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The presence of *Aspergillus fumigatus* is associated with worse respiratory quality of life in cystic fibrosis

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ABSTRACT

Background: The clinical effects of Aspergillus fumigatus in the cystic fibrosis (CF) airway, with the exception of allergic bronchopulmonary aspergillosis, is unclear.
Methods: CF adolescents and adults (age 14 years and older) underwent bacterial and semi-selective fungal culture testing to determine the prevalence of fungi in the CF respiratory tract and the independent association between the presence of Aspergillus fumigatus and clinical characteristics.
Results: Aspergillus fumigatus (10.3%) and Candida species (57.8%) were the most common filamentous fungi and yeast seen respectively in the sputa of 206 individuals with CF. Inhaled corticosteroid (ICS) use was more common in Aspergillus fumigatus-positive than Aspergillus fumigatusnegative (100% versus 75.8%, $p = .01$). Aspergillus fumigatus was significantly associated with lower respiratory domain score (β −8.74, 95% CI −16.6, −0.88, $p = .03$), representing worse respiratory-related quality of life, accounting for demographics, disease characteristics, and the presence of a pulmonary exacerbation.
Conclusion: The presence of Aspergillus fumigatus in CF sputum was associated with worse respiratory quality of life in CF in a crosssectional, single center study. Longitudinal analysis examining the clinical implications of Aspergillus fumigatus on respiratory health over time is needed.

1. Background

Cystic fibrosis (CF), an autosomal recessive life-limiting genetic disease, is classically characterized by chronic airway infections that lead to progressive respiratory disease and ultimately respiratory failure [1]. The epidemiology of pathogens found in the CF airway has historically focused on the bacterial inhabitants; however, *Aspergillus fumigatus* (*Af*), the most common filamentous fungi in CF patients, is becoming more frequently isolated in sputum cultures [2,3]. Yet, the clinical significance of *Af* in the CF host in the absence of allergic bronchopulmonary aspergillosis (ABPA) remains unclear.

Previous studies have suggested that *Af* colonization or persistence in the CF airways is associated with increased pulmonary exacerbations requiring hospitalization, mosaic attenuation and bronchiectasis on chest computed tomography, and failure of clinical improvement of pulmonary exacerbations despite intravenous (IV) antibiotics [4–7]. Other studies have shown no association between *Af* and lung function decline [8,9]. The interpretation of these data is limited by retrospective study design, small sample size, and ascertainment bias. We aimed to determine the relationship between *Af* detected in fungal culture and disease characteristics in individuals with CF. We hypothesized the presence of *Af* in CF sputum was associated with lower respiratory-related quality of life (QOL). In addition, description of other clinically reported fungi in CF, including *Scedosporium boydii* complex, other *Aspergillus* species, *Trichosporon mycotoxinivorans*, and *Exophiala dermatidis* were conducted. Preliminary data for this study were presented previously in abstract form [10].

Abbreviations: CF, cystic fibrosis; *Af*, *Aspergillus fumigatus*; ABPA, allergic bronchopulmonary aspergillosis; FEV_1, forced expiratory volume in one second; IV, intravenous; *Pa*, *Pseudomonas aeruginosa*; MRSA, methicillin-resistant *Staphylococcus aureus*; BMI, body mass index; CI, confidence intervals.

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2. Methods

2.1. Study design and participants

We conducted a cross-sectional study of adolescents and adults with CF (age 14 years and greater) receiving clinical care at the Children's Hospital of Philadelphia (CHOP) and Hospital of University of Pennsylvania (HUP) from March 2017 through October 2018. We included subjects with a diagnosis of CF according to the CF Foundation diagnosis guidelines [11] and excluded patients who had received solid organ transplantation. The study was approved by the University of Pennsylvania and CHOP institutional review boards (825979).

