



Ultrasound assessment of diaphragm function in patients with late-onset Pompe disease

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Abstract

Introduction Late-Onset Pompe Disease (LOPD) is characterized by progressive limb-girdle muscle weakness and respiratory dysfunction. Diaphragm is the most impaired muscle in LOPD and its dysfunction cause major respiratory symptoms. The aim of this study was to evaluate the correlation between diaphragm thickness and mobility assessed by ultrasonography and respiratory function and muscle strength tests in patients with LOPD.

Methods 17 patients with LOPD (9 female, 47 ± 15 years) and 17 age and gender-matched healthy controls underwent spirometry, muscle strength testing, and ultrasound evaluation of diaphragm excursion and thickness.

Results The following parameters were significantly reduced in LOPD patients versus controls (all $p < 0.001$): forced vital capacity (FVC) in seated and supine position, maximum inspiratory and expiratory pressure (MIP and MEP), diaphragm excursion, thickness at functional residual capacity (FRC) and total lung capacity (TLC), and thickness fraction (TF). Ultrasound studies of diaphragm thickness at FRC correlated with MIP ($r = 0.74$; $p < 0.0001$) and seated FVC ($r = 0.73$; $p < 0.05$). Diaphragm thickness at TLC correlated with MIP ($r = 0.85$; $p < 0.0001$) and FVC in both seated ($r = 0.77$; $p < 0.0001$) and supine position ($r = 0.68$; $p < 0.05$). TF correlated significantly with MIP ($r = 0.80$; $p < 0.001$), FVC in both seated ($r = 0.66$; $p < 0.005$) and supine position ($r = 0.61$; $p < 0.05$). Interestingly diaphragm thickness at FRC correlated with disease duration (years) in LOPD patients ($r = -0.53$; $p < 0.05$). Ultrasound diaphragm mobility correlated with diaphragm thickness at TLC ($r = 0.87$; $p < 0.0001$), FRC ($r = 0.84$; $p < 0.005$) and TF ($r = 0.73$; $p < 0.05$). Moreover diaphragm mobility correlated with FVC in seated ($r = 0.79$; $p < 0.005$) and supine position ($r = 0.74$; $p < 0.05$) and MIP ($r = 0.81$; $p < 0.005$).

Conclusion Diaphragm ultrasonography is a simple and reproducible technique for manage respiratory dysfunction in LOPD patients.

Keywords Diaphragm · Late-onset Pompe disease · Lung function · Ultrasonography · Respiratory muscle strength

PR and LLM have contributed equally to the study conception and design. Material preparation, data collection, and analysis were performed by PR, LLM, OM, and GT. The first draft of the manuscript was written by PR and LLM and all authors commented on previous versions of the manuscript. MG, GC, and AT provided a critical review, commentary, and revision. All authors read and approved the final manuscript.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Introduction

Late-Onset Pompe Disease (LOPD), also known as glycogen storage disease type II (GSDII), is an autosomal recessive inherited disorder, due to a largely reduced activity of the lysosomal acid alpha-glucosidase (GAA). The deficiency of GAA results in a failure of lysosomal glycogen degradation, leading to a progressive accumulation in several tissues and organs [1, 2]. Progressive limb-girdle weakness and respiratory dysfunction characterize LOPD patients [3]. Enzyme replacement therapy (ERT) with recombinant human acid α -glucosidase (rhGAA) is available since 2006 [4, 5] and may improve or stabilize motor and respiratory function [6]; it has also been suggested that an early initiation of ERT could delay muscle derangement [7]. New therapeutic strategies such as new generation ERT or gene therapy are underway [8]. Diaphragm is the most impaired respiratory muscle in LOPD and its dysfunction causes major respiratory symptoms and later in the course of the disease, the necessity of a ventilation support [9]. Invasive and non-invasive methods can be applied to evaluate diaphragm function [10]. The former are laborious and difficult to be used in a follow-up program whereas the latter are preferable because are repeatable although dependent on patient cooperation. Diaphragm ultrasonography (DUS) is a relatively recent, easy to perform and reproducible technique that was utilized to evaluate diaphragm dysfunction in several respiratory diseases [11]. Considering the neuromuscular disorders, few studies have utilized DUS to investigate diaphragm function [12–14]. Since diaphragm weakness is one of the main LOPD features, DUS could become an easy way to investigate diaphragmatic dysfunction in these patients. We perform a cross-sectional case-control study to evaluate DUS in 17 LOPD compared to healthy control volunteers, trying to match the results with traditional lung function tests actually used in routine clinical practice.

Material and methods

Study participants

The study was conducted from January 2019 to July 2019. It was approved by the Research Ethics Committee of the University of Messina School of Medicine Hospital Policlinico “G. Martino” (protocol n° 118). All patients and controls provided written informed consent prior to enrolment in the program. This study was a part of a wider project investigating diaphragmatic function by ultrasonography in neuromuscular patients. All recruited patients were adults and had a proven LOPD diagnosis according to the current recommendations [15]. All patients harbored the common mutation c.-32-13 T>G (IVS1-13 T>G), associated with a second

pathogenic mutation detected in the second allele (Table 1). Control subjects were matched for age, gender, and body mass index. For assessment of neurological disability, the Walton Medwin-Gardner scale (WMG) was applied [16]. The WGMS score ranges from 0 to 10, with a higher score indicating a greater disability. The modified Medical Research Council dyspnea scale (mMRC-DS) was used for self-assessment of breathlessness [17]. It stratifies severity of dyspnea in respiratory diseases ranges from 0 to 4, with a higher score indicating more breathless.

