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Title:

Chronic Pseudomonas aeruginosa Infection and Respiratory Muscle Impairment in Cystic Fibrosis

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Abstract

Background - Chronic infection with *Pseudomonas aeruginosa* (*Pa*) in subjects with Cystic Fibrosis (CF) is associated with increased morbidity. Chronic infection can cause limb and respiratory muscle compromise. Respiratory muscle function can be assessed by maximal inspiratory pressure (Pi_{max}), maximal expiratory pressure (Pe_{max}) and pressure-time index of the respiratory muscles (PTI_{mus}). Our aim was to study the effect of chronic *Pa* infection on respiratory muscle function in CF subjects.

Methods - This cross-sectional study assessed Pi_{max}, Pe_{max}, PTI_{mus}, FEV_1, FVC, Maximal Expiratory Flow between 25 and 75% of VC, Body Mass Index and Upper Arm Muscle Area (UAMA) in 122 CF subjects who formed four groups matched for age and sex at different stages of *Pa* infection according to the Leeds criteria. Respiratory muscle function parameters were then compared in the ensuing groups according to *Pa* infection state.

Results - Median Pi_{max} was significantly lower in CF subjects with chronic *Pa* infection (Pi_{max}= 62 cmH_2O) compared to subjects that were never infected (Pi_{max}=86 cmH_2O, p=0.016), free of infection (Pi_{max}=74 cmH_2O, p=0.014) or intermittently infected (Pi_{max}=72 cmH_2O, p=0.016). Median PTI_{mus} was significantly increased in CF subjects with chronic *Pa* infection (PTI_{mus}=0.142) compared to subjects that were free of infection (PTI_{mus}=0.102, p=0.006). Median UAMA was significantly lower in CF subjects with chronic Pa infection (UAMA=2219) compared to subjects that were never infected (UAMA=2754, p=0.032), free of infection (UAMA=2678, p=0.011) or intermittently infected (UAMA=2603, p=0.037). Multivariate logistic regression revealed *Pa* state of infection as a significant determinant of PTI_{mus} (p=0.025) independently of gender, UAMA and FEV_1.

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Conclusions - These findings suggest that CF subjects with chronic *Pa* infection exhibit impaired respiratory muscle function and decreased inspiratory muscle strength and that chronic *Pa* infection independently impacts on respiratory muscle function in subjects with CF.

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Introduction

Pseudomonas aeruginosa (*Pa)* infection in Cystic Fibrosis (CF) is a major determinant of lung disease and is associated with severe pulmonary disease[1] and increased morbidity and mortality.[2] Acquisition of *Pa* is associated with gradually declining pulmonary status in children and young adults with CF as assessed by lung function studies.[3],[4] Furthermore, chronic pulmonary infection with *Pa* has been associated with deteriorating nutritional state to the point of malnourishment.[5] Gender differences relating to the natural history of *Pa* infection have been described in the literature with women suffering higher rates of colonization and younger age of conversion to the more aggressive mucoid phenotype compared to men.[6, 7] Chronic infection has been linked to compromised diaphragm function in animal models[8] and human patients.[9]

Respiratory failure in CF is caused by a number of parameters, such as parenchymal destruction, recurrent infections and bronchiectasis while respiratory muscle dysfunction might also play a critical role. Respiratory muscle compromise in CF can lead to respiratory muscle fatigue and thus contribute to the establishment of respiratory failure. Respiratory muscle strength can be assessed by measurement of maximal inspiratory (Pi_{max}) and maximal expiratory (Pe_{max}) pressures.[10] Respiratory muscle strength has been assessed in numerous studies and conflicting evidence have been presented on whether it is decreased, maintained or decreased in subjects with CF, as recently highlighted.[11] While some studies advocate that the chronically increased workload against which the respiratory muscles are forced to operate in CF exerts a conditioning effect,[12] other studies support that chronic malnutrition and hyperinflation impact on respiratory muscle strength.[13, 14] Hyperinflation, airway obstruction and malnutrition have been recognised as the major determinants of respiratory muscle compromise in CF patients.[13-15]

To our knowledge, the effect of chronic *Pa* infection on respiratory muscle function has not been previously studied in CF subjects. We hypothesized that CF subjects chronically-infected with *Pa* would have impaired respiratory muscle function compared to non-chronically-infected CF subjects. Our aim was to compare respiratory muscle function by measurement of Pi_{max}, Pe_{max} and

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PTI_{mus} between CF subjects at different stages of *Pa* infection in a large cohort comprising of children and young adults.

