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Association of Chronic *Candida albicans* Respiratory Infection With a More Severe Lung Disease in Patients With Cystic Fibrosis

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Summary. Background: Despite the increase in fungal isolates, the significance of chronic *Candida albicans* airway colonization in CF is unclear. Aim: To investigate the impact of *C. albicans* airway colonization on CF disease severity. Methods: Longitudinal analysis of clinical data from CF patients followed during 2003–2009 at our CF center. Patients were stratified based on their *C. albicans* colonization status – chronic, intermittent, and none. Results: A total of 4,244 cultures were obtained from 91 patients (mean age 19.7 years, range 5–68). The three colonization groups were similar in age, gender,and body mass index (BMI). Compared to the non-colonized group (n = 27, 30%), the chronic *C. albicans* colonization group (n = 34, 37%), had a significantly lower FEV_1 percent predicted (74.3 ± 23.1% vs. 93.9% ± 22.2) with a higher annual rate of FEV_1 decline (− 1.9 ± 4.2% vs. 0.7 ± 4.5%). The patients who were intermittently colonized with *C. albicans* had intermediate values. Conclusions: Chronic respiratory colonization of *C. albicans* is associated with worsening of FEV_1 in CF. Prospective studies are needed to confirm this finding and to corroborate whether indeed *C. albicans* drives a deleterious lung phenotype. **Pediatr Pulmonol. 2015;50:1082–1089.**

Key words: Cystic fibrosis; disease progression; forced expiratory volume/physiology; sputum/microbiology.

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INTRODUCTION

Cystic fibrosis (CF) is characterized by chronic inflammation and infection of the airways resulting in progressive damage leading to respiratory failure and early death. *P. aeruginosa* and *S. aureus* are the leading respiratory pathogens, however recent studies looking into the CF airway microbiome have uncovered other microorganisms.[1] Increased prevalence of fungi in CF respiratory cultures has been reported over the last decade,[2–4] *Candida albicans* the most common of them, with prevalence reaching 75% in some studies.[2,3] Other Candida species, including *C. glabrata*, *C. parapsilosis*, *C. dubliniensis*, and *C. tropicalis*, have also been reported, though with much lower prevalence.[4,5] Frequent use of antibiotics, as well as oral and inhaled steroids, potentially predisposes CF patients to colonization by *Candida* spp.[2] Clinical manifestations range in severity from asymptomatic colonization, to oral thrush, genital candidiasis, and bloodstream infections.[6,7] However, the consequences of chronic respiratory colonization by *C. albicans* in patients with CF remain unknown.

The aims of this study were to investigate the prevalence and persistence of *C. albicans* in the

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Conflict of interest: None.

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respiratory tract of patients with CF, and to assess possible relationship between *C. albicans* colonization and CF lung disease severity.

MATERIALS AND METHODS

Study Design and Patients

Patients with a confirmed diagnosis of CF,[8] who were followed at the Hadassah-Hebrew University Medical Center between 2003 and 2009 and were able to perform pulmonary function tests, were included in the study. No exclusion criteria were used. Most of the patients undergo routine assessment of nutritional status, pulmonary function, and sputum culture each month. The clinical data used for this study were continuously and prospectively collected, and retrospectively analyzed. The study was approved by the Hadassah Medical Center Institutional Review Board and written informed consent was waived (Committee on Research Involving Human Subjects of the Hebrew University—Hadassah Medical School, 0324-08-HMO). All patients or their legal guardians signed informed consent to be included in the CF registry database for research purposes.

Laboratory Methods

The routine in our center is a monthly visit in which anthropometric measurements, sputum culture, and pulmonary function are obtained. Sputum cultures are considered to be of good quality in cases where there are >25 leukocytes and ≤ 10 squamous cells per low power field. Samples that do not meet this definition are considered contaminated by saliva and rejected. Patients were grouped based on modified criteria from the original criteria published for *Pseudomonas* spp.[9] and for *C. albicans*.[10] Patients with three or more consecutive growth of *Candida* in sputum cultures or in $\geq 50\%$ of cultures obtained within a 12-month period were considered to have chronic *Candida* colonization, those with positive cultures in 25–49% of sputum samples obtained within 12 months were classified as having intermittent colonization, and those with no positive cultures or positive findings in $< 25\%$ of cultures obtained over 12 months were defined as not colonized. Throughout the study period, *C. albicans*, *C. glabrata*, and *C. tropicalis* were identified using Candida CHROMagar. Other *Candida* species were identified using sugar assimilation tests. The patients were considered to be colonized with *Candida* based on evaluation of sputum samples, and no patient met the requirements to fulfill the criteria for "proven," "probable," or "possible" invasive fungal disease, as proposed in the revised definitions of invasive fungal disease from the EORTC/MSG Consensus Group.[11]

