



Original Article

Risk factors for persistent *Aspergillus* respiratory isolation in cystic fibrosis

Gina Hong^{a,*}, Kevin J. Psoter^b, Mark T. Jennings^c, Christian A. Merlo^c, Michael P. Boyle^{c,d},
Denis Hadjiliadis^a, Steven M. Kawut^a, Noah Lechtzin^c

^a University of Pennsylvania Perelman School of Medicine, Division of Pulmonary, Allergy and Critical Care Medicine, Philadelphia, PA, United States

^b Johns Hopkins School of Medicine, Department of Pediatrics, Division of General Pediatrics and Adolescent Medicine, Baltimore, MD, United States

^c Johns Hopkins School of Medicine, Department of Medicine, Division of Pulmonary and Critical Care Medicine, Baltimore, MD, United States

^d Cystic Fibrosis Foundation, Bethesda, MD, United States

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Abstract

Background: *Aspergillus* species are increasingly detected in the respiratory tracts of individuals with cystic fibrosis (CF), and chronic *Aspergillus fumigatus* is associated with more frequent hospitalizations for pulmonary exacerbations. However, patient and clinical factors that may contribute to the acquisition of persistent *Aspergillus* infection have yet to be identified. The objective of this study was to identify risk factors for development of *Aspergillus* respiratory isolation in CF.

Methods: A retrospective cohort study of participants in the CF Foundation Patient Registry between 2006 and 2012 was conducted. Generalized estimating equation models were used to evaluate the association between the development of persistent *Aspergillus* respiratory isolation and individual level demographic and clinical characteristics.

Results: Among 16,095 individuals with CF followed from 2006 to 2012, 1541 (9.6%) subjects developed persistent *Aspergillus* isolation. White race (Odds Ratio [OR] 1.74, 95% confidence interval 1.23, 2.48, $p < 0.001$) and pancreatic insufficiency (OR 1.50, 95% CI 1.09, 2.06, $p < 0.001$) were found to be risk factors for persistent *Aspergillus* isolation. Chronic therapies, including inhaled antibiotics (OR 1.33; 95% CI 1.21, 1.46), macrolides (OR 1.23, 95% CI 1.14, 1.32, $p < 0.001$), and inhaled corticosteroids (OR 1.13, 95% CI 1.04, 1.20, $p < 0.001$) were also independently associated with an increased risk for persistent *Aspergillus* isolation.

Conclusions: We identified macrolides and inhaled antibiotics, which individually have been shown to improve CF outcomes, and inhaled corticosteroids as risk factors for developing persistent *Aspergillus* isolation. Further work is needed to determine whether these associations are causal or due to confounding by other factors.

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Keywords: *Aspergillus*; Fungus; Cystic fibrosis; Epidemiology; Inhaled antibiotics

1. Introduction

Cystic fibrosis (CF) is the most common life-shortening autosomal recessive disorder in Caucasians, affecting >30,000

individuals in the United States [1]. As a result of recurrent pulmonary infections, respiratory failure and death occurs [1]. Increased understanding of the epidemiology of microbial pathogens in the CF airways has contributed to improvements in treatment of infections. Traditionally, study has focused on the most common bacterial pathogens, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*. However, the prevalence of filamentous fungi in respiratory cultures of individuals with CF has significantly increased in the past two decades, with *Aspergillus fumigatus* (*A. fumigatus*) being the predominant species [2]. The prevalence of *Aspergillus* detected in the sputum of CF patients rose from approximately 6% in 1995 to

Abbreviations: CF, cystic fibrosis; CFF, cystic fibrosis foundation; ABPA, allergic bronchopulmonary aspergillosis; CFFPR, Cystic Fibrosis Foundation Patient Registry; GEE, generalized estimating equations; FEV1, forced expiratory volume in 1 s; IV, intravenous; *Pa*, *Pseudomonas aeruginosa*; MRSA, methicillin-resistant *Staphylococcus aureus*; BMI, body mass index; CI, confidence intervals.