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Materials and Methods

Subjects

Patients with CF attending their follow-up appointments in the Department of CF of the "Aghia Sophia" Children's Hospital, Athens, Greece were recruited. CF diagnosis was confirmed by abnormal sweat-test results and expanded mutation analysis. Individuals with CF received standard daily chest physical-therapy. CF subjects unable to perform reproducible lung function tests, subjects with acute exacerbation during the month prior to assessment[16] and subjects on steroids, anti-inflammatory agents and azithromycin were excluded from the study. Subjects who had positive respiratory cultures for *Aspergillus* spp., *Stenotrophomonas* spp., *Scedosporium* spp., *Burkholderia* spp., Methicillin-resistant *Staphylococcus aureus* or any pathogen other than *Staphylococcus aureus* and *Haemophilus influenzae* in the five years prior to the study were also excluded.

The study protocol was approved by the Hospital-Ethics Committee and all subjects, parents or legal guardians provided informed written consent prior to the study.

Sample size

Power analysis was conducted to determine the sample size required to identify PTI_{mus} differences between the groups of CF subjects according to *Pa* infection state. PTI_{mus} standard deviation was set at 0.03.[15] The power analysis indicated that in order to detect an increase in PTI_{mus} of 0.05 at a power of 0.9 and a level of statistical significance of 0.05, a sample size of 8 subjects would be required for each group of subjects according to *Pa* infection state.

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Measurements

Equipment

Flow was recorded with a pneumotachograph (Mercury F100L, GM Instruments, Kilwinning, Scotland) connected to a differential pressure transducer (DP45, range ±3.5 cmH_2O, Validyne Engineering, Northridge, California). Airway pressure (Paw) was measured from a side port on the pneumotachograph, with a differential pressure transducer (DP45, range ±225 cmH_2O, Validyne Engineering, Northridge, California). A carrier amplifier (Validyne CD 280, Validyne Engineering, Northridge, California) was used to amplify the signals from the differential pressure transducers. The amplified signals were recorded and displayed in real time on a computer with data analysis software (Labview, National Instruments, Austin, Texas) with analog-to-digital sampling at 100 Hz (16-bit NI PCI-6036E, National Instruments, Austin, Texas).

Measurement of respiratory pressures

Pi_{max}, Pe_{max}, airway-occlusion pressure at 0.1 s after the start of inspiration ($P_{0.1}$), breathing frequency, tidal volume (TV), minute ventilation (MV), inspiratory time (Ti) and total time for each breath (Ttot) were recorded. $P_{0.1}$ was calculated as the airway pressure generated 0.1s after an occlusion while the subject was breathing quietly. At least five airway occlusions were performed and the average $P_{0.1}$ value was calculated. Pi_{max} was measured from residual volume performing a maximal inspiratory effort against an occluded airway.[17] Pe_{max} was measured from total lung capacity performing a maximal expiratory effort against an occluded airway.[17] Five maximal reproducible respiratory efforts were performed and the maximum value achieved was recorded.[10] The occlusions were performed with a unidirectional valve connected to the mouthpiece (total deadspace 8 ml). Care was taken to eliminate any leak around the mouthpiece; a small leak allowed for avoidance of artificial glottic closure.[10] Only Pi_{max} and Pe_{max} manoeuvres with plateau pressure for at least 1 s were accepted for subsequent analysis.[10] Pi_{max} and Pe_{max} were also presented as percentage of the predicted values.[18]

Calculation of Pressure-Time Index of Respiratory muscles

Respiratory muscle function was evaluated by the pressure-time index of the respiratory muscles (PTI_{mus}). The pressure–time index of the respiratory muscles (PTI_{mus}), was calculated from the formula: $PTI_{mus}=(Pi_{mean}/Pi_{max})\times(Ti/Ttot)$,

where Pi_{mean} stands for the average airway pressure during inspiration, obtained from the formula $Pi_{mean}=5\times P_{0.1}\times Ti$,

Pi_{max} stands for the maximum inspiratory airway pressure, Ti for the inspiration time, and Ttot for the total time for each breath, as derived from the airway flow signal.[15, 19] PTI_{mus} is a composite index of respiratory muscle function which describes the efficiency of the respiratory muscles and the balance between neuromuscular competence and respiratory load. Higher PTI_{mus} values indicate inefficient respiratory muscle function and are related to increased risk of respiratory muscle fatigue.[20]

Lung Function Tests

FEV_1, maximal expiratory flow between 25% and 75% of VC ($MEF_{25–75}$) and FVC were measured (MasterScreen, Jaeger/CareFusion, San Diego, California) and are expressed as percentage of the predicted values.[21] The tests were performed in accordance to European Respiratory Society guidelines.[22] The values recorded were the ones achieved before the use of bronchodilator drugs.

