Pulmonary function patterns and their association with genotype and phenotype in adult cystic fibrosis patients

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PULMONARY FUNCTION PATTERNS AND THEIR ASSOCIATION WITH GENOTYPE AND PHENOTYPE IN ADULT CYSTIC FIBROSIS PATIENTS.

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Abstract

Background: While Cystic fibrosis (CF) lung disease is generally considered to be an obstructive disorder, other pulmonary function patterns (PFP) may occur. Furthermore, little is known about possible associations between PFP and genotype or phenotypical characteristics.

Methods: Cross-sectional study including CF patients aged 16 years or more, identifying different PFP and exploring associations between PFP and genotype or phenotypical characteristics.

Results: Obstructive PFP was most prevalent in our population (n=80), comprising obstructive lung disease (62.5%), small airway (obstructive) disease (11.2%) and mixed obstructive-restrictive disorder (1.3%). However, one in four adult CF patients did not show any obstruction at all: normal (13.7%) or restrictive (8.8%) lung disease and isolated diffusion disorder (2.5%). Obstructive PFP was associated with a greater proportion of cystic fibrosis related diabetes mellitus (CFRD) (P=0.04), *Pseudomonas aeruginosa* colonization (P=0.02) and frequent exacerbators (P=0.04). We observed no association between PFP and genotype.

Conclusions: Obstructive PFP remains the most common pulmonary function pattern in adult CF and is associated with CFRD, *Pseudomonas aeruginosa* colonization and frequent exacerbators.

Keywords: cystic fibrosis, pulmonary function patterns, genotype-phenotype associations.

Abbreviations

BCFR: Belgian cystic fibrosis registry

BMI: body mass index

CF: Cystic Fibrosis

CFRD: cystic fibrosis related diabetes

CFTR gene: cystic fibrosis transmembrane regulator gene

$FEF_{25-75\%}$: forced expiratory flow 25-75%

FEV_1: forced expiratory volume in one second

FVC: forced vital capacity

F508del: deletion of phenylalanine at position 508

HbA_{1C}: hemoglobin A1C

LLN: lower limit of normal

MRSA: *methicillin resistant Staphylococcus aureus*

PA: *Pseudomonas aeruginosa*

PFP: pulmonary function pattern

TLC: total lung capacity

TL_{CO}: transfer factor of carbon monoxide

ULN: upper limit of normal

Introduction

Cystic Fibrosis (CF) is a multi-systemic lethal autosomal recessive disorder caused by a mutation in the CFTR gene, which encodes for the cystic fibrosis transmembrane regulator protein, with the deletion of phenylalanine at position 508 (F508del) being the most common mutation in Northern Europe and North America [1,2]. CF lung disease is an important predictor of survival and remains the main reason for morbidity and mortality [3]. CFTR dysfunction causes defective mucociliary clearance of thickened mucus which potentially leads to obstruction of the airways [2]. Such obstructions create a hypoxic environment that can harbor different bacteria [4]. The predominant microorganisms colonizing the airways of CF patients are *Haemophilus influenzae* and *Staphylococcus aureus* early in life, and *Pseudomonas aeruginosa (PA)* later on. Besides these pathogens, *methicillin resistant Staphylococcus aureus (MRSA)* and *Achromobacter xylosoxidans* also plays an important role in more advanced lung disease. These bacteria can potentially cause infectious exacerbations, inflammation, and finally lung functional degradation with structural abnormalities due to a vicious circle of inflammation [3].

In terms of lung function, CF is considered to be a chronic progressive obstructive disorder [4] with the forced expiratory volume in one second (FEV_1) being an important indicator of lung disease severity [5]. Decline in FEV_1 has indeed been shown to be associated with different phenotypic characteristics of disease severity such as pancreatic insufficiency, cystic fibrosis related diabetes (CFRD), colonization with *PA* and *MRSA* [6-7]. However, limiting CF lung disease to its spirometric assessment (which can only establish airway obstruction) seems inadequate since restrictive pulmonary disorders may occur in CF as well [8]. Also, to confirm normal lung function in some adult CF patients [9], a full lung function evaluation, including diffusing capacity and lung volume measurements is required. The goal of the present study was to scrutinize the different pulmonary function patterns in a

CF population from the adult CF center at University Hospital UZ Brussel. Secondly, we aimed to explore possible associations between distinct pulmonary function patterns and genotype and certain phenotypical characteristics.