* Corresponding author at: 3600 Spruce Street, Room 9046 Gates Building, Philadelphia, PA 19104, United States.

E-mail address: gina.hong@uphs.upenn.edu (G. Hong).

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12% in 2013 in the United States according to Cystic Fibrosis Foundation (CFF) national registry data and is reported to be as high as 36 to 57% in some centers [2–7]. Given this trend, understanding the role of *Aspergillus* in CF lung disease has become increasingly important.

There is increased evidence that *Aspergillus* is detrimental to the CF airway [8]. The presence of *Aspergillus* can perpetuate inflammation and lung injury in animal and in-vitro models [9,10]. *A. fumigatus* is associated with more rapid decline in lung function and increased mortality in cases of allergic bronchopulmonary aspergillosis (ABPA) and disseminated infection after lung transplant in CF patients respectively [11–16]. In addition, *Aspergillus* bronchitis and sensitization have been described, yet classification is still ill-defined [17–19]. In the absence of ABPA, chronic *A. fumigatus* respiratory infection or *Aspergillus* bronchitis is associated with increased risk for hospitalization for pulmonary exacerbations, respiratory symptoms, and bronchiectasis in CF patients [20–22]. Notably, the nomenclature for persistent isolation of *Aspergillus* is obscure as repeated *Aspergillus* positive cultures may be interpreted as colonization to some clinicians and infection to others. Furthermore, in contrast to chronic *Pseudomonas aeruginosa* infection, there is no universally accepted definition of chronic *Aspergillus* infection or colonization [23]. However, many investigators have adopted the definition of chronic *Aspergillus* infection or colonization as two or more *Aspergillus* positive cultures during a 12-month period [17,20,22,24–27].

Although improved detection methods may contribute to the apparent rise of fungi in CF, individual patient factors may play a key role in the development of repeated isolation of *Aspergillus* and other fungi. Age and antibiotic exposure are potential risk factors; fungi in the airways is uncommon in young children [24,28–31]. To our knowledge, no large, multicenter study has investigated patient characteristics and clinical risk factors for the development of *Aspergillus* respiratory infections. Our objective was to identify potential risk factors for persistent *Aspergillus* isolation in children and adults with CF. Such risk factors could suggest potential mechanisms contributing to chronic *Aspergillus* isolation and approaches to prevention and management of chronic infection.

2. Materials and methods

2.1. Study design and participants

This was a retrospective cohort study using data from the Cystic Fibrosis Foundation Patient Registry (CFFPR), a longitudinal, encounter-based registry of individuals with CF seen at CFF accredited centers in the United States [3,32]. Individuals followed in the CFFPR between January 1, 2004 and December 31, 2012 comprised the study population. We excluded patients under 6 years of age (unreliable pulmonary function data) and patients older than 45 years of age (milder CF phenotype who are not representative of the general CF population). We also excluded patients with prevalent *Aspergillus* isolation (one or more positive *Aspergillus* cultures two years prior to study period), insufficient respiratory culture data defined by an average of one or fewer

respiratory culture per year, and history of solid organ transplant. For subjects who underwent lung transplant during the study, encounters after transplantation were excluded. In order to identify *Aspergillus*-prevalent subjects for exclusion, the first two years of observation were utilized. Our analytic period for our statistical model starts in 2006 due to absence of recorded data for important independent variables, such as inhaled and oral antibiotics, which the CFFPR has collected since 2006.

The primary outcome of interest of this study was incident, persistent *Aspergillus* respiratory isolation. Similar to Amin et al., we defined persistent *Aspergillus* isolation as the presence of *Aspergillus* species in two or more separate occurrences during the 12 month period after the first positive result [20]. The isolation of *Aspergillus* in less than two encounters was considered transient *Aspergillus*. The “*Aspergillus* species” variable in the CFFPR encompasses both *Aspergillus fumigatus* and non-*fumigatus* species. Respiratory tract culture data included oropharyngeal swab, expectorated sputum, and bronchoalveolar lavage results.

