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ORIGINAL ARTICLE

Is the type of chronic pulmonary infection a determinant of lung function outcomes in adult patients with cystic fibrosis?

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ABSTRACT: ***Is the type of chronic pulmonary infection a determinant of lung function outcomes in adult patients with cystic fibrosis? A.J. Lopes, T.T. Mafort, A. de Sá Ferreira, M.C. Santos de Castro, M. de Cássia Firmida, E. de Andrade Marques.***

Background and Aim. **Lung function abnormalities are the main factors responsible for the high mortality of cystic fibrosis (CF) patients. It is not yet clear whether *Burkholderia cepacia* infection causes more pronounced loss of lung function than *Pseudomonas aeruginosa* infection. Our primary objective was to compare the lung function of adult CF patients with different chronic pulmonary infections. Our second objective was to compare the microbiology using patients' genetic status.**

Methods. **Fifty-two adult CF patients were divided into 3 groups according to their chronic pulmonary infection profile. All subjects underwent clinical evaluation, pulmonary function tests (PFT) and genetic analysis.**

Results. **The PFT parameters of chronically infected patients were significantly different from those of subjects without pulmonary infection ($p < 0.0001$). FVC was significantly more altered in patients infected with *B. cepacia* complex ($p < 0.0001$); in contrast, $FEF_{25-75\%}$ was significantly more altered in patients with *P. aeruginosa* infection ($p < 0.0001$). In the groups with chronic *P. aeruginosa* infection and chronic *B. cepacia* complex infection, 58.1% and 10% of patients were homozygous for ΔF508, respectively. In addition to chronic infections, pancreatic insufficiency was also associated with lung function deterioration.**

Conclusion. **Chronic pulmonary infection and pancreatic insufficiency are critical processes in lung function deterioration in adult CF patients. Although chronic *B. cepacia* complex infection causes a more pronounced lung volume reduction, chronic *P. aeruginosa* infection causes a more pronounced obstruction of small airways. Our results also suggest that ΔF508-homozygous patients are more susceptible to chronic *P. aeruginosa* infection.**

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Introduction

Cystic fibrosis (CF) is the most common inherited disease in the Caucasian population [1]. Although it is debilitating, improvements in knowledge and treatment in recent years have led to a dramatic increase in the life expectancy of individuals with CF. The median predicted survival of 16 years in 1970 has now reached approximately 38 years in most developed countries, and infants born with CF in the 21st century are expected to live beyond 50 years of age [2]. The number of middle-aged or older individuals with CF is also increasing [3].

Lung disease is recognised as the factor with the greatest impact on the morbidity and mortality in older people with CF. Lung disease develops as a consequence of diminished chloride and water secretion, which increases the viscosity of secretions in the affected airways. This impaired mucociliary clearance can facilitate chronic bacterial infections [4].

Adult CF patients are predominantly infected with specific microbial species, including *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex. Oxacillin-resistant *S. aureus*, *Stenotrophomonas maltophilia*, *Alcaligenes xylosoxidans*, *Mycobacterium spp*. but not *Mycobacterium tuberculosis*, and *Aspergillus fumigatus* are being observed with increasing frequency in CF patients [5].

P. aeruginosa is the most important pathogen in CF lung disease, infecting 70 to 80% of adult patients [6]. Patients with CF who are chronically infected with *P. aeruginosa* have been shown to have a two- to three-fold increased risk of death compared with those who are uninfected [7]. Sev-

eral studies have found an association between chronic *P. aeruginosa* respiratory tract infections and a faster decline in lung function [8, 9].

Over the last decades, *B. cepacia* complex has emerged as an important respiratory pathogen in adult CF patients. Although only 2.8% of American CF patients and 4% of the British CF population are colonized with *B. cepacia* complex, the consequences of infection are extensive and can cause a rapid clinical deterioration associated with severe respiratory inflammation [10]. Several studies have shown lung function decline in patients after *B. cepacia* complex infection [11, 12]. However, it remains uncertain whether *B. cepacia* infection causes more pronounced loss of lung function than *P. aeruginosa* infection.

The CF genotype of the patient is also significantly related to survival. Several investigators have found that patients who are homozygous for ΔF508 have greater lung damage than patients with other mutations [13, 14]. However, an association between genotype and microbiology in CF patients has not been well established [14, 15].

The primary objective of our study was to compare lung function in groups of adult CF patients with different chronic pulmonary infections. Our second objective was to compare the microbiology using patients' genetic status.