2.2. Study procedures

All participants (or their parents) provided written informed consent (or assent, when age appropriate). Subjects underwent routine clinical evaluation by a CF physician, performed spirometry, and underwent bacterial culture evaluation of sputum. If subjects were unable to expectorate, sputum induction with 3% hypertonic sodium chloride was performed. Spirometry was performed according to the American Thoracic Society guidelines; forced expiratory volume in 1 s (FEV_1) percent predicted reported according to the National Health and Nutrition Examination Survey III prediction equations. The Cystic Fibrosis Questionnaire-Revised (CFQ-R), a validated CF-specific health-related QOL survey, was administered [12]. Demographics, comorbidities, medications, past spirometry, microbiological data, and antibiotic usage for pulmonary exacerbation (PEx) episodes over the 12 months previous to the visit were collected.

2.3. Laboratory processing

The sputum samples underwent bacterial culture and semi-selective fungal culture evaluation within 8 h of collection in the HUP clinical microbiology laboratory using a research protocol. Whole sputum was directly inoculated on MacConkey agar, sheep blood agar, chocolate agar, *Burkholderia cepacia* selective agar (BCSA), and mannitol salt agar and incubated at 35 degrees Celsius for 3 days (with the exception of BCSA incubation for 5 days). In addition, inhibitory mold agar, brain-heart infusion agar, Mycosel, and CHROMagar (Becton, Dickinson, Sparks, Maryland) were incubated at 30 degrees Celsius for two weeks for the culture of fungi. Morphologic identification of macroscopic and microscopic features of fungal isolates and if necessary, DNA sequencing of the D1/D2 region of the 28S ribosomal subunit were performed when morphologic identification alone was insufficient. Assessments of the cultures and DNA sequencing were performed by individuals blinded to all clinical information.

2.4. Dependent and independent variables

The primary independent variable of interest was *Af* isolation on culture. The primary dependent variable was the CFQ-R respiratory domain score, which ranges from 0 to 100 with a higher score reflecting better respiratory-related QOL (minimal clinically important difference of 4 points) [13]. Other dependent variables included FEV_1 percent predicted and diagnosis of PEx (defined as clinician diagnosis of PEx at time of study visit). Covariates of interest included age, female sex, white race, F508del cystic fibrosis transmembrane conductance regulator (CFTR) homozygosity, pancreatic insufficiency, body mass index (BMI), CF related diabetes mellitus (CFRD), allergic bronchopulmonary aspergillosis (ABPA), number of PEx (oral and/or IV antibiotic use) in previous 12 months, and number of severe PEx (IV antibiotic use) in previous 12 months. BMI was calculated based in weight and heigh for absolute value in kg/m^2. Pancreatic insufficiency, CFRD, and ABPA status were defined by clinician diagnosis in the medical chart. Additional covariates included microbiological data: presence of *Pseudomonas aeruginosa* (*Pa*), methicillin-resistant *Staphylococcus aureus* (MRSA), and *Burkholderia cepacia* complex at the study visit. In additional secondary analyses, the following independent variables were explored: *Scedosporium boydii* complex, non-*fumigatus Aspergillus* species, *Trichosporon mycotoxinivorans*, *Exophiala dermatidis, Candida* species, and clinically important fungi defined as isolation of *Af*, *Scedosporium boydii* complex, non-*fumigatus Aspergillus* species, *Trichosporon mycotoxinivorans*, or *Exophiala dermatidis.*

Prevalence was determined by fungal isolation and identification on culture. Recurrent *Af* was defined as detection of *Af* at the study assessment with a history of positive *Af* cultures in the previous two years.