Spirometry and tests of respiratory muscle strength

Forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) were measured from flow-volume curves obtained with a pneumotachograph (Ganshorn Medizin Electronic GmbH, Germany) according to ATS/ERS standards in both upright and supine positions [18]. Values were expressed as absolute values and percentage of predicted normal values. Reference values were derived from published data [19]. A drop in the percentage of predicted FVC upon changing posture from the upright to the supine position > 20% was considered as expression of diaphragmatic weakness [20, 21]. Maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP) tests were, respectively, performed by an experienced operator with MicroRPM (Micro Medical, Kent, UK), who strongly advised the subjects to produce maximum inspiratory (Mueller maneuver) and expiratory (Valsalva maneuvers) efforts at near residual volume and total lung capacity, respectively. All tests were repeated at least three times and highest value measured was recorded. Reference values were taken into account according to previously published data [22].

Diaphragm ultrasound

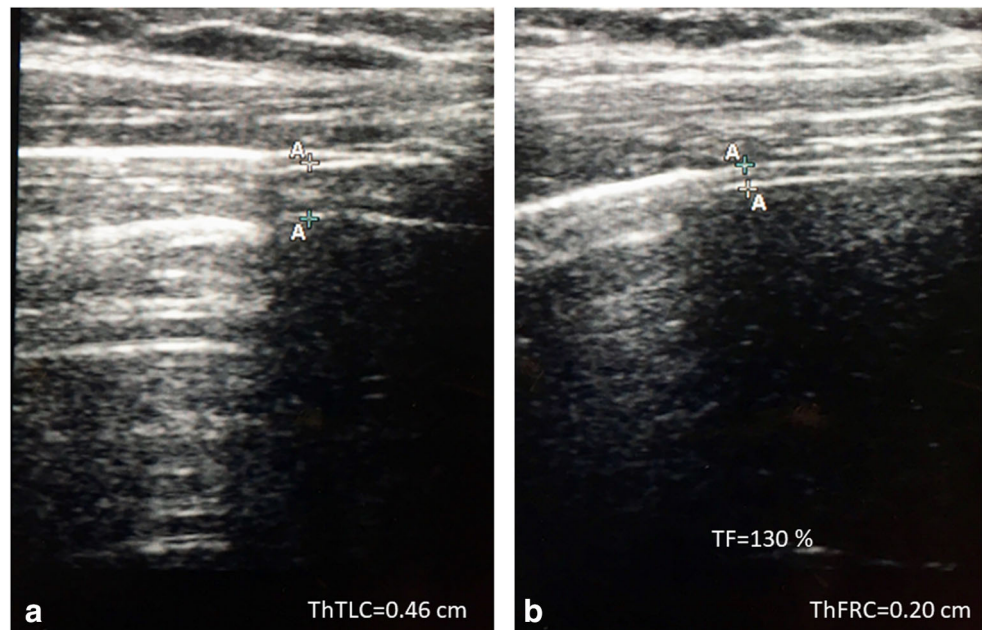
Subjects were examined in the semi-recumbent position with the bed slope at 45°. A high resolution portable ultrasound machine (MicroMaxx; Sonosite Inc., Bothell, WA, USA) was used, with a 7–13 MHz linear array transducer. Real time ultrasound was used to identify the zone of apposition where the diaphragm was most easily visualized. Typically, the eighth or ninth intercostal space was chosen, between the antero-axillary and mid-axillary lines [23]. The diaphragm was identified by its characteristic 3-layered appearance, location beneath the intercostal muscles and subcutaneous tissue, and extension deep to the ribs (as opposed to the intercostal muscles which span the ribs). The normal diaphragm is composed of a relatively thick layer of hypoechoic (dark) muscle tissue encased between 2 hyperechoic (bright) lines of pleural and peritoneal fascia. The hypoechoic muscle will thicken significantly during deep inspiration (Fig. 1a) compared to thickness at end expiration (Fig. 1b). At more cephalad