Methods

Patients

This study was cross-sectional and included adult CF patients treated in our CF centre at the Piquet Carneiro Polyclinic of the University of the State of Rio de Janeiro. The diagnosis of CF was based on at least two of the following criteria: sweat chloride concentration > 60 mEq/mL; two clinical features consistent with CF; or genetic testing demonstrating two mutations associated with CF [16]. We included individuals who were ≥ 18 years of age. We excluded patients who had more than one microbial species in respiratory cultures collected within a one-year period, and patients with acute intercurrent respiratory infection during the 3 weeks preceding enrollment.

The protocol was approved by the Research Ethics Committee of the University of the State of Rio de Janeiro, and written informed consent was obtained from all participants.

Measurements

Chronic infection was defined as 3 consecutive positive respiratory cultures (oropharyngeal, sputum, and/or bronchoalveolar lavage) collected within a 6-month period and with an interval of at least 1 month between them [17]. Pancreatic insufficiency was considered when fecal elastase level was ≤ 100 μg/g stool [18]. Cystic fibrosis related diabetes was diagnosed by a plasma glucose ≥ 11 mmol/L at a 2 h oral 75 g glucose tolerance test [19]. Cystic fibrosis-related liver disease was defined if at least 2 of the following conditions were present on at least 2 consecutive examinations spanning a 1-year period: (1) elevated serum liver enzyme levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, or gamma-glutamyltransferase; (2) ultrasound confirmed hepatomegaly; (3) ultrasound abnormalities other than hepatomegaly [20].

Pulmonary function tests (PFT) were conducted using the Collins Plus Pulmonary Function Testing Systems (Warren E. Collins, Inc., Braintree, MA, USA), following the American Thoracic Society's standards for the procedure and interpretation [21]. Forced vital capacity (FVC), forced expiratory volume in the first second (FEV_1), FEV_1/FVC, and forced expiratory flow between 25 and 75% of FVC ($FEF_{25-75\%}$) measurements were obtained for all subjects. The results are expressed as the percent of the predicted values for the Brazilian population [22].

Blood samples were collected for genetic analysis. The DNA was amplified using the polymerase chain reaction followed by gel electrophoresis to identify the ΔF508 mutation.

Data analysis

To check the homogeneity of the sample, a Shapiro-Wilk test was used; if a meaningful number of variables did not have a normal distribution, nonparametric tests were selected. The results were expressed as the median and interquartile range values or frequencies (percentage). The study subjects were divided into three groups according to their chronic pulmonary infection profile, as follows: Group A = patients with no chronic pulmonary infection; Group B = patients with chronic *P. aeruginosa* infection; and Group C = patients with chronic *B. cepacia* complex infection. Comparisons of pulmonary function parameters and lung infection profiles were examined using the nonparametric Kruskal-Wallis test followed by Dunn's posttest. To analyse genetic status, we grouped the patients according to whether they were ΔF508 homozygous or had other mutations. The differences between those who were homozygous for ΔF508 and those who were carriers of other mutations were compared using the chi-squared test. Multifactorial variance analysis was performed to investigate the association between pulmonary function, microbiology and the possible confounders. It allowed the inclusion of predictor variables (covariates) and allowed assessment of the impact of multiple covariates in the same model. This model was designed to analyse FVC or $FEF_{25-75\%}$ as the dependent variable with covariates of age, body mass index (BMI), chronic *P. aeruginosa* infection (yes/no), chronic *B. cepacia* complex infection (yes/no), *S. aureus* infection history (yes/no), time of colonisation for *P. aeruginosa* or *B. cepacia* complex, ΔF508 homozygous (yes/no), pancreatic insufficiency (yes/no), diabetes mellitus (yes/no), and liver disease (yes/no). Data analysis was performed using SAS 6.11 software (SAS Institute, Inc., Cary, NC, USA). The statistical significance level was set at $p < 0.05$.

Results

Fifty-two patients were included in this study. Overall, 26 (50%) patients were male; the median age was 26 years (range, 19-40 years). In our sample, 59.6% of subjects were chronically infected with *P. aeruginosa*, 19.2% were chronically infected with *B. cepacia* complex, and 21.2% of patients had no chronic pulmonary infection. All of the patients infected with *B. cepacia* complex were infected with *Burkholderia cenocepacia*. Only two of the infected cultures had multidrug-resistant *P. aeruginosa* (samples with simultaneous resistance to at least three classes of antimicrobials); all cultures with *B. cepacia* complex were multidrug-resistant. Based on our genotyping results, no transmissible strains of *P. aeruginosa* were detected.

