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Analysis of motor and respiratory function in Duchenne muscular dystrophy patients

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ABSTRACT

Introduction: Duchenne muscular dystrophy(DMD) shows motor and respiratory impairment.
Methods: 19 DMD patients (DMDG) (nine ambulatory and 10 non-ambulatory) were evaluated through motor function measure (MFM), 6-minute walk test (6MWT), respiratory muscle strength, cough peak flow, spirometry and volumetric capnography (VCap) tools. Control group that performed spirometry and VCap (CG1-n = 17) were different from those that performed the 6MWT (CG2-n = 8).
Results: The follow tools were assessed ($p < 0.05$): (i) MFM: Ambulatory patients showed higher values than non-ambulatory patients; (ii) 6MWT: DMDG walked a shorter distance and showed higher respiratory rate at rest and heart rate (HR) at rest than CG2; (iii) Spirometry: DMDG and non-ambulatory patients had minor values achieved in spirometry when compared with CG1 and ambulatory patients, respectively; (iv) VCap: DMDG when compared with CG1 showed: (< 11 years-old) lower values in VCap parameters; (> 11 years-old): higher HR and lower slope 2. There was correlation between spirometry, mainly for $zFEV_1$/FVC, and MFM.
Conclusion: DMDG showed motor (MFM/6MWT) and respiratory (spirometry/VCap) deterioration when compared with CG. Non-ambulatory condition was associated with worse MFM and spirometry.

1. Introduction

Duchenne muscular dystrophy (DMD) is an inherited and X-linked disease that affects ˜1:3500 male newborns (Söderpalm et al., 2013; Machado et al., 2012). The deficiency in dystrophin protein production causes inadequate muscle responses to mechanical stretching and contraction, progressive degeneration in muscle fibers and fibrosis in the contractile tissue, causing progressive muscular dysfunction (Zhou and Lu, 2010).

Commonly, children with DMD aged between three to five years

Abbreviations: %, percentage; 6MWT, six-minute walk test; 95%CI, confidence interval of 95%; ATS, American Thoracic Society; BMI, body mass index; bpm, breath per minute and beats per minute; CAPES, Coordination for the Improvement of Higher Education Personnel; CC, correlation coefficient; CG1, control group 1; CG2, control group 2; cmH_2O, centimeters of water; CO_2, carbon dioxide; CPF, cough peak flow; D1, dimension 1; D2, dimension 2; D3, dimension 3; DMD, Duchenne muscular dystrophy; DMDG, Duchenne muscular dystrophy group; DMDG-6MWT, Duchenne muscular dystrophy group that performed six-minute walk test; DS/TV, airway dead space/tidal volume ratio; DS, airway dead space; ERS, European Respiratory Society; EV, expiratory volume; FAPESP, São Paulo Research Foundation; $FEF_{25–75\%}$, forced expiratory flow between 25–75% of forced vital capacity; FEF_{max}, maximum forced expiratory flow; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; HR, heart rate; IV, inspiratory volume; L/min, liter per minute; LVR, lung volume recruitment; MEP, maximal expiratory pressure; MFM, motor function measure; MIP, maximal inspiratory pressure; mL, milliliters; MVV, maximal voluntary ventilation; N, number of cases; RR, respiratory rate; SD, standard deviation; Sec, seconds; SpO_2, peripheral capillary oxygen saturation; SPSS, Statistical Package for the Social Sciences; TV, tidal ventilation; TV_{alv}, tidal alveolar ventilation; VCap, volumetric capnography; VCO_2, carbon dioxide production; VM, ventilation per minute; VM_{alv}, alveolar ventilation per minute

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exhibit progressive muscle weakness that evolves to scapular girdle and other muscular groups, culminating with ambulation loss in age between nine to 12 years (Humbertclaude et al., 2012). Historically, cardiorespiratory issues lead to death (˜20 years old) Humbertclaude et al., 2012). However, when we consider DMD with all the interventions to date (Ricotti et al., 2015), over the past few years the survival for ages close to 20 years and beyond increased from 0 to close to 50% (Eagle et al., 2002).

After ambulation loss, numerous issues show major attention (Bach et al., 2010). Wheelchair restriction is associated with fastest scoliosis progression, spine and thoracic chest malformations, which impair the respiratory status, accelerating the evolution of respiratory insufficiency and the process of hypercapnia (Finder et al., 2004).

Although there are limitations in the DMD treatment, the management of motor and respiratory deterioration has improved in recent decades. Moreover, corticosteroids showed an improvement in the quality of life being a viable therapy (Guglieri et al., 2017; Ryder et al., 2017). Also, new treatments have emerged as the use of Eteplirsen in the DMD treatment. The Eteplirsen is a drug that acts to promote dystrophin production by restoring the translational reading frame of DMD through specific skipping of exon 51 in defective gene variants and it is applicable for ˜14% of patients with DMD mutations (Irwin and Herink, 2017; Lim et al., 2017; Aartsma-Rus and Krieg, 2017).

Nowadays, more children with DMD are transitioning into adulthood (Schrans et al., 2013). A randomized controlled trial demonstrated that dynamic training approach with low intensity (assisted bicycle training) is beneficial for motor maintenance (Jansen et al., 2013). However, in relation to the respiratory dysfunction management, the results of muscular training are controversial (Finder et al., 2004).

Lung volume recruitment and cough efficiency techniques have been studied to minimize the deterioration of lung function in DMD patients (Brito et al., 2009; McKim et al., 2012). The study performed by McKim et al. (2012) was conducted in Canada with 22 DMD patients, when one evaluated the long-term effect on measures of forced vital capacity (FVC) before and after the introduction of regular lung volume recruitment (LVR) maneuvers (breath stacking). The rate of FVC decline in DMD patients improved with initiation of regular LVR (McKim et al., 2012). Also, the study performed by Brito et al. (2009) evaluated cough efficiency using two manually-assisted cough techniques in 28 DMD patients. In the study, chest compression and air stacking techniques were efficient in increasing cough peak flow (CPF). Besides, the combination of these two techniques (chest compression and CPF) had additional effect (Brito et al., 2009).

After the DMD diagnosis, the loss of motor function was verified (Buckon et al., 2016; Nunes et al., 2016). Also, the loss of lung function showed association with loss of motor function (Mayer et al., 2015; Meier et al., 2017; Finder, 2015), once the respiratory dysfunction worsened slowly and progressively with age (Finder et al., 2004). In several circumstances, the DMD patient and/or their caregivers fail to notice the deterioration in lung function, but, everyone recognizes a deterioration in cough, and this fact should be highlighted as a marker for severity progression. Cough, which is initially a defense mechanism, becomes progressively insufficient over time until it becomes ineffective in eliminating airway secretions (Brito et al., 2009; McKim et al., 2012). According to Brito et al. (2009), in ˜90% cases of respiratory failure in DMD patients, there are accompanying episodes of pulmonary illnesses, mainly by bacterial pneumonias, and the ineffective cough worsen the severity of the pulmonary status (Brito et al., 2009). To verify the deterioration of motor and respiratory function, nowadays, we have a great number of tools for assessment of ventilatory and motor function in DMD (Buckon et al., 2016; Nunes et al., 2016; Diniz et al., 2012; de Lattre et al., 2013; de Carvalho et al., 2015; LoMauro et al., 2015).

For DMD patients to enjoy the therapeutic benefits, the health professional must be aware of motor and respiratory demands during disease progression. Empirically, these dysfunctions coexist in the DMD patient since very early. However, the approach to assessment and management is done systematically, professionals often do not think of how much the dysfunction in one system affects the other. Although not studied or evaluated together, they are seen in clinical practice quite frequently. In our study emerged the hypothesis about the high severity in DMD patients with non-ambulatory condition when compared with ambulatory patients for motor and respiratory function. Also, we have an early motor and respiratory function impairment in DMD patients when compared with control subjects. In this context, the study aimed at to establishing motor and respiratory function impairment in DMD patients by the analysis of numerous tools.

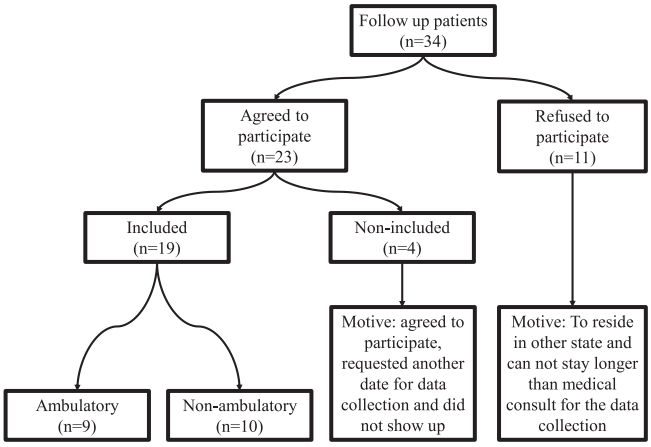

Fig. 1. Inclusion flowchart of patients in the study.