Table 1 Baseline characteristics for individual patients

Patient ID	Age	Sex	BMI	Genetics	Disease duration (years)	Clinical presentation	Smoking habit	Pack-years	WMG	mMRC-DS	NIV	ERT
1	42	M	40	c.-32-13 T > G (IVS1-13 T > G) c.1802C>G	8	HyperCKemia	former smoker	10	0	2	No	No
2	55	M	32	c.-32-13 T > G (IVS1-13 T > G)	15	HyperCKemia	former smoker	8	0	2	No	No
3	58	M	24	c.-32-13 T > G (IVS1-13 T > G) c.2395C>T	25	LGMW+RI	never smoker	-	6	3	Yes	Yes
4	50	M	25	c.-32-13 T > G (IVS1-13 T > G) c.784G>A	10	LGMW+RI	current smoker	10	5	3	Yes	Yes
5	52	F	25	c.-32-13 T > G (IVS1-13 T > G) c.2560C>T	8	LGMW+RI	never smoker	-	5	3	Yes	Yes
6	64	F	25	c.-32-13 T > G (IVS1-13 T > G) c.2560C>T	26	LGMW+RI	never smoker	-	4	3	Yes	Yes
7	37	M	35	c.-32-13 T > G (IVS1-13 T > G) c.1551+1G>C	7	LGMW+RI	never smoker	-	6	2	Yes	Yes
8	48	F	26	c.-32-13 T > G (IVS1-13 T > G) c.2530_2541del	16	LGMW+RI	never smoker	-	5	2	Yes	Yes
9	50	F	23	c.-32-13 T > G (IVS1-13 T > G) c.2530_2541del	3	LGMW+RI	former smoker	7	5	2	Yes	Yes
10	19	M	23	c.-32-13 T > G (IVS1-13 T > G) c.2481+102_2646+31del	4	LGMW+RI	never smoker	-	6	2	No	Yes
11	62	F	22	c.-32-13 T > G (IVS1-13 T > G) c.2481+102_2646+31del	6	LGMW+RI	never smoker	-	1	3	Yes	Yes
12	49	M	34	c.-32-13 T > G (IVS1-13 T > G) c.1561G>A	3	LGMW+RI	former smoker	15	4	3	No	Yes
13	19	F	20	c.-32-13 T > G (IVS1-13 T > G) c.1551+1G>C	9	LGMW	never smoker	-	2	2	No	Yes
14	21	F	19	c.-32-13 T > G (IVS1-13 T > G) c.1802C>G	14	HyperCKemia	never smoker	-	0	2	No	No
15	63	F	27	c.-32-13 T > G (IVS1-13 T > G) c.1551+1G>C	20	HyperCKemia	current smoker	10	0	3	No	No
16	50	M	21	c.-32-13 T > G (IVS1-13 T > G) c.1771C>T	30	LGMW+RI	current smoker	12	4	3	No	Yes
17	55	M	34	c.-32-13 T > G (IVS1-13 T > G) c.2481+102_2646+31del	6	LGMW	never smoker	-	2	3	No	Yes
Mean	46.7	-	26.8	-	12.3	-	-	10	3.2	2.2	-	-
SD	14.6	-	6	-	8.4	-	-	2	2.3	0.5	-	-

BMI, body mass index; ERT = enzyme replacement therapy; F, female; HyperCKemia, increased serum creatine kinase; LGMW, limb-girdle muscle weakness; M, male; mMRC-DS, modified medical research council dyspnoea scale; NIV, noninvasive ventilation (for nocturnal hypoventilation); RI, respiratory involvement; SD, standard deviation; WMG, walton medwin-garden scale

Fig. 1 Ultrasonography imaging of the diaphragm obtained in a healthy control to measure thickness at functional residual capacity (ThFRC) (1a), thickness at total lung capacity (ThTLC) (1b). Thickness fraction % is calculated as follow:
 $TF = ((ThTLC - ThFRC) / ThFRC) \times 100$



intercostal spaces, the diaphragm will become obscured by the very hyperechoic shadow of the lung coming into the field of view with deep inspiration (curtain sign). The thickness of diaphragm was measured near the zone of identification of curtain sign. Once the most appropriate intercostal space was identified, the subject was instructed to breathe quietly, while 3 images were captured at functional residual capacity (FRC). The subject was then instructed to take slow deep breaths in and out, and 3 images were captured at total lung capacity (TLC). Electronic calipers were used to measure the thickness of the diaphragm muscle at the aspect closest to the more caudal rib, where the 2 fascial lines outlining the diaphragm were parallel, and the 3 images for each position were then averaged to give a thickness at FRC (ThFRC) and a thickness at TLC (ThTLC). We also calculated the thickening fraction (TF), as the proportional thickening of the diaphragm from FRC to TLC. TF represents an index of diaphragmatic thickening as is defined by the following equation: $TF = ((ThTLC - ThFRC) / ThFRC) \times 100$. To evaluate diaphragm mobility echocardiograph was connect to a 2.5 to 3.5 MHz transducer array and M-mode was selected. Right hemidiaphragm was chosen for best liver acoustic view [24]. The probe was placed between the midclavicular and anterior axillary lines, in the subcostal area, and directed medially, cranially, and dorsally, so that the ultrasound beam reached perpendicularly the posterior third of the right hemidiaphragm (Fig. 2). Diaphragm movements were recorded in M-mode. Ultrasonographic measurements were performed during quiet breathing (QB) and deep breathing (DB). This maneuver began at the end of normal expiration, and the volunteers were asked to breathe in as deeply as they possibly could. At least 3 measurements for each parameter were taken. Again, the averaged value of three consecutive measurements was recorded

for each. Images were stored in electronic format by an examiner blinded to the analysis and to the results of lung tests.

Statistical analysis

All analyses and graphical illustrations were performed using GraphPad Prism 7™ (GraphPad Software, San Diego, California). Results are expressed as mean and standard deviation for parametric data. Categorical variables are expressed as percentages, unless otherwise specified. Differences between groups were analyzed using the unpaired t-test or the Mann-Whitney U test as appropriate. The Pearson correlation coefficient was used to evaluate correlations between different parameters. For all analyses, a *p* value of <0.05 was considered statistically significant.