2.5. Statistical analysis

Continuous and categorical clinical variables were compared between subjects with and without *Af* status by Wilcoxon rank sum test and Fisher's exact test respectively. Variables were also compared between *Af*-positive, *Af*-negative, and non-expectorators by Kruskal-Wallis analysis of variance and Fisher's exact test. Simple linear regression was performed to explore the unadjusted associations between independent variables and outcomes, CFQ-R respiratory domain score and FEV_1 percent predicted. Univariate logistic regression was performed to assess association between *Af*-positive status and PEx diagnosis. We assessed the independent association between *Af* and CFQ-R respiratory domain score using multivariable linear regression with adjustment for confounders. Model selection of covariates was based on existing medical knowledge and/or univariate analyses with $p < .10$. Multiple imputation with chained equations was performed to account for missing data. Additional sensitivity analyses were performed, [1] complete case analysis, [2] imputation of *Af*-status in subjects without sputum (non-expectorators), [3] accounting for ABPA, [4] defining recurrent *Af* as the primary exposure, and [5] exploring relationships between other clinically relevant fungi (*Scedosporium* species, other *Aspergillus* species, *Trichosporon mycotoxinivorans*, *Exophiala dermatidis*) individually and collectively. *P* values are 2-sided, with alpha of 0.05. Statistical analyses were performed using STATA 15 (StataCorp LP, College Station, TX).

3. Results

3.1. Subject characteristics

A total of 206 participants were enrolled in the study. Twenty-four (11.7%) subjects were unable to expectorate sputum or undergo sputum induction. *Af* was detected in 21 (10.3%, 95% CI 6.4,15.2%) individuals (Fig. 1). Characteristics of the cohort stratified by *Af*-status are shown in Table 1. Inhaled corticosteroid use was more commonly seen in participants with *Af*-positive culture. Lower respiratory domain score was seen in the *Af*-positive

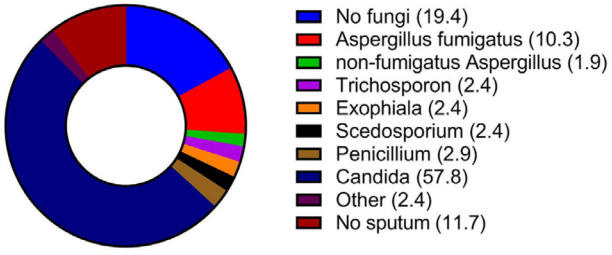

Fig. 1. Prevalence of fungi ($n = 206$).

Table 1
Cohort characteristics ($n = 182$).

	No *Aspergillus fumigatus* ($n = 161$)[a]	*Aspergillus fumigatus* ($n = 21$)[a]	*p*-value
Demographics			
Age	30.9 ± 12.1	30.1 ± 13.0	0.75
Female sex	76 (47.2)	11 (52.4)	0.82
White race (versus non-white)	150 (93.2)	20 (95.2)	1.00
Disease characteristics			
F508del homozygous	72 (44.7)	8 (38.1)	0.65
Pancreatic insufficiency	141 (87.6)	20 (95.2)	0.48
BMI (kg/m^2)	22.6 ± 3.67	22.6 ± 4.45	0.62
FEV_1 percent predicted (%)	67.8 ± 25.2	62.4 ± 28.3	0.41
CFQ-R respiratory quality-of-life (points)	60.9 ± 19.6	49.2 ± 17.8	0.02
Comorbidities			
CF related diabetes	50 (31.1)	6 (28.6)	1.00
ABPA	11 (6.8)	4 (19.0)	0.08
Medications			
CFTR modulators	56 (34.8)	8 (38.1)	0.81
Inhaled antibiotics	97 (60.3)	14 (66.7)	0.64
Azithromycin	83 (51.6)	11 (52.4)	1.00
Chronic oral antibiotics	61 (37.9)	10 (47.6)	0.48
Inhaled corticosteroids	122 (75.8)	21 (100.0)	0.01
Prednisone	28 (17.4)	7 (33.3)	0.14
Antifungal	13 (8.1)	4 (19.1)	0.11
Oral and IV antibiotics in previous 12 months (number)	2.37 ± 2.03	2.10 ± 2.10	0.52
IV antibiotics in previous 12 months (number)	0.86 ± 1.24	1.05 ± 1.63	0.67
Visit microbiology			
Pseudomonas aeruginosa	96 (59.6)	13 (61.9)	1.00
MRSA	25 (15.5)	3 (14.3)	1.00
Burkholderia cepacia complex	10 (6.2)	0	0.61
Microbiology history in previous 24 months			
Pseudomonas aeruginosa	117 (72.7)	17 (81.0)	0.11
MRSA	34 (21.1)	8 (38.1)	0.17
Burkholderia cepacia complex	9 (5.6)	0	0.57