Nutritional Parameters

Height and weight were measured and the corresponding body mass index (BMI) and BMI z-score were calculated.[23] Midarm-muscle-circumference (MAMC) was measured halfway between the acromion and the olecranon to the nearest centimetre, right hand hanging relaxed.[24] Triceps-skinfold-thickness (TST) was measured to the nearest millimetre halfway over the triceps muscle (Harpenden Skinfold Caliper, Baty International, West Sussex, United Kingdom), skinfold parallel to the upper arm longitudinal axis.[24] Upper arm muscle area (UAMA) was calculated from these indices.[25]

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Definition of chronic *Pa* infection

The Leeds criteria were used to classify *Pa* infection state.[26] The infection was classified as **chronic** when more than 50% of months when samples were taken were *Pa* culture positive, **intermittent** when 50% or less of months when samples were taken were *Pa* culture positive, **free of infection** when no growth of *Pa* occurred over the previous twelve months having previously been *Pa* culture positive and **never infected** when *Pa* was never cultured from sputum or cough swab. At least 6 airway cultures were acquired in separate months over the year before the assessment.[26] Sputa were collected in sterile disposable containers, stored at ambient temperature and processed within 4h from collection. Sputa were inoculated and incubated for the isolation of *Pa* and other pathogens. *Pa* positive cultures included both mucoid and non-mucoid phenotypes. The media were incubated aerobically at 37uC for 48 hours.

All subjects chronically infected with *Pa* were regularly treated with inhaled antibiotics.

Protocol

Patients were all reviewed in the same setting using the same medical instruments. They were all in a stable clinical condition and had received their medication, as usual. They were evaluated in the morning hours by the following order: nutritional assessment, pulmonary function testing, respiratory muscle assessment. All participants were evaluated in a sitting position while a nose clip was utilized for both pulmonary function and respiratory muscle studies.

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Statistics

Data were checked for normality using the Kolmogorov–Smirnoff and Shapiro–Wilk tests. Differences between groups were assessed for significance using the Kruskal–Wallis rank sum test and Pearson's Chi-squared test, as appropriate. In case significant differences were detected, the Mann-Whitney rank sum test was used for subsequent pairwise comparisons between the groups according to *Pa* infection state. Multivariate logistic regression was performed to determine which variables contribute to alterations of PTI_{mus}. P values<0.05 were accepted as significant. Statistical analysis was performed using statistics software (SPSS 17.0, SPSS, Chicago, Illinois).

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Results

Patients:

Between October 2009 and June 2010, 122 subjects (68 male) were included in the study. Median (Interquartile range) age was 13 (10-17) years (13 subjects were older than 19 years). Median (IQR) BMI z-score was 0.22 (-0.49 to 0.84), median (IQR) FEV_1%predicted was 99 (75-119).

Four groups of subjects were formed, matched for age and gender: Group 1 consisted of 11 subjects that had never been infected by *Pa*, group 2 consisted of 33 subjects who were free of *Pa* infection at the time of assessment, group 3 consisted of 39 subjects with intermittent *Pa* infection and group 4 consisted of 39 subjects with chronic *Pa* infection. As the power analysis showed that 8 subjects in each group were required, the sample size per group was deemed appropriate. In group 4, 17 of 39 patients (43.6%) were chronically infected with the mucoid *Pa* phenotype. Since data were not normally distributed, non-parametric tests were applied to compare values within different groups of patients according to *Pa* infection state.

Anthropometry and nutrition

No significant differences were detected for height and weight between the four groups (table 1). Significant differences in BMI z-score were detected between the 4 groups of subjects (p=0.015). Post hoc analysis revealed that BMI z-score was significantly decreased in subjects of group 4 compared to subjects of group 1 (p=0.009) and group 3 (p=0.016). Significant differences between the 4 groups were detected for MAMC (p=0.001), TST (p=0.024) and UAMA (p=0.028). Post hoc analysis revealed significantly decreased MAMC in group 4 compared to group 1 (p=0.003), group 2 (p=0.003) and group 3 (p=0.002), and significantly decreased TST in group 4 compared to group 2 (p=0.05) and group 3 (p=0.006).

