“Pulmonary dysfunction in children with Duchenne muscular dystrophy may appear earlier than we thought – analysis using novel methodology based on z-scores.”

**Type**
Research paper

**Keywords**
pulmonary function, Pulmonary Function Testing (PFT), Duchenne muscular dystrophy, z-score

**Abstract**

**Introduction**
Respiratory status is one of the main factors affecting the length of survival in patients with Duchenne muscular dystrophy (DMD) – the most common, severe, progressive muscular dystrophy. The aim: (1) to assess pulmonary function in DMD patients using the z-score method and (2) to identify factors affecting it, irrespective of the disease progress.

**Material and methods**
We evaluated 55 boys (aged 5 – 18 years) with DMD. The spirometry was performed with: forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1), peak expiratory flow (PEF) analysis as absolute values (in litres or litres/min), % predicted value (%pv) and z-scores [z]. The need of ventilation support, ambulatory status, steroid therapy were collected.

**Results**
25(45%) subjects were non-ambulatory, 38(69%) used steroid therapy. Mean FVC[z] -2.4±2.2, FEV1[z] -2.0±1.9, PEF[z] -1.5±1.3 value significantly decrease with age (r=-0.62/-0.65/-0.55; p<0.001 respectively), after reaching the peak values between 9-12 or 6-9 years of age depending on analysis method (absolute, %pv or z-score). The results fell below normal range (z-score<-1.64) at the age of 9.8/10.4/11.6 years and below 80%pv at 10.7/13.2/13.2 for FVC/FEV1/PEF, respectively. The pulmonary function test results were significantly lower in non-ambulant (p<0.001) and non-steroid patients (p<0.02).

**Conclusions**
Analysis of pulmonary function test based on z-score shows that deterioration of pulmonary function in DMD males may appear earlier than we thought measured by %pv and absolute values. Early loss of ambulation, lack of or delayed steroid therapy are risk factors for worse pulmonary outcomes. To confirm these findings cohort longitudinal studies are necessary.
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Conclusion: Analysis of pulmonary function test based on z-score shows that deterioration of pulmonary function in DMD males may appear earlier than we thought measured by %pv and absolute values. Early loss of ambulation, lack of steroids and delayed steroid therapy are risk factors for worse pulmonary outcomes. To confirm these findings cohort longitudinal studies are necessary.
1. Introduction

Duchenne muscular dystrophy (DMD) is a severe, progressive, X-linked disorder affecting 1 in every 3500-5000 male births [1]. Mutations in the dystrophin gene lead to an absence or defect in the protein product resulting in progressive muscle weakness and, in consequence, loss of ambulation, cardiac and respiratory failure [2,3]. The respiratory status is among the main factors determining the length of survival in DMD patients. Initially, patients have no difficulties in breathing or coughing and their pulmonary function test (PFT) results are within normal limits. The progression of the disease causes gradually increasing muscular weakness, that leads to respiratory complications [4-7]. Typically it is manifested by nocturnal hypoventilation, followed by day-time hypoxemia, hypercapnia, and finally respiratory failure. Additionally, from the moment when the patient becomes non-ambulant (usually by the age of 10-12 years), and spends more time in a wheelchair in a forced sitting position, the impairment of lung function increases. This is particularly evident in patients who suffer from scoliosis [3,8-10]. Historically, DMD results in death in the teens. Currently, through appropriate supportive treatment, the average life expectancy of a patient with DMD is approximately 30 years [6,7]. A careful and systematic approach to patients’ respiratory management may prolong survival [4,11], thus it is important to find the moment of deterioration of pulmonary function in DMD patients.

American Thoracic Society (ATS) recommends baseline pulmonary function assessment optimally between 4 and 6 years of age or before confinement to a wheelchair [10]. Patients should visit a paediatric pulmonologist twice yearly or while on mechanically assisted ventilation even every 3 months [5]. Although Global Lung Initiative (GLI) and European Respiratory Society (ERS) Taskforce Report recommend analysis of PFT based on z-score, this method is still not used in DMD patients [12,13]. Besides, large-scale, randomized controlled trials are rare in this field, so knowledge of the pulmonary status of DMD patients is obtained often from small clinical groups [14,16]. Therefore, subsequent studies of pulmonary function in DMD patients remain constantly up-to-date and important.

The aim of this study was: (1) to assess pulmonary function in DMD patients using the z-score method and (2) to identify factors affecting it, irrespective of the disease progress.