The anthropometric characteristics and clinical data of the studied subjects according to their chronic pulmonary infection profiles are summarized in table 1. Regarding genetic status, 36.5% patients were homozygous for ΔF508; there was significant difference in Groups A, B and C (p = 0.006). The median time of colonisation for *P. aeruginosa* and *B. cepacia* complex were 142.3 and 111.5 months, respectively; there was no significant difference between the two groups (p = 0.13). In the sample studied, 61.5% patients had *S. aureus* infection history; however there was no significant difference in Groups A, B and C (p = 0.62). Pancreatic insufficiency, diabetes mellitus and liver disease were observed in 82.7%, 40.4% and 11.5% of patients, respectively; for these variables, there was significant difference only for the percentage at pancreatic insufficiency between the three groups (p = 0.01).

For spirometry, 48.1% of patients had an FEV_1 of ≥ 70%pred, 7.7% had an FEV_1 of between 40% and 70% pred, and 44.2% had an FEV_1 of < 40%pred. Table 2 shows the lung function parameters according to the chronic pulmonary infection profile. There were significant differences among the groups for PFT variables (p = 0.001 to FVC, FEV_1, and $FEF_{25-75\%}$). The PFT results for patients with chronic infections (either *P. aeruginosa* or *B. cepacia* complex) were significantly different from those for subjects without chronic pulmonary infection (p < 0.0001) (figs. 1-3). Importantly, FVC was significantly more altered in patients who were chronically infected with *B. cepacia* complex than in those with chronic *P. aeruginosa* infection. In contrast, $FEF_{25-75\%}$ was significantly more altered in patients with chronic *P. aeruginosa* infection than in those with chronic *B. cepacia* complex infection.

Multivariable analysis was performed to determine whether anthropometric characteristics and clinical data was associated with pulmonary function parameters after considering other factors that may influence the severity of CF (table 3). All variables were assessed in a multifactorial variance model. Covariates with significant associations with FVC were chronic *B. cepacia* complex infection and pancreatic insufficiency. In this model, chronic *P. aeruginosa* infection, ΔF508 homozygous, and pancreatic insufficiency were associated with $FEF_{25-75\%}$.

Discussion

In adult CF patients, chronic infection of the respiratory tract appears to be an important contributor to the loss of lung function and eventual demise [5-7]. Given the polymicrobial nature of CF-related lower airway disease, it is difficult to assess the exact roles of each pathogen in CF lung disease. Thus, we examined only those patients who had chronic infections with *P. aeruginosa* or *B. cenopacia*; other microbial species, including *Haemophilus influenza* and *Staphylococcus aureus*, have not been associated with impaired pulmonary status [1]. Recruitment was facilitated by the fact that our CF center schedules patients on different days according to their chronic infection type.

Table 1. - Anthropometric characteristics and clinical data of the studied subjects according to the profile of chronic pulmonary infection (Kruskal-Wallis test or chi-squared)

	Group A (n = 11)	Group B (n = 31)	Group C (n = 10)	*p* value
Male/female, n	4/7	20/11	6/4	-
Age, years	22 (21-26)	25 (22-30)	29 (27-31)	0.08
Height, cm	165 (160-172)	167 (160-175)	164 (156-169)	0.81
Weight, kg	67.0 (61.5-73.0)	52.2 (49.7-74.0)	58.3 (45.5-64.9)	0.45
BMI, kg/m^2	21.1 (19.7-25.4)	20.7 (18.0-24.3)	21.6 (17.5-22.7)	0.30
ΔF508 homozygous, %	–	58.1	10.0	0.006
Time of colonisation, months	–	142.3 (118.6-177.1)	111.5 (79.0-126.7)	0.13
S. aureus infection history, %	54.5	64.5	60.0	0.62
Pancreatic insufficiency, %	54.5	96.8	70.0	0.01
Diabetes mellitus, %	36.4	41.9	40.0	0.74
Liver disease, %	9.09	12.9	10.0	0.91

Data are presented as medians (interquartile range) or frequencies (percentage).
Group A: patients with no chronic pulmonary infection. Group B: patients with chronic *P. aeruginosa* infection. Group C: patients with chronic *B. cepacia* complex infection.