2. Methods

A cross-sectional study was conducted in a University Hospital and approved by the Research Ethics Committee (#500/2011). Written Informed consent was obtained from all individuals included in the study. During a 2-year study, 34 DMD patients were monitored. Twenty-three DMD patients, out of the 34 invited to join the study, have participated in the study (Fig. 1).

The DMD diagnostic criteria were: elevated serum creatine kinase levels; muscular biopsy tested via immunohistochemistry indicating DMD (the test was done in daily practice at the reference center to indicate the location abundance of dystrophin, but in a diagnostic setting was most frequently used to show the presence or absence of dystrophin); presence of deletions, duplications and point mutations in dystrophin gene; clinical features and/or presence of DMD in the family (Rall and Grimm, 2012). Thus, the total of patients in DMD group (DMDG) was 19. All DMD patients showed elevated serum creatine kinase levels, altered muscular biopsy tested via immunohistochemistry and clinical features. Also, in three cases there are presence of previous DMD diagnosis in the family. Finally, for 11/19 (10/11 deletions and 1/11 duplication) DMD patients, we are able to identify the mutation in the dystrophin gene. For the remaining patients (8/19) no genetic studies were available.

The DMD patients were classified by disease progression: (i) ambulatory phase; (ii) transition phase – the patient uses wheelchair for long distances but maintaining independent ambulation in short distances; (iii) restricted to wheelchair (non-ambulatory); (iv) end-stage disease.

The age-matched control group enrolled healthy male children and adolescents, without chronic disease, randomly selected from a database at a Pulmonary Physiology Laboratory. The database was created by the same protocols used to evaluate the DMDG. The control group subjects that performed spirometry and volumetric capnography (VCap) (CG1, n = 17) were different from those subjects that

performed the six-minute walk test (6MWT) (CG2, n = 8). In this context, CG1 and CG2 were unpaired.

The nutritional assessment was analyzed by z-score of height, weight and body mass index (BMI). To calculate height of non-ambulatory DMDG, the arm-span was divided by 1.06, as indicated by the European Respiratory Society (ERS) and American Thoracic Society (ATS) (Miller et al., 2005). To calculate DMDG weight, we weighed the caregiver holding the child, weighed the caregiver alone, and then subtracted the weight of the caregiver from the sum of weight of both.

Motor function was assessed by motor function measure scale (MFM) and 6MWT. The MFM is adequate to patients with preserved walking ability and those with partial or total restriction of walking (Iwabe et al., 2008). The MFM assesses proximal, distal and axial motor dysfunctions, on the broad spectrum of neuromuscular diseases. The scale is composed by 32 items (scored from zero to three points), divided into three dimensions that assesses: [D1 – 13 items (%)] standing position and postural transfers; [D2 – 12 items (%)] proximal and axial motor function; [D3 – seven items (%)] distal motor function – six items comprehend the upper limbs. The three dimensions give the information of a total score in percentage.

The 6MWT was performed by ambulatory DMDG subjects, who could freely walk distances equal to or greater than 10 m, considering ATS recommendations with modifications (Mazzone et al., 2010; McDonald et al., 2010a). Predicted distance value was obtained by standardized equation for pediatric population (Priesnitz et al., 2009). In the 6MWT, the following parameters were analyzed: walking distance (meters); predicted distance (meters); at rest, 6-min and 9-min peripheral capillary oxygen saturation (SpO_2) (%); at rest, 6-min and 9-min respiratory rate at rest (RR) (bpm); at rest, 6-min and 9-min heart rate (HR) (bpm).

Respiratory assessment comprehends: respiratory muscle strength, CPF, spirometry, VCap and other tools (e.g. inspiratory and expiratory pressure generating capacity are indices of respiratory muscle strength).

Respiratory muscle strength was assessed by maximal inspiratory pressure (MIP) (cmH_20) and maximal expiratory pressure (MEP) (cmH_20). For the evaluation, a pressure-meter with a connected mouthpiece was used (MV-120 model, Ger-Ar Comércio e Equipamentos Ltda, Brazil). Each maneuver was performed at least five times so that to consider the highest value, between three values with variation < 10%. Maneuvers sustained for at least one second were considered (Gayraud et al., 2010; Park et al., 2010).

A peak flow meter was used (ASTECH®, Center Laboratories, NY, USA) for CPF measure (L/min). The maneuvers were performed three times and the highest value was considered (LoMauro et al., 2015).

The spirometry was conducted with a spirometer (CPFS/D model, Med Graphics®, MN, USA; BREEZE PF software Version 3.8 B for Windows 95/98/NT) considering the ERS and ATS recommendations. Predicted values of FVC, forced expiratory volume in one second (FEV_1), FEV_1/FVC ratio, forced expiratory flow between 25% to 75% of FVC ($FEF_{25–75\%}$), maximum forced expiratory flow (FEF_{max}), maximal voluntary ventilation (MVV), peak inspiratory flow (L/min), peak expiratory flow (L/min), inspiratory time (sec) and expiratory time (sec) were evaluated according to Polgar and Promadhat (1971) reference. We also analyzed the z-score for admixture population according to Global Lung Initiative for the following parameters: FVC, FEV_1, FEV_1/FVC and $FEF_{25–75\%}$ (ERS task force report, 2012).

To perform the VCap, a CO_2SMO®Plus! respiratory profile monitor was used (DX-8100 model, Novametrix Inc., CT, USA; Analysis Plus!® software). The carbon dioxide (CO_2) sensor, flow sensor and pulse oximeter were connected to non-invasive monitor. All participants breathed through the mouthpiece quietly for five minutes, with nasal clip, seated and without visual feedback. For data debug, the first minute was excluded, and all cycles were corrected to accommodate a variation < 25% for expiratory volume. From the data achieved in the VCap, the following parameters were used: alveolar ventilation per minute (VM_{alv}); ventilation per minute (VM); tidal alveolar volume (TV_{alv}); tidal volume (TV); airway dead space (DS); DS/TV ratio; carbon dioxide production (VCO_2); inspiratory volume (IV); expiratory volume (EV); RR; HR; SpO_2; Slope 2; Slope 3; Slope 2/TV; Slope 3/TV.

Statistical analysis was performed by Statistical Package for the Social Sciences software version 16.0 (SPSS Inc. Released 2007, Chicago, USA). A descriptive analysis was performed considering the number of cases (n), mean, standard deviation (SD), median, minimum, maximum and 95% confidence interval (95%CI). The parameters values achieved in the study showed non-normal distribution, or we had a low number of subjects in each category of parameters (n < 25). In this context, to compare the groups [DMDG versus CG1; DMDG that performed the 6MWT (DMDG-6MWT) versus CG2; ambulatory versus non-ambulatory DMD patients], we performed the Mann–Whitney test, except in relation to height z-score, which was analyzed regarding the *T*-test assuming equal variance between the groups (DMDG and CG1). Besides, we performed the correlation test using the Spearman rank test so that to compare MFM and spirometry. All statistical tests performed were two-tailed tests with alpha equal to 0.05.

3. Results

3.1. Clinical and demographic data

The DMD patients were classified into I-phase [ambulatory (n = 9)] or III-phase [non-ambulatory (n = 10)]. The classification per walking status showed association with patient's age, at an arbitrary cutoff for 11 years old (p = 0.003).

A total of 16/19 DMD patients were using oral glucocorticoid medications (prednisone). The mean time of use of oral prednisone was 2.74 ± 1.94 years, median of 2.12 years (amplitude of 0.08–6.33 years). Two DMD patients suspended the treatment without medical order for at least three years and one patient has never used the medication. To have a standard operating procedure, the DMD patients enrolled did not show cardiac involvement, pulmonary hypertension, use of oxygen therapy, invasive/non-invasive mechanical ventilation or recurrent pneumonia.

Age, height z-score, weight z-score and BMI z-score are shown in Table 1 for DMDG, DMDG-6MWT (n = 7), CG1 and CG2. DMDG and CG1 did not differ in age, height z-score, weight z-score and BMI z-score. Also, DMDG-6MWT were heavier (p = 0.041) and presented higher BMI (p = 0.021) than CG2. However, DMDG-6MWT and CG2 did not differ regarding age and height z-score.

3.2. Motor function measure

Ambulatory DMD patients showed higher values of MFM when compared with non-ambulatory DMD patients [D1 (p < 0.001), D2 (p < 0.001), D3 (p = 0.043) and total score (p < 0.001)]. In this case, the medians for D1, D2, D3 and total score were, respectively, 56.41%, 99.44%, 85.71% and 76.04% for ambulatory DMD patients, and 0, 55.55%, 66.66% and 36.98% for non-ambulatory DMD patients (p < 0.05) (Table 2 and Fig. 2).