ABBREVIATIONS:

ABPA	Allergic bronchopulmonary aspergillosis
BMI	Body mass index
CF	Cystic fibrosis
CFRD	Cystic fibrosis-related diabetes mellitus
FEV_1	Forced expiratory volume in 1 sec
MSSA	Methicillin-sensitive staphylococcus aureus
MRSA	Methicillin-resistant staphylococcus aureus
PFTs	Pulmonary function tests
PI	Pancreatic insufficiency
VAP	Ventilator-associated pneumonia

Pulmonary Function Testing

Pulmonary function tests (PFTs) were performed according to American Thoracic Society/European Respiratory Society guidelines.[12] Patients able to complete spirometry were included in the study. Forced expiratory volume in 1 sec (FEV_1) was presented as a percent of the predicted value according to Wang et al.[13] for children and Hankinson et al.[14] for adults. The best $FEV_1\%$ of predicted value over a calendar year was considered for the study.[15] The average rate of annual decline in FEV_1 was calculated as the difference between the FEV_1 at the beginning and at the end of the study period for each subject, divided by the number of years in the study for each subject.[16]

Pancreatic and Nutritional Status

Pancreatic function was defined in all patients based on 3-day stool fat collection and/or fecal elastase assessment. Pancreatic insufficiency (PI) was defined as stool elastase less than 100 μg/g of stool or as a coefficient of fat absorption less than 93%. Body mass index (BMI) was calculated for all patients. To facilitate comparisons, BMI percentile was calculated for all patients below and for those above the age of 20, as previously described.[17] CF-related diabetes (CFRD) was defined according to the Cystic Fibrosis Foundation guidelines.[18]

Statistical Analysis

Descriptive analysis was used for all variables based on patients' *C. albicans* colonization status at the endpoint of the study, during the year 2009, and all data relate to this time point. Means and standard deviations and/or 95% confidence intervals are presented for continuous variables, and proportions for categorical data. Continuous variables were checked for normality, and nonparametric tests were applied for the data that was not normally distributed. Student's *t*-test or Mann–Whitney *U*-test was used to compare between two groups. Type 3 analysis of effects using linear or logistic regression models was used to compare variables between the three colonization groups, as appropriate. Multiple logistic regression

analysis was built to predict colonization with *C. albicans* from clinically relevant factors. All analyses were performed with SAS software (v9.3, SAS Institute Inc., Cary, NC). Statistical significance was defined as $P < 0.05$ using two-tailed analysis. Data were presented as mean $\pm$ standard deviation unless stated otherwise.

Results

Patients' Characteristics

A total of 4,244 cultures were obtained from 91 patients that met the inclusion criteria and followed for 3.8 ± 2.4 years. Sputum was obtained in most of the patients during their monthly visit, more often in those with severe disease and during exacerbations and hospitalizations, and less often in those with a mild disease. The mean patients' age was 19.8 ± 11.8 years (range: 5.3–68), 54 (59%) were males, 63 (69%) had PI and 24 (26%) had CFRD. The mean FEV_1 was $82.3 \pm 25\%$ predicted (range: 20–132) (Table 1). A total of 26 patients (28%) were treated chronically with corticosteroids (inhaled in most patients) during the study period. About 34 patients (37%) met criteria for chronic colonization with *Candida*, 30 (33%) were intermittently colonized, and 27 (30%) were not colonized. The three groups did not differ in age, gender distribution, or nutritional status (Table 1). The most frequent chronic bacterial pathogens were *Staphylococcus aureus*, detected in 64 patients (70%) and *Pseudomonas aeruginosa*, found in 40 patients (44%). The most common chronic fungal microorganism was *C. albicans* in 34 patients (37%), followed by *A. fumigatus* in 15 (16.5%), *C. glabrata* in 12 (13%), and *C. parapsilosis* in 7 (7%) patients.