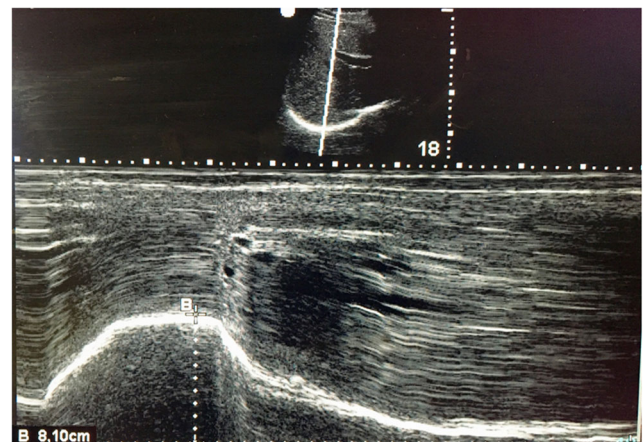


Fig. 2 Ultrasonography imaging of the diaphragm obtained in a healthy control to measure mobility of diaphragm from functional residual capacity (FRC) to total lung capacity (TLC)

Results

Subjects

17 patients with LOPD (age 47 ± 15 years, 9 female, BMI 27.0 ± 6 kg/m²) and 17 healthy control subjects (age 47 ± 14 years, 8 male, BMI 26 ± 5 kg/m²) were enrolled (Table 2). Each patient underwent DUS examination as well as pulmonary function tests (PFTs) in the same day. Mean disease duration was 12 ± 8 years and age at disease diagnosis was 34 ± 14 years. When the clinical evaluation was performed, 13/17 patients presented a limb-girdle muscle weakness with a prominent involvement of axial and proximal muscles at lower limbs. Four of 17 had only increased serum CK and myalgia. Respiratory involvement was more evident in 10/17 patients with history of exercise intolerance and exertional dyspnea (mean MMRC 2.5, interquartile range 2–3). Eight of 10 patients required noninvasive ventilation (NIV) (mean usage time: 8 h/night) for nocturnal hypoventilation. Thirteen of 17 patients were receiving ERT according to standard recommendations (biweekly, 20 mg/kg body weight). Duration of ERT ranged from 1 year to 10 years.

Spirometry and tests of respiratory muscle strength

The results of spirometry and respiratory strength tests in LOPD patients and healthy controls are summarized in Table 2. The following parameters were significantly reduced in LOPD patients versus controls: FEV₁ (both expressed as absolute value and % of predicted) in seated and supine position, FVC (both expressed as absolute value and % of predicted) in seated and supine position. Mean drop in FVC passing from seated to supine position was significantly different in LOPD patients compared to healthy controls [-0.56 ± 0.41 L ($27 \pm 19\%$ pred) vs 0.120 ± 0.06 L ($3 \pm 1\%$ pred), $p < 0.0001$]. LOPD patient n. 1,3,6 did not perform supine spirometry due to increased dyspnea and inability to maintain the supine position. Evaluation of muscle strength revealed a significant reduction of MIP [45 ± 24 cmH₂O ($52 \pm 23\%$ pred) vs 98 ± 16 cmH₂O ($112 \pm 10\%$ pred), $p < 0.0001$] and MEP [60 ± 31 cmH₂O ($60 \pm 24\%$ pred) vs 134 ± 16 cmH₂O ($109 \pm 28\%$ pred), $p < 0.0001$] comparing LOPD patients and healthy control. LOPD patient n. 6 did not perform MIP and MEP maneuvers for incapacity to sealing the mouthpiece.

Table 2 Demographic, basic spirometry, and respiratory muscle strength data in LOPD patients and healthy controls

	LOPD patients ($n = 17$)	Healthy controls ($n = 17$)	p value
Male, n (%)	9 (53%)	8 (47%)	n.s.
Age (years)	46.7 ± 14.6	47 ± 14.3	n.s.
BMI (Kg/m ²)	26.8 ± 6	25.7 ± 5.1	n.s.
mMRC dyspnea score	2.2 ± 0.5	1	< 0.0001
Pulmonary Function Tests			
FEV ₁ (L)	2.4 ± 1.2	3.5 ± 0.6	< 0.005
FEV ₁ (% pred)	76 ± 25.3	98 ± 4.1	< 0.005
FEV _{1sup} (L)	1.7 ± 1.1	3.4 ± 0.6	< 0.0001
FEV ₁ drop (L)	0.55 ± 0.3	0.15 ± 0.04	< 0.0005
FEV ₁ drop (%)	19.5 ± 5.4	4.4 ± 1.4	< 0.0001
FVC (L)	2.9 ± 1.2	4.3 ± 0.7	< 0.005
FVC (% pred)	78 ± 21.1	97 ± 2.8	< 0.005
FVC _{sup} (L)	2.3 ± 1.2	4.1 ± 0.7	< 0.0001
FVC drop (L)	0.56 ± 0.4	0.12 ± 0.06	< 0.005
FVC drop (%)	26.7 ± 19.5	2.8 ± 1.4	< 0.0001
PEF (L/s)	6.1 ± 3.1	7.5 ± 1.6	n.s.
PEF (% pred)	78 ± 31.6	96 ± 2.1	< 0.05
Respiratory muscle strength			
MIP (cmH ₂ O)	45.5 ± 24	97.7 ± 16.2	< 0.0001
MIP (% pred)	52.4 ± 23.3	111.8 ± 9.9	< 0.0001
MEP (cmH ₂ O)	60.3 ± 30.6	134.3 ± 15.8	< 0.0001
MEP (% pred)	60.1 ± 24.3	108.8 ± 27.7	< 0.0001