Lung function and breathing cycle components

No significant differences in lung-function were detected between the four groups. Breathing frequency, tidal volume (TV), tidal volume per kilogram (TV/kg), minute ventilation (MV), inspiratory flow (TV/Ti), inspiratory time (Ti), total time of respiration (Ttot) and Ti/Ttot ratio were not significantly different between the four groups.

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Respiratory pressures

$P_{0.1}$, Pi_{mean} and Pe_{max} were not significantly different between the four groups. Significant differences in Pi_{max} (figure 1) and Pi_{max}%predicted were detected between the 4 groups (p=0.043 and 0.037 respectively) and post-hoc analysis revealed significantly decreased Pi_{max} values in group 4 compared to group 1 (p=0.044), group 2 (p=0.014) and group 3 (p=0.046) and significantly decreased Pi_{max}%predicted values in group 4 compared to group 2 (p=0.009) and group 3 (p=0.024). Nonparametric testing revealed significant differences in Pi_{mean}/Pi_{max} and PTI_{mus} between the 4 groups (p=0.023 and p=0.032 respectively). Pi_{mean}/Pi_{max} ratio was significantly decreased in group 4 compared to group 2 (p=0.005), and PTI_{mus} (figure 2) was significantly increased in group 4 compared to group 2 (p=0.006). Patients of group 4 infected with the mucoid *Pa* phenotype had median Pi_{max} of 59 cmH_20 compared to patients of group 4 infected with the non-mucoid *Pa* phenotype who had a median Pi_{max} of 66 cmH_20 (p=0.148).

Multivariate logistic regression analysis revealed that *Pa* infection state was significantly related to PTI_{mus} values independently of FEV_1, UAMA and gender (table 2).

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Discussion

Our study demonstrated that PTI_{mus} was significantly increased and Pi_{max} was significantly decreased in CF subjects with chronic *Pa* infection compared to CF subjects who were free, never-infected or intermittently-infected with *Pa*. Furthermore, CF subjects who were chronically-infected with *Pa* were found to have compromised somatic muscular indices such as MAMC and UAMA.

Our results suggest that chronic *Pa* infection impacts on respiratory muscle function in the context of normal lung-function parameters, probably identifying chronic infection with *Pa* as an independent determinant of respiratory muscle compromise in CF. Our findings emphasize the multifactorial origin of respiratory muscle impairment in the pathophysiology of respiratory failure in CF.

Chronic pulmonary infection with *Pa* affects the majority of CF subjects by adulthood[27] while chronic infection with *Pa* causes increased mortality and morbidity in CF subjects[2] and declining pulmonary status in children with CF.[3] *Pa* plays a central role in the vicious cycle of pulmonary infection, pulmonary inflammation, lung tissue damage and consequent respiratory failure.[28]

Although systemic inflammation is not a major component of CF disease, pulmonary inflammation has been suspected to cause limb and respiratory muscle wasting and weakness via “spill-over” of inflammatory mediators. Pulmonary inflammation and subsequent injury has been linked to systemic inflammatory phenomena in subjects with COPD[29] while increased circulating inflammatory markers have been detected in CF subjects.[30, 31] Infection has been shown to induce respiratory muscle weakness in animal models[32] while respiratory muscle weakness has been associated with upper-respiratory-tract infections in human patients.[33] Induced chronic bronchopulmonary infection by *Pa* resulted in significant decrease in diaphragmatic and limb strength in infected mice[28] while TNF-α depressed the diaphragmatic tetanic force in murine diaphragm and limb muscle preparations.[8] Furthermore, endotoxin administration in mice resulted in diaphragm weakness and contractile dysfunction.[34]

Respiratory muscle function is compromised in CF, according to some studies which have reported decreased maximal respiratory pressures in hyperinflated, malnourished CF patients with airway obstruction.[13-15, 35] PTI_{mus} attained abnormal values in CF subjects indicating respiratory muscle impairment related to nutritional compromise, decreased somatic muscular mass, hyperinflation and airway obstruction.[13-15]

In our study, PTI_{mus} was selected to describe respiratory muscle function because it is measured non-invasively, it is a global respiratory-muscles index while it incorporates properties of time, respiratory load and neuromuscular competence.[20] As a limitation of PTI_{mus} in CF we should mention that measurement of $P_{0.1}$ might be affected by the increased time-constant of the CF lung and the ensuing delayed transmission of the pressure changes from the alveoli to the mouth, as exhibited in COPD subjects.[36] Sniff nasal inspiratory pressure has been alternatively proposed as a noninvasive test of respiratory muscle function in CF but it has been shown to underestimate esophageal pressure, probably because of nasal obstruction and dampening of the pressure changes secondary to the increased time-constant of the CF lung.[37]