2. Patients and methods

2.1 Study design
In this prospective, single-centre, cross-sectional study, pulmonary status of muscular dystrophy patients was evaluated. Boys visited the regional reference Rare Disease Centre in Gdansk for the first time, between the years 2015-2017. The study was performed in compliance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). Approval for the study was obtained from The Committee of Ethics no NKBBN/105/2018 and informed written consent was obtained from all subjects. This study was funded by the Medical University of Gdańsk, Poland (Institutional Grant ST 554).

2.2 Patients

Inclusion criteria were: (1) male aged 5-18 years old, (2) confirmed diagnosis of DMD based on guidelines [6] - the presence of typical clinical symptoms, genetic testing, muscle biopsy results, (3) the ability to perform PFT.

Patients were evaluated according to the study protocol by a multidisciplinary team, including a paediatric pulmonologist. Interview, physical examination and spirometry was performed in all patients. The need of ventilation support, ambulatory status, steroid therapy - ST (the presence, the type, the dose, the length and the age of onset) were collected from interview and medical records. Anthropometrical data: weight (a calibrated weighing chair -AXIS, model B 150 K, with accuracy up to 50g), height (in non-ambulatory patients as recumbent segmental length: head to hip, hip to knee, and knee to foot) were measured according to the guidelines [6,7].

2.3 Pulmonary function test

Spirometry was performed using the calibrated, computerized spirometer Pneumo Screen, Jaeger, Germany, according to the ATS recommendations, by a certificated, experienced with children, teenagers’ physical therapist [10]. A minimum of three, and up to five, manoeuvres with maximum effort were attempted by each subject. The highest value of forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1) expressed as litres [L], and peak expiratory flow (PEF) in litres per minute [L/min], from correct acceptable attempts were included into the analysis. Corresponding % predicted value (%pv) and z-scores [z] for age, ethnicity, gender and height were calculated, based on the GAMLSS (Generalized Additive Model for Location, Scale and Shape) method in accordance with 2012 GLI and ERS Taskforce Report, and recommendations using GLI-2012 Excel Sheet Calculator (Version 4, 25 May 2014) [13].
2.4 Statistical analysis

Statistical analyses were performed using Wizard Pro 1.9.16 (Evan Miller, Chicago, IL), data is given as mean (±SD) or median (min – max) dependently on the distribution. Normality of the distribution was tested using the Shapiro-Wilk test. Student t-test and Mann-Whitney test were used as indicated. For multiple comparisons, proper ANOVA or Friedman tests were used. Categorical data was compared with the chi-square test and chi-square test for trend (Cochran-Armitage). Pearson correlations, linear regression and multivariable linear models were additionally employed. To compare the rates of the disease progression between two groups we have used the test for equality of two regression coefficients as recommended [17,18]. Results having p-value < 0.05 were considered statistically significant.

3. Results

3.1 Patients characteristics

Out of 78 patients recognized earlier as muscular dystrophies, 55 children met the inclusion criteria. Twenty-three patients were excluded for the following reasons: age below 5 years, mutation other than DMD (Becker muscular dystrophy), inability to perform PFT (9, 9, 5 males respectively). All patients were male Caucasians, their characteristics are presented in Table 1.

3.2 Pulmonary function tests

PFT results are shown in Table 2. Absolute FVC[L], FEV₁[L], PEF [L/min] value increased with age, reaching peak numbers in children age of 9-12 years and then subsequently decreased in older ones (Fig. 1A-C). Normalized FVC[z], FEV₁[z], PEF[z] values start to decline earlier after reaching the highest z-score values in subjects age of 6-9 years (Fig. 1D-E).

While the correlation between %pv and z-score values was strong (R=0.96, R=0.98, R=0.68 p=0.0001 for FVC, FEV₁, PEF respectively, analysis of the linear regressions for the whole sample revealed that the results fell below normal range (z-score < -1.64) at the age of 9.8, 10.4, 11.6 years for FVC[z], FEV₁[z], PEF[z], respectively and fell below normal range (value < 80%pv) at the age of 10.7, 12.2, 13.2 years for FVC%pv, FEV₁%pv, PEF%pv, respectively.

There was a negative correlation between PFT described by z-scores of measured parameters and age: FVC[z] (r=-0.62; p<0.001), FEV₁[z] (r=-0.65, p<0.001), and PEF[z] (r=-0.55; p<0.001). A multiple linear regression was calculated to predict PFT results based on age. Significant regression equations were found for FVC[z], FEV₁[z], PEF[z] (see Table 3).
Participants’ predicted FVC[z] was equal to 3.73, predicted FEV1[z] - 3.47 and PEF[z] was equal to 1.57. Therefore annual decline z-score rates were 0.55, 0.49, 0.28 for FVC, FEV1, PEF respectively (Fig 1 D-E, regressions lines for the overall sample not depicted).