Abbreviations: BMI = body mass index, kg/m^2 = kilogram divided by meter squared, CF = cystic fibrosis, ABPA = allergic bronchopulmonary aspergillosis, FEV_1 = forced expiratory volume in one second, CFQ-R = Cystic fibrosis questionnaire-revised, CFTR = cystic fibrosis transmembrane conductance regulator, IV = intravenous, MRSA = methicillin-resistant *Staphylococcus aureus*.

[a] Continuous variables are described in mean ± standard deviation and categorical variables are described in number (percentage).

group compared to *Af*-negative. Age, sex, white race, F508del homozygosity, pancreatic insufficiency, BMI, FEV_1 percent predicted, CFRD, and other CF-related medications did not differ between *Af*-positive and *Af*-negative groups. Antifungal therapy, chronic prednisone use (greater than one month of continuous use), and history of *Pa* were more commonly trended in *Af*-positive group, but were not statistically significantly different. Supplemental Table 1 characterizes the full cohort, including individuals unable to expectorate sputum. Non-expectorators ($n = 24$) represented an older and potentially healthier group (pancreatic sufficient and lower *Pa* prevalence).

3.2. Prevalence

Thirty-seven individuals (18.0%, 95% CI 13.0, 23.9%) of the study population had a history of Af in the two-year period before enrollment. Yet, recurrent *Af* was seen in only 11 subjects (5.3%, 95% CI 2.70, 9.35%). Fig. 1 shows prevalence of all fungi recovered in the cohort. *Candida* species (57.8%) was the most common fungus detected in the study population. We found additional clinically relevant fungi, *Scedosporium boydii* complex (2.4%), *Trichosporon mycotoxinivorans* (2.4%), non-fumigatus *Aspergillus* species (1.9%), and *Exophiala dermatidis* (2.4%). We detected 22 new cases of clinically relevant fungi using semi-selective fungal culture media in 20 individual subjects without history of fungal isolation (Fig. 2). We identified four subjects with co-infection: two subjects with *Af* and *Exophiala dermatitidis*, *Af* and non-*fumigatus Aspergillus*, and *Af* and *Scedosporium boydii* complex.

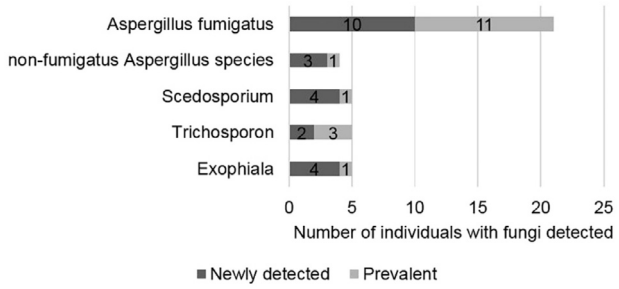

Fig. 2. Newly detected and prevalent fungal diagnoses.

3.3. Af status and health-related quality of life

Associations between clinical characteristics and CFQ-R respiratory domain score are depicted in Table 2 (unadjusted). Table 3 represents the imputed multivariable model of participants who produced sputum for evaluation on the day of the assessment. *Af* culture positivity was associated with a respiratory domain score 11.7 points lower in the univariate analysis, and 8.43 points lower than individuals without *Af* after accounting for age, sex, lung function, pancreatic insufficiency, *Pa* status, and a PEx diagnosis at the time of specimen collection (β −8.43, 95% CI −16.3, −0.53, $p = .04$). We did not find interactions between *Af* and *Pa*-positive culture at visit or history of *Pa* (defined as any present or previous isolation of *Pa* on culture).

