore) of the ALSFRS-R scale.

|                              | ALS—R-subscore                                                                 |
|------------------------------|--------------------------------------------------------------------------------|
|                              | AUC (95% CI) | Optimal cutoff (95% CI) | Sensitivity (%) | Specificity (%) | p     |
| MRR (ms⁻¹)                  | 0.654 (0.485 to 0.799) | 0.0065 (0.0053 to 0.0086) | 64.29          | 72             | 0.130 |
| MRPD (cmH₂O·ms⁻¹)           | 0.721 (0.555 to 0.853) | −0.300 (−0.500 to −0.288) | 71.43          | 72             | 0.01  |
| τ (ms)                      | 0.824 (0.669 to 0.927) | 89.1 (70.1 to 168) | 85.71          | 72             | <0.0001|
| 1/2 RT (ms)                 | 0.720 (0.553 to 0.852) | 160 (158 to 256) | 92.88          | 52             | 0.01  |
| CT (ms)                     | 0.657 (0.488 to 0.801) | 232 (199 to 280) | 78.57          | 64             | 0.08  |
| SNIP (%pred)                | 0.614 (0.445 to 0.765) | 67 (60 to 67) | 100            | 32             | 0.216 |
| FVC (%pred)                 | 0.700 (0.532 to 0.836) | 67.5 (63.7 to 106) | 85.71          | 52             | 0.03  |

ALSFRS-R, amyotrophic lateral sclerosis functional rating scale-revised; AUC, area under curve; CI, confidence interval; MRR, maximum relaxation rate; MRPD, maximum rate of pressure development; τ, tau; 1/2RT, half-relaxation time; CT, contraction time; SNIP, sniff nasal inspiratory pressure; FVC, forced vital capacity; ES, effect size; %pred, percentage of predicted; cmH₂O, centimeter of water; ALS, amyotrophic lateral sclerosis.

### Table 6: Receiver operating characteristic analysis between ALS subjects classified according to a decrease in FVC.

|                              | ALS—FVC %pred                                                                 |
|------------------------------|--------------------------------------------------------------------------------|
|                              | AUC (95% CI) | Optimal cutoff (95% CI) | Sensitivity (%) | Specificity (%) | p     |
| MRR (ms⁻¹)                  | 0.572 (0.485 to 0.799) | 0.0086 (0.0053 to 0.0086) | 95.83          | 26.67          | 0.467 |
| MRPD (cmH₂O·ms⁻¹)           | 0.781 (0.555 to 0.853) | −0.460 (−0.500 to −0.288) | 95.83          | 53.33          | <0.001 |
| τ (ms)                      | 0.794 (0.669 to 0.927) | 73.1 (70.1 to 168) | 79.17          | 73.33          | 0.0001|
| 1/2 RT (ms)                 | 0.632 (0.553 to 0.852) | 174 (158 to 256) | 62.50          | 73.33          | 0.162 |
| CT (ms)                     | 0.536 (0.488 to 0.801) | 304 (199 to 280) | 100            | 73.33          | 0.713 |
| SNIP (%pred)                | 0.769 (0.445 to 0.765) | 46.7 (60 to 67) | 79.17          | 73.33          | 0.002 |

AUC, area under curve; CI, confidence interval; MRR, maximum relaxation rate; MRPD, maximum rate of pressure development; τ, tau; 1/2RT, half-relaxation time; CT, contraction time; SNIP, sniff nasal inspiratory pressure; FVC, forced vital capacity; ES, effect size; %pred, percentage of predicted; cmH₂O, centimeter of water; ALS, amyotrophic lateral sclerosis.
effect. Because of this, we chose to subdivide the ALS subjects into those with ALSFRS-R of ≤30 and >30 points (decline of 15 points—37.5%) and with the R-subscore of ≤9 and >9 points (decline of 3 points—25%). Moreover, as changes in FVCpred over time strongly predicts respiratory muscle weakness, ventilatory failure and death in ALS (2, 38, 39), subjects were also subdivided into ≤70 and >70% FVCpred subgroups.