BMI z-score, MAMC and UAMA were significantly decreased in CF subjects with chronic *Pa* infection in our study. Muscular indices and lean body mass have been shown to correlate well with respiratory muscle indices.[15] It has been suggested that decreased muscular synthesis and impaired muscle regeneration occur in the presence of abundant pro-inflammatory cytokines in the chronically-infected lung which spill-over to cause chronic systemic inflammation.[38]

Recent work has addressed gender differences in the course of *Pa* infection in patients with CF, highlighting that estrogens induce mucoid conversion of *Pa* in women with CF and are associated with increased exacerbations.[6] On the other hand, it has been highlighted that male CF patients with impaired skeletal muscle strength have normal testosterone levels.[39] Mucoid conversion of *Pa* in CF has been associated with resistance to antibiotics and increased morbidity and mortality.[40] In the present study, which was clearly not designed to address these questions, gender and mucoid state of *Pa* were not significant determinants of respiratory muscle function in CF subjects. The lack of statistical difference in Pi_{max} between subjects infected with the mucoid and the non-mucoid

state raises the question as to whether muscle weakness is the result of the chronic disease itself rather than the *Pa* infection state. Of note, Pi_{max} in CF patients chronically infected with the non-mucoid strain was non-significantly higher compared to CF subjects chronically infected with the mucoid strain. This might represent the course of disease progression in CF, reflecting subsequent mucoid conversion and increasing disease severity with age.

Compared to previous studies our study reports similar values of maximal respiratory pressures and PTI_{mus}. Pe_{max} was not significantly different in the four groups of subjects according to *Pa* infection. This might indicate a preferential action of chronic infection with *Pa* to the diaphragm compared to the expiratory muscles which might be explained by way of proximity. A preferential-to-the-diaphragm weakness has been demonstrated during induced sustained *Pa* infection in animal models.[28]

The population of this study was in a good lung function condition, probably due to idiosyncratic reasons. This was not deliberately done, although enrolment was limited to stable outpatients. Furthermore, our study included younger subjects compared to previous studies,[14,15,38] who probably due to limited disease progression had milder lung disease, implying that further investigation in older patients with lower baseline lung function may demonstrate an even more pronounced effect.

Adult CF patients with severe lung disease have increased diaphragm thickness and inspiratory strength,[38] probably as a result of the training effect that the chronically increased respiratory load imposes upon the respiratory muscles. In these patients inflammation was not identified as a predictor of respiratory muscle compromise, while fat free mass and airway resistance were.[38] In our cohort of CF subjects with mild lung disease, diaphragm thickening might have not yet occurred and the respiratory muscles might have been exposed to the deleterious effects of chronic infection and malnutrition in the absence of a counterbalancing diaphragm thickening mechanism which comes as a result of increased respiratory load.

Our study has some limitations that need to be considered. Presently, no definition of chronic *Pa* infection has been universally accepted. In keeping with defining chronic infection as an infectious process which persists in spite of appropriate therapy, chronic infection could be more accurately described by persistent pathological and immune marker abnormalities.[26] Due to technical limitations we were unable to collect data on Functional Residual Capacity, precluding the possibility to incorporate hyperinflation as another factor that probably contributes to respiratory muscle impairment in CF as suggested in previous studies.[14, 15] Furthermore, it is possible that the lack of difference between the groups as far as lung function and breathing cycle components are concerned, was to a large extend due to the fact that all groups consisted of subjects in good pulmonary condition and that, had more debilitated subjects been included, more differences might have emerged. The authors acknowledge this as probable bias, since CF subjects with more severe lung disease could have respiratory muscle impairment secondary to marked airway obstruction and hyperinflation, on top of the impairment caused by chronic *Pa* infection. Thus, the selection of a lung-mild population in the present study might theoretically have affected the results and their subsequent applicability in the CF community. Finally, although subjects with various other pathogens were excluded, for logistical reasons we did not exclude subjects infected with *Staphylococcus aureus* and *Haemophilus influenzae* as the respective cohort would not be empowered for statistical analysis, nor did we apply PCR techniques in the assessment of sputum samples. The relative contribution of these pathogens to our results cannot thus be safely refuted, while in practical terms the formulation of such a cohort would be particularly challenging for any CF centre. Although, it is possible that co-infected CF subjects have worse outcomes, both *Staphylococcus aureus* and *Haemophilus influenzae* are pathogens which are frequently cultured early-on from respiratory cultures of CF subjects, while their relative contribution to lung disease progression and respiratory muscle impairment has not been clearly delineated.