3.4. Af status and other clinical outcomes

PEx was diagnosed in 91 (44.2%) of patients at the study visit. *Af* status was not associated with the clinical diagnosis of a PEx

Table 2
Unadjusted association between patient characteristics and CFQ-R respiratory domain score ($n = 205$).[a]

	β	95% CI	p-value
Age	−0.20	−0.43, 0.03	0.08
Female sex	−6.85	−12.3, −1.37	0.02
Pancreatic insufficiency	−10.5	−18.3, −2.81	0.01
BMI (kg/m^2)	0.60	−0.11, 1.32	0.10
CF related diabetes	−2.67	−8.61, 3.27	0.38
ABPA	−3.48	−14.1, 7.19	0.52
Mean FEV_1 percent predicted in the past 12 months (10% intervals)	2.25	1.19, 3.32	<0.001
FEV_1 percent predicted at visit (10% intervals) ($n = 204$)	2.54	1.52, 3.56	<0.001
Pulmonary exacerbation diagnosis	−15.4	−20.5, −10.2	<0.001
Pseudomonas aeruginosa ($n = 181$[b])	−5.75	−11.6, 0.11	0.05
MRSA (n = 181[b])	0.95	−7.14, 9.05	0.82
Burkholderia cepacia complex (n = 181[b])	7.71	−5.16, 20.6	0.24
Aspergillus fumigatus (n = 181[b])	−11.7	−20.6, −2.85	0.01

Abbreviations: CFQ-R = Cystic Fibrosis Questionnaire-revised, BMI = body mass index, CF = cystic fibrosis, ABPA = allergic bronchopulmonary aspergillosis, FEV1 = forced expiratory volume in 1 s, MRSA = methicillin-resistant *Staphylococcus aureus*.

[a] One subject did not complete CFQ-R.

[b] 24 subjects did not expectorate sputum and did not have sputum culture performed at visit.

Table 3
Association between *Aspergillus fumigatus* isolation and CFQ-R respiratory domain score in subjects with sputum culture, adjusted for covariates[a] with multiple imputation with chained equations ($n = 182$).

	β	95% CI	p-value
Age	−0.23	−0.49, 0.03	0.08
Female sex	−3.48	−8.65, 1.69	0.19
Pancreatic insufficiency	−8.15	−17.1, 0.77	0.07
BMI (kg/m^2)	0.22	−0.53, 0.96	0.56
FEV_1 percent predicted at visit (10% intervals)	1.60	0.42, 2.78	0.01
Pulmonary exacerbation diagnosis at visit	−12.6	−17.9, −7.33	<0.001
Pseudomonas aeruginosa	−0.50	−5.96, 4.95	0.86
Aspergillus fumigatus	−8.43	−16.3, −0.53	0.04

Abbreviations: CFQ-R = Cystic Fibrosis Questionnaire-revised, BMI = body mass index, FEV1 = forced expiratory volume in 1 s.
[a] Adjusted for age, female sex, pancreatic insufficiency, BMI, FEV1 percent predicted, presence of pulmonary exacerbation, and Pseudomonas aeruginosa positive culture.

(odds ratio 1.82, 95% CI 0.73, 4.57 $p = .20$). *Af* status was not associated with FEV_1 percent predicted at visit (β −5.35 95% CI −17.1, 6.36, $p = .37$) or change in FEV1 percent predicted from average lung function over 12 months previous to visit (β −2.00, 95% CI −5.27, 1.28, $p = .23$).

