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**Effect of Long Term Steroids on Cough Efficiency and Respiratory Muscle Strength
in Patients with Duchenne Muscular Dystrophy**

A dissertation submitted to the graduate school of the University of Cincinnati

In partial fulfillment of the requirements for the degree of

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By

AMEET SHIRISH DAFTARY

MBBS (MD)

University of Bombay, India, December 1995

Committee Chair: Dr. Kim N. Dietrich, Ph.D.

Professor, Epidemiology & Biostatistics, Department of Environmental Health

College of Medicine, University of Cincinnati

Abstract

The effect of long-term steroid (≥ 1 year) therapy on peak cough flow (PCF) in patients with Duchenne muscular dystrophy (DMD) is assessed alongside the pulmonary function (PFT) variable most predictive of PCF. Medical records of 10 steroid treated and 25 untreated DMD patients are analyzed. Forced vital capacity (FVC), maximum expiratory pressure (MEP), maximum inspiratory pressure (MIP), maximum voluntary ventilation (MVV), and PCF data were collected. Groups were compared with Wilcoxon test and predictive relationships assessed with multiple linear regression. PCF ($P = 0.047$) & MEP ($P = 0.021$) were higher in the treated patients [PCF values 27 L/min higher than the untreated (95% CI = 2 L/min to 52 L/min, $P = 0.033$)]. MVV was most predictive of PCF. We concluded that long term steroid therapy may be beneficial to PCF and respiratory muscle strength in DMD. MVV may be a useful predictor of lung function in DMD.

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Introduction:

Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder with an incidence of 1:3000 live male births. It is characterized by progressive loss of muscle function eventually causing respiratory insufficiency and death frequently from respiratory failure.^{1,2} Patients often succumb to acute or chronic respiratory failure brought on by respiratory tract infections.³ Typically these exacerbations result from impaired airway clearance by a weakened cough and further weakening of respiratory muscles. Bach et al. have demonstrated prolongation of survival in patients with DMD with cough augmentation and non-invasive ventilatory support.⁴ Since the commencement of steroid therapy for treatment of DMD, several studies have shown higher forced vital capacity predicted, an excellent measure of respiratory function, in treated patients.⁵⁻⁷ Our objective was to determine if DMD patients treated with long-term steroids have improved peak cough flow (PCF) and respiratory muscle strength as compared to the untreated group. We also sought to determine which pulmonary function test (PFT) variable was most predictive of PCF.

Patients and Methods:

A case-control study was proposed and approved by the institutional review board. A retrospective review of the medical records of DMD patients treated at our institution in the past two years was performed. DMD was diagnosed by DNA testing or demonstration of lack of dystrophin in the patients' muscle biopsy specimen. Only patients who had PFTs performed at our institution which were acceptable per American Thoracic Society (ATS) criteria were included.^{8,9} As DMD patients are weak and thus often unable to

sustain exhalation for 6 seconds, as required by ATS to meet PFT acceptability criteria, we arbitrarily chose a ≥ 3 second exhalation criterion for acceptability. For the steroid treated patients, informed decisions regarding therapy were obtained from the caregivers after reviewing the benefits and risks of therapy. Patients had to have been on therapy for at least one year for inclusion in the study. As all of the patients were commenced on steroids prior to publication of the practice parameter statement from the American Academy of Neurology,¹⁰ the steroid regimen (intermittent vs daily) was not standardized. Prednisone was commenced at a dose of 0.75mg/kg/day and deflazacort at 0.9mg/kg/day. As patients grew, the dose was not increased for weight gain but clinically adjusted for optimal motor function. Forced vital capacity (FVC), maximum voluntary ventilation (MVV), maximum inspiratory pressure (MIP), maximum expiratory pressure (MEP) and PCF data were collected. PFTs were performed by qualified respiratory therapists using a Sensormedics® (VIASYS Healthcare Inc.) pneumotachograph that is calibrated daily as per ATS recommendations. PCF was performed using a portable peak flow meter (Astech®, NY, USA). Tests were conducted in the sitting position, over 30 minutes to one hour, to allow for patient recovery from each maneuver. The order of testing was respiratory muscle strength & PCF, followed by spirometry and lastly MVV. For muscle strength testing, patients performed repeat maneuvers until a 10% reproducibility criterion was met (up to three maneuvers, with the highest value reported). An unassisted PCF value was measured on our patients. Patients on average had PFTs tested twice a year; however to avoid biases from missed follow up visits, we chose the most recent PCF and PFT results on record for group comparison.