Effect of *C. albicans* Colonization on Clinical Outcomes

Pulmonary Function

Patients chronically colonized with *C. albicans* had significantly lower baseline FEV_1 and lower FEV_1 at the end of the study period with higher annual decline of FEV_1 compared to those non-colonized (Table 1 and Figs. 1 and 2). The patients who were intermittently colonized with *C. albicans* had intermediate values of FEV_1 and annual FEV_1 decline. Since patients with lower FEV_1, might have also higher annual decline rate irrespective of their colonization status, we analyzed the association between the baseline FEV_1 in all the studied patients, and the rate of FEV_1 decline during the study period. There was no statistically significant correlation between baseline FEV_1 and annual FEV_1 decline ($r = 0.07$, $P = 0.57$). We then analyzed our cohort according to *P. aeruginosa* colonization and non-colonization. Within the *P. aeruginosa* non-colonized group, the chronic *C. albicans* group had significantly

TABLE 1— Demographic and Clinical Data Based on *C. albicans* Colonization Status

	C. albicans colonization status			
	None (n = 27)	Intermittent (n = 30)	Chronic (n = 34)	*P*-Value[1]
Age, years (range)	19.9 ± 14.2 (6–68)	18.9 ± 10.7 (5.3–49)	20.3 ± 11 (6.4–64.8)	0.891
BMI %	52.3 ± 26.6	38.1 ± 30.3	45.1 ± 26.5	0.217
Male/female	17/10	16/14	21/13	0.713
FEV_1% predicted at enrollment (range)	97.2 ± 11.2 (80–118)	86 ± 23.4 (42–122)	80.2 ± 16.1 (42–106)	0.005
FEV_1% predicted at the end of the study (range)	93.9 ± 22.2 (27–132)	80.8 ± 26.3 (20–124)	74.3 ± 23.1 (29–106)	0.006
ΔFEV_1%/yr (range)	0.7 ± 4.5 (− 9.7–13)	− 1.5 ± 7.2 (− 29–10)	− 1.9 ± 4.2 (− 14.5–7)	0.03
CFRD, n (%)	2 (7)	6 (20)	16 (47)	0.001
PI, n (%)	12 (44)	22 (73)	29 (85)	0.001
Corticosteroid use, n (%)	4 (15)	7 (23)	15 (44)	0.01
Chronic *P. aeruginosa*, n (%)	5 (18)	17 (56)	18 (53)	0.006
Chronic *A. fumigatus*, n (%)	0 (0)	3 (10)	12 (35)	< 0.001
Aspergillus spp. colonization, n (%)	2 (7)	8 (27)	19 (56)	< 0.001

[1]Comparison of chronic versus non-chronic colonizers.
Data are presented as mean ± SD (range) or No. (%). BMI, body mass index; FEV_1, forced expiratory volume in 1 sec; CFRD, cystic fibrosis-related diabetes mellitus; PI, pancreatic insufficiency.