3.5. Sensitivity analyses

Several sensitivity analyses were conducted to evaluate the rigor of our findings. We found a similar relationship between *Af* and poor respiratory quality of life (β −8.47, 95% CI −16.4, 0.53, $p = .04$) in the complete case analysis (Supplemental Table 2). We then performed multiple imputation with chained equations to all subjects (expectorators and non-expectorators), which exhibited consistent results (Supplemental Table 3). We also forced in ABPA into our final multivariable model described in the primary analysis; *Af* was still associated with lower respiratory domain score, β −8.06, 95% CI −16.1, −0.05, $p = .049$ (Supplemental Table 4). With excluding the 15 subjects with ABPA, the effect estimate of the association between *Af* and respiratory QOL remained unchanged with borderline statistical significance (−8.17, 95% CI −17.0, 0.65, $p = .07$). Recurrent *Af* was associated with lower respiratory domain score, β −12.9, 95% CI −24.9, −0.90 $p = .04$ in univariate analysis; however, when adjusting for covariates, the association was attenuated and was no longer statistically significant (β −9.07, 95% CI −19.8, 1.61, $p = .10$). Other fungi, including *Scedosporium boydii* complex, non-*fumigatus Aspergillus* species, *Trichosporon mycotoxinivorans*, *Exophiala dermatidis,* and *Candida* species were not associated with lower patient reported respiratory QOL (data not shown).

4. Discussion

We found that *Af* was significantly associated with worse patient-reported respiratory-related QOL in a cross-sectional study of adolescents and adults receiving care in a large CFF-accredited center and semi-selective fungal culture evaluation. Subjects with *Af* had a clinically meaningful difference of −8.43 points in respiratory domain score compared to *Af*-negative subjects. A trend between recurrent *Af* and worse respiratory QOL was seen; though, the classification of recurrent *Af* should be cautiously interpreted, as subjects' historical sputum cultures may not have used selective fungal culture media. The presence of *Af* has been postulated to be a marker of disease severity and antibiotic exposure in CF; however, clinical markers of more advanced CF, such as *Pa* colonization, BMI, and FEV_1 percent predicted did not differ between the *Af*-positive and *Af*-negative groups. Unlike previous studies [14–17], chronic inhaled and oral antibiotics were not associated with the presence of *Af*. Whereas, subjects using inhaled corticosteroid use were more likely to recover *Af* in sputum; a relationship that has been previously reported [14,18,19].

Changes in CF respiratory-related health status over time are highly correlated with significant changes in the CFQ-R respiratory domain score and have exhibited high discrimination of "stable" and "sick" clinical states [13,20–22]. Although our analysis is cross-sectional and cannot inform causality or changes in health over time, these data suggest that *Af* isolation may be associated with a perception of overall poorer respiratory health independent of other indicators of illness severity. Furthermore, *Af* may have significant respiratory impact on patients in the setting of stable lung function and absence of an exacerbation. Increased inflammatory responses (neutrophil elastase and IL-8) have been implicated in children with *Aspergillus* positive bronchoalveolar lavage cultures, suggesting a biological mechanism for the observed relationship [23]. Yet, the question remains; is *Af* contributing to poor respiratory health or a consequence of poor respiratory health? Longitudinal analyses of these issues are needed and may yield further insights.

To our knowledge, our study is the first investigation of the relationship between *Af* and patient-reported outcomes in a general CF population. Lung function decline, pulmonary exacerbations, and radiographic findings of lung disease have been the outcomes of previous studies examining the clinical effects of *Aspergillus* species [4,24–29]. Aaron et al. conducted a randomized, placebo-controlled trial of itraconazole for chronic *Af* in CF patients which did not find a difference in change in respiratory domain score of CFQ-R and other clinical endpoints over 24-week treatment period. Subtherapeutic itraconazole drug levels in 43% of patients in the treatment arm of the study and small sample size limit the interpretation of these data [30]. In contrast, Coughlan et al. demonstrated an improvement of respiratory symptoms (CFQ-R) after six-weeks of itraconazole therapy in an observational study of 13 *Af*-colonized CF patients; however, therapeutic drug monitoring was not described in these data and this study was not randomized or controlled [7].