3.3. Six-minute walk test

Two (2/9) ambulatory DMD patients were not able to complete the 6MWT due to excessive muscle fatigue. DMDG-6MWT subjects (median of 393 m) walked a shorter distance than CG2 subjects (median of 585 m) (p < 0.001).We also verified higher values in RR at rest (median for DMDG-6MWT equal to 26 and median for CG2 equal to 20) (p = 0.04), 9-minute RR (median for DMDG-6MWT equal to 24 and median for CG2 equal to 20) (p = 0.04) and HR at rest for DMDG-6MWT (median for DMDG-6MWT equal to 98 and median for CG2 equal to 80) (p = 0.009) when compared with CG2 (Table 3).

Table 1
Subjects characterization for age, z-score of height, weight and Body Mass Index. The data are shown as number of cases; mean ± standard deviation; median (minimum to maximum); 95% confidence interval for the mean.

Clinical marker	Duchenne muscular dystrophy group	Control group 1	p-value
Age (months)	19; 128.68 ± 31.75; 119 (93 to 205); 113.38 to 143.99	17; 130.76 ± 29.36; 134 (78 to 186); 115.67 to 145.86	0.925[a]
Height z-score	19; 0.34 ± 1.74; −0.14 (−2.1 to 3.64); −0.5 to 1.18	17; 0.33 ± 0.92; 0.45 (−1.67 to 1.6); −0.14 to 0.8	0.991[b]
Weight z-score	10; 0.99 ± 1.78; 0.86 (−1.77 to 3.45); −0.29 to 2.26	17; 0.43 ± 1.01; 0.47 (−0.74 to 1.54); −1.17 to 2.04	0.539[a]
Body Mass Index z-score	19; 0.16 ± 2.31; 0.78 (−5.34 to 3.3); −0.95 to 1.27	17; 0.73 ± 1.16; 0.81 (−1.1 to 2.91); 0.13 to 1.33	0.925[a]
Clinical marker	**Duchenne muscular dystrophy group-6MWT**	**Control group 2**	**p-value**
Age (months)	7; 105.71 ± 18.26; 97 (93 to 143); 88.83 to 122.6	8; 106.63 ± 20.04; 102 (86 to 143); 89.87 to 123.38	1[a]
Height z-score	7; 1.16 ± 2.05; 1.75 (−2.1 to 3.64); −0.74 to 3.06	8; -0.23 ± 0.84; −0.06 (−1.43 to 0.91); −0.93 to 0.48	0.152[a]
Weight z-score	6; 1.94 ± 1.35; 2.19 (0.19 to 3.45); 0.53 to 3.35	8; 0.03 ± 0.80; 0.17 (−1.49 to 0.74); −0.80 to 0.87	**0.041**[a]
Body mass index z-score	7; 1.42 ± 1.04; 0.83 (0.42 to 3.3); 0.46 to 2.38	8; -0.14 ± 1.25; 0.07 (−2.08 to 1.64); −1.18 to 0.91	**0.021**[a]

6MWT, six-minute walk test. Positive data ($p < 0.05$) are shown in bold type. The control group subjects that performed spirometry and volumetric capnography were designed as control group 1 and were different from those subjects that performed the 6MWT, designed as control group 2. [a], Statistical analysis was performed by Mann-Whitney test. [b], Statistical analysis was performed by *T*-test assuming equal variance between the groups. Alpha = 0.05. To calculate height of non-ambulatory DMDG, the arm-span was divided by 1.06, as indicated by the European Respiratory Society (ERS) and American Thoracic Society (ATS) (Miller et al., 2005).

3.4. Spirometry

When comparing the values of spirometry between DMDG and CG1, we observed lower values for FVC [%, median for DMDG = 76.5 and median for CG1 = 98 ($p < 0.001$) and z-score, median for DMDG = −1.74 and median for CG1 = 0.38 ($p < 0.001$)], FEV_1 [%, median for DMDG = 81.5 and median for CG1 = 102 ($p < 0.001$) and z-score, median for DMDG = −1.12 and median for CG1 = 0.8 ($p < 0.001$)], $FEF_{25–75\%}$ [%, median for DMDG = 87.5 and median for CG1 = 108 ($p = 0.013$)], FEF_{max} [%, median for DMDG = 88.5 and median for CG1 = 106 ($p < 0.001$)] and higher values for FEV_1/FVC ratio [%, median for DMDG = 100 and median for CG1 = 97 ($p = 0.004$) and z-score, median for DMDG = 1.22 and median for CG1 = 0.73 ($p = 0.032$)] in the DMDG.

We compared the ambulatory and non-ambulatory DMD patients as well, and the non-ambulatory patients presented lower FVC [%, median for ambulatory = 87 and median for non-ambulatory = 70 ($p = 0.001$) and z-score, median for ambulatory = 0.79 and median for non-ambulatory = -2.39 ($p < 0.001$)], FEV_1 [%, median for ambulatory = 85 and median for non-ambulatory = 78 ($p < 0.001$) and z-score, median for ambulatory = −0.29 and median for non-ambulatory = −1.33 ($p < 0.001$)], FEF_{max} (%, median for ambulatory = 99 and median for nonambulatory = 88) ($p = 0.001$), and higher values for FEV_1/FVC ratio [%, median for ambulatory = 95.6 and median for non-ambulatory = 105 ($p = 0.004$) and z-score, median for ambulatory = 0.63 and median for non-ambulatory = 2.33 ($p = 0.003$)], $FEF_{25–75\%}$ (%, median for ambulatory = 74 and median for non-ambulatory = 92) ($p = 0.035$) (Table 4 and Fig. 3).

In addition, we did not observe any difference between the groups (DMDG versus CG1 or ambulatory and non-ambulatory DMD patients) for peak expiratory flow, peak inspiratory flow, inspiratory time and expiratory time ($p > 0.05$).

3.5. Volumetric capnography

To compare the VCap findings for DMDG and CG1, we used an arbitrary age cutoff for 11 years. Younger DMD patients were in I-phase, while DMD patients > 11 years old were in III-phase.

DMD patients and CG1 subjects matched by age were compared between them, and in the group aged < 11 years old, DMD patients presented lower values for VM_{alv} (median for DMDG = 4.69 and median for CG1 = 6.98; $p = 0.002$), VM (median for DMDG = 6.43 and median for CG1 = 9.3; $p = 0.004$), TV_{alv} (median for DMDG = 238.23 and median for CG1 = 318.61; $p = 0.016$), TV (median for DMDG = 275.65 and median for CG1 = 398.03; $p = 0.016$), DS (median for DMDG = 63.69 and median for CG1 = 80.7; $p = 0.012$), VCO_2 (median for DMDG = 156.42 and median for CG1 = 213.17; $p = 0.006$), IV (median for DMDG = 292.13 and median for CG1 = 385.96; $p = 0.043$), EV (median for DMDG = 274.26 and median for CG1 = 395.84; $p < 0.001$) and slope 2/TV (median for DMDG = 1.73 and median for CG1 = 1.18; $p = 0.027$). In the group with age > 11 years old, the DMD patients presented higher values for HR (median for DMDG = 98.34 and median for CG1 = 83.53; $p = 0.019$) and lower for slope 2 (median for DMDG = 368.06 and median for CG1 = 521.45; $p = 0.024$) (Table 5).

Our data showed that lung development in DMD patients occurs at a slower pace in comparison with healthy control group subjects in early ages. In this context, a DMD patient reach their matched control healthy subjects by lung function in a more advanced age, in brief, an oldest DMD patient will have the same lung function value than a newest control subject by age.

3.6. Respiratory muscle strength

There was no difference between ambulatory and non-ambulatory

Table 2
Association between motor function measure for ambulatory and non-ambulatory Duchenne muscular dystrophy patients. The data are shown as mean ± standard deviation; median (minimum to maximum); 95% confidence interval for the mean.

Marker	Ambulatory – 9 patients	Non-ambulatory – 10 patients	p-value
Dimension 1 (%)	56.12 ± 15.19; 56.41 (38.46 to 82.05); 44.44 to 67.8	1.28 ± 1.81; 0 (0 to 5.12); −0.01 to 2.57	< **0.001**
Dimension 2 (%)	93.21 ± 5.75; 94.44 (83.33 to 100); 88.79 to 97.62	57.77 ± 17.36; 55.55 (33.33 to 88.88); 45.35 to 70.19	< **0.001**
Dimension 3 (%)	80.42 ± 12.47; 85.71 (57.14 to 95.23); 70.83 to 90	67.14 ± 15.46; 66.66 (38.09 to 85.71); 56.08 to 78.2	**0.043**
Total score (%)	75.34 ± 9.66; 76.04 (60.41 to 88.54); 67.92 to 82.76	36.87 ± 9.54; 36.98 (20.83 to 53.12); 30.05 to 43.69	< **0.001**

Positive data ($p < 0.05$) are shown in bold type. Statistical analysis was performed by Mann-Whitney. Alpha = 0.05. The MFM assesses proximal, distal and axial motor dysfunctions, on the broad spectrum of neuromuscular diseases. The scale is composed by 32 items (scored from zero to three points), divided into three dimensions that assesses: [D1 – 13 items (%)] standing position and postural transfers; [D2 – 12 items (%)] proximal and axial motor function; [D3 – seven items (%)] distal motor function – six items comprehend the upper limbs. The three dimensions give the information of a total score in percentage.