Our study has potential clinical consequences. Identifying chronic *Pa* infection as an independent predictor of respiratory muscle compromise implies that aggressive eradication of *Pa* might contribute to avoiding respiratory muscle fatigue and respiratory failure in CF. CF subjects

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chronically-infected with *Pa* could be targeted for respiratory muscle function testing especially if other risk factors coexist, such as malnutrition, hyperinflation and airway obstruction. Treatment modalities that aim to alleviate the respiratory load and decrease the work of breathing in CF subjects such as non-invasive ventilation,[41] inspiratory muscle training and aerobic exercise could then be initiated on the basis of the respiratory muscle function condition.

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Conclusions

In conclusion, this study demonstrated that CF subjects with chronic *Pa* infection exhibit impaired respiratory muscle function. Maximal inspiratory pressure was found to be significantly decreased and PTI_{mus} was found to be significantly increased in CF subjects with chronic *Pa* infection compared to CF subjects who were not chronically-infected with *Pa*. Somatic muscular indices such as MAMC and UAMA were found to be significantly decreased in CF subjects with chronic *Pa* infection compared to CF subjects who were never infected, free of infection or suffered intermittent infection with *Pa*. Chronic infection with *Pa* might be an independent determinant of respiratory muscle compromise in CF.

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Figure legends

Figure 1

Pi_{max} in CF groups according to *P. aeruginosa* infection state. Horizontal lines represent the 5th, 25th, 50th, 75th and 95th percentile of Pi_{max} values. The level of significance for non-parametric comparison between all groups is presented.

Figure 2

PTI_{mus} in CF groups according to *P. aeruginosa* infection state. Horizontal lines represent the 5th, 25th, 50th, 75th and 95th percentile of PTI_{mus} values. The level of significance for non-parametric comparison between groups 2 and 4 is presented.

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Table 1: Anthropometric, nutrition, pulmonary function and respiratory muscle function data according to different stages of *P. aeruginosa* infection

	Group 1 N=11	Group 2 N=33	Group 3 N=39	Group 4 N=39
Gender (male)	6 (54.5)	19 (57.6)	23 (58.9)	20 (51.3)
Age (years)	15 (8-26)	13 (11-17)	13 (10-15)	14 (11-17)
Height (cm)	160 (130-166)	159 (140-165)	153 (135-162)	156 (138-168)
Weight (kg)	52 (32-61)	48 (36-61)	48 (31-58)	44 (34-55)
BMI z-score*	0.81 (0.10-1.22)	0.04 (-0.61-0.87)	0.29 (-0.15-1.07)	-0.13 (-0.88-0.15)†
MAMC (cm)*	24 (21-27)	23 (19-25)	23 (20-25)	20 (17-22)**
TST (mm)*	13 (9-15)	13 (8-15)	12 (10-17)	11 (7-13)§
UAMA (mm^2)*	2754 (2111-3359)	2678 (2063-3522)	2603 (1784-3257)	2219 (1578-2670)**
FVC (%pred)	112 (81-125)	105 (96-115)	109 (90-119)	96 (74-112)
FEV_1 (%pred)	107 (88-125)	99 (80-118)	99 (72-124)	95 (63-116)
MEF_{25-75} (%pred)	98 (70-124)	71 (47-102)	78 (39-115)	68 (34-105)
BF	18 (16-19)	17 (15-22)	16 (15-21)	19 (16-23)
TV (L)	0.72 (0.37-0.82)	0.52 (0.41-0.62)	0.48 (0.37-0.70)	0.48 (0.36-0.69)
TV/kg (ml/kg)	13.9 (6.0-17.1)	10.9 (8.8-14.7)	10.3 (7.7-14.8)	10.3 (8.8-14.0)
MV (L/min)	8.64 (7.22-13.68)	9.69 (7.25-11.03)	8.63 (6.56-11.33)	9.31 (6.80-11.52)
TV/T_i (L/s)	0.323 (0.255-0.525)	0.344 (0.282-0.435)	0.318 (0.270-0.408)	0.350 (0.257-0.427)
T_i (s)	1.49 (1.22-1.52)	1.50 (1.20-1.84)	1.53 (1.31-1.84)	1.39 (1.10-1.56)
T_{tot} (s)	3.29 (3.06-3.56)	3.37 (2.65-4.16)	3.40 (2.82-4.16)	3.15 (2.62-3.64)
T_i/T_{tot}	0.45 (0.43-0.47)	0.44 (0.42-0.47)	0.45 (0.43-0.47)	0.44 (0.41-0.46)
$P_{0.1}$ (cmH_2O)	2.18 (1.37-3.74)	2.28 (1.78-3.04)	2.51 (1.76-3.88)	2.76 (1.95-3.91)
Pi_{mean} (cmH_2O)	17.8 (8.9-24.9)	17.9 (12.9-24.5)	18.6 (13.5-26.4)	20.5 (13.2-28.4)
Pi_{max} (cmH_2O)*	86 (56-103)	74 (62-93)	72 (55-97)	62 (48-78)**
Pi_{max} (%pred)*	100 (82-124)	96 (85-123)	99 (85-126)	82 (63-114)§
Pi_{mean}/Pi_{max}*	0.208 (0.137-0.307)	0.220 (0.146-0.335)	0.261 (0.179-0.376)	0.317 (0.213-0.460)#
PTI_{mus}*	0.097 (0.067-0.131)	0.102 (0.063-0.149)	0.116 (0.080-0.175)	0.142 (0.097-0.190)#
Pe_{max} (cmH_2O)	71 (50-108)	75 (50-95)	66 (49-96)	62 (46-84)
Pe_{max} (%pred)	69 (60-119)	75 (59-89)	78 (57-101)	69 (51-80)