Table 2. - Lung function parameters according to the profile of chronic pulmonary infection (Kruskal-Wallis test followed by Dunn's post-test)

	Group A (n = 11)	Group B (n = 31)	Group C (n = 10)	*p* value
FVC*	95.2 (88.2-112.0)	64.6 (44.0-98.0)†	42.0 (24.0-91.0)‡§	0.001
FEV_1*	90.0 (81.2-99.0)	38.6 (27.2-76.8)†	31.9 (21.3-73.0)‡	0.001
FEV_1/FVC	80.0 (77.1-81.5)	60.0 (51.8-71.0)	73.9 (62.0-79.0)	0.18
$FEF_{25-75\%}$*	73.0 (56.1-81.0)	12.4 (9.8-37.8)†	23.5 (12.1-74.0)‡§	0.001

Data are presented as medians (interquartile range).
FVC: forced vital capacity. FEV_1: forced expiratory volume in the first second. $FEF_{25-75\%}$: forced expiratory flow between 25 and 75% of FVC. Group A: patients with no chronic pulmonary infection. Group B: patients with chronic *P. aeruginosa* infection. Group C: patients with chronic *B. cepacia* complex infection.
* Results expressed as percentages of predicted values. † p value < 0.0001 compared to Group A by using Dunn's post-test. ‡ p value < 0.0001 compared to Group A by using Dunn's post-test. § p value < 0.0001 compared to Group B by using Dunn's post-test.

This study showed that compared with adult CF patients persistently infected with *P. aeruginosa*, adult CF patients with chronic *B. cepacia* complex infection have a more pronounced reduction in FVC. In contrast, patients with chronic *P. aeruginosa* infection show a more pronounced reduction in $FEF_{25-75\%}$. Interestingly, we also observed a higher proportion of ΔF508-homozygous patients with chronic *P. aeruginosa* infection; this mutation was rare in subjects not infected with *P. aeruginosa*.

The negative impact of chronic *B. cepacia* complex infection on lung function in patients with CF has long been recognized [1, 23]. Sanders *et al* [23] demonstrated an association between chronic *B. cepacia* complex infection and failure to recover baseline pulmonary function after CF pulmonary exacerbations. Navarro *et al* [1], using a cross-sectional analysis, identified a strong relationship between *B. cepacia* infection and poor respiratory function, mostly in the older age groups. The bacterial determinants related to the tissue damage caused by *B. cenopacia* have been minimally investigated, but may include cytotoxicity to airway epithelial cells and the capability of releasing tissue factor-bearing microparticles from infected cells [24]. This release of pro-inflammatory mediators may explain the intense fibrosis process that contributes to a restrictive lung function pattern. In fact, compared with patients who are persistently infected with *P. aeruginosa*, our results showed a more pronounced FVC decline in patients with chronic *B. cepacia* complex infection ($p = 0.001$).

In this investigation, subjects who were chronically infected with *P. aeruginosa* had a significantly greater rate of $FEF_{25-75\%}$ decline ($p = 0.001$), highlighting the clinical impact of *P. aeruginosa* in CF. In a longitudinal analysis, Ren *et al* [25] found a significant association between

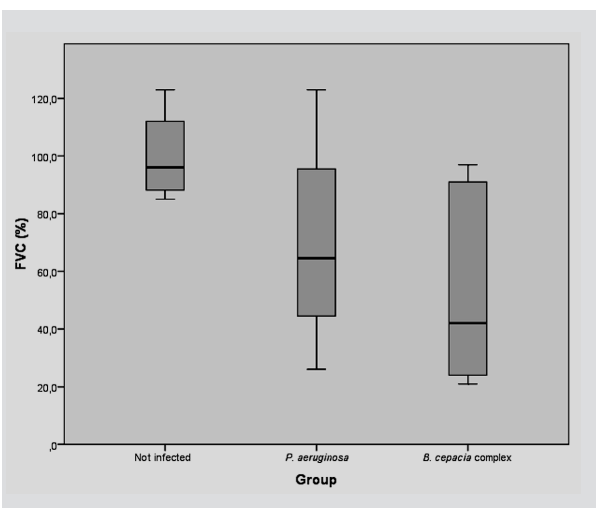

Fig. 1. - Box plot descriptions of the values of forced vital capacity (FVC). Patients were divided in three groups: not infected; chronic *P. aeruginosa* infection; chronic *B. cepacia* complex infection. The top and the bottom of the box plot represent the 25th- to 75th-percentile values, while the bar across the box represents the 50th-percentile value. The whiskers outside the box represent the 10th- to 90th-percentile values. A significant difference ($p = 0.001$) was found between the three groups.