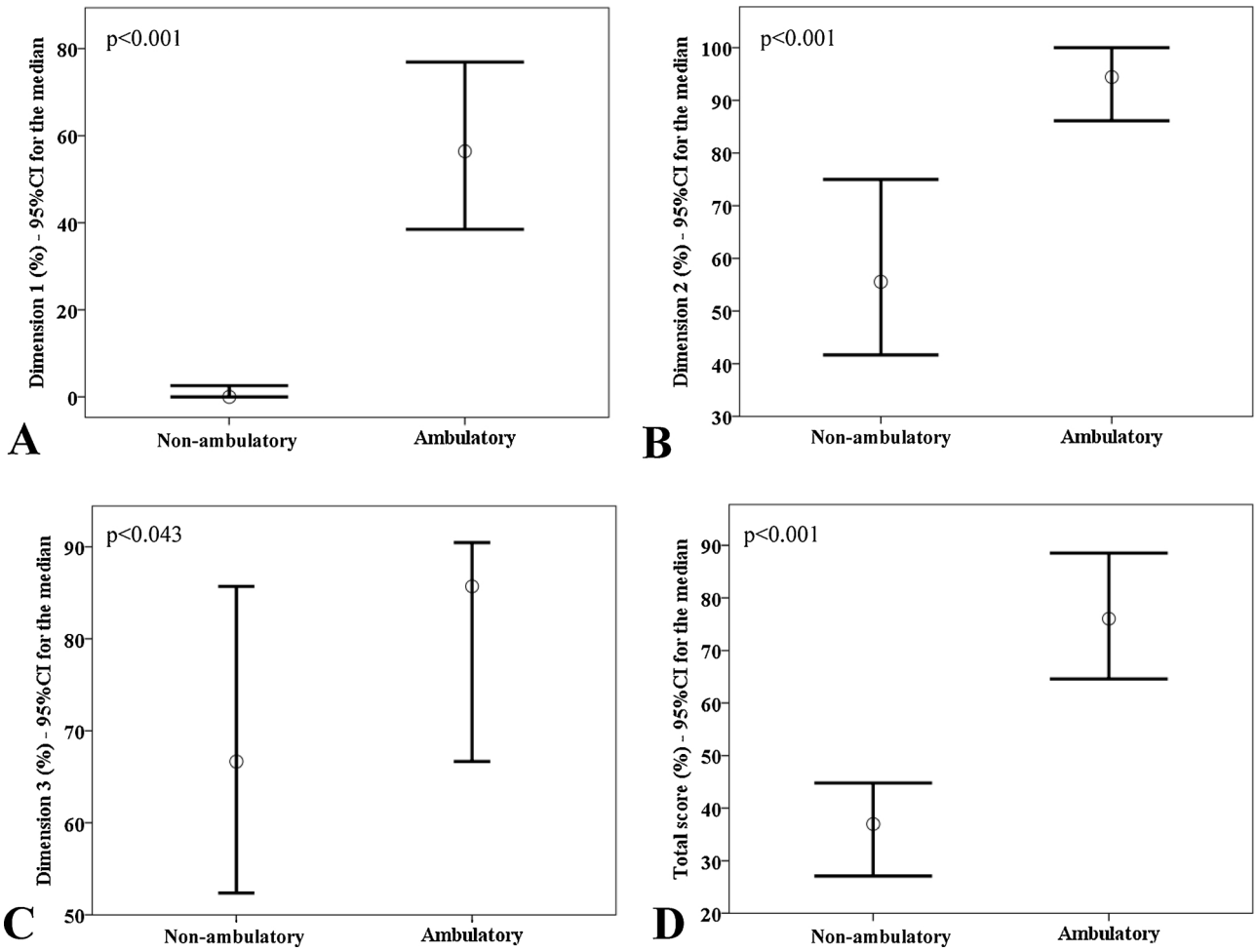

Fig. 2. Association between motor function measure for ambulatory (n = 9) and non-ambulatory (n = 10) Duchenne muscular dystrophy patients. The data are shown as median and 95% confidence interval for the median. (A) dimension 1 between ambulatory (median = 56.41) and non-ambulatory (median = 0) Duchenne muscular dystrophy patients ($p < 0.001$). (B) dimension 2 between ambulatory (median = 94.44) and non-ambulatory (median = 55.55) Duchenne muscular dystrophy patients ($p < 0.001$). (C) dimension 3 between ambulatory (median = 85.71) and non-ambulatory (median = 66.66) Duchenne muscular dystrophy patients ($p = 0.043$). (D) total score between ambulatory (median = 76.04) and non-ambulatory (median = 36.98) Duchenne muscular dystrophy patients ($p < 0.001$). The statistical analysis was performed by Mann–Whitney test. Alpha = 0.05.

Table 3
Association between the six-minute walk test variables and control group. The data are shown as mean ± standard deviation; median (minimum to maximum); 95% confidence interval for the mean.

Marker	DMDG-6MWT – 7 patients*	Control group 2 – 8 patients	p-value
Walking distance (meters)	389.29 ± 42.12; 393 (333 to 443); 350.33 to 428.24	602.13 ± 86.64; 586 (450 to 723); 529.69 to 674.56	< **0.001**
Predicted distance (meters)	549.46 ± 29.54; 548.74 (501.49 to 582.93); 522.14 to 576.78	573.33 ± 46.2; 554.46 (535.02 to 669.46); 534.7 to 611.95	0.536
SpO_2 at rest (%)	97.14 ± 1.68; 98 (94 to 99); 95.59 to 98.69	98.13 ± 0.84; 98 (97 to 100); 97.43 to 98.82	0.336
6-minute SpO_2 (%)	96 ± 5.77; 98 (83 to 99); 90.66 to 101.34	98.38 ± 0.92; 98 (97 to 100); 97.61 to 99.14	0.463
9-minute SpO_2 (%)	97.86 ± 0.38; 98 (97 to 98); 97.51 to 98.21	97.75 ± 1.17; 98 (95 to 99); 96.78 to 98.72	0.779
Respiratory rate at rest (bpm)[a]	26.43 ± 6.58; 26 (15 to 36); 20.34 to 32.51	20.25 ± 3.11; 20 (16 to 26); 17.65 to 22.85	**0.040**
6-minute respiratory rate (bpm)[a]	32.57 ± 15.09; 30 (12 to 60); 18.62 to 46.52	24.25 ± 5.81; 22 (16 to 32); 19.4 to 29.1	0.189
9-minute respiratory rate (bpm)[a]	25.14 ± 8.24; 24 (10 to 36); 17.53 to 32.76	20 ± 2.14; 20 (18 to 24); 18.21 to 21.79	**0.040**
Heart rate at rest (bpm)[b]	104.43 ± 19.48; 98 (94 to 148); 86.41 to 122.45	82 ± 11.61; 80 (70 to 100); 72.29 to 91.71	**0.009**
6-minute heart rate (bpm)[b]	109.86 ± 12.62; 105 (99 to 136); 98.19 to 121.52	106.38 ± 29.1; 106.5 (62 to 150); 82.05 to 130.7	0.867
9-minute heart rate (bpm)[b]	97.86 ± 5.11; 95 (93 to 105); 93.13 to 102.59	86.50 ± 12.41; 88 (65 to 99); 76.13 ± 96.87	0.152

SpO_2, peripheral capillary oxygen saturation; DMDG-6MWT, Duchenne muscular dystrophy group and six-minute walk test. The control group 2 performed spirometry and six-minute walk test. Positive data ($p < 0.05$) are shown in bold type. Statistical analysis was performed by Mann-Whitney test. Alpha = 0.05. [a], bpm, breath per minute; [b], beats per minute; *, two (2/9–22.22%) ambulatory DMD patients were not able to complete the 6MWT due to excessive muscle fatigue. The 6MWT was performed considering ATS recommendations with modifications (Mazzone et al., 2010; McDonald et al., 2010a). Predicted distance value was obtained by standardized equation for pediatric population (Priesnitz et al., 2009).

Table 4
Association of spirometry markers between ambulatory and non-ambulatory Duchenne muscular dystrophy patients, and between Duchenne muscular dystrophy patients and control group. The data are shown as mean ± standard deviation; median (minimum to maximum); 95% confidence interval for the mean.