Methods

A retrospective cross-sectional study was conducted using the patient registry of the Adult Cystic Fibrosis clinic at the University Hospital UZ Brussel, obtaining informed consent from all eligible patients. This study was approved by the ethics committee at the University Hospital UZ Brussel (B.U.N.143201422734). CF diagnosis was obtained as described by the European Cystic Fibrosis Society as the combination of clinical characteristics, a positive sweat test and/or two disease causing mutations [10]. Inclusion criteria were: conclusive CF diagnosis and complete pulmonary function test including diffusing capacity and plethysmography during the study period. Exclusion criteria were: lung function tests performed during infectious exacerbation, incomplete genetic or sweat test information, active smoking, lung transplantation and age under 16. Data were collected from the period between February 2013 and November 2014.

The following data were used for all patients: gender, age, weight, height and body mass index (BMI), genotype, pancreatic function, CFRD, presence of colonization with *PA*, *MRSA*, *Burkholderia species* and *Stenotrophomonas maltophilia*. Genotypes were classified as F508del homozygous or other mutations. Pancreatic insufficiency was defined by pancreatic enzyme replacement therapy and faecal elastase 1 below 200 microgram/gram faeces [11]. Diagnostic criteria for CFRD were fasting plasma glucose of at least 200 mg/dl in symptomatic patients, or fasting plasma glucose of at least 126 mg/dl and/or positive oral glucose tolerance test and/or HbA_{1C} of at least 6,5% in asymptomatic patients [12]. Colonization with bacteria was defined as having at least three positive sputum cultures over at least six months. We also identified frequent exacerbators, whereby a pulmonary

exacerbation was defined by Bilton et al. [13] as a recent change in at least two of the following: change in sputum volume or colour, increased cough, increased malaise, fatigue or lethargy, anorexia or weight loss, decrease in FEV_1 by 10% or more, radiographic changes or increased dyspnoea. Frequent exacerbators were then identified as having received at least three treatments with antibiotics (oral or intravenous) for respiratory symptoms in the past year.

All eligible patients from the CF clinic of the University Hospital UZ Brussel had performed lung function in the adult lung function laboratory UZ Brussel, performed by the same lung function technician and with the same commercial equipment (Vmax Encore VE models 20c, 22 and 22d; Cardinal Health, Dublin, OH, USA). This included spirometry, body plethysmography and a single breath carbon monoxide diffusing capacity test, performed according to ERS/ATS guidelines for standardisation of lung function testing [14-17]. Spirometry was performed before and after inhalation of 400 microgram salbutamol by metered dose inhaler. In patients receiving therapy with bronchodilators (short and long acting), these were not stopped before pulmonary function testing. Except for the determination of spirometric reversibility of obstruction, only post-bronchodilator values of pulmonary function parameters were considered. All lung function parameters were scrutinized for abnormality using lower and upper limits of normal (LLN and ULN) based on reference values from the Global Lung Function Initiative [18] for spirometric parameters, Stocks et al. [19] and Quanjer et al. for the static lung volumes [20] and Cotes et al. for the diffusion parameters [21].

We first considered the following lung function subgroups:

1. *Normal lung function*: all lung function variables within limits of normal.

2. *Small airway (obstructive) disease:* end-expiratory flows $FEF_{25-75\%}$ below the LLN, in the presence of a normal Tiffeneau-index (LLN<FEV_1/FVC<ULN) [17] and no restriction (LLN<TLC<ULN).
3. *Reversible obstructive pulmonary disease*: pre-dilator forced expiratory volume in one second over forced expiratory volume (FEV_1/FVC) below LLN [17], and reversibility of obstruction defined by an increase in FEV_1 and/or FVC after bronchodilation of at least 200 ml and 12 percent of baseline FEV_1 and/or FVC [17].
4. *Non-reversible obstructive pulmonary disease*: pre-dilator FEV_1/FVC below LLN [17], and no reversibility of obstruction defined by an increase in FEV_1 and/or FVC after bronchodilation of less than 200 ml or 12 percent of baseline FEV_1 and FVC [17].
5. *Restrictive lung disease*: total lung capacity (TLC) below LLN [17].
6. *Mixed obstructive and restrictive lung disease:* a combination of obstruction and restriction as defined under 2, 3 and 4.
7. *Isolated diffusion disorder:* Diffusing capacity (TL_{CO}) < LLN in the absence of obstruction (LLN<FEV_1/FVC<ULN) or restriction (TLC>LLN).