ABPA has a distinct Th-2 mediated pathophysiology and associated with accelerated lung function decline in CF [31,32]. While we did not exclude ABPA in the primary analysis, sensitivity analysis adjusting for ABPA in the multivariable model were performed and did not alter our findings. With omitting ABPA from the study sample, the relationship between *Af* isolation and lower QOL remained with borderline statistical significance (β −8.17, 95% CI -17.0, 0.65, $p = .07$), most likely due to sample size reduction.

There was an unexpectedly high proportion of PEx diagnosed at the time of the research study visit. Individuals were recruited in the outpatient clinic, regardless of a routine follow-up visit versus sick visit. A plausible explanation for this finding could be that an individual would be more likely to have a clinical encounter when sick. The median number of antibiotic courses (oral or IV) for a diagnosis of PEx in the previous 12 months was 2 (interquartile range [IQR] 1,3) and IV antibiotic courses were lower with median 0 (IQR 0,1), which is reflective of the general CF population in the U.S. [33] PEx was significantly associated with lower respiratory QOL, β −15.4 points, 95% CI -20.5, −10.2. Therefore, we adjusted PEx status in our final model and the relationship between *Af* and worse respiratory symptom score remained.

We investigated the interaction between *Af* and *Pa* given the evidence of *Pa* inhibition of *Af* growth and mutual antagonism of *Af* towards *Pa* in vitro [34–38]. Interestingly, epidemiologic data suggest *Pa* are associated with higher odds for concurrent and subsequent *Aspergillus* infection [39]. We also saw a trend for history of *Pa* colonization to observed more in the *Af*-group compared to *Af*-negative group (Table 1). The presence of *Pa* colonization may reflect more diseased airways environment for inhaled *Af*

conidia to germinate. Also, in-vivo microbial interactions between *Pa* and *Af* could be contributing; although this is not well-explained by the known in-vitro interactions. In addition, worse clinical outcomes in children and adults with CF, including lower lung function and greater PEx episodes, are seen in co-infection of *Pa* and *Af* [4,40]. There was no statistical interaction between *Af* and *Pa* on respiratory-related QOL in our data.

Our study has several limitations. The cross-sectional design of the study precludes any inference of directionality in the relationship discovered between *Af* and respiratory QOL. Therefore, *Af* may represent a bystander of advanced CF lung disease rather than a factor in the causal pathway of disease progression. Longitudinal studies could shed light on this question. We were also unable to classify *Af* status as chronic or persistent, previously described as two or more *Af*-positive culture in one year, due to the study design [4,5,14,16,30]. Chronic or persistent *Af* may be more clinically relevant compared to transient isolation [4]. Our study attempted to overcome this by including previous microbiological culture data. We found a trend for the association of recurrent *Af* with decreased respiratory QOL. Yet, the risk of misclassification of recurrent *Af* exists as selective fungal culture procedures were not conducted for all historical sputum cultures. Based on our previous work, our study protocol inclusive of selective fungal culture media likely facilitates detection and recovery for fungi in CF sputa [41]. Despite this, culture-based testing exhibits some limitations in detecting the presence of *Af*, compared to culture-independent methods [24,42]. Our data also lacked the assessment of *Af*-specific immunoglobin (Ig) levels, including IgE and IgG, which prevented the differentiation of the previously described *Aspergillus* bronchitis and *Aspergillus* sensitization; however, the clinical impact of these phenotypes is poorly defined [24].

In conclusion, these data support the correlation of *Af* recovery on culture and poor respiratory health in CF, accounting for individual level factors and acute PEx. The study design prevents from inferring that *Af* directly causes worse respiratory health in people with CF, but these data suggest a relationship which warrants further investigation. Prospective longitudinal analyses examining the clinical implications of *Af* culture positivity on respiratory health over time is clearly needed.

Contributions

GH had access to the full data in the study and take responsibility for the integrity of the data and data analysis. GH, KA, SN, VF, CK, RCR, DJD, SMK, and DH contributed to the study design and conduct and writing of the manuscript. DH and SMK contributed to the data analysis and writing of the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcf.2019.08.008.

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