Consequently, the number of subjects with abnormal absolute and normative FVC, FEV1, PEF value increased with age (p=0.001, p=0.002, p=0.048; respectively, Cochran-Armitage test) (Fig 2 A-C).

All children older than 14.0 years had abnormal PFT with significant variability in the results, interestingly absolute FVC was ranging from 0.6L to 2.5L (3 patients presented FVC under 1 L) (Fig 1 D-E).

3.2.1 Pulmonary function test and ambulatory status

Patients who were able to walk on their own had significantly higher PFT results than non-ambulatory patients (Fig 3A). The ambulatory transitional period is established between 10 and 14 years of age (n=24). In our study group 10 (42%) patients in this period were ambulant. Ambulatory patients in transitional age had significantly higher FCV[z], FEV1[z] (p<0.05 for both variables), but not PEF[z] (p=0.190) in comparison to the non-ambulatory (Fig 3B). Similar results were obtained for FVC %pv, FEV1 %pv, PEF %pv: 87.3vs 57.7; 94.5 vs 63.6 and 79.7 vs 60.7, respectively (p=0.0001).

3.2.2 Pulmonary function test and steroid therapy

Patients treated with steroid therapy (on-ST) presented significantly higher values of FVC %pv (81.1±27.1 vs 61.1±22.2 p=0.002), FEV1 %pv (87.1±27.8 vs 69.1±25.1 p=0.02), PEF %pv (74.5±21.8 vs 65.1±20.1 p=0.11) and also, FVC[z] (-1.7±2.3 vs -3.4±2.0; p=0.005), FEV1[z] (-1.5±2.0 vs -2.8±1.9; p=0.016), but not PEF[z] (-1.4±1.4 vs -1.8±1.1; p=0.257) than patients without steroid therapy (non-ST). This difference did not reach statistical significance within age subgroups (Fig 4 A-C), most probably as a result of small sample sizes.

Analysing the possible effect of the type of steroid used (deflazacort vs prednisone) we didn’t find any significant difference in FVC[z] (-1.4±2.1 vs -2.4±2.5; p=0.189), FEV1[z] (-1.2±1.9 vs -2.1±2.2; p=0.246), PEF[z] (-1.3±1.3 vs -1.6±1.6; p=0.478) nor in the proportion of ambulant and non-ambulant patients (56% vs 45%; p=0.511). Similarly, there are no statistical differences for FVC %pv (75.1 vs 84.9; p=0.125), FEV1 %pv (80.9 vs 91.7; p=0.253), PEF %pv (71.7 vs 76.5; p=0.510).
A positive correlation between use of steroids and FVC[z], and FEV$_1$[z] (R Spearman =0.55; $p<0.05$ for both variables) and PEF (R Spearman =0.31 ; $p<0.05$) was noticed. Also, we observed the positive correlation between use of steroids and FVC %pv, FEV$_1$ %pv, (R Spearman = 0.36 for both variables; $p<0.05$), but not for PEF.

Additionally, the duration of ST had a positive effect on PFT outcomes correcting for age (multiple linear model; Table 3).

To determine the effect of ST on the disease progression rate we have fitted linear regression models separately for the on-ST and non-ST patients. The predicted levels of FVC[z], FEV$_1$[z] and PEF[z] are presented in the Table 4.

Comparing the slopes of the annual decline rate of PFT they were similar in both on-ST and non-ST subgroups (Fig 4), for FVC[z] (-0.55 and -0.48; $p=0.637$), FEV$_1$[z] -0.48 and -0.45; $p=0.863$), PEF[z] (-0.25 and -0.29 respectively; $p=0.660$). Thus, we found no significant differences in the rate of PFTs decline between the two subgroups.

However, the regression lines are shifted and intersect the threshold for a lower level of normal (z-score < -1.64) at different ages for on-ST vs non-ST patients: FVC (10.7 vs 7.9), FEV$_1$ (11.2 vs 9.2 years of age respectively). This could be interpreted as 3 years of advantage for FVC and 2 years of advantage for FEV$_1$ of the treated vs untreated patients. The results presented as %pv showed slower progression with steroid treatment, and the regression lines are shifted the threshold for a lower level of normal (<80%pv) later than presented in z-score (on-ST vs non-ST patients: FVC 11.0 vs 8.1, FEV$_1$ 11.5 vs 9.6 years of age respectively, Fig 1.G-J).