All subgroups with an obstructive component: s*mall airway (obstructive) disease, reversible and non-reversible obstructive pulmonary disease* and *mixed obstructive-restrictive lung disease* were pooled as a pulmonary function pattern labelled "obstructive PFP". Normal lung function, restrictive lung disease and isolated diffusion disorder were grouped as "non-obstructive PFP". Using these two PFP groups, we assessed the potential associations with genotype and phenotype.

Statistical analysis was obtained with MedCalc (version16.4.3, Mariakerke, Belgium) using the Fisher-exact test for categorical parameters and the Mann Whitney U test for the quantitative parameters. P-values below 0.05 were considered statistically significant.

Results

Of the 91 patients registered in the database, 11 patients (4 male/7 female) were excluded: 5 patients had no complete lung function test available during the inclusion period, 2 patients had previously been transplanted, 2 patients were active smokers and 2 patients were under the age of 16 years. A breakdown of the cohort is shown in Figure 1. The remaining patients (n=80) had a median age of 28 years (62.5% male) and a median BMI of 21.3 kg/m² (Table 1).

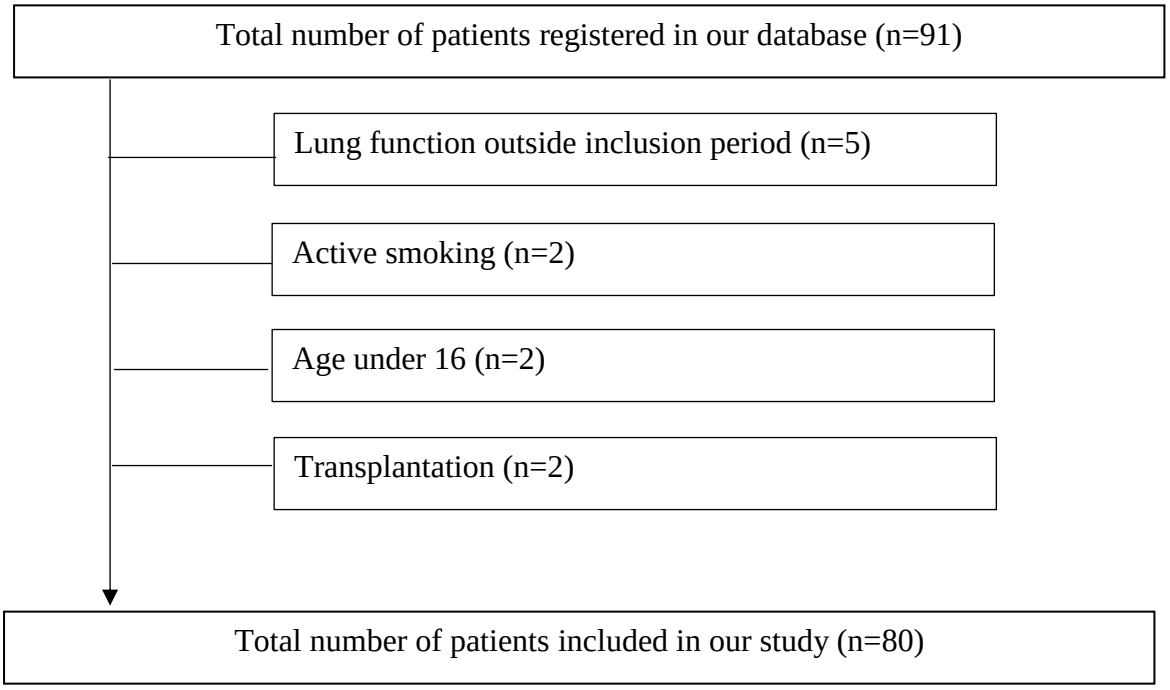

Figure 1: Flow chart with reasons for exclusion

One out of four patients were homozygous for F508del. Exocrine pancreatic function was compromised in most patients (87.5%), while endocrine pancreatic function was more often preserved with a CFRD prevalence of 27.5%. Colonization with *Pseudomonas aeruginosa* was present in 42.5% of patients followed by *Burkholderia species* (5%), *Methicillin resistant Staphylococcus aureus* (2.5%) and *Stenotropohomons maltophilia* (1.2%). The combination of *PA* and *MRSA* colonization was not found in any of our CF

patients. 41.3% of patients had frequent exacerbations. A summary of the anthropometric, genotypic and phenotypic characteristics is shown in Table 1.