Statistical Analysis:

Data analysis was performed using SAS® (version 9.1) software. A Wilcoxon rank sum test was performed to evaluate whether any of the PFT variables differed between treatment groups (one-sided test with $\alpha = 0.05$). Multiple linear regression analysis was used to identify which combination of our six independent variables (MIP, MEP, FVC, MVV, age, and treatment group) was most predictive of PCF. The Pearson adjusted- r^2 values for each of the 63 possible one-factor through six-factor models were obtained using the ADJRSQ selection option of PROC REG. The final model was chosen by selecting the model that had the highest adjusted- r^2 value and the fewest explanatory variables. The assumptions of normality and homogeneity of variance were tested using the Shapiro-Wilk and the Brown-Forsythe tests, respectively. Influential observations were detected using Cook's distance measure. Iteratively re-weighted least squares (IRLS) regression was applied using the Huber weight function to dampen the influence of outlying observations. The IRLS assigned lower weights to those observations that had higher error estimates. Model coefficients whose t-statistics had p-values < 0.05 were considered to be statistically significant.

Results:

A total of 49 patients, 15 steroid-treated and 34 untreated, were initially identified. A total of 35 patients (10 steroid-treated and 25 steroid untreated) met inclusion criteria and were included in the final analysis. The age range of the steroid treated group was 7-21 years. The mean duration of steroid therapy was 8.2 years (range 1-14 years). Three patients had been treated exclusively with prednisone and five with exclusively deflazacort. Two patients had initially been started on intermittent prednisone and were

then switched to daily deflazacort. Two patients who were on steroid therapy, were commenced on a regimen at a different medical center, prior to transfer of care to our program. The age range of the untreated group was 8-26 years. MVV and MEP data were missing on two patients in the steroid treated group. MVV data was missing on four patients and peak cough flow as well as muscle strength data in one, in the untreated group. Based on flow-volume loop analysis and FEV1/FVC ratio, none of the patients had evidence of obstructive ventilatory limitation. Our results (Table- 1) show that two of the pulmonary function variables measured, PCF ($P = 0.047$) and MEP ($P = 0.021$), were significantly higher in the steroid-treated patients than in the untreated patients.

The linear correlation between PCF and each of the pulmonary function variables was significant. Pearson correlation coefficients between PCF and MVV, MIP, FVC, and MEP had values of 0.545 ($P = 0.003$), 0.537 ($P = 0.002$), 0.527 ($P = 0.002$) and 0.430 ($P = 0.016$), respectively. A multiple linear regression model was used to identify the combination of variables that best predicted PCF. The model having the highest adjusted- r^2 value (0.430) and the fewest explanatory variables included only two variables—MVV and a categorical variable corresponding to the treatment group (1 = steroid-treated; 0 = untreated). The best three-variable model (MVV, steroid treatment group, MIP) decreased the adjusted- r^2 value to 0.418, indicating that no additional benefit was obtained by adding more than two explanatory variables to the model.