3.2.3. Multivariable regression model

Finally, including all the identified risk factors (patient age, ambulatory status, ST) we have calculated multiple-linear regression models to predict FVC[z], FEV$_1$[z], PEF[z] for DMD patients. The age was considered a continuous variable, ambulatory status and ST were coded 0=no, 1=yes. FVC[z] and FEV$_1$[z] were significantly dependent on age, ambulatory status and ST, PEF[z] only on age and ambulatory status (Table 3). Specifically, FVC[z], FEV$_1$[z], PEF[z] decreased with age by 0.359, 0.303, 0.166 respectively annually but were 1.188, 1.233, 1.619 higher if the patient was ambulant. ST influenced FVC[z] and FEV$_1$[z], by 1.525 and 1.125 respectively.
4. Discussion

Our study provides an insight on respiratory system condition assessed by PFT in 5-18 years old, male Caucasians, DMD patients in different stages of the disease, addressing for the first time the reference centre in northern Poland. More than half (55%) were non-ambulatory, and the majority (69%) were treated with glucocorticoids. We have used state-of-the-art methodology to interpret PFT results in children, that is calculating z-scores based on the GAMLSS method. This method has been recently recommended by GLI 2012 and ERS, as is considered to be superior to the still commonly-used method of expressing the results as a percentage of predicted value [10,13].

4.1 Pulmonary function parameters in different stages of the disease

The most important finding of our study was the fact that using GAMLSS method we show the difference in time of PFT peak between absolute values, percentage of predicted volume and z-scores values. Noticeably, the measured parameters still increase until age of 9-12 years, but with normalized data it becomes apparent that pulmonary status deceivingly starts to decline much earlier – from the age of 6–9 years after reaching the highest z-score values. While the correlation between %pv and z-score values was strong our analysis showed that the z-score could predict faster decline PFT value than %pv in patients with DMD. It is an interesting finding that may change everyday practice focusing clinician’s attention to z-score values of PFT results. Earlier pulmonary function decline should lead to earlier introduction of respiratory physiotherapy in DMD patients. It also means that their pulmonary capacity never develops to its full potential of healthy peers. The observation above further underlines the necessity to use normalized data in interpretation of the results in children as they account for the effects of development (growth) and body size among others.

Generally, all PFT values significantly decrease with age after reaching the peak values which is consistent with the natural history of pulmonary function in DMD patients described in two prospective studies of a longitudinal observational cohort: The Cooperative International Neuromuscular Research Group (CINRG) Duchenne natural history study (DNHS) and an Institutional Review Board approved United Dystrophinopathy Project (UDP) cohort study [19,20].

Interestingly, out of all analysed z-score parameters, FVC started to fall in the earliest age, and faster than others. This indicates that the FVC is a good and sensitive parameter for monitoring
the progression of the disease which is confirmed by the updated guidelines [5]. Also, the decline in FVC is historically the best validated pulmonary function predictor of mortality in DMD patients [10,21].

The next interesting fact is that all children over the age of 14.0 years had FVC values below normal range for age, but at the same time, there was a significant variability in the measured FVC in Litres. Phillips et al. evaluated a threshold of FVC of 1L as a predictor of unfavourable outcome (5-year survival - 8%) [21]. Similar lung function deterioration rate and course of the disease has been reported in the literature review [22-27]. To validate these findings, we need to recruit more patients and continue with a longitudinal study.

4.2 Effect of loss of ambulation on PFT

Per subgroup analysis we concluded that patients who were not able to walk on their own had lower PFT results than ambulatory patients at the same age. The moment of loss of ambulation coincides with the onset of decline of measured FVC and FEV1. It is unsure whether to treat it as a consequence of the natural course of the disease or as a complication of immobilization together with progressive scoliosis, which may worsen conditions for ventilation by itself. Such conclusions were drawn by some authors previously [20,28,29]. Moreover, Philips et al. concluded that a reduction in FVC to less than 1 L increased the risk of a faster transition to a wheelchair and also predicted median survival length of 3.1 years [21].

4.3 Pulmonary function parameters and ST

Results of our study indicated that steroids positively influence pulmonary outcome; steroid treatment delays, but does not prevent, the progression of the disease. Independently of steroid regimens (prednisone or deflazacort), we found better PFT results, both absolute and normalized. The onset of pulmonary function loss was delayed from 2 to 3 years in patients on-ST compared to non-ST.

Our PFT results performed in the Polish cohort are similar to other authors’ findings. Two randomized controlled trials studies described impact of ST on pulmonary function assessed by FVC [16,30]. Griggs et al. reported an improvement in FVC about 0.16 L in subjects treated with prednisone (0.3 mg/kg/day) for six months compared to placebo group [16]. Mendell et al. evaluated treatment with prednisone 1.5 mg/kg/day versus placebo for six months showing a mean improvement in FVC in the treatment group similarly to the Griggs study (0.14 L more
than in the placebo group) [30]. Similar results are available from non-randomized cohort studies which showed improvement of FVC in patients on ST [31-34].