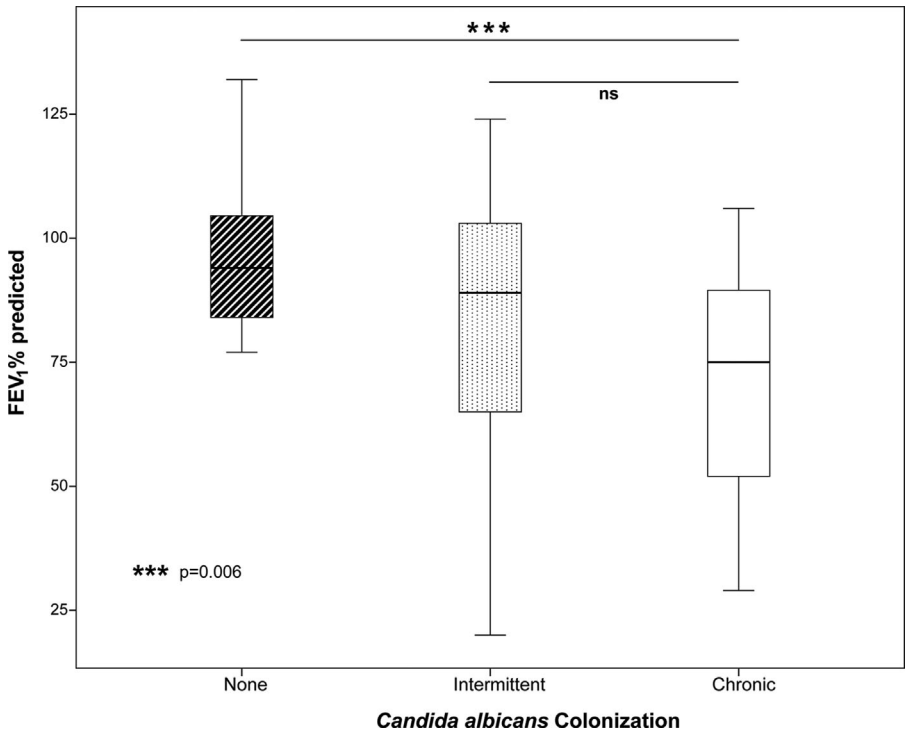

Fig. 1. Comparison of the FEV_1% predicted between the *C. albicans* colonization groups at the end of the study period.

lower FEV_1 as compared to *C. albicans* non-colonized group (83.4% ± 24.8% vs. 96.9% ± 19.3%, respectively, $P = 0.04$). In the *P. aeruginosa* colonized group, the same trend appeared, but it was not statistically significant, possibly due to the small sample number (65.4% ± 17.8% vs. 70.5% ± 24.8%, respectively, $P = 0.5$).

Predictors of Chronic *C. albicans* Colonization

In a univariate analysis, the presence of PI and CFRD significantly increased the risk for chronic *C. albicans* colonization: PI as compared to pancreatic sufficiency (PS) (OR = 3.9, CI_{95}: 1.3–11.6, $P = 0.01$), and a diagnosis of CFRD versus no CFRD (OR = 5.4, CI_{95}: 2–14.9, $P = 0.001$). Chronic corticosteroid use similarly increased the likelihood of chronic *C. albicans* colonization (OR = 2.7, CI_{95}: 1–6.9, $P = 0.03$). Although chronic colonization with *P. aeruginosa* was associated with higher rate of overall (chronic and intermittent) *C. albicans* colonization (OR = 5.3, CI_{95}: 1.7–15.7, $P = 0.003$), it did not convey a statistically significant risk for chronic *C. albican*s (OR = 1.8, CI_{95}: 0.7–4.2, $P = 0.18$). Moreover, chronic methicillin-sensitive *S. aureus* (MSSA) did not convey a statistically significant risk for chronic *C. albicans* (OR = 1.5, CI_{95}: − 1.4–0.3, $P = 0.21$). However, methicillin-resistant *S. aureus* (MRSA) colonization increased the risk for chronic *C. albicans* (OR = 6.5, CI_{95}: 1.6–26.3, $P = 0.008$). Finally, colonization with *Aspergillus* spp. (chronic + intermittent) was associated with a significant risk of chronic *C. albicans* (OR = 9.8, CI_{95}: 2.5–38.2 $P = 0.001$) (Table 2).

A multivariate logistic regression model was used to identify independent predictors of chronic *C. albicans* colonization. Variables entered into the regression model included age, gender, BMI, PI, CFRD, FEV_1, corticosteroid use, chronic *P. aeruginosa*, chronic MRSA colonization, and overall (chronic and intermittent) *Aspergillus* spp. colonization. Three parameters independently predicted chronic *C. albicans*: co-colonization with *Aspergillus* spp. (OR = 7.5, CI_{95}: 1.8–30.2, $P = 0.004$), FEV_1 below 60% (OR = 5.2, CI_{95}: 1.1–24.5, $P = 0.03$) and BMI below 20 (OR = 4.9, CI_{95}: 1.1–21, $P = 0.02$) (Table 3).