Data are presented as mean and standard deviation or number of patients (%). BMI, body mass index; FEV₁, forced expiratory volume in one second; FVC, forced vital capacity; LOPD, late-onset Pompe disease; MEP, maximum expiratory pressure; MIP, maximum inspiratory pressure; mMRC, modified medical research council; n.s., not significant; PEF, peak expiratory flow; pred, predicted; sup, supine position

Table 3 Diaphragm ultrasound measures in LOPD patients and control subjects

	LOPD patients (n = 17)	Healthy controls (n = 17)	p value
Diaphragm excursion			
Mobility from FRC to TLC (cm)	5.34 ± 2	7.82 ± 0.4	< 0,0001
Diaphragm thickness			
ThFRC (cm)	0.16 ± 0.06	0.20 ± 0.02	< 0,0001
ThTLC (cm)	0.30 ± 0.18	0.54 ± 0.06	< 0,0001
TF	72.26 ± 40.05	181.4 ± 49.27	< 0,0001

FRC, functional residual capacity; ns, not significant; LOPD, late-onset Pompe disease; RV, residual volume; ThFRC, thickness fraction at FRC; ThTLC, thickness fraction at TLC; TF, thickness fraction calculated by following equation: $TF = \{[(ThTLC - ThFRC) / ThFRC] \times 100\}$; TLC, total lung capacity

Diaphragm ultrasonography

Whereas diaphragm thickness has been measured in all subjects except one LOPD patient for lack of cooperation, quantitative assessment of diaphragm excursion was hampered by an insufficient acoustic windows in a subset of 5 LOPD patients. Diaphragm mobility during deep breathing, ThTLC, ThFRC, and TF were significantly reduced in LOPD patients versus healthy controls ($p < 0.0001$) (Table 3 and Fig. 3). As an example ThFRC, ThTLC, and diaphragm mobility images in a LOPD patient are reported in Fig. 4.

Correlation between diaphragm ultrasonography, functional scales, and disease duration

Statistically significant correlations were found between mMRC-DS and diaphragm mobility ($r = -0.64$; $p < 0.05$), ThFRC ($r = -0.20$; $p < 0.05$), MIP ($r = -0.58$; $p < 0.05$), FVC ($r = -0.53$; $p < 0.05$), FVC_{sup} ($r = -0.71$; $p < 0.05$), and FVC drop from sitting to supine position ($r = -0.75$; $p < 0.05$). The WGMS was correlated significantly with MIP% pred ($r = -0.68$; $p < 0.05$), FVC%pred ($r = -0.55$; $p < 0.05$) and FVC drop from sitting to supine position ($r = 0.59$; $p < 0.05$). Finally disease duration expressed in years was correlated with ThFRC ($r = -0.53$; $p < 0.05$).

Correlations between diaphragm ultrasonography, spirometry, and respiratory muscle strength tests

Statistically significant correlations were found between ThTLC with MIP ($r = 0.85$; $p < 0.0001$), FVC both in seated ($r = 0.77$; $p < 0.0001$) and supine position ($r = 0.68$; $p < 0.05$). ThFRC correlated significantly with MIP ($r = 0.74$; $p < 0.0001$) and FVC ($r = 0.73$; $p < 0.05$). TF correlated significantly with MIP ($r = 0.80$; $p < 0.001$), FVC in seated ($r = 0.66$; $p = 0.005$), and supine position ($r = 0.61$; $p = 0.034$). Ultrasound diaphragm mobility correlated with diaphragm thickness at TLC ($r = 0.87$; $p < 0.0001$), FRC ($r = 0.84$; $p < 0.005$), and TF ($r = 0.73$; $p < 0.05$). Moreover diaphragm mobility correlated with FVC in seated ($r = 0.79$;

$p < 0.005$) and supine position ($r = 0.74$; $p < 0.05$) and MIP ($r = 0.81$; $p < 0.005$). Significant correlations between diaphragm ultrasonography, spirometry, and respiratory muscle strength tests in LOPD patient are reported in Figs. 5 and 6.