Although the Cochrane review confirmed the positive effect of steroids on pulmonary function, administration of ST in DMD is still controversial due to multiple adverse reactions associated with chronic uptake [14,35,36]. Further, the optimal standard regimen is still under discussion, there are questions about the frequency of administration, the optimum dose – adequate to be efficient, but at the same time not provoking significant side-effects. Bushby et al. recommended starting the treatment during the plateau phase – while there is no longer progress in motor skills, but prior to the decline, as described by medical history and exercise testing [4]. So far it is proven that 0.75mg/kg of prednisone daily or deflazacort equivalent of 0.9 mg/kg stabilize strength in DMD [37]. In our study the data comes from a first-time visit in our Center - consequently, the cohort is heterogenous in the prior approach to the ST. Also, frequently the prior follow-up intervals were longer than recommended and thus the ST doses were not updated adequately for growth. Surprisingly, despite that fact, we were able to show statistically significant effects of the treatment on the PFT, delaying pulmonary function decrease by 2-3 years.

Despite the limitation of the study in the form of a relatively small sample size, our results show the benefits of using normalized values (z-score) for parameters determining pulmonary function. Additionally, our study is the first to analyse pulmonary function of the DMD population in northern Poland. Furthermore, according to our best knowledge, this is also the first study of pulmonary function in DMD patients to employ the GLI 2012 guidelines and recommended normative values of z-scores and GAMLSS methodology.

Clinical implications

Deterioration of respiratory function in DMD boys may appear even earlier than previously thought. Patients, their careers and health care providers should be informed about the necessity of frequent assessment of the respiratory system. PFT results should be interpreted also as z-score values. Better respiratory system evaluation may lead to earlier medical intervention resulting in improved quality of life for DMD patients.

5. Conclusions

The results of the current study show the difference in the time of peak of PFT between measured as absolute, % predicted and z-scores values, which decrease with the progression
of the disease. Interpretation of PFT results based on z-score, earlier and more specifically, reflects changes in pulmonary function than other values. As a consequence, appropriate physiotherapy will be applied sooner. This is especially important for patients with risk factors for worse pulmonary outcomes, which were: early loss of ambulation and lack of or delayed steroid therapy.

**Compliance with Ethical Standards**

**Conflict of interest**

The authors declare that they have no conflict of interest.

**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.

**Informed consent**

Informed consent was obtained from all individual participants included in the study.

**References:**

[1] Emery AE. Population frequencies of inherited neuromuscular diseases--a world survey. Neuromuscul Disord 1991;1:19–29.

[2] Hoffman EP, Brown RH, Kunkel LM. Dystrophin: the protein product of the Duchenne muscular dystrophy locus. Cell 1987;51:919–28.

[3] Roberto R, Fritz A, Hagar Y, Boice B, Skalsky A, Hwang H, et al. The natural history of cardiac and pulmonary function decline in patients with Duchenne muscular dystrophy. Spine 2011;36:E1009-1017. doi:10.1097/BRS.0b013e3181feae1ed.

[4] Bushby K, Finkel R, Birnkrant DJ, Case LE, Clemens PR, Cripe L, et al. Diagnosis and management of Duchenne muscular dystrophy, part 2: implementation of multidisciplinary care. Lancet Neurol 2010;9:177–89. doi:10.1016/S1474-4422(09)70272-8.

[5] Birnkrant DJ, Bushby K, Bann CM, Alman BA, Apkon SD, Blackwell A, et al. Diagnosis and management of Duchenne muscular dystrophy, part 2: respiratory, cardiac, bone health, and orthopaedic management. Lancet Neurol 2018. doi:10.1016/S1474-4422(18)30025-5.

[6] Birnkrant DJ, Bushby K, Bann CM, Apkon SD, Blackwell A, Brumbaugh D, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and
neuromuscular, rehabilitation, endocrine, and gastrointestinal and nutritional management. Lancet Neurol 2018;17:251–67. doi:10.1016/S1474-4422(18)30024-3.

[7] Bushby K, Finkel R, Birnkrant DJ, Case LE, Clemens PR, Cripe L, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and pharmacological and psychosocial management. Lancet Neurol 2010;9:77–93. doi:10.1016/S1474-4422(09)70271-6.