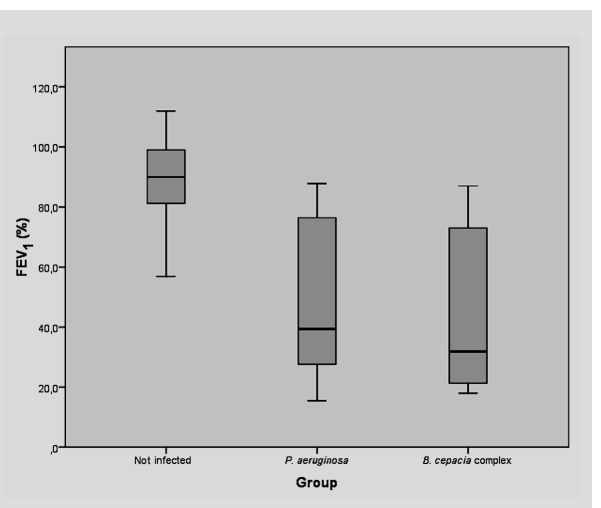

Fig. 2. - Box plot descriptions of the values of forced expiratory volume in the first second (FEV_1). Patients were divided in three groups: not infected; chronic *P. aeruginosa* infection; chronic *B. cepacia* complex infection. The top and the bottom of the box plot represent the 25th- to 75th-percentile values, while the bar across the box represents the 50th-percentile value. The whiskers outside the box represent the 10th- to 90th-percentile values. A significant difference ($p = 0.001$) was found between the three groups.

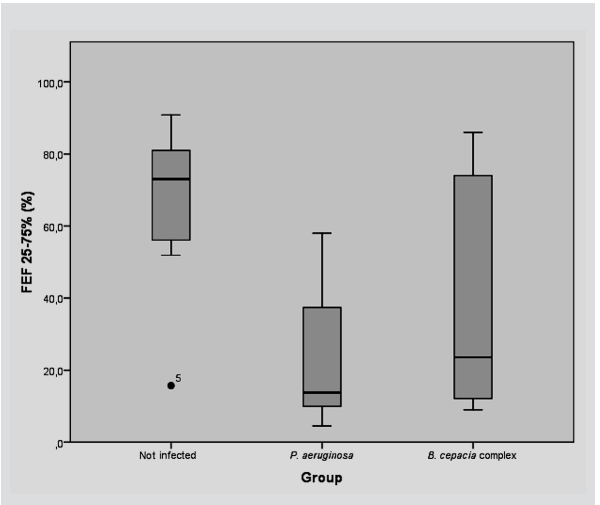

Fig. 3. - Box plot descriptions of the values of forced expiratory flow between 25 and 75% of FVC ($FEF_{25-75\%}$). Patients were divided in three groups: not infected; chronic *P. aeruginosa* infection; chronic *B. cepacia* complex infection. The top and the bottom of the box plot represent the 25th- to 75th-percentile values, while the bar across the box represents the 50th-percentile value. The whiskers outside the box represent the 10th- to 90th-percentile values. A significant difference ($p = 0.001$) was found between the three groups.

$FEF_{25-75\%}$ decline and *P. aeruginosa* infection ($p = 0.024$); there were no other significant longitudinal associations between any other lung function and clinical characteristics. These findings suggest that small airway inflammation may be a critical component leading to flow limitation in CF patients with chronic *P. aeruginosa* infection. This role of this organism has prompted suggestions that the surface properties of CF airways are altered in a way that promotes adherence and the formation of *P. aeruginosa* biofilms [26, 27].

Courtney *et al* [28] suggest that the effect of microbiology on outcome may be more important than the effect of cystic fibrosis transmembrane regulator (CFTR) genetics. In addition to a simple comparison, there seems to be a relationship between genotype and microbiology in CF. In our study, 58.1% of patients with chronic *P. aeruginosa* infection were homozygous for the ΔF508 mutation; in contrast, only one *B. cepacia* complex-infected or noncolonised patient had this mutation. An analysis of CFTR using an *in vitro* experiment showed that *P. aeruginosa* binds significantly more to the epithelial cells of patients homozygous for the ΔF508 mutation than to those of patients with other CF mutations [29]. According to Courtney *et al* [28], a possible explanation for the increased *P. aeruginosa* adherence to epithelial cells in ΔF508-homozygous patients is the larger number of asialylated glycolipids, which serve as receptors for *Pseudomonas* pili on the surface of these cells. Another *in vitro* study suggests that the ΔF508 mutation increases iron availability in the airway surface liquid of the CF lung, which contributes to the exuberant formation of *P. aeruginosa* [27].