Values are presented as median (interquartile range), values for age are presented as numbers (percentage)

* Significant difference at P<0.05 in the Kruskal-Wallis test when used to check if there were any differences between the P. aeruginosa groups

** Significant difference between group 4 and all other groups at P<0.05 in the Mann-Whitney test when used as post hoc test

Significant difference between group 4 and group 2 at P<0.01 in the Mann-Whitney test when used as post hoc test

† Significant difference between group 4 and groups 1 and 3 at P<0.05 in the Mann-Whitney test when used as post hoc test

§ Significant difference between group 4 and groups 2 and 3 at P<0.05 in the Mann-Whitney test when used as post hoc test

MAMC: Mid arm muscle circumference, TST: Triceps skinfold thickness, UAMA: Upper arm muscle area, FVC: Forced vital capacity, FEV_1: forced expiratory volume in 1 s, MEF_{25-75}: maximal expiratory flow between 25% and 75% of VC, BF: Breathing Frequency, TV: Tidal volume, TV/kg: Tidal volume per kilogram of body weight, MV: Minute ventilation, TV/T_i: inspiratory flow, T_i: inspiratory time, T_{tot}: Total time of respiration, $P_{0.1}$: inspiratory pressure 0.1s after onset of inspiration, Pi_{mean}: Inspiratory pressure, Pi_{max}: Maximal inspiratory pressure, PTI_{mus}: Pressure time index of the respiratory muscles, Pe_{max}: Maximal expiratory pressure.

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Table 2: Multivariate logistic regression analysis with PTI_{mus} as the outcome variable. The analysis incorporated the whole CF population of the present study.

Parameter	95% Confidence intervals		Standardized coefficient	p value
FEV_1 (% predicted)	0.000	0.000	-0.128	0.165
UAMA	0.000	0.000	-0.087	0.371
Gender	-0.043	0.009	-0.120	0.197
P. aeruginosa infection state	0.002	0.029	0.210	0.025