[8] Kurz LT, Mubarak SJ, Schultz P, Park SM, Leach J. Correlation of scoliosis and pulmonary function in Duchenne muscular dystrophy. J Pediatr Orthop 1983;3:347–53.

[9] Bach JR. Update and perspective on noninvasive respiratory muscle aids. Part 2: The expiratory aids. Chest 1994;105:1538–44.

[10] Finder JD. A 2009 perspective on the 2004 American Thoracic Society statement, “respiratory care of the patient with Duchenne muscular dystrophy.” Pediatrics 2009;123 Suppl 4:S239-241. doi:10.1542/peds.2008-29521.

[11] Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J 2005;26:319–38. doi:10.1183/09031936.05.00034805.

[12] Manzur AY, Kuntzer T, Pike M, Swan A. Glucocorticoid corticosteroids for Duchenne muscular dystrophy. Cochrane Database Syst Rev 2008:CD003725. doi:10.1002/14651858.CD003725.pub3.

[13] Clogg CC, Petkova E, Haritou A. Statistical Methods for Comparing Regression Coefficients Between Models. American Journal of Sociology 1995;100:1261–93. doi:10.1086/230638.

[14] Paternoster Ray, Brame Robert, Mazerolle Paul, Piquero Alex. Using the correct statistical test for the equality regression coefficients. Criminology 1998;36:859–66.

[15] Henricson EK, Abresch RT, Cnaan A, Hu F, Duong T, Arrieta A, et al. The cooperative international neuromuscular research group Duchenne natural history study:
glucocorticoid treatment preserves clinically meaningful functional milestones and reduces rate of disease progression as measured by manual muscle testing and other commonly used clinical trial outcome measures. Muscle Nerve 2013;48:55–67. doi:10.1002/mus.23808.

[20] Mayer OH, Finkel RS, Rummey C, Benton MJ, Glanzman AM, Flickinger J, et al. Characterization of pulmonary function in Duchenne Muscular Dystrophy. Pediatr Pulmonol 2015;50:487–94. doi:10.1002/ppul.23172.

[21] Phillips MF, Quinlivan RC, Edwards RH, Calverley PM. Changes in spirometry over time as a prognostic marker in patients with Duchenne muscular dystrophy. Am J Respir Crit Care Med 2001;164:2191–4. doi:10.1164/ajrccm.164.12.2103052.

[22] Humbertclaude V, Hamroun D, Bezzou K, Béard C, Boespflug-Tanguy O, Bommelaer C, et al. Motor and respiratory heterogeneity in Duchenne patients: implication for clinical trials. Eur J Paediatr Neurol 2012;16:149–60. doi:10.1016/j.ejpn.2011.07.001.

[23] Rideau Y, Jankowski LW, Grellet J. Respiratory function in the muscular dystrophies. Muscle Nerve 1981;4:155–64. doi:10.1002/mus.880040213.

[24] Gayraud J, Ramonatxo M, Rivier F, Humberclaude V, Petrof B, Matecki S. Ventilatory parameters and maximal respiratory pressure changes with age in Duchenne muscular dystrophy patients. Pediatr Pulmonol 2010;45:552–9. doi:10.1002/ppul.21204.

[25] LoMauro A, D’Angelo MG, Aliverti A. Assessment and management of respiratory function in patients with Duchenne muscular dystrophy: current and emerging options. Ther Clin Risk Manag 2015;11:1475–88. doi:10.2147/TCRM.S55889.

[26] Khirani S, Ramirez A, Aubertin G, Boulé M, Chemouny C, Forin V, et al. Respiratory muscle decline in Duchenne muscular dystrophy. Pediatr Pulmonol 2014;49:473–81. doi:10.1002/ppul.22847.

[27] Tangsrud S, Petersen IL, Lodrup Carlsen KC, Carlsen KH. Lung function in children with Duchenne’s muscular dystrophy. Respir Med 2001;95:898–903.

[28] Hsu JD. The development of current approaches to the management of spinal deformity for patients with neuromuscular disease. Semin Neurol 1995;15:24–8. doi:10.1055/s-2008-1041003.

[29] Shapiro F, Zurakowski D, Bui T, Darras BT. Progression of spinal deformity in wheelchair-dependent patients with Duchenne muscular dystrophy who are not treated with steroids: coronal plane (scoliosis) and sagittal plane (kyphosis, lordosis) deformity. Bone Joint J 2014;96-B:100–5. doi:10.1302/0301-620X.96B1.32117.

[30] Mendell JR, Moxley RT, Griggs RC, Brooke MH, Fenichel GM, Miller JP, et al. Randomized, double-blind six-month trial of prednisone in Duchenne’s muscular dystrophy. N Engl J Med 1989;320:1592–7. doi:10.1056/NEJM198906153202405.