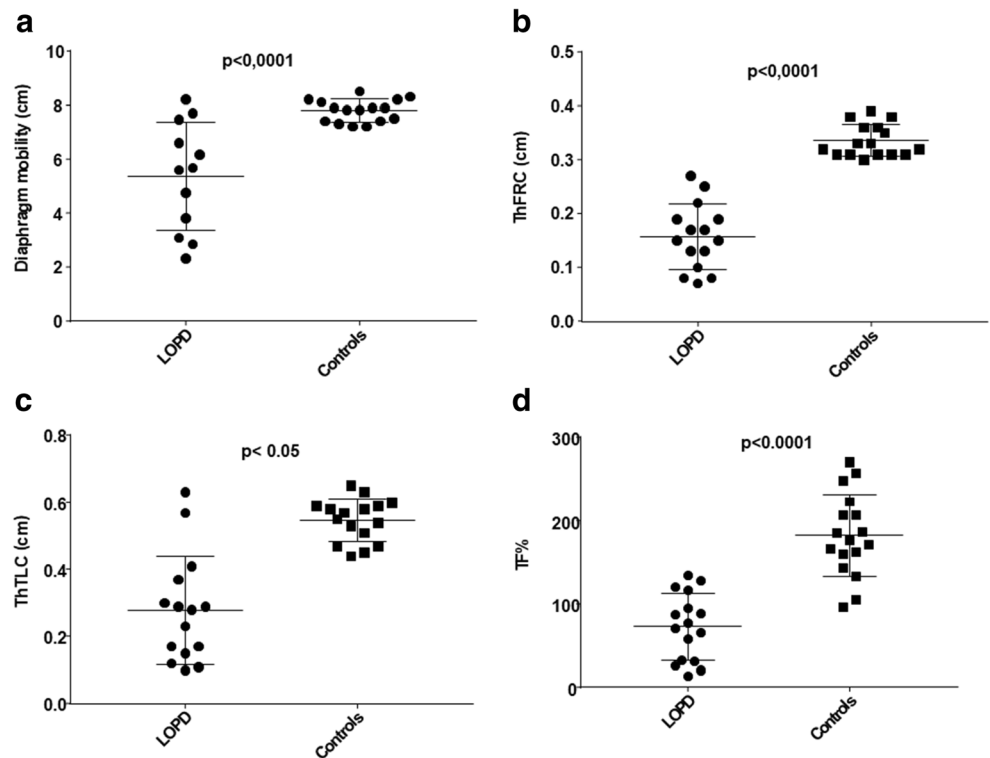
Role of NIV

Of the 17 LOPD, 8 patients used NIV (mean usage time: 8 h/night) for nocturnal hypoventilation. Statistical analysis of patients divided by the use of NIV or not showed that patients on NIV had a significant reduction ($p < 0.05$) of ultrasound diaphragm mobility, ThTLC ($p < 0.05$), ThFRC, and TF. Moreover LOPD patients using NIV had a significant reduction ($p < 0.05$) of FVC in seated and supine position and its variation in percentage compared with LOPD patients with no NIV. Finally LOPD on NIV patients had a significant reduction ($p < 0.05$) of inspiratory muscle strength evaluated by MIP, but no statistically significant variation was found for MEP. Differences between diaphragm ultrasonography, spirometry, and respiratory muscle strength tests in LOPD patients with and without NIV are reported in Fig. 7.

Discussion

This cross-sectional case-control study comprehensively explored, in patients with LOPD, diaphragm function evaluated by ultrasonography. The results showed that ultrasonography assessment of diaphragm function is a rapid and easy technique to perform even at bedside with a significant correlation with the traditional respiratory tests used in daily clinical practice. Several authors focused attention on a potential role of DUS in the evaluation of diaphragm activity. Determination of diaphragm thickness and real-time diaphragm excursions by ultrasonography is an easy and reproducible method and need minimum patient cooperation [11, 25]. Considering its use in neuromuscular disorders, ultrasonography has already been utilized to evaluate diaphragm muscle weakness in patients with amyotrophic lateral sclerosis (ALS) [12], Duchenne muscular dystrophy (DMD) [14], and other muscle disorders [26].

Fig. 3 Diaphragm mobility (a), thickness at functional residual capacity [ThFRC (cm)] (b), thickness at total lung capacity [ThTLC (cm)] (c), and thickness fraction % (TF%) (d) in LOPD patients and healthy controls



Recently, in a case of Facio-Scapulo-Humeral Distrophy (FSHD) there was an evidence of a significant reduction of diaphragm excursion amplitude and thickening ratio compared with an healthy control group [27]. Unfortunately, routine PFTs, including FVC reduction between upright and supine position as well as MIP and MEP reflects an indirect way to assess diaphragm muscle dysfunction in LOPD. Whereas the findings obtained from a small subgroup of 4 presymptomatic patients (2 females) in absence of respiratory symptoms suggest that diaphragm weakness could be evaluated by DUS as an early and initial sign. Therefore, this technique can be applied in routine clinical practice for evaluation of diaphragm function at the early stage of the disease. However, diaphragmatic CT and MRI are reference imaging tests to explore diaphragm integrity. We have previously demonstrated by CT and MRI studies that diaphragm involvement

represents the predominant cause of respiratory failure in LOPD usually with preservation of other respiratory muscles [28]. However these procedures have some limitations: CT exposes patients to radiations whereas for MRI evaluation, although is quite fast and not invasive, is difficult to be performed in patients with severe respiratory insufficiency that cannot stand long supine. On the other hand ultrasonography can be used at bedside [29]. Recently Spiesshoefer and co-workers [30] investigated diaphragm morphology and function by ultrasonography in 13 LOPD patients. They primarily investigated the neural contributions to diaphragm dysfunction but evidence of phrenic nerve involvement was not found, but the study was not designed to correlate diaphragm function evaluated by ultrasonography with routine PFTs. In the present study we confirm that thickness and mobility of diaphragm are significantly reduced in LOPD patients

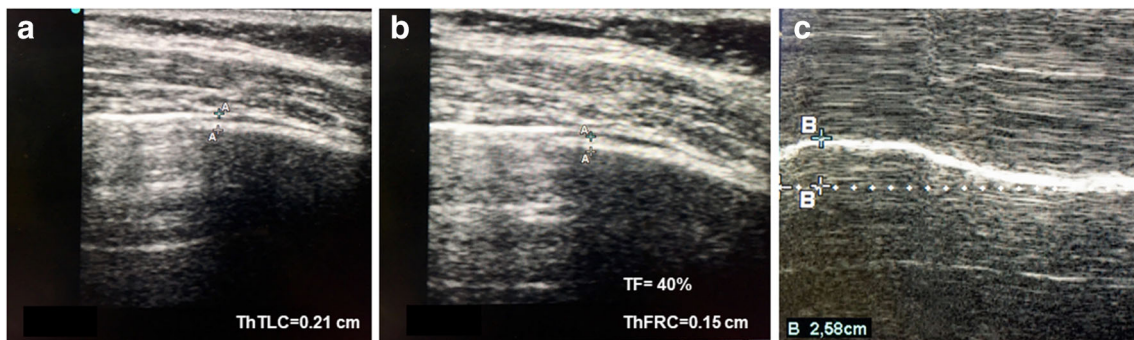


Fig. 4 Ultrasonography imaging of the diaphragm obtained in a LOPD patient to measure thickness at functional residual capacity (ThFRC)