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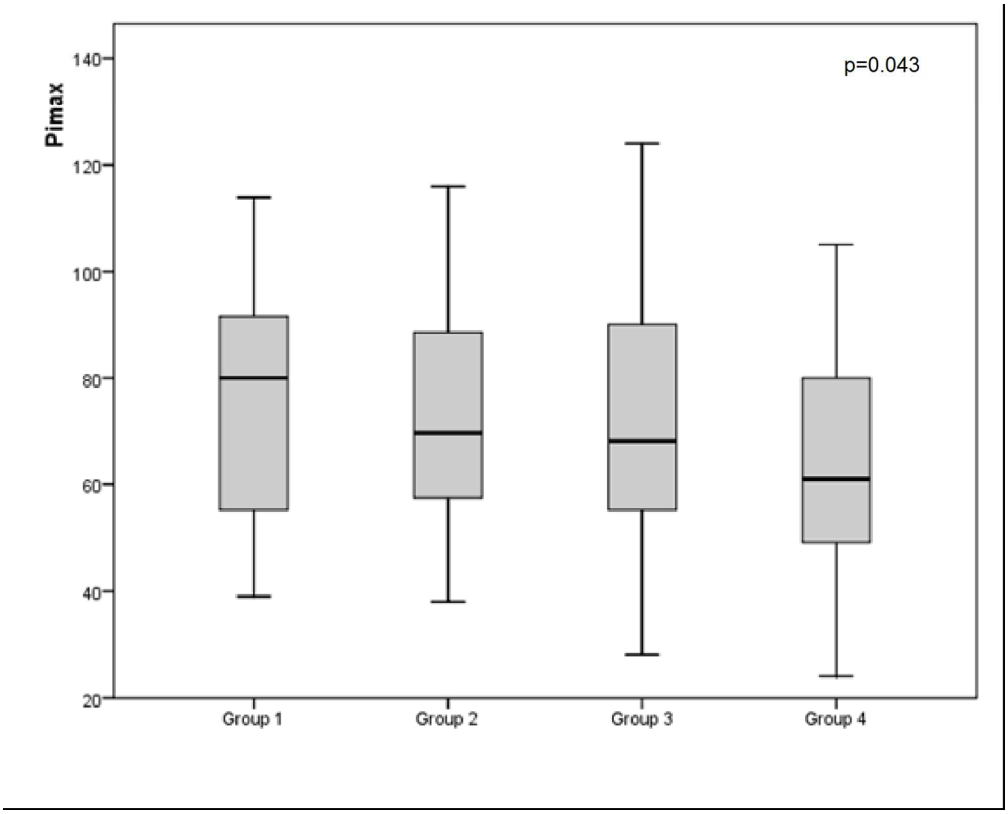

Pi_{max} in CF groups according to *P. aeruginosa* infection state. Horizontal lines represent the 5th, 25th, 50th, 75th and 95th percentile of Pi_{max} values. The level of significance for non-parametric comparison between all groups is presented.
152x122mm (300 x 300 DPI)

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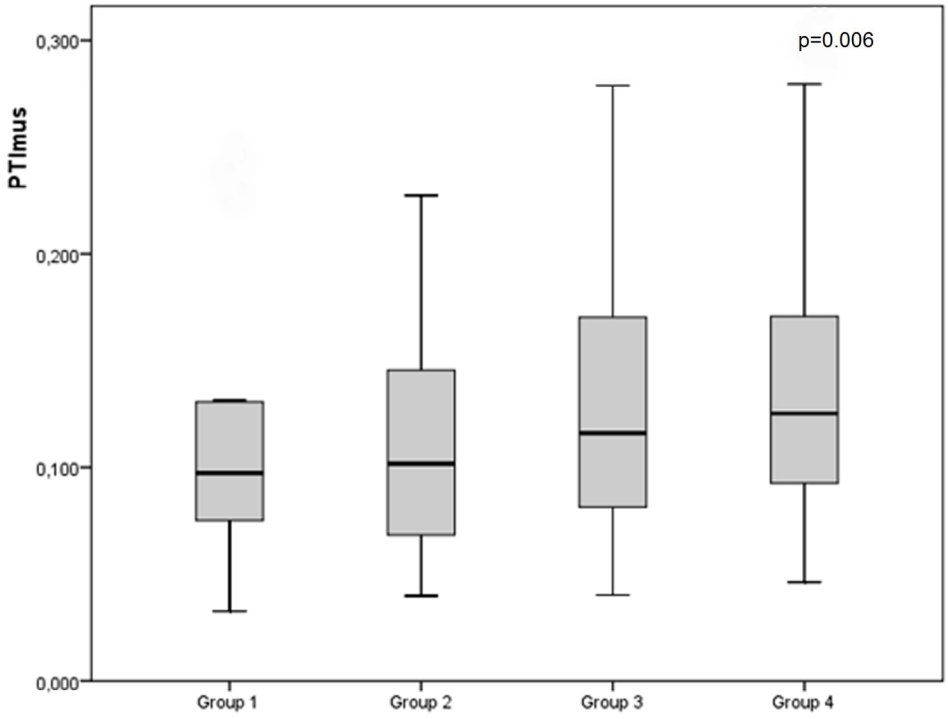

PTI_{mus} in CF groups according to *P. aeruginosa* infection state. Horizontal lines represent the 5th, 25th, 50th, 75th and 95th percentile of PTI_{mus} values. The level of significance for non-parametric comparison between groups 2 and 4 is presented.
152x122mm (300 x 300 DPI)