Marker	DMDG – 14 patients	Control group – 17 patients	p-value
FVC (% predicted)	71.64 ± 24.48; 76.5 (26 to 102); 57.51 to 85.78	99.94 ± 9.95; 98 (84 to 116); 94.82 to 105.06	< **0.001**
FEV_1 (% predicted)	72.36 ± 23.73; 81.5 (30 to 101); 58.66 to 86.06)	102.29 ± 10.37; 102 (83 to 122); 96.96 to 107.62	< **0.001**
FEV_1/FVC	100.57 ± 5.6; 100 (90 to 107); 97.34 to 103.8	96.12 ± 4.82; 97 (84 to 102; 93.64 to 98.6	**0.004**
$FEF_{25-75\%}$ (% predicted)	88.14 ± 23.1; 87.5 (56 to 139); 74.8 to 101.48	106.65 ± 18.81; 108 (72 to 143); 96.98 to 116.32	**0.013**
FEF_{max} (% predicted)	84.5 ± 19.62; 88.5 (46 to 115); 73.17 to 95.83	110.12 ± 18.28; 106 (80 to 163); 100.72 to 119.51)	< **0.001**
MVV (% predicted)	73 ± 28.04; 75 (34 to 141); 56.81 to 89.19	90.42 ± 27.4; 90 (61 to 170); 73.01 to 107.83[*]	0.106
FVC z-score	−2.27 ± 2.37; -1.74 (-6.45 to 1.13); -3.64 to -0.9	0.57 ± 0.91; 0.38 (-0.6 to 2.37); 0.06 to 2.37	< **0.001**
FEV_1 z-score	−1.59 ± 2.04; -1.12 (-5.14 to 1.5); -2.77 to 0.42	0.81 ± 0.91; 0.8 (-0.71 to 2.66); 0.35 to 1.28	< **0.001**
FEV_1/FVC z-score	1.4 ± 1.11; 1.22 (-0.7 to 2.95); 0.76 to 2.05	0.49 ± 0.69; 0.73 (-1.44 to 1.44); 0.13 to 0.85	**0.032**
$FEF_{25-75\%}$ z-score	−0.07 ± 1.03; 0.02 (-1.69 to 1.63); -0.66 to 0.53	0.47 ± 0.83; 0.66 (-0.88 to 1.59); 0.04 to 0.89	0.128
Peak expiratory flow (L/min)[c]	24.95 ± 7.77; 24.02 (15.23 to 45.71); 21.21 to 28.7	28.15 ± 8.87; 24.76 (16.34 to 45.86); 23.58 to 32.71	0.300
Peak inspiratory flow (L/min)[c]	27.75 ± 6.87; 25.48 (19.82 to 44.36); 23.44 to 30.07	28.45 ± 7.81; 26.65 (17.69 to 43.39); 24.44 to 32.47	0.684
Expiratory time (sec)[c]	1.45 ± 0.43; 1.35 (0.88 to 2.7); 1.25 to 1.66	1.63 ± 0.61; 1.5 (0.92 to 3.16); 1.32 to 1.94	0.100
Inspiratory time (sec)[c]	1.17 ± 0.31; 1.15 (0.76 to 1.82); 1.02 to 1.32	1.45 ± 0.52; 1.4 (0.92 to 2.98); 1.18 to 1.72	0.363
Marker	**Ambulatory – 5 patients[a]**	**Nonambulatory – 09 patients[b]**	**p-value**
FVC (% predicted)	87.2 ± 14.76; 87 (66 to 102); 68.88 to 105.52	63 ± 25.1; 70 (26 to 95); 43.71 to 82.29	**0.001**
FEV_1 (% predicted)	86 ± 16.52; 85 (61 to 101); 65.48 to 106.52	64.78 ± 24.44; 78 (30 to 91); 45.99 to 83.57	< **0.001**
FEV_1/FVC	95.6 ± 4.28; 96 (90 to 101); 90.29 to 100.91	103.33 ± 4.21; 105 (96 to 107); 100.09 to 106.57	**0.004**
$FEF_{25-75\%}$ (% predicted)	81.4 ± 15.44; 74 (69 to 105); 62.23 to 100.57	91.89 ± 26.53; 92 (56 to 139); 71.5 to 112.28	**0.035**
FEF_{max} (% predicted)	96.4 ± 18.16; 99 (71 to 115); 73.85 to 118.95	77.89 ± 17.98; 88 (46 to 99); 64.07 to 91.71	**0.001**
MVV (% predicted)	78.6 ± 7.8; 79 (71 to 91); 68.92 to 88.28	69.89 ± 34.88; 61 (34 to 141); 43.08 to 96.7	0.213
FVC z-score	−0.6 ± 1.37; 0.79 (-2.59 to 1.13); -2.3 to 1.1	−3.2 ± 2.35; -2.39 (-6.45 to -0.68); -5 to -1.39	< **0.001**
FEV_1 z-score	−0.36 ± 1.35; -0.29 (-2.1 to 1.5); -2.04 to 1.31	−2.27 ± 2.09; -1.33 (-5.14 to 0.07); -3.88 to -0.67	< **0.001**
FEV_1/FVC z-score	0.38 ± 0.69; 0.63 (-0.7 to 1); -0.48 to 1.23	1.97 ± 0.87; 2.33 (0.37 to 2.95); 1.3 to 2.64	**0.003**
$FEF_{25-75\%}$ z-score	−0.15 ± 0.89; -0.43 (-1.2 to 1.14); -1.25 to 0.96	−0.02 ± 1.14; 0.28 (-1.69 to 1.63); -0.9 to 0.86	0.287
Peak expiratory flow (L/min)[c]	25.2 ± 9.22; 23.03 (15.23 to 45.71); 18.11 to 32.29	24.73 ± 6.7; 24.18 (15.58 to 34.97); 19.93 to 29.52	0.601
Peak inspiratory flow (L/min)[c]	28.1 ± 8.39; 27.08 (19.82 to 44.36); 21.65 to 34.55	25.54 ± 5.33; 24.32 (20.09 to 34.32); 21.73 to 29.35	0.780
Expiratory time (sec)[c]	1.34 ± 0.3; 1.34 (0.94 to 2.7); 1.11 to 1.57	1.56 ± 0.51; 1.52 (0.94 to 2.7); 1.19 to 1.93	0.315
Inspiratory time (sec)[c]	1.06 ± 0.3; 1.04 (0.76 to 1.73); 0.83 to 1.29	1.27 ± 0.3; 1.21 (0.8 to 1.82); 1.05 to 1.48	0.079

DMDG, Duchenne muscular dystrophy group; FVC, forced vital capacity; FEV_1, forced expiratory volume in one second of FVC; $FEF_{25–75\%}$, forced expiratory flow between 25% and 75% of FVC; FEF_{max}, maximum forced expiratory flow; MVV, maximum voluntary ventilation; L/min, liter per minute; sec, second. Positive data ($p < 0.05$) are show in bold type. [*], MVV marker was analyzed in 12 controls subjects. The control group 1 performed spirometry and volumetric capnography. Statistical analysis was performed by Mann–Whitney test. Alpha = 0.05. [a], Four DMD patients did not perform the spirometry; [b], one DMD patient did not perform the spirometry; [c], all DMD patients were analyzed. Spirometry was conducted considering ERS and ATS recommendations. Predicted values were evaluated according to Polgar and Promadhat reference (1971). Moreover, we analyzed the z-score for admixture population according to Global Lung Initiative (ERS task force report, 2012).

DMD patients ($p > 0.05$) (Table 6). This fact can be associated with the study sample size that limits the findings in biomarkers with high variability.

3.7. Peak cough flow

Among ambulatory DMD patients, the median CPF was 200 L/min, with minimum 100 L/min and maximum 330 L/min. For non-ambulatory DMD patients, the median CPF was 225, with minimum 80 L/min and maximum 360 L/min. There was no difference between ambulatory and non-ambulatory DMD patients ($p = 0.05$) (Table 6).

The walking status of DMDG did not interfere with their cough ability ($p > 0.05$) (Table 6).

The CPF showed 8/19 DMD patients with values < 160 L/min (ineffective cough), 4/19 with values between 160 to 270 L/min (intermediate cough efficiency) and 7/19 with values > 270 L/min (efficient cough). There were ambulatory and non-ambulatory DMD patients in all CPF groups.

3.8. Correlation between motor function measure and spirometry

In our data, we measured the correlation between spirometry and MFM regarding the previous significant associations described, mainly for FEV_1/FVC z-score and all parameters achieved in MFM [D1(%) correlation coefficient (CC) = −0.631, p = 0.016; D2(%) CC = −0.56, p = 0.037; D3(%) CC = −0.563, p = 0.036; total score, CC = −0.596, p = 0.025). Other significant correlations were measured and are described in Table 7.