Our results showed that FEV_1 values in chronically infected patients (those with either *P. aeruginosa* or *B. cepacia* complex infections) were significantly lower than those observed in subjects without chronic pulmonary infections ($p < 0.0001$). Similarly, Navarro *et al* [1], in a cross-sectional analysis of data from the European Epidemiologic Registry of Cystic Fibrosis, demonstrated that sputum cultures that were positive for *P. aeruginosa* or *B. cepacia* complex were associated with a > 10% lower FEV_1 in all age groups, compared with uninfected patients. In the study by Steinkamp and Wiedemann [30] that evaluated a cohort of 3,298 patients, FEV_1 decline was observed in 85.6% of *P. aeruginosa*-positive adults, whereas only 66.3% of noncolonized patients had abnormal FEV_1 values ($p < 0.001$). In fact, FEV_1 has been shown to be one of the most significant predictors of survival [31], and attempts should always be made, to eradicate chronic *P. aeruginosa* or *B. cepacia* complex infections.

Interestingly, we did not observe transmissible strains of *P. aeruginosa* in the population of our CF center. One possible explanation is the strict segregation of CF patients in our CF center. The emergence of highly prevalent, transmissible *P. aeruginosa* strains has been described, and its prevention has become a target of infection control policies [32]. It has been hypothesised that such transmissible strains have specific characteristics that enable them to be either more virulent or to be more resistant to antibiotics [33].

Table 3. - Multifactorial variance analysis: relationship between the pulmonary function, anthropometric characteristics and clinical data of the studied subjects (only statistically significant associations are shown)

Outcome variable	Independent variables	Unstandardised coefficient			*p*-value
		B	SE	Wald *F*-test	
FVC	Chronic *B. cepacia* complex infection	4.21	1.65	8.94	0.001
	Pancreatic insufficiency	2.98	1.58	5.50	0.026
$FEF_{25-75\%}$	Chronic *P. aeruginosa* infection	3.87	1.69	7.75	0.001
	ΔF508 homozygous	2.73	1.46	4.91	0.031
	Pancreatic insufficiency	2.01	1.52	5.03	0.047

B: regression coefficient. SE: standard error. FVC: forced vital capacity. $FEF_{25-75\%}$: forced expiratory flow between 25 and 75% of FVC.

We also investigated whether clinical data and anthropometric characteristics would demonstrate an independent role in predicting the pulmonary function. In this investigation, chronic *P. aeruginosa* infection and chronic *B. cepacia* complex infection were associated with FVC and $FEF_{25-75\%}$, respectively. In addition to chronic infections, pancreatic insufficiency was a covariate for both FVC and $FEF_{25-75\%}$. The association of pancreatic insufficiency with lung function deterioration in CF patients is well established [34, 35]. Pancreatic insufficiency may affect the energy balance, contributing to malnutrition with decrease in lean body mass and reduction in the contraction of the diaphragm [30, 36, 37]. Malnutrition may also be associated with impaired immune response and antioxidant deficit, easing the way for the onset of infection and inflammation [38]. In our multifactorial variance model, it is not surprising the lack of association between the measuring of the BMI and pulmonary function parameters. Some authors highlight the benefit of using the measuring of the lean body mass rather than the measuring of the BMI as an indicator of nutritional status. Unlike BMI, lean body mass is highly correlated with pulmonary function in CF patients [39, 40]. In the multivariable analysis of the current investigation, the results show also that ΔF508 homozygous and lung function ($FEF_{25-75\%}$) are co-dependent variables in CF patients. Interestingly, Corey *et al* [34] found direct association between ΔF508 homozygous and the rate of decline in pulmonary function in CF.

Our study has several limitations. First, the investigation is limited by its single-center design, and our results may not reflect results from other CF centers. However, we thought it was important to perform this study because results have been variable, depending on the population analysed. Second, we did not collect information about age at diagnosis, which may influence baseline lung function. In addition, the present study is a cross-sectional analysis. It only indicates associations, not allowing the establishment of cause-effect relationship. Thus, longitudinal studies may better complement these results.

In conclusion, our results suggest that chronic pulmonary infection and pancreatic insufficiency are critical processes in lung function deterioration in adult CF patients. Although chronic *B. cepacia* complex infection causes a more pronounced volume reduction (decrease in FVC), chronic *P. aeruginosa* infection causes a more pronounced obstruction of small airways (decrease in $FEF_{25-75\%}$). These findings reveal the possibility of different mechanisms of lung injury. Our study also suggests that patients who are homozygous for ΔF508 are more susceptible to chronic *P. aeruginosa* infection.