[31] Biggar WD, Politano L, Harris VA, Passamano L, Vajsar J, Alman B, et al. Deflazacort in Duchenne muscular dystrophy: a comparison of two different protocols. Neuromuscul Disord 2004;14:476–82. doi:10.1016/j.nmd.2004.05.001.
[32] Biggar WD, Gingras M, Fehlings DL, Harris VA, Steele CA. Deflazacort treatment of Duchenne muscular dystrophy. J Pediatr 2001;138:45–50. doi:10.1067/mpd.2001.109601.

[33] Biggar WD. Long-term benefits of deflazacort treatment for boys with Duchenne muscular dystrophy in their second decade. Neuromuscul Disord 2006 Apr;16(4):249-55 Epub 2006 Mar 20 n.d.

[34] Silversides CK, Webb GD, Harris VA, Biggar DW. Effects of deflazacort on left ventricular function in patients with Duchenne muscular dystrophy. Am J Cardiol 2003;91:769–72.

[35] Matthews E, Brassington R, Kuntzer T, Jichi F, Manzur AY. Corticosteroids for the treatment of Duchenne muscular dystrophy. Cochrane Database Syst Rev 2016:CD003725. doi:10.1002/14651858.CD003725.pub4.

[36] Gloss D, Moxley RT, Ashwal S, Oskoui M. Practice guideline update summary: Corticosteroid treatment of Duchenne muscular dystrophy: Report of the Guideline Development Subcommittee of the American Academy of Neurology. Neurology 2016;86:465–72. doi:10.1212/WNL.0000000000002337.

[37] Bonifati MD, Ruzza G, Bonometto P, Berardinelli A, Gorni K, Orcesi S, et al. A multicenter, double-blind, randomized trial of deflazacort versus prednisone in Duchenne muscular dystrophy. Muscle Nerve 2000;23:1344–7.
Table 1. Anthropometric and baseline characteristics of subjects.

| Variables                        | value                       |
|----------------------------------|-----------------------------|
| **Anthropometric**               |                             |
| Age [y]                          | 10.6±2.9 [5-18]             |
| Weight [kg]                      | 36 ±3.9 [17.5 – 94]         |
| Height [cm]                      | 133±4.2 [108 – 170]        |
| BMI [kg/m²]                      | 20.2±5.3 [19.2-30.3]      |
| **Stage of disease**             |                             |
| Ambulatory/ Non-ambulatory [n]   | 25/30                       |
| Ambulatory/ Non-ambulatory with Steroid therapy [n] | 14/25                     |
| Non-invasive ventilation [n(%)]  | 1 (2%)                      |
| **Steroid Therapy**              |                             |
| ST [n(%)]                        | 38 (69%)                    |
| Duration of ST [y]               | 3.4±1.2 [0.5 – 13.1]       |
| Age at the beginning ST [y]      | 6.2± 0.3 [3 – 14]          |
| Prednisone/Deflazacort [n]       | 22/16                       |
| Mean dose [mg/kg body mass/day]  |                             |
| prednisone                       | 0.28±0.06 [0.05-0.45]      |
| deflazacort                      | 0.40±0.09 [0.05-0.65]      |

Anthropometric and duration, start, doses of ST are shown as mean:SD [range] values; ST- steroid therapy; n-number of patients; BMI- body mass index
Table 2. Descriptive statistics [mean±SD (range)] of spirometric variables by age group.