(a), Thickness at total lung capacity (ThTLC) (b). Thickness Fraction (c) is calculated as follows: $TF = ([ThTLC - ThFRC] / ThFRC) \times 100$

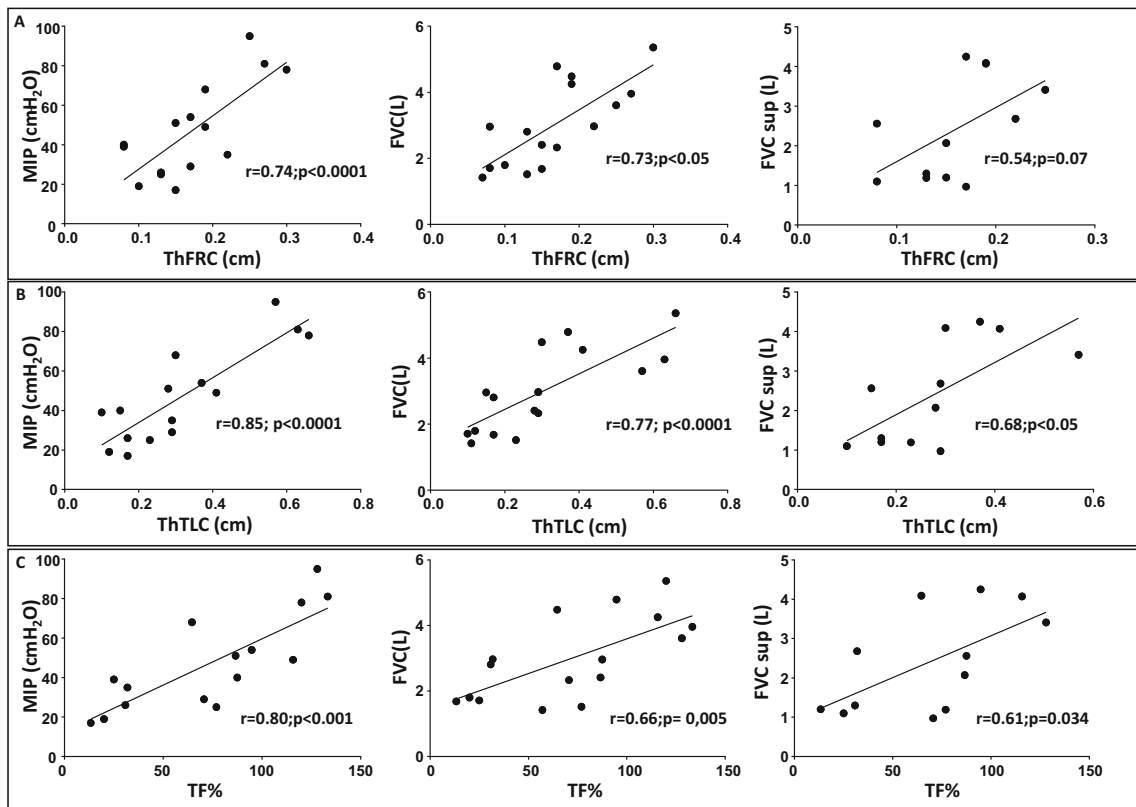


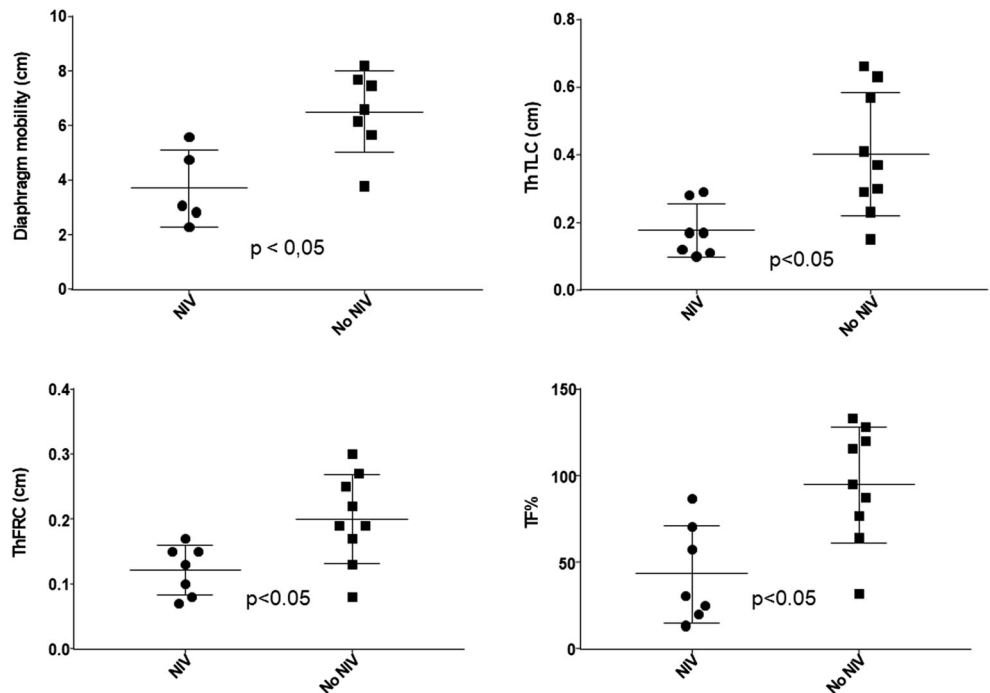
Fig. 5 Significant correlations between diaphragm ultrasonography thickness, spirometry, and muscle strength. MIP, maximal inspiratory pressure; FRC, functional residual capacity; TLC, total lung capacity;