DISCUSSION

This study demonstrates that chronic respiratory colonization by *C. albicans* is associated with lower pulmonary function and a higher rate of annual decline in FEV_1. The strength of this study is that it was performed in a single CF center having standard treatment strategies with frequent routine visits and with sputum cultures analysis in almost each clinic visit. The prospectively acquired data from a long-term follow-up period increases the validity of the findings. It is one of the very few published large cohort studies that assess the

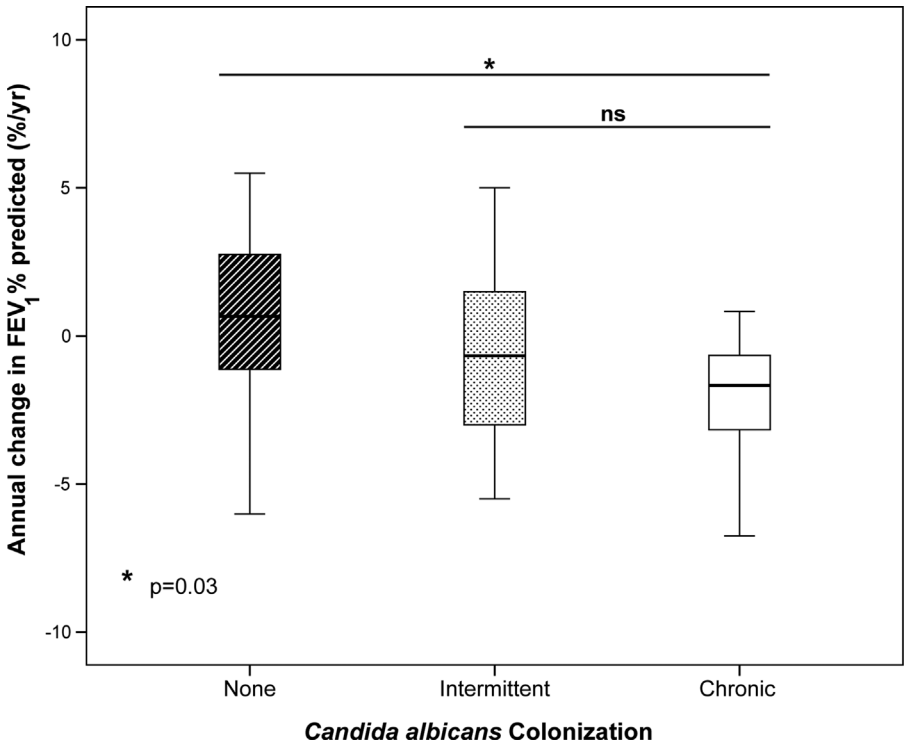

Fig. 2. Comparison of the annual change in FEV_1% between the *C. albicans* colonization groups.

prevalence and persistence of *C. albicans* colonization in patients with CF and its association with disease severity.

Historically, *C. albicans* is considered a colonizer rather than a pathogen. Accordingly, several small studies with non-stringent criteria for chronicity did not show effect of *C. albicans* colonization on pulmonary function.[4,5,19] Conversely, in a large cross-sectional study of 7,010 CF patients from the European Epidemiologic Registry of CF, found that *Candida* spp. colonization was associated with a moderately lower values of FEV_1 (5–10%),[20] although this association was not examined longitudinally. To the best of our knowledge, there is only one other systematic longitudinal study from Ireland examining *C. albicans* influence on PFTs in patients with CF.[10] Defining chronic colonization similarly to our current work, Chotirmall and colleagues followed 89 patients over 11 years and found that *C. albicans* colonization was associated with a more rapid decline in FEV_1 and a higher exacerbation rate. The current retrospective analysis found similarly increased decline of PFTs. Since our study is retrospective in nature, possible explanation to such finding could be due to lower FEV_1 at baseline and not due to the chronic *C. albicans* colonization. Lack of correlation between the initial FEV_1 and the annual rate of decline, argues against such explanation. Furthermore, in the longitudinal CFF Epidemiological Study of Cystic Fibrosis of young adults with CF followed for 5.5 years,[21] slower decline rate of FEV_1 was noted in patients with lower baseline FEV_1.

Rates of *C. albicans* colonization may differ between studies due to varying definitions of colonization, or differences in patient age, culture protocols, or laboratory

TABLE 2— Risk Factors for Chronic *C. albicans* Colonization (Univariate)

	OR (95%CI)	*P*-Value
Age	0.85 (0.3–2)	0.72
Gender	0.85 (0.3–2)	0.71
BMI < 20	0.61 (0.2–1.4)	0.27
$FEV_1 < 60\%$	3.3 (1.1–9.9)	0.03
CFRD	5.4 (2–14.9)	0.001
PI	3.9 (1.3–11.6)	0.01
Corticosteroids	2.7 (1–6.9)	0.03
P. aeruginosa	1.8 (0.7–4.2)	0.18
Aspergillus spp.	5.9 (2.2–15.6)	0.0003
MRSA	6.5 (1.6–26.3)	0.008

BMI, body mass index; FEV_1, forced expiratory volume in 1 sec; CFRD, cystic fibrosis-related diabetes mellitus; PI, pancreatic insufficiency; MRSA, methicillin-resistant *Staphylococcus aureus*.