| Variables | [5-6] | [6-9] | [9-12] | [12-15] | [15-18] | All patients |
|-----------|-------|-------|--------|---------|---------|-------------|
| n         | 6     | 14    | 16     | 10      | 9       | 55          |
| FVC (L)   | 0.96 ± 0.21 (0.67 – 1.29) | 1.40 ± 0.35 (0.73 – 1.99) | 1.90 ± 0.39 (1.40 – 2.52) | 1.70 ± 0.61 (0.85 – 2.83) | 1.63 ± 0.71 (0.64 – 2.47) | 1.59 ± 0.55 (0.64 – 2.83) |
| FVC %pv   | 74.1 ± 17.4 53.1-99.2 | 88.8 ± 25.7 39.6-122.0 | 84.9 ± 14.54 61.32-113.1 | 58.24 ± 25.89 29.01-104.1 | 44.34 ± 16.11 16.87-67.43 | 73.87 ± 27.01 16.8-122.01 |
| FVC (z-score) | -2.00 ± 1.18 (-3.36 –0.29) | -0.94 ± 1.94 (-5.12 –1.37) | -1.30 ± 1.21 (-3.42 –1.08) | -3.82 ± 2.10 (-6.64 –0.41) | -5.33 ± 1.58 (-7.65 –3.29) | -2.40 ± 2.30 (-7.65 –1.37) |
| FEV1 (L)  | 0.91 ± 0.21 (0.66 – 1.28) | 1.28 ± 0.29 (0.72 – 1.67) | 1.69 ± 0.33 (1.29 – 2.32) | 1.59 ± 0.53 (0.84 – 2.58) | 1.47 ± 0.64 (0.60 – 2.38) | 1.45 ± 0.47 (0.60 – 2.58) |
| FEV1 %pv  | 82.5 ± 19.9 61-116.3 | 91.65 ± 26.11 40.72-136.41 | 91.83 ± 14.53 69.4-117.21 | 65.23 ± 26.67 34.76-105.91 | 47.72± 20.5 19.12-78.4 | 80.57 ± 28.09 19.1-136.43 |
| FEV1 (z-score) | -1.76 ± 1.24 (-2.99 –0.43) | -0.71 ± 1.77 (-4.56 –1.55) | -1.10 ± 1.17 (-2.83 –1.19) | -3.14 ± 1.83 (-5.60 –0.63) | -4.65 ± 1.40 (-6.63 –2.65) | -2.02 ± 2.05 (-6.63 –1.55) |
| PEF (L/min) | 2.04 ± 0.74 (1.37 – 3.30) | 2.69 ± 0.57 (1.56 – 3.75) | 3.20 ± 0.49 (2.39 – 4.38) | 3.18 ± 0.93 (1.98 – 4.43) | 3.59 ± 1.06 (2.19 – 4.92) | 2.99 ± 0.83 (1.37 – 4.92) |
| PEF %pv   | 73.3 ± 26.5 51.6-118.7 | 84.4 ± 20.22 42.76-124.23 | 74.89 ± 10.41 60.1-91.76 | 60.6 ± 25.23 33.01-108.74 | 53.8 ± 16.89 38.92-74.33 | 71.11 ± 21.45 33.1-124.21 |
| PEF (z-score) | -1.31 ± 1.30 (-2.37 –0.91) | -0.71 ± 1.11 (-2.81 –1.18) | -1.22 ± 0.52 (-1.96 –0.40) | -1.94 ± 1.24 (-3.27 –0.42) | -2.95 ± 1.64 (-5.20 –0.99) | -1.53 ± 1.33 (-5.20 –1.18) |

FVC - forced vital capacity, FEV₁ - forced expiratory flow in 1 second, PEF - peak expiratory flow, age groups given in left-closed, right-open intervals - that is including the left and excluding the right boundaries.
Table 3. Multivariate regression models predicting the PFT.

| Variables                  | FVC[z] Coefficient | p-value | FEV1[z] Coefficient | p-value | PEF[z] Coefficient | p-value |
|----------------------------|--------------------|---------|---------------------|---------|--------------------|---------|
| Age (years)                | -0.551             | <0.001  | -0.303              | <0.001  | -0.166             | <0.001  |
| Ambulation                 | -1.188             | 0.004   | 1.233               | <0.001  | 0.619              | 0.021   |
| Steroids yes/no           | 1.525              | <0.001  | 1.125               | 0.008   | -0.13              | 0.410   |
| Steroid therapy duration /years | 0.334           | 0.036   | 0.340               | 0.021   | 0.334              | 0.072   |
| Constant                   | 3.400              | 0.091   | 3.822               | 0.043   | 2.401              | 0.110   |
| R²                         | 0.805              | <0.001  | 0.784               | <0.001  | 0.747              | <0.001  |
Table 4. The predicted statistically significant (p<0.001) levels of FVC[z], FEV1[z] and PEF[z] by age in ST and non-ST patients.

| Parameters | ST patients | Non-ST patients |
|------------|-------------|-----------------|
| FVC[z]     | 4.23        | 2.13            |
| FEV1[z]    | 3.68        | 2.50            |
| PEF[z]     | 1.80        | 1.14            |
Figure 1. Comparison the PFT measured as absolute values (Litres or Litres/min, top panel A-C), normalized as z-scores (panel D-F) and % predicted volume (panel G-J) by age.
Figure 2. Number of patients with normal and abnormal results of PFT z-score by age group (n given inside the bars).
Figure 3. Pulmonary function test (normalized value) in ambulatory and non-ambulatory in all patients and in transitional subgroup of 10-14 years.
Figure 4. Mean normalized PFT values by age group and current steroid treatment (ST) status.