4. Discussion

A few studies have assessed motor and respiratory deterioration simultaneously to date. In our study, we observed that DMD patients have motor and respiratory deterioration when compared with healthy subjects, and between them, regarding different conditions (ambulatory and non-ambulatory status). Moreover, it is the first study to analyze the association between VCap and DMD severity.

4.1. Motor force measure

Previous studies have not compared MFM between ambulatory and non-ambulatory DMD patients. However, revaluation each six-month showed decrease in D1 scores (Mazzone et al., 2010) and maintenance or increase in D2 scores in ambulatory and non-ambulatory DMD patients going through glucocorticoid therapy (Finder et al., 2004). In our data, all dimensions analyzed in MFM presented motor deterioration in non-ambulatory DMD patients when compared with ambulatory DMD patients. The scores achieved in MFM are important to verify the response to drug therapy and validate clinical trials; it was also observed that the scores, mainly D1 and total score, are associated with the disease progression (Diniz et al., 2012; Silva et al., 2012).

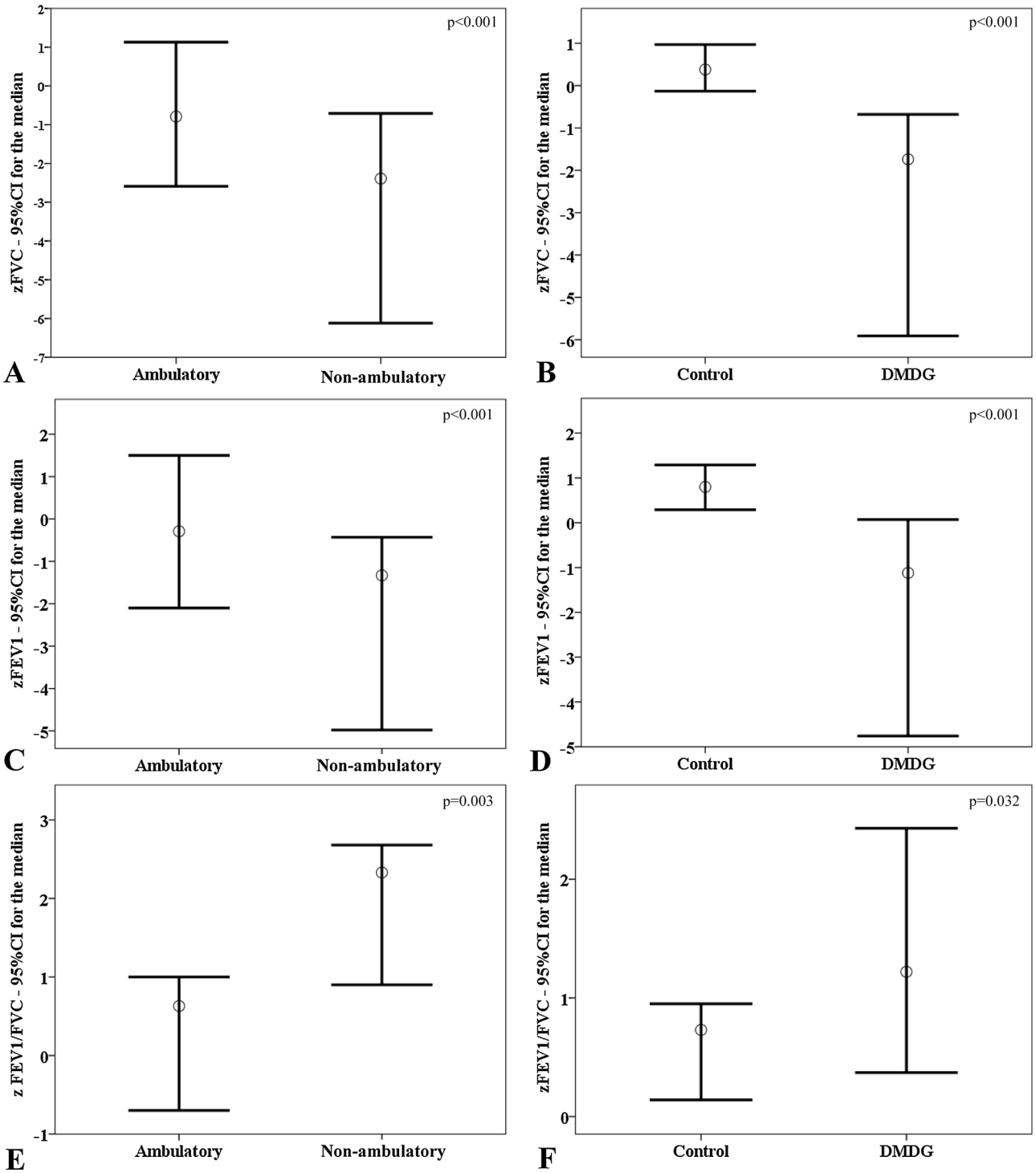

Fig. 3. Association of spirometry parameters between ambulatory (n = 5), and non-ambulatory (n = 9) Duchenne muscular dystrophy patients, and between Duchenne muscular dystrophy patients (n = 14) and control gruop (n = 17). The data are shown as median and 95% confidence interval for the median. (A) FVC z-score between ambulatory (median = 0.79) and non-ambulatory (median = −2.39) Duchenne muscular dystrophy patients ($p < 0.001$). (B) FVC z-score between Duchenne muscular dystrophy patients (median = −1.74) and control group (median = 0.38) Duchenne muscular dystrophy patients ($p < 0.001$). (C) FEV_1 z-score between ambulatory (median = −0.29) and non-ambulatory (median = −1.33) Duchenne muscular dystrophy patients ($p < 0.001$). (D) FEV_1 z-score between Duchenne muscular dystrophy patients (median = −1.12) and control group (median = 0.8) Duchenne muscular dystrophy patients ($p < 0.001$). (E) FEV_1/FVC z-score between ambulatory (median = 0.63) and non-ambulatory (median = 2.33) Duchenne muscular dystrophy patients ($p = 0.003$). (F) FEV_1/FVC z-score between Duchenne muscular dystrophy patients (median = 1.22) and control group (median = 0.73) ($p = 0.032$). FVC, forced vital capacity; FEV_1, forced expiratory volume in one second of FVC. Statistical analysis was performed by Mann–Whitney test. Alpha = 0.05. Spirometry was conducted considering ERS and ATS recommendations. The z-score was analyzed for admixture population according to Global Lung Initiative (ERS task force report, 2012).

Table 5
Association between volumetric capnography markers from Duchenne muscular dystrophy (< 11 years old and > 11 years old) and age-matched control gruop. The data are shown as mean ± standard deviation; median (minimum to maximum); 95% confidence interval for the mean.