The value of the FVCpred was the only parameter that differed between middle stage ALS subjects of both ALSFRS-R and R-subscore subgroups, possibly because the decrease of this parameter is not related only to respiratory musculature function (36, 60). On the other hand, when subdividing according to the R-subscore, 1/2RT, and τ values were significantly different between middle stage ALS subjects which demonstrate that these diaphragmatic properties (32, 61) are probably related to the respiratory function assessed by this subdomain. Presumably, the most interesting fact is that SNIPpred, a parameter that reflects the diaphragmatic strength and predicts survival in ALS (62), only differ between those middle stage ALS subjects which demonstrate that these diaphragmatic properties (32, 61) are probably related to the respiratory function assessed by this subdomain. Therefore, the most interesting fact is that SNIPpred, a parameter that reflects the diaphragmatic strength and predicts survival in ALS (62), only differ between those middle stage ALS subjects which demonstrate that these diaphragmatic properties (32, 61) are probably related to the respiratory function assessed by this subdomain. Although data were collected in a single point of the disease stage, it is known that the peak pressure of sniff test declines less when compared to the decline in ALSFRS-R (8) leading us to consider that SNIPpred is not a parameter that is sensitive to small changes in the ALSFRS-R and R-subscore. Regarding MRPD and τ, the results were not surprising since the first is related to respiratory muscle function (11) as well as related to neural adaptations (19, 20, 63) and the second increases well before diaphragmatic pressure is reduced during respiratory muscle weakness or fatigue (17, 34).

The results of the ROC curves show that all parameters extracted from the sniff curve can highly discriminate middle stage ALS from healthy subjects. When taking into account the functional decline of ALS subjects, only MRPD and FVCpred could predict a fall in 37.5% of the ALSFRS-R score. Among all parameters, τ provides the highest discriminative power in predicting a decline of 25% in the R-subscore. This power was even higher than FVCpred, possibly because the R-subscore is less sensitive in predicting a fall in FVCpred (57). Moreover, as ALS patients with R-subscore <11 points are considered with relevant symptoms of respiratory distress as well as at risk of respiratory insufficiency (57, 58) and peak pressure of sniff test could not detect a fall in the ALSFRS-R and R-subscore, we believe that the SNIPpred might not be a parameter as reliable as some parameters extracted from the SNIP curve (i.e., τ, MRPD, and 1/2RT) or FVCpred in detecting a clinically meaningful decline in functional and respiratory status. The SNIPpred was reliable in detecting respiratory muscle weakness (39, 60) in our middle stage ALS subjects only when considering the FVCpred classification; nevertheless, MRPD and τ parameters were still more sensitive than SNIPpred.

It is unlikely that the results found are investigator related since all measurements were performed by the same experienced respiratory physiotherapist. We believe that four are the main limitations of the study. First, even with a calculated statistical power of 1 − β = 0.99, our ALS cohort may be limited in terms of sample size; second, the mean age of ALS included is lower than those of the main epidemiological studies (64, 65); third, we included only ALS patients at middle stage of the disease; and fourth, not all subjects could be paired by the same exact age and BMI. Further studies including patients with other motor neuron disorders are needed. Finally, ongoing longitudinal studies are already investigating these parameters during varying levels of disease progression in order to identify differences between patients with and without the need for non-invasive ventilation as well as the optimal parameter and its cutoff point able to predict an appropriate timing for the initiation of non-invasive ventilation.

In terms of clinical applicability, the calculation of the SNIP curve parameters can be easily performed and give more information about the state of the respiratory muscles, thus possibly allowing an early detection of weakness or fatigue before respiratory failure is reached (35, 53) as well as early implementation of new therapeutic interventions before the beginning of the peak pressure decay of the SNIP curve (17, 31, 51).
CONCLUSION

The contractile properties and relaxation rates of the diaphragm are altered in middle stage spinal onset ALS when compared with healthy subjects. When assessed through the nasal inspiratory sniff test, these parameters are able to discriminate between those ALS subjects with early decline in inspiratory muscle function and those who not. In addition, despite the limitations of our cohort and especially the lack of longitudinal data, we suggest ALS subjects with early decline in inspiratory muscle function sniff test, these parameters are able to discriminate between those healthy subjects. When assessed through the nasal inspiratory pressure or flow training in humans.

ETHICS STATEMENT

All procedures performed in studies involving human participants were in accordance with the recommendations of the National Health Council in Brazil according to the resolution 466/12. The protocol was approved by the Research Ethics Committee of the Hospital Universitário Onofre Lopes under number 1.344.512/2015. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

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AUTHOR CONTRIBUTIONS

AS, GF, and VR designed the study. GF, VR, and AA supervised the clinical study. AS, LM, and MD-J collected the data and performed clinical assessment. AS, GF, FP, VR, and AA analyzed/interpreted the data. AS wrote the manuscript, and GF, LM, FP, MD-J, VR, and AA revised the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at https://www.frontiersin.org/articles/10.3389/fneur.2018.00306/full#supplementary-material.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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