Diagnostic tests indicated that both the assumptions of the normality of error terms and the homogeneity of variance were met. However, Cook's distance measure identified two observations as outlying cases. One of these cases was in the steroid-treated group and had an unusually high PCF (340 L/min), while the other case was in the steroid untreated

group and had an unusually low PCF (140 L/min) for its associated MVV. Consequently, we used IRLS regression to produce more conservative estimates of the influence of steroid treatment on PCF. The results for the IRLS regression model containing MVV and the treatment group as explanatory variables are shown in Table 2. The model suggests that the steroid-treated group had PCF values approximately 27 L/min higher than the steroid untreated group (95% CI = 2 to 52 L/min; $P = 0.0328$). The multiple r^2 value for the model was 0.526, indicating that MVV and steroid treatment could account for over 50% of the variance in PCF. While we tested for a possible interaction between MVV and treatment group, this interaction was not found to be significant ($P = 0.591$). Thus, a model with two parallel lines, one for each treatment group, was found to best describe the data, indicating that the influence of steroid treatment remained approximately constant over the range of MVV values observed (Figure 1).

The median age of the untreated group (13 years) was higher than that of the steroid-treated group (10 years). Consequently, we introduced age as a third explanatory variable into the model to investigate its possible influence as a confounder. The estimated coefficient for the age variable was not statistically significant ($P = 0.418$), indicating that age explained little of the variance in PCF. Additionally, the introduction of age into the model failed to alter the coefficients for MVV and treatment group by more than 10% (i.e., a 4% increase for MVV and a 7% decrease for treatment group). This finding suggested that age was not confounded with either MVV or treatment group and thus, we eliminated age from our final model as an explanatory variable.

Discussion:

Cough is a vital protective reflex and plays an important role in airway clearance with prevention of atelectasis and pulmonary infection. Inhaling to large lung volumes followed by isometric contraction of expiratory muscles against a closed glottis results in high intra-thoracic pressures with dynamic compression of airways. Glottic release initiates the expiratory phase characterized by high airflow velocity which clears the airways of secretions.¹¹ Integrity of cough requires adequate inspiratory and expiratory muscle strength with an intact cough pathway. As modest increases in intra-thoracic pressures are sufficient to cause dynamic airway compression with augmentation of cough flow velocity, it is possible to preserve such airway clearance with mild to moderate respiratory muscle weakness¹² Pulmonary morbidity has been shown to be improved in DMD patients by supporting respiratory muscles.⁴

DMD results from absence of the muscle protein dystrophin.¹³ Increased calcium permeability of dystrophin depleted cells has been observed in cell cultures^{14,15} and elevated inflammatory mediators have been observed in dystrophin deficient muscles.¹⁶ The abnormal calcium homeostasis in DMD is believed to activate signaling cascades involved in inflammation¹⁷. Motor and respiratory outcomes have been shown to be preserved by steroid therapy in DMD patients¹⁸ and immune modulation to improve ventilatory function has been suggested in patients with muscular dystrophy.¹⁹ Biggar et al. demonstrated improved forced vital capacity predicted in DMD patients treated with long-term steroids.⁶ We therefore attempted to ascertain if the beneficial effects of steroid therapy translated to preservation of cough efficiency and respiratory muscle strength. Our results show that respiratory muscle strength as well as peak cough flow, in the

steroid treated group of DMD patients was higher than untreated patients. The correlations of MIP and MEP with PCF suggest that preservation of respiratory muscle strength should translate into better cough efficiency.

Respiratory outcome studies of DMD patients treated with steroids have shown improved forced vital capacity predicted. Since the forced vital capacity maneuver requires integrity of both inspiratory and expiratory muscles, it is an excellent measure of respiratory function reserve and correlated with PCF in our data (Pearson $r = 0.526$, $P = 0.002$). The limitation of FVC predicted is that its value is subject to error when the reference is changed from height to arm span as patients become non-ambulatory. We therefore attempted to establish correlation of PCF with other PFT variables and found MVV to be most predictive. Since both MVV and FVC require adequate inspiratory and expiratory muscle function, they both represent respiratory function reserve, however MVV is probably a better measure of endurance. As against the assisted peak cough flow technique described by Bach et al., we test unassisted peak cough flow as we believe it reflects real life cough for our patients. Dystrophic muscle demonstrates a predominance of type I muscle fibers (slow twitch/oxidative)^{20,21} which are recruited for submaximal endurance activity. As cough mediated airway clearance can be accomplished with mild to moderate muscle weakness, it is likely that type I muscle fibers participate to a greater extent in the weak cough, characteristic of DMD patients. Given the histopathologic bias towards type I fibers in dystrophic muscle and our study results, MVV may be a useful addition to FVC in monitoring respiratory function in DMD.