Marker (> 11 years old)	DMDG – 9 patients	Control group 1 – 9 patients	p-value
VM_{alv} (L/min)	5.81 ± 2.57; 4.82 (3.49 to 10.46); 3.83 to 7.79	6.43 ± 2.18; 6.52 (4.23 to 10.91); 4.76 to 8.11	0.387
VM (L/min)	7.96 ± 2.9; 7.14 (4.41 to 12.88); 5.73 to 10.2	8.19 ± 2.43; 8.01 (5.92 to 13.73); 6.32 to 10.06	0.605
TV_{alv} (mL)	277.23 ± 89.23; 284.71 (186.97 to 447.93); 208.64 to 345.82	425.83 ± 262.64; 365.54 (151.24 to 919.52); 223.95 to 627.7	0.222
TV (mL)	356.33 ± 99.68; 311.64 (257.96 to 538.25); 279.70 to 432.95	493.38 ± 266.46; 439.37 (213.82 to 999.38); 288.56 to 698.2	0.340
DS (mL)	94.26 ± 16.05; 91.67 (72.2 to 125.3); 81.93 to 106.6	93.51 ± 22.28; 88.06 (67.9 to 126.1); 76.39 to 110.63	0.730
DS/TV	0.28 ± 0.06; 0.29 (0.17 to 0.35); 0.23 to 0.33	0.22 ± 0.07; 0.2 (0.13 to 0.32); 0.17 to 0.28	0.094
VCO_2 (mL)	182.74 ± 63.39; 158.18 (126.35 to 298.25); 134.01 to 231.47	231.72 ± 73.21; 221.8 (150.29 to 342.57); 175.44 to 288	0.136
IV (mL)	377.73 ± 77.04; 371.28 (297.41 to 507.84); 318.52 to 436.95	510.26 ± 287.31; 449.52 (193.68 to 1035.45); 289.4 to 731.1	0.436
EV (mL)	360.13 ± 78.53; 339.50 (270.24 to 494.23); 299.76 to 420.5	516.4 ± 276.69; 472.88 (209.97 to 1061.71); 303.72 to 729.08	0.258
RR (bpm)[a]	22.79 ± 4.96; 24.21 (14.57 to 28.79); 18.98 to 26.6	19.13 ± 6.06; 17.61 (9.98 to 31.66); 14.47 to 23.79	0.136
HR (bpm)[b]	99.9 ± 12.54; 98.34 (82.5 to 116.92); 90.26 to 109.54	84.15 ± 10.6; 83.53 (66.13 to 102.74); 76 to 92.3	**0.019**
SpO_2 (%)	97.9 ± 1; 97.97 (96.28 to 99.84); 97.14 to 98.68	97.82 ± 0.5; 97.94 (96.73 to 98.38); 97.44 to 98.2	0.796
Slope 2	410.67 ± 110.19; 368.06 (306.7 to 633.28); 325.97 to 495.38	535.23 ± 108.3; 521.45 (368.29 to 701.08); 451.98 to 618.48	0.024
Slope 3	16.92 ± 8.09; 16.17 (8.16 to 28.34); 10.71 to 23.14	16.69 ± 9.35; 12.6 (4.63 to 30.6); 9.5 to 23.87	1
Slope 2/TV	1.11 ± 0.51; 1.29 (0.41 to 1.65); 0.69 to 1.53	1.47 ± 0.95; 1.07 (0.37 to 3.28); 0.74 to 2.2	0.863
Slope 3/TV	0.08 ± 0.04; 0.07 (0.03 to 0.13); 0.05 to 0.1	0.06 ± 0.04; 0.05 (0.02 to 0.11); 0.03 to 0.08	0.863
Marker (< 11 years old)	**DMDG – 10 patients**	**Control group 1 – 8 patients**	**p-value**
VM_{alv} (L/min)	4.87 ± 0.84; 4.69 (3.57 to 6.69); 4.27 to 5.47	7.92 ± 2.73; 6.98 (5.09 to 12.46); 5.64 to 10.2	**0.002**
VM (L/min)	6.76 ± 1.36; 6.43 (5.33 to 10.26); 5.79 to 7.74	10.1 ± 3.04; 9.3 (6.3 to 14.52); 7.57 to 12.64	**0.004**
TV_{alv} (mL)	235.7 ± 29.58; 238.23 (188.87 to 294); 214.54 to 256.86	352.55 ± 138.23; 318.61 (221.13 to 617.6); 236.99 to 468.12	**0.016**
TV (mL)	278.25 ± 34.34; 275.65 (231.96 to 333.14); 253.68 to 302.81	415.98 ± 144.68; 398.03 (263.06 to 679.4); 295.03 to 436.94	**0.016**
DS (mL)	67.6 ± 7.62; 63.69 (60.7 to 80.9); 62.15 to 73.05	80.46 ± 11.06; 80.7 (66.8 to 99.1); 71.22 to 89.71	**0.012**
DS/TV	0.24 ± 0.02; 0.24 (0.21 to 0.29); 0.23 to 0.26	0.22 ± 0.06; 0.23 (0.13 to 0.33); 0.16 to 0.27	0.203
VCO_2 (mL)	157.37 ± 22.73; 156.42 (118.48 to 206); 141.11 to 173.33	225.3 ± 63.04; 213.17 (148.49 to 329.37); 172.6 to 278	**0.006**
IV (mL)	298.48 ± 39.42; 292.13 (239.25 to 365.5); 270.28 to 326.68	408.71 ± 141.17; 385.96 (241.89 to 688.13); 290.69 to 526.74	**0.043**
EV (mL)	279.8 ± 33.53; 274.26 (233.25 to 355.67); 255.8 to 303.78	433.91 ± 146; 395.84 (306.73 to 723.66); 311.85 to 555.96	**< 0.001**
RR (bpm)[a]	24.23 ± 3.55; 25.27 (19.47 to 30.71); 21.69 to 26.77	25.5 ± 6.67; 24.31 (15.45 to 34.07); 19.93 to 31.09	0.762
HR (bpm)[b]	97.63 ± 7.76; 96.42 (86.66 to 110.28); 92.07 to 103.18	91.55 ± 19.3; 87.38 (67.87 to 127.74); 75.42 to 107.69	0.315
SpO_2 (%)	97.7 ± 0.86; 97.79 (96.34 to 99.58); 97.09 to 98.32	97.82 ± 0.73; 98 (96.46 to 98.87); 97.22 to 98.43	0.515
Slope 2	481.18 ± 99.58; 452.44 (374.46 to 638.13); 409.94 to 552.41	406.26 ± 136.72; 391.33 (264.37 to 674.21); 291.96 to 520.56	0.203
Slope 3	20.09 ± 7.61; 18.66 (10.58 to 30.09); 14.65 to 25.53	14.17 ± 5.95; 13.51 (6.86 to 24.37); 9.2 to 19.14	0.122
Slope 2/TV	1.75 ± 0.4; 1.73 (1.12 to 2.32); 1.47 to 2.03	1.26 ± 0.54; 1.18 (0.57 to 2.03); 0.85 to 1.67	**0.027**
Slope 3/TV	0.04 ± 0.03; 0.04 (0.01 to 0.09); 0.02 to 0.07	0.05 ± 0.05; 0.03 (0.005 to 0.14); 0.01 to 0.09	0.055

DMDG, Duchenne muscular dystrophy group; VM_{alv}, alveolar ventilation per minute; VM, ventilation per minute; TV_{alv}, tidal alveolar volume; TV, tidal volume; DS, dead space; VCO_2, carbon dioxide production; IV, inspiratory volume; EV, expiratory volume; RR, respiratory rate; HR, heart rate; SpO_2, oxygen saturation using pulse oximetry; %, percentage; mL, milliliter. Positive data ($p < 0.05$) are show in bold type. The control group 1 performed the spirometry and volumetric capnography. Statistical analysis was performed by Mann–Whitney test. Alpha = 0.05. [a], bpm, breath per minute; [b], beats per minute.

Table 6
Association of respiratory muscle strength and peak cough flow markers between ambulatory and non-ambulatory Duchenne muscular dystrophy patients. The data are shown as mean ± standard deviation; median (minimum to maximum); 95% confidence interval for the mean.

Respiratory muscle strength	Ambulatory – 10 patients	Nonambulatory – 9 patients	p-value
Inspiratory muscle strength (cmH_2O – %)	32.44 ± 13.32; 30 (16 to 54); 22.21 to 42.68	28.6 ± 11.11; 31.5 (9 to 47); 20.65 to 36.55	0.842
Expiratory muscle strength (cmH_2O – %)	22.56 ± 9.3; 20 (9 to 41); 15.41 to 29.71	15.1 ± 6.33; 16 (7 to 24); 10.57 to 19.63	0.053
Peak cough flow	**Ambulatory – 10 patients**	**Nonambulatory – 9 patients**	**p-value**
Peak cough flow meter (L/min)	211.11 ± 85.51; 200 (100 to 330); 145.39 to 276.84	234 ± 97.32; 225 (80 to 360); 164.38 to 303.62	0.604

cmH_2O, centimeters of water; %, percentage; L/min, liter per minute. Statistical analysis was performed by Mann–Whitney test. Alpha = 0.05.

4.2. Six-minute walk test

DMDG-6MWT subjects were heavier and presented higher BMI than CG2, and this fact maybe explained by the continuous use of prednisone to maintain walking status in ambulatory DMD patients (Bach et al., 2010). Besides, DMD children are less active when matched by age with healthy control subjects in relation to motor restriction (Söderpalm et al., 2013).

The 6MWT has been analyzed in DMD by numerous studies (Mazzone et al., 2010; McDonald et al., 2010a; McDonald et al., 2010b; Henricson et al., 2012; Mazzone et al., 2011; Goemans et al., 2013; Mazzone et al., 2013). Our DMD patients walked an average distance of 389.29 ± 42.12 m (median = 393). The walking distance shows a decline that is associated with disease progression, and it is age-related, being a useful tool to determine motor deterioration and the progression to wheelchair dependency, as well as being used in clinical trials (Goemans et al., 2013; Mazzone et al., 2013). Moreover, one study described that a maximum walking distance of 330 m was a predictive value to indicate ambulation loss within the next two years (Mazzone et al., 2013). None DMD patients, in our study, walked a distance < 330 m.

In our data, Priesnitz equation was used to analyze the 6MWT. This equation is suited to South American population (Priesnitz et al., 2009; Henricson et al., 2012). The comparison among the studies is limited regarding the use of different equations to analyze the 6MWT and the high variability among the DMD patients considering disease progression.

The longitudinal studies may give us a response about the disease

Table 7
Correlation between motor function dimension and spirometry marker Duchenne muscular dystrophy patients.