TABLE 3—Independent Predictors of Chronic *C. albicans* Colonization (Multivariate)

	OR (95%CI)	*P*-Value
Aspergillus spp.	7.5 (1.8–30.2)	0.004
$FEV_1 < 60\%$	5.2 (1.1–24.5)	0.03
BMI < 20	4.9 (1.1–21)	0.02

Variables entered into the model: age, gender, BMI < 20, $FEV_1 < 60\%$, pancreatic insufficiency, CF-related diabetes, corticosteroid use, chronic *P. aeruginosa*, chronic MRSA, overall (chronic + intermittent) *Aspregillus* spp. colonization. BMI, body mass index; FEV_1, forced expiratory volume in 1 sec.

techniques. We defined chronic *C. albicans* colonization using stringent criteria similar to the Leeds criteria defining chronic *P. aeruginosa* colonization,[9] with the goal of differentiating between patients with incidental isolation versus chronic or intermittent colonization. The rate of *C. albicans* colonization in our study was 70%, with 37% of the study population meeting the definition for chronic colonization. The use of multiple antibiotics is common in patients with CF[22] and higher rates of colonization with *C. albicans* have been reported in patients heavily treated with anti-pseudomonal antibiotics.[10] Similarly, higher rates of *C. albicans* colonization in nasopharynx and stool were reported in healthy subjects and CF patients with a history of previous antibiotic treatment.[23] We were unable to examine the relationship between the use of antibiotics and the rate of *C. albicans* colonization, but it is plausible to assume that such relationship exists in our study population as well, since patients with lower FEV_1 at our center are treated more intensively with antibiotics. In the current study, patients with chronic *P. aeruginosa* colonization, as compared to non-colonized, had significantly lower FEV_1 ($68.3\% \pm 21.8\%$ vs. $92.6 \pm 21.9\%$, $P < 0.001$), however, the adverse effect of chronic *C. albicans* on FEV_1 remained significant after adjusting for *P. aeruginosa* infection. Interestingly, only in the *P. aeruginosa* non-colonized group, the detrimental effect of chronic *C. albicans* on PFTs was statistically significant. The association we describe between *C. albicans* colonization and chronic *P. aeruginosa* corroborates similar findings from the Irish longitudinal study,[10] although a cross-sectional study from Turkey did not find such relationship.[24] Several in vitro studies investigating the interaction between *P. aeruginosa* and *C. albicans* provide some insight into a possible mechanistic link. The two species co-exist in many clinical settings of impaired host immunity and there is a complex interplay between them. It has been shown that *P. aeruginosa* promotes morphogenic change in *C. albicans* and the production of its virulence factors, but also inhibits *C. albicans* biofilm formation.[25,26] On the other hand, in the presence of *C. albicans* there is an up regulation of *P. aeruginosa* virulence factors such as exotoxin A, as well as massive induction of iron-acquisition systems.[27]

The strong independent correlation between *Aspergillus* spp. and chronic *C. albicans* has been previously described.[10] *Aspergillus fumigatus* is the most common filamentous fungus involved in CF lung disease, with reported prevalence rates ranging from 6% to nearly 45%.[28,29] The most documented clinical effects of *Aspergillus* in patients with CF are ABPA[30] and *Aspergillus* bronchitis.[31] However, apart from these two entities, it remains unclear whether the presence of *A. fumigatus* or other *Aspergillus* species in CF sputum simply reflects colonization or whether it is driving deleterious respiratory pathology.[4,10,32,33] In a recent clinical trial from Canada, prolonged treatment with itraconazole of patients with CF chronically infected with *A. fumigatus* did not alter the rate of pulmonary exacerbations or the degree of FEV_1 decline.[34] However, this study was underpowered and itraconazole levels were sub-therapeutic, thus the true beneficial effect of antifungal therapy might have been undetected. In another study from Ireland,[35] *A. fumigatus* eradication by itraconazole resulted in improvement in markers of Th2 inflammation measured in bronchoalveolar lavage fluid, as well as improvement in radiological parameters, suggesting benefits to antifungal treatment beyond the traditional PFT based outcomes. Interestingly, *Aspergillus* was one of the strongest predictors of chronic *C. albicans*. To the best of our knowledge, no study examined the biology of combined colonization in the context of lung inflammation. On the other hand, potentially similar mechanisms of pathogen-host interaction, such as activation of innate immunity through C-lectin (Dectin-1), and Toll-like receptors recognizing fungal components,[6] might be augmented when the two fungal species co-exist.