References

1. Navarro J, Rainisio M, Harms HK, *et al*. Factors associated with poor pulmonary function: cross-sectional analysis of data from the ERCF. *Eur Respir J* 2001; 18: 298-305.
2. Simmonds NJ. Cystic fibrosis in the 21st century. *Respir Med* 2010; 24: 85-96.
3. Hodson ME, Simmonds NJ, Warwick WJ, *et al*. An international/multicentre report on patients with cystic fibrosis (CF) over the age of 40 years. *J Cyst Fibros* 2008; 7: 537-542.
4. Heijerman H, Westerman E, Conway S, *et al*. Inhaled medication and inhalation devices for lung disease in patients with cystic fibrosis: A European consensus. *J Cyst Fibros* 2009; 8: 295-315.
5. Miller MB, Gilligan PH. Laboratory aspects of management of chronic pulmonary infections in patients with cystic fibrosis *J Clin Microbiol* 2003; 41: 4009-4015.
6. Burkett A, Vandemheen KL, Giesbrecht-Lewis T, *et al*. Persistency of Pseudomonas aeruginosa in sputum cultures and clinical outcomes in adult patients with cystic fibrosis. *Eur J Clin Microbiol Infect Dis* 2011; doi: 10.1007/s10096-011-1483-1488.
7. Emerson J, Rosenfeld M, McNamara S, *et al*. Pseudomonas aeruginosa and other predictors of mortality and morbidity in young children with cystic fibrosis. *Pediatr Pulmonol* 2002; 34: 91-100.
8. McPhail GL, Acton JD, Fenchel MC, *et al*. Improvements in lung function outcomes in children with cystic fibrosis are associated with better nutrition, fewer chronic *Pseudomonas aeruginosa* infections, and dornase alfa use. *J Pediatr* 2008; 153: 752-757.
9. Konstan MW, Schluchter MD, Xue W, *et al*. Clinical use of ibuprofen is associated with slower FEV_1 decline in children with cystic fibrosis. *Am J Respir Crit Care Med* 2007; 176: 1084-1089.
10. Vrankrijker AMM, Wolfs TFW, van der Ent CK. Challenging and emerging pathogens in cystic fibrosis. *Paediatr Respir Rev* 2010; 11: 246-254.
11. Whiteford ML, Wilkinson JD, McColl JH, *et al*. Outcome of *Burkholderia (Pseudomonas) cepacia* colonization in children with cystic fibrosis following a hospital outbreak. *Thorax* 1995; 50: 1194-1198.
12. Liou TG, Adler FR, FitzSimmons SC, *et al*. Predictive 5-year survivorship model of cystic fibrosis. *Am J Epidemiol* 2001; 153: 345-352.
13. O'Connor GT, Quinton HB, Kahn R, *et al*. Case-mix adjustment for evaluation of mortality in cystic fibrosis. *Pediatr Pulmonol* 2002; 33: 483-491.
14. Johansen HK, Nir M, Hoiby N, *et al*. Severity of cystic fibrosis in patients homozygous and heterozygous for delta F508 mutation. *Lancet* 1991; 337: 631-634.
15. Borgo G, Gasparini P, Bonizzato A, *et al*. Cystic fibrosis: the delta F508 mutation does not lead to an exceptionally severe phenotype. A cohort study. *Eur J Pediatr* 1993; 152: 1006-1011.
16. Farrel PM, Rosenstein BJ, White TB, *et al*. Guidelines for diagnosis of cystic fibrosis in newborns through older adults: Cystic Fibrosis Consensus Report. *J Pediatr* 2008; 153: S4-S14.
17. Frederiksen B, Koch C, Høiby N. Changing epidemiology of Pseudomonas aeruginosa infection in Danish cystic fibrosis patients. *Pediatr Pulmonol* 1999; 28: 159-166.
18. Borowitz D, Stevens C, Brettman LR, Campion M, Wilschanski M, Thompson H. Litrotamase long-term safety and support of nutritional status in pancreatic-insufficient cystic fibrosis. *J Pediatr Gastroenterol Nutr* 2012; 54: 248-257.
19. Hillman M, Eriksson L, Mared L, Helgesson K, Landin-Olsson M. Reduced levels of active GLP-1 in patients with cystic fibrosis with and without diabetes mellitus. *J Cyst Fibros* 2012; 11: 144-149.
20. Karlas T, Neuschulz M, Oltmanns A, *et al*. Non-invasive evaluation of cystic fibrosis related liver disease in adults with ARFI, transient elastography and different fibrosis scores. *PLoS One* 2012; 7: e42139.