2.2. Statistical analysis

Comparisons of baseline demographic and clinical variables between subjects who developed persistent *Aspergillus* and those who remained persistent *Aspergillus* negative were performed using *t*-tests for continuous variables and chi-square for categorical variables. Kaplan Meier estimates were used to determine cumulative incidence. Unadjusted associations of potential risk factors were explored using simple logistic regression with generalized estimating equations (GEE) [33]. Independent variables included in the multivariable model were: age, gender, race (white or non-white), forced expiratory volume in 1 s (FEV₁) percent predicted, pancreatic insufficiency (categorized according to the use of pancreatic enzymes), CF related diabetes, inhaled antibiotic use (includes inhaled tobramycin, colistin, aztreonam, and other inhaled aminoglycoside), chronic macrolide use, oral antibiotic use, number of intravenous (IV) antibiotic courses per year, inhaled corticosteroid use, oral corticosteroid use, respiratory culture results positive for *Pseudomonas aeruginosa* (*Pa*), methicillin-resistant *Staphylococcus aureus* (MRSA) and *Burkholderia cepacia* complex (*B. cepacia*). Demographic factors, including baseline age (age at entry into the study), female sex, and race were treated as fixed variables. F508del genotype was omitted from the final model due to high correlation with white race. Annual pulmonary function (FEV₁ percent predicted, converted into 10 percentage units) and IV antibiotic courses per year were treated as time-varying continuous variables. The remaining variables, CF-related diabetes, pancreatic insufficiency, inhaled antibiotic, oral antibiotic, inhaled corticosteroid, oral corticosteroid, *Pa*, MRSA, and *B. cepacia* were time-varying binary variables (yes/no) based on annual registry data. The model employed multiple logistic regression with GEE (exchangeable correlation structure) to determine independent risk factors for incident persistent *Aspergillus* isolation. To account for potential surveillance bias, the number of cultures per year during follow-up was included in the multivariable analysis as a time-varying variable.

In the subgroup analyses performed, baseline body mass index (BMI) percentile and BMI were included for age stratified groups children (age 6 through <20 years) and adults (≥ 20 years and <46 years) respectively. Also, the relationship between host factors and any *Aspergillus* isolation (including persistent and transient) was explored in a sensitivity analysis.

Pearson goodness-of-fit test was performed for the full model with $p > 0.05$. Results of logistic regression models using GEE are presented as odds ratios (OR) with corresponding 95% confidence intervals (CIs). Statistical significance was defined as a p -value of <0.05 . Analyses were performed using Stata version 11.0 (StataCorp, College Station, Texas). The study was reviewed and approved by the institutional review board of Johns Hopkins School of Medicine (IRB number NA_00088963).

3. Results

3.1. Study population

A total of 34,079 individuals with CF were followed in the CFFPR from January 1, 2004 to December 31, 2012, of which 16,095 individuals met the inclusion criteria and comprised the final analytic population (Fig. 1). Subjects entered into the cohort at any point during the study period and were followed until solid organ transplantation, death, or December 31, 2012. The median follow-up time during the study period was 6.66 years [interquartile range (IQR) 4.79, 6.87]. The median number of clinical encounters for an individual subject was 18 [IQR 11, 26] with an average of 3.48 encounters per year (sd 1.46).

The figure illustrates the number of subjects, reasons for exclusion and development of the final analytic population.

3.2. Incidence of persistent *Aspergillus*

A total of 4495 (27.9%) patients developed incident *Aspergillus* respiratory isolation during follow-up. Persistent *Aspergillus* isolation was seen in 1541 (9.6%) subjects and transient *Aspergillus* isolation seen in the 2954 (18.4%) remaining individuals. The cumulative incidence of persistent *Aspergillus* isolation was 11.1% (95% CI 10.5, 11