Does the association of *C. albica*ns colonization with worse PFTs we describe is driven by *Candida* itself? Our study does confirm that *C. albicans* colonization is associated with worsening pulmonary status in cystic fibrosis patients; however, it is possible that *C. albicans* colonization rather may just be a surrogate marker of patients requiring frequent antibiotics. Whether *C. albicans* colonization is the driving force or just a surrogate marker of disease severity is difficult to establish due to the retrospective nature of our study. However, there's biological plausibility to hypothesize that *C. albicans* might lead to increased inflammatory process in CF airways, a well described modulator of pulmonary function in CF.[36] Supporting evidence can be inferred from several lines of research in other disease models and from in vitro studies: For example, *C. albicans* cell wall components induce production of pro-

inflammatory cytokines, such as TNF-alpha, in mononuclear cells. In addition, *C. albicans* is able to suppress phagocytosis and to evade the host immune response,[37] in addition to formation of drug-resistant biofilm.[38] In line with these observations in vitro, several studies in ventilator-associated pneumonia (VAP) patients admitted to ICU, found increased levels of systemic inflammatory markers, greater mortality and increased incidence of multi-drug resistant bacteria in patients with *Candida* airway colonization.[39,40]

In our cohort, independent risk factors for chronic *C. albicans* infection were *Aspergillus* colonization, poor nutritional status and lower PFTs. In the study of Chotirmall et al.,[10] among 89 patients, independent risk factors for *C. albicans* colonization were pancreatic insufficiency, Pseudomonas colonization and osteopenia. A possible explanation for the differences between the two studies could be related to different cohort characteristics (such as older age and worse PFTs), although all the predictors are associated with more severe clinical phenotype, thus re-confirming the association of chronic *C. albicans* with advanced disease.

This work has several limitations that should be acknowledged: First, as mentioned, this is a retrospective study, thus the question of the timing of *C. albicans* acquisition in respect to clinical deterioration of lung function remains unanswered. Secondly, patients' records did not include complete data on the amount of antibiotic use, which could potentially mediate the association between *C. albicans* chronic colonization, and the lower FEV_1 seen in this study. Nonetheless, since this is a single center study with a uniform treatment protocol, it is reasonable to assume that patients with chronic *P. aeruginosa* received similar amount of antibiotics. This idea is supported by the fact that *P. aeruginosa* did not predict *C. albicans* chronic colonization in the multivariate analysis. Moreover, the effect of *C. albicans* on PFTs remained significant within *P. aeruginosa* non-colonized group. Thirdly, there's a possibility that *C. albicans* isolates originated from the oropharynx, and thus do not reflect a true lower airway colonization. Another possible limitation is the association of chronic *C. albicans* colonization with several risk factors, like corticosteroids treatment, in the univariate analysis. Although any of them could potentially moderate the association of *C. albicans* with PFT deterioration, most were not predictive of chronic colonization in the multivariate analysis. Finally, there was a significant correlation between *A. fumigatus* and chronic *C. albicans*, which might indicate that acquisition of *A. fumigatus* is in fact responsible for the deleterious effect we describe in the chronic *C. albicans* group.

In conclusion, the current study demonstrated an association of chronic *C. albicans* airway colonization with lower FEV_1, and a more rapid decline of FEV_1 in patients with CF. Despite the biological plausibility of causal relationship between the two observations, we are unable to determine causality. Further prospective longitudinal studies are needed to define the time-course of *C. albicans* acquisition, serially collect clinical and laboratory markers associated with it (including examination of airway inflammation status), and determine the effect of this colonization on disease severity.

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