The prevalence of the seven different pulmonary function subgroups is depicted in Figure 2. A large portion of patients had non-reversible obstruction (46.2%), followed by the patient subgroup with reversible obstruction (16.2%), and the subgroup with normal lung function (14%). When including all patients with some degree of obstruction - obstructive pulmonary disorder, mixed obstructive and restrictive lung disease and small airway (obstructive) disease - as "obstructive PFP", these constituted the majority of patients (73.7%). A Spearman Rank correlation between FEV_1 (%predicted) and age was highly significant in the obstructive PFP group (rho= -0.43; P<0.001; n=60) and not significant at all in the non-obstructive PFP group (P=0.6; n=20). Compared to the non-obstructive PFP group, the obstructive PFP group had more cystic fibrosis related diabetes mellitus (P=0.04), were more colonized with *Pseudomonas aeruginosa* (P=0.02) and were more frequent exacerbators (P=0.003) (Table 2).

Table 1: Anthropometric, genotypic and phenotypic characteristics in the adult cystic fibrosis population at the University Hospital UZ Brussel (n=80).

Parameter	Number (percentage of population: n =80)
ANTROPOMETRIC	
Gender: male	50 (62.5%)
Age (years)	28 (18-38)[i]
Weight (kg)	63(52-74)[i]
Height (cm)	171 (162-179)[i]
Body Mass Index (kg/m²)	21.3 (18.6-24.0)[i]
GENOTYPE	
Homozygous for F508del	32 (40%)
Other mutation	48 (60%)
PANCREATIC FUNCTION	
Pancreatic insufficiency	70 (87.5%)
CFRD	22 (27.5%)
COLONIZATIONS	
PA	34 (42.5%)
MRSA	2 (2.5%)
PA + MRSA	0 (0.0%)
Burkholderia	4 (5.0%)
Stenotrophomonas maltophilia	1 (1.2%)
EXACERBATIONS	
Frequent exacerbations	33 (41.3%)

Abbreviations: CFRD: cystic fibrosis related diabetes mellitus, PA: *Pseudomonas aeruginosa*, MRSA: *Methicillin resistant Staphylococcus aureus*.

[i]: median value (95% confidence interval)

Figure 2: Pulmonary function subgroups in the adult cystic fibrosis population at the University Hospital UZ Brussel (n=80): see text for definition of the 7 subgroups.