Our study's limitations are the retrospective design and thus risk of selection bias, small patient number in each of the study groups as well as the non-standardized steroid

regimen. The longitudinal effect of age on peak cough flow could not be assessed due to the study design. The small number of patients, differing duration and regimens of therapy also limit comparisons between prednisone and deflazacort. A larger prospective study needs to be conducted to confirm our findings. Our results show meaningful interpretation using a shortened exhalation criterion for PFTs and thus it maybe worthwhile to consider a 3 second exhalation criterion when assessing PFTs in patients with neuromuscular disorders.

In conclusion, steroid therapy appears to be beneficial in preserving respiratory muscle strength and cough efficiency in DMD. In the event of patients being unable to perform ATS acceptable FVC maneuvers, MVV may be a useful additional parameter to monitor for a global perspective on respiratory function in DMD patients. A study with larger patient numbers is warranted to reproduce our demonstrated benefits in respiratory muscle strength and cough efficiency and establish the magnitude of effect.

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Table 1. Descriptive Statistics for the Steroid-Treated and Untreated Groups*

Measure	Steroid Group (n=10)	Untreated Group (n=25)
Age, y	10.0 (8.0, 15.0)	13.0 (10.0,17.0)
FVC, L	2.02 (1.66, 2.13)	1.73 (1.06, 2.09)
MEP, cm H ₂ O ^	62.5 (54.5, 75.5)	44.5 (37.0, 59.0)
MIP, cm H ₂ O	63.0 (58.0, 85.0)	57.0 (31.0, 77.0)
MVV, L/min	56.5 (47.5, 75.5)	49.0 (44.0, 64.0)
PCF, L/min ^	215.0 (160.0, 250.0)	177.5 (147.5, 215.0)

* Values are medians (IQR); ^ Difference in these variables was statistically significant P<0.05

Table 2. Estimated Coefficients for the Multiple Linear Regression Model*

Variable	Estimated Coefficient	Standard Error	t	P
Intercept	123.3	16.9	7.31	< 0.0001
MVV	1.24	0.29	4.34	0.0002
Treatment Group	27.3	12.1	2.26	0.0328

* Model: PCF = 123.3 + 1.24* MVV + 27.3 *(Steroid Group=1; Untreated Group = 0)

Figure 1. Correlation between peak cough flow and maximum voluntary ventilation by treatment group. Lines indicate the iteratively re-weighted least squares fits to data for the steroid-treated (solid line) and untreated (dashed line) groups.

List of Abbreviations:

1. DMD: Duchenne muscular dystrophy
2. PFT: Pulmonary function test
3. PCF: Peak cough flow
4. FVC: Forced vital capacity
5. MIP: Maximum inspiratory pressure
6. MEP: Maximum expiratory pressure
7. MVV: Maximum voluntary ventilation
8. ATS: American Thoracic Society

Contributions:

1. Project conception: Ameet S. Daftary, MD
2. IRB application, Primary Investigator: Ameet S. Daftary, MD
3. Data Collection: Ameet S. Daftary, MD
4. Data Analysis: Ameet S. Daftary, MD, Mark M. Crisanti, PhD
5. Data Interpretation: Ameet S. Daftary, MD, Mark M. Crisanti, PhD
6. Manuscript: Ameet S. Daftary, MD
7. Scholarly Contribution/Input: Raouf Amin, MD; Maninder Kalra, MD, Brenda Wong, MD