ThFRC, thickness at FRC; ThTLC, thickness at TLC; TF, thickness fraction

compared with healthy controls resulting in a good clinical correlation with the traditional PFTs. 4 LOPD patients included in this study were not receiving ERT because still

presymptomatic and presenting only with hyperCKemia. This small number of patients without ERT were not comparable with the symptomatic patients, but when related to the

Fig. 6 Significant correlations between diaphragm ultrasonography mobility, spirometry, and muscle strength. MIP, maximal inspiratory pressure; FRC, functional residual capacity; TLC, total lung capacity; ThFRC, thickness at FRC; ThTLC, thickness at TLC; TF, thickness fraction



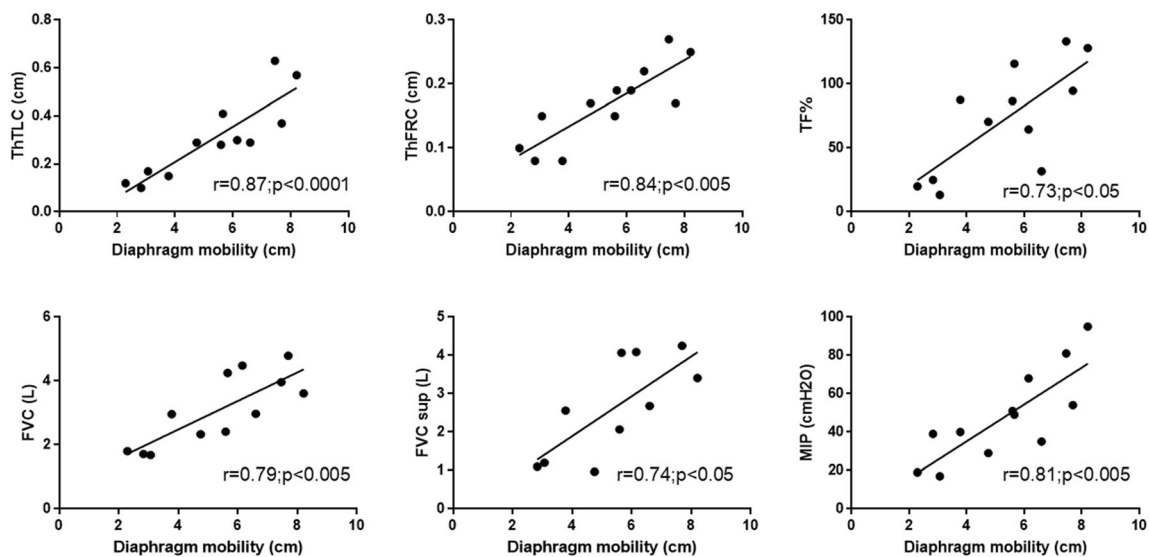


Fig. 7 Diaphragm mobility, ThFRC, ThTLC, and TF in LOPD ventilated and non-ventilated patients. NIV, noninvasive ventilation; ThFRC, thickness at FRC; ThTLC, thickness at TLC; TF, thickness fraction

healthy control group they showed a reduction in thickness fraction (110 ± 22 vs 181 ± 49 , respectively, $p < 0,05$) and diaphragm mobility (6.5 ± 1 mm vs 7.8 ± 0.4 , respectively, $p < 0,05$). Consequently, our data confirm that diaphragm ultrasonography is an alternative useful and risk-free technique to supply clinical, functional, and neurophysiological assessment of respiratory muscle weakness in LOPD patients. Despite the comprehensive protocol applied in this study, some limitations have to be recognized. Firstly, intra-observer variability of diaphragm ultrasonography could affect the results. However we minimized this bias by an extensive operator training and by performing up to three reproducible tests per patient. Secondly, we did not perform a comparison with other methods that may be considered a gold standard in the assessment of diaphragmatic function as transdiaphragmatic pressures because, in our opinion, those are invasive procedures and quite difficult to be performed in a routine clinical practice. However a previous study found a good correlation between transdiaphragmatic pressure time product and diaphragm thickness [31].

In conclusion, we suggested that DUS should become a routine method to evaluate diaphragm dysfunction in LOPD patients. Interestingly, DUS could help physicians in anticipating diagnosis of subclinical dysfunction of the diaphragm in LOPD patients. If these data are confirmed in further clinical trials, DUS could be part of the diagnostic pathway in LOPD patients for the management of respiratory failure, including indication for noninvasive ventilation as well as a useful outcome measure for future diagnostic and therapeutic trials.

Compliance with ethical standards

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

Ethical approval Approved by the local Research Ethics Committee.

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