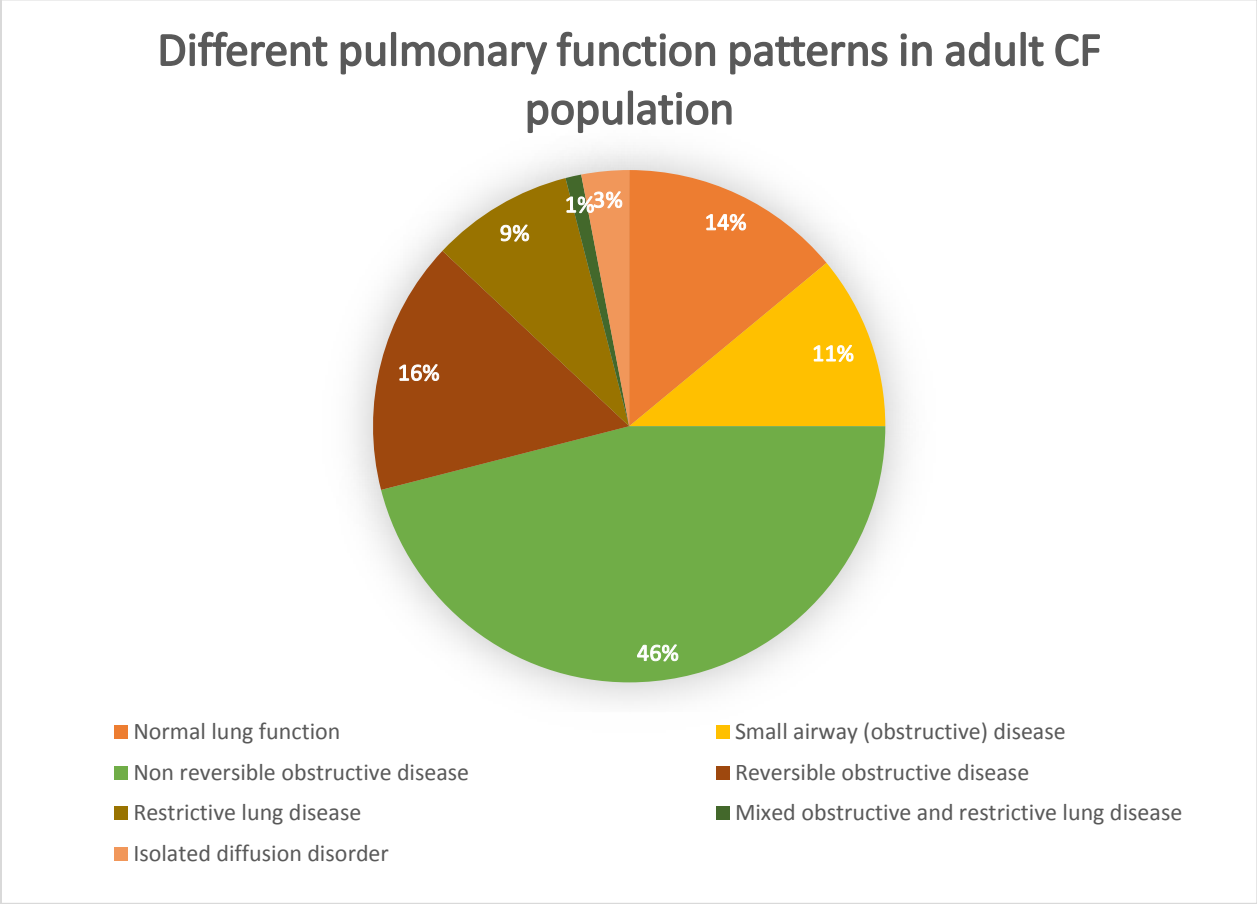

Table 2: Anthropometric, genotypic and phenotypic characteristics in the obstructive and non-obstructive population at the adult CF clinic at the University Hospital UZ Brussel.

Parameter	Obstructive (n= 60)	Non-obstructive (n= 20)	P-value
	ANTROPOMETRIC DATA		
Gender: male	37 (61.7%)	13 (65.0%)	1.00
Age (years)	29 (19-39)[i]	24 (15-33)[i]	0.09
Body Mass Index (kg/m²)	20.9 (18.4-23.4)[i]	22.2 (19.2-25.2)[i]	0.08
	GENOTYPE		
F508del homozygous	27 (45.0%)	5 (25.0%)	0.19
	PANCREATIC FUNCTION		
Pancreatic insufficiency	55 (91.7%)	15 (75%)	0.11
CFRD	**20 (33.3%)**	**5 (25%)**	**0.04**
	COLONIZING MICRO-ORGANISMS		
PA	**30 (50.0%)**	**4 (20.0%)**	**0.02**
MRSA	2 (3.3%)	0 (0.0%)	1.00
PA + MRSA	0 (0.0%)	0 (0.0%)	1.00
Burkholderia	4 (6.7%)	0 (0.0%)	0.57
Stenotrophomonas	1 (1.7%)	0 (0.0%)	1.00
	EXACERBATIONS		
Frequent exacerbations	**29 (48.3%)**	**4 (20.0%)**	**0.03**

Abbreviations: CFRD: cystic fibrosis related diabetes mellitus, PA: *Pseudomonas aeruginosa*, MRSA: *Methicillin resistant Staphylococcus aureus*.
[i]: median value with 95% confidence interval

Discussion

This mono-centric observational study examined the prevalence of different pulmonary function patterns in CF patients from the adult CF clinic at the University Hospital UZ Brussel. Our study confirmed the presence of obstructive disease in the majority of CF patients (62.5%), increasing to 75% when also including small airway (obstructive) disease and mixed obstructive-restrictive disorder into an "obstructive PFP" group. However, 25% of the CF patients had either normal lung function (13.7%), restrictive disorder (8.8%) or an isolated diffusion disorder (2.5%). To the best of our knowledge, this is the first time that the link between different pulmonary function patterns (obstructive PFP or not), genotype and phenotypic characteristics was investigated. In particular, we showed that the patients with obstructive PFP had more CFRD, were more frequently colonized with *PA,* and had more frequent exacerbators. We did not observe an association with genotype.

We compared our study population for demographic characteristics with the most recent data from the Belgian CF registry (BCFR) [22]. Considering that the adult CF population in the BCFR was made up of 644 patients, the proportion of male patients in our study was found to be very similar (62.5 % versus 52% in the BCFR; P=0.07). Also, our population had a very similar prevalence of F508del homozygous **(**P>0.1), pancreatic insufficiency (P>0.18) and CFRD (P>0.1). Given that the single-center CF population is so similar to that of the BCFR, we believe our results may be more widely applicable to the adult Belgian CF population.