Spirometry marker		Motor function measure			
		Dimension 1 (%)	Dimension 2 (%)	Dimension 3 (%)	Total dimension (%)
FVC (% predicted)	Correlation coefficient	0.479	0.493	0.518	0.554*
	P-value	0.083	0.073	0.058	**0.040**
FEV_1 (% predicted)	Correlation coefficient	0.561*	0.520	0.612*	0.615*
	P-value	**0.037**	0.057	**0.020**	**0.019**
FEV_1/FVC	Correlation coefficient	−0.522	−0.505	−0.576*	−0.551*
	P-value	0.055	0.065	**0.031**	**0.041**
$FEF_{25-75\%}$ (% predicted)	Correlation coefficient	0.138	0.348	0.376	0.376
	P-value	0.637	0.223	0.186	0.185
FEF_{max} (% predicted)	Correlation coefficient	0.534*	0.607*	0.525	0.637*
	P-value	**0.049**	**0.021**	0.054	**0.014**
MVV (% predicted)	Correlation coefficient	0.369	0.470	0.482	0.548*
	P-value	0.195	0.090	0.081	**0.043**
FVC z-score	Correlation coefficient	0.543*	0.531	0.579*	0.604*
	P-value	**0.045**	0.051	**0.030**	**0.022**
FEV_1 z-score	Correlation coefficient	0.499	0.514	0.565*	0.596*
	P-value	0.069	0.060	**0.035**	**0.025**
FEV_1/FVC z-score	Correlation coefficient	−0.631*	−0.560*	−0.563*	−0.596*
	P-value	**0.016**	**0.037**	**0.036**	**0.025**
$FEF_{25-75\%}$ z-score	Correlation coefficient	0.131	0.386	0.323	0.380
	P-value	0.655	0.173	0.261	0.180

FVC, forced vital capacity; FEV_1, forced expiratory volume in one second of FVC; $FEF_{25–75\%}$, forced expiratory flow between 25% and 75% of FVC; FEF_{max}, maximum forced expiratory flow; MVV, maximum voluntary ventilation. Spearman's rank correlation coefficient was used in all correlations tests performed. * The correlation showed level significance of 0.05 (two tails values); **The correlation showed level significance of 0.01 (two tails values). Positive data ($p < 0.05$) are show in bold type. Spirometry was conducted considering ERS and ATS recommendations. Predicted values were evaluated according to Polgar and Promadhat reference (1971). Moreover, we analyzed the z-score for admixture population according to Global Lung Initiative (ERS task force report, 2012).

progression and the use of 6MWT. Currently, we know that the walking distance has been reduced year by year slowly and progressively, but there is no agreement among how many meters are lost per year (Mazzone et al., 2011; Henricson et al., 2012; Mazzone et al., 2013).

The HR increased in rest condition, in our DMDG, as previously described for 6MWT (McDonald et al., 2010a). The HR increased and its delay to return to basal values may indicate an early overload in cardiac system (Shirokova and Niggli, 2013).

4.3. Spirometry

DMD lung function is early altered, being detected around eight years old, when spirometry measures are more reliable (Phillips et al., 2001). FVC and FEV_1 may characterize decreased lung function in DMDG (Finder et al., 2004); in addition, the peak expiratory flow may be used to show lung disease progression, despite ambulation status or steroid treatment (Mayer et al., 2015; Gauld and Boynton, 2005).

For the best of our knowledge, no other study has performed the comparison of spirometry parameters between DMDG and age-matched control group. Besides, lung function decline in DMDG, with restrictive characteristics, is mainly present in non-ambulatory DMD patients according to the cited literature (Humbertclaude et al., 2012; Gayraud et al., 2010).

Gauld and Boyton showed a correlation between FVC and FEV_1 with CPF, and lower CPF was correlated with higher spirometry values (Goemans et al., 2013). In our data, such association was not found.

4.4. Volumetric capnography

It has been described that DMD children present an initial ascending respiratory pattern due to their growth and development (Humbertclaude et al., 2012; Gayraud et al., 2010). Our data showed that growth occurs at a slower pace, in comparison with age-matched control group, and they reach their matched control healthy subjects in a more advanced age. However, this finding is limited in terms of subjects enrolled.

The VCO_2 was lower in DMDG < 11 years old, and this fact may occur more likely due to a metabolic imbalance rather than a ventilatory imbalance (Söderpalm et al., 2013). At that early age, motor dysfunctions are more evident than respiratory muscle dysfunction. Moreover, the same result did not appear in DMDG > 11 years old, leading the authors to believe that VCO_2 exhibits an ascending pattern before overtaking normal values. The overtaking could be visualized in IV-phase. Other parameters from VCap point to the opportunity to include new tools to evaluate lung function in regard to degrees of DMD severity.

At last, HR was higher for DMDG > 11 years old when compared with age-matched control group. This fact may be correlated with elevated levels of end-tidal carbon dioxide during sleep and should be better assessed with transcutaneous carbon dioxide monitoring. It was already shown that pulmonary care needs to be more effective after the loss of independent ambulation (Bushby et al., 2009). VCap might emerge as new tool to be used in the monitoring of DMD patients.

The VCap is a tool that can be used to determine the lung function without high costs and it is a tool where patient effort is not required being measured at tidal volume. However, clinicians and researchers can lack complete knowledge about it and, unfortunately, its subuse is common.

4.5. Maximal inspiratory pressure and maximal expiratory pressure

In our data, MIP and MEP presented no difference between ambulatory and non-ambulatory DMD patients. Nevertheless, all data were below the predicted values (Shirokova and Niggli, 2013) and previously published data for DMD patients (Park et al., 2010; Nève et al., 2013).

4.6. Cough peak flow

For our DMDG, walking status did not interfere in cough ability. Also, our DMDG presented higher values than a previous study (Park et al., 2010).

A previous study established a standardized curve of CPF values per age and gender (Bianchi and Baiardi, 2008). For male children > seven years old, the 200 L/min of CPF was set as percentile 5. Also, the

strong correlation of CPF with age, height and weight guarantees that all CPF values were increasing up to percentile 95 (944 L/min) until 17 years old. Considering the previous data, in our casuistic including DMD patients, 14/19 were < percentile 5 and the whole DMDG was < percentile 50 for age and sex by the standardized curve of CPF.

DMDG classification for cough efficiency was previously used to associate spirometry, respiratory muscle strength and CPF with plethysmography in order to determine if ineffective cough is associated with low operational chest wall and pulmonary volumes, secondary to DMD muscle weakness (LoMauro et al., 2014). The results from cough peak flow do not differ between ambulatory and non-ambulatory patients with DMD, and in this way it does not appear to account for changes in body size due to growth and development.

4.7. Perspectives

The quality of life and life expectancy presented improvement in DMD, but the main cause of death regarding this disease is cardio-respiratory failure, without major alteration in the management over the past years (Van Ruiten et al., 2016). This fact should be studied in order to improve the patient management.

We also need to consider that DMD affects the patients and the caregivers, being a "familiar disease" (de Moura et al., 2015), and the psychological aspects should be better studied.

Additionally, the gene editing by clustered regularly interspaced short palindromic repeat/Cas9 (CRISPR/Cas9) may be a useful tool to permanent gene correction in DMD, and human studies should be done in the next years, including the lung function and motor function analysis after to promote the gene editing (Xu et al., 2016; El Refaey et al., 2017).

4.8. Limitations

Our study had limitations, mainly caused by the very DMD characteristics. DMD is a rare condition and the ideal sample size would require a multicentered study to assess a substantial number of patients. Even in a Reference Center and recruiting all DMD patients that were monitored, our sample could not generalize our findings to all patients. A better study design would include a DMDG with adolescents in IV-phase, for it would be of great interest to evaluate how the lung function behaves after a greater period of wheelchair restriction. Longitudinal studies and genetic screening could highlight new insights regarding the DMD to identify different phenotypes of the disease. In future, to evaluate pulmonary deterioration of DMD, we should include other tools as lung clearance index e exhaled breath condensate.

5. Conclusion

The DMD patients presented motor and respiratory deterioration when compared with control subjects, mainly for MFM/6MWT and spirometry/VCap, respectively. Moreover, the non-ambulatory condition was associated with worse MFM and spirometry. The deterioration of motor and respiratory function showed correlation between each other. In this context, motor and respiratory deterioration may be evaluated by MFM, 6MWT, spirometry and VCap. The assessments should be performed on a routine basis. Multicenter prospective longitudinal studies may contribute to better understanding of motor and respiratory dysfunction progression and DMD management.

Authors' contribution

LCL conceived the study, carried out the collection, analysis and interpretation of data, performed statistical analysis and drafted the manuscript. CCBA participated in the design of the study, carried out the analysis and interpretation of data and drafted the manuscript. AADCT participated in the design of the study, carried out the analysis and interpretation of data and drafted the manuscript. AN conceived the study, participated in the collection of data and revised the manuscript critically for important intellectual content. FALM/JDR conceived the study, participated in the interpretation of data and revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

Conflict of interest

None of the authors has any conflict of interest to disclose.

Ethical publication statement

We have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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