21. Miller MR, Hankinson J, Brusasco V. ATS/ERS Task Force. Standardization of spirometry. *Eur Respir J* 2005; 26: 319-338.
22. Pereira CAC, Sato T, Rodrigues SC. New reference values for forced spirometry in white adults in Brazil. *J Bras Pneumol* 2007; 33: 397-406.
23. Sanders DB, Bittner RCL, Rosenfeld M, *et al*. Failure to recover to baseline pulmonary function after cystic fibrosis pulmonary exacerbation. *Am J Respir Crit Care Med* 2010; 182: 637-632.
24. Cunha Jr LG, Assis MC, Machado GB, *et al*. Potential mechanisms underlying the acute lung dysfunction and bacterial extrapulmonary dissemination during *Burkholderia cenocepacia* respiratory infection. *Respir Res* 2010; 11: doi: 10.1186/1465-9921-11-4.
25. Ren CL, Rosenfeld M, Mayer OH. Analysis of the associations between lung function and clinical features in preschool children with cystic fibrosis. *Pediatr Pulmonol* 2012; 47: 574-81.
26. Peterson-Carmichael SL, Harris WT, Goel R, *et al*. Association of lower airway inflammation with physiologic findings in young children with cystic fibrosis. *Pediatr Pulmonol* 2009; 44: 503-511.
27. Moreau-Marquis S, Bomberger JM, Anderson GG, *et al*. The ΔF508-CFTR mutation results in increased biofilm formation by *Pseudomonas aeruginosa* by increasing iron availability. *Am J Physiol Lung Cell Mol Physiol* 2008; 295: L25-L37.
28. Courtney JM, Bradley J, Mccaughan J, *et al*. Predictors of mortality in adults with cystic fibrosis. *Pediatr Pulmonol* 2007; 42: 525-532.
29. Zar H, Saimann L, Quittell L, *et al*. Binding of *Pseudomonas aeruginosa* to respiratory epithelial cells from patients with various mutations in the cystic fibrosis transmembrane regulator. *J Pediatr* 1995; 126:230-233.
30. Steinkamp G, Wiedemann B. Relationship between nutritional status and lung function iin cystic fibrosis: cross sectional and longitudinal analyses from the German CF quality assurance (CFQA). *Thorax* 2002; 57: 596-601.
31. Kerem E, Reisman J, Corey M, *et al*. Prediction of mortality in patients with cystic fibrosis. *N Engl J Med* 1992; 32: 1187-1191.
32. Saiman L. Microbiology of early CF lung disease. *Paediatr Respir Rev* 2004; 5 (Suppl A): S367-S369.
33. Salunkhe P, Smart CH, Morgan JA, *et al*. A cystic fibrosis epidemic strain of *Pseudomonas aeruginosa* displays enhanced virulence and antimicrobial resistance. *J Bacteriol* 2005; 187: 4908-4920.
34. Corey M, Edwards L, Levison H, Knowles M. Longitudinal analysis of pulmonary function decline in patients with cystic fibrosis. *J Pediatr* 1997; 131: 809-814.
35. Schaedel C, de Monestrol I, Hjelte L, *et al*. Predictors of deterioration of lung function in cystic fibrosis. *Pediatr Pulmonol* 2002; 33: 483-491.
36. Stallings VA, Stark LJ, Robinson KA, Feranchak AP, Quinton H. Evidence-based practice recommendations for nutrition-related management of children and adults with cystic fibrosis and pancreatic insufficiency: results of a systematic review. *J Am Diet Assoc* 2008; 108: 832-839.
37. Konstan MW, Butler SM, Wohl MEB, *et al*. Growth and nutritional indexes in early life predict pulmonary function in cystic fibrosis. *J Pediatr* 2003; 142: 624-630.
38. Hart N, Tounian P, Clément A, *et al*. Nutritional status is an important predictor of diaphragm strength in young patients with cystic fibrosis. *Am J Clin Nutr* 2004; 80: 1201-1206.
39. Rochat T, Slosman D, Pichard C, Belli D. Body composition analysis by dual-energy X-ray absorptiometry in adults with cystic fibrosis. *Chest* 1994; 106: 800-805.
40. King S, Wilson J, Kotsimbos T, Bailey M, Nyulasi I. Body composition assessment in adults with cystic fibrosis: comparison of dual-energy X-ray absorptiometry with skinfolds and bioelectrical impedance analysis. *Nutrition* 2005; 21: 1087-1094.