CF lung disease: more than a purely obstructive lung disease

Our study showed that one out of four adult CF patients did not have any airway obstruction. The proportion of CF patients with either normal lung function (13.7%) or restrictive lung function (8.8%) were in agreement with previous studies by Ziegler and Ries

[9,10]. Isolated diffusion disorder was found in 2.5% of patients (diffusion is known to be normal or increased until late in the course of the disease due to claustration with inhomogenic ventilation [23]). Importantly, the patients with normal or restrictive lung disease and isolated diffusion disorder were not significantly younger than patients with obstructive lung disease (Table 2), and in fact their FEV_1(%predicted) was also independent of age, in contrast to the obstructive PFP group. Based on the association between progressing age and lower FEV_1 (%predicted) in obstructive PFP, obstructive CF lung disease seems to be a progressive disease, but because of the cross-sectional design of this retrospective study, we are not able to prove this. Concerning lung function assessment, we also have to be aware that this is always a snapshot of the pulmonary condition of the patient. Inter test variability, for example because of seasonal variation, is possible but this concerns all the PFP subgroups The presence of an obstructive PFP in a large portion of CF patients could be expected based on the pathophysiology with a vicious circle of infection and inflammation caused by CFRT dysfunction, defective mucociliary clearance and infection leading to obstruction of the airways [2] and structural damage. The fact that the majority of patients with obstruction did not show reversibility of obstruction (Figure 1), may be partly due to the fact that most of our CF patients were treated with bronchodilators (82.1%). In the most recent study reporting reversibility of obstruction in CF patients by Levine et al. [24], 39% of 109 patients were found to have reversible airway obstruction, but this was mainly true for the pediatric CF patients, the older CF patients in that study showing less reversibility. This was interpreted as children having considerable bronchomotor tone and bronchospasm, which gets attenuated as disease progresses because of chronic inflammation and destruction of the airways.

Genotype and phenotype associations

Genotype-pulmonary phenotype associations reported in an earlier study where pulmonary phenotype was considered in terms of FEV_1, were found to be weak in cystic fibrosis [25]. Nevertheless, Geborek and Hjelte [25] did observe some associations between lower FEV_1 and class I (biosynthesis problem with no CFTR protein), II (protein maturation problem with potentially some residual CFTR activity) and III (ion channel regulation with normal amount of non-functional CFTR) mutations. We were not able to demonstrate a link between obstructive of non-obstructive pulmonary function pattern and genotype.

The prevalence of *Pseudomonas aeruginosa* was low in our cohort (42.5%), compared to prevalences reported for other centers of the Belgian CF registry (ranging 45.0-70.0% in adult patients) [22]. The proportion of patients with *PA* was significantly greater in the obstructive versus non-obstructive PFP. It had been previously demonstrated that the risk of having severe lung disease (in terms of FEV_1) increases with a factor 2.4 when patients are chronically infected with *PA* [7]. Colonization with *MRSA* in our center was very low (2.5%), compared to the prevalence in Belgium (up to 15% in adults) [22], which can be explained by a recent eradication study performed in our CF center [26]. Despite the high detection rate in our laboratory, where subtypes of *Burkholderia* species (*multivorans* and *vietnamiensis*) are also identified, prevalence of *Burkholderia* colonization was 5.0%, which is similar (P=0.12) to that in the Belgian CF register (3.6%). Finally, the frequent exacerbators were more represented in obstructive PFP. The number of exacerbations can be used as a marker for CF lung disease severity [27]. Previous research by Sanders et al. has shown that having three or more exacerbations a year is associated with a greater FEV_1 decline in adults, and that there is a linear relationship between the number of exacerbations and decrease in lung function (FEV_1) [28].

Limitations of the study

Because this was a single-center study, some PFP subgroups contained very few patients. The small sample sizes are the biggest limitation of our study. We therefore pooled the seven different lung function subtypes into two larger groups: the obstructive and the non-obstructive lung disease group. To remedy for this we can only recommend that the data of the Belgian CF Registry be analyzed in a similar way to what was done here.

Conclusion

CF lung disease is a heterogeneous disease in terms of pulmonary function, with almost half of the patients inflicted with non-reversible obstructive pulmonary disease. One in 4 CF patients in our center did not show any obstructive lung function pattern. We also found significant associations between the type of lung function abnormality and certain phenotypical characteristics. Indeed, an obstructive pulmonary function pattern was associated with increased cystic fibrosis related diabetes mellitus, *Pseudomonas aeruginosa* colonization and more frequent exacerbations.. A similar investigation could be conducted in a larger population such as the Belgian CF Registry, provided that lung function parameters beyond spirometry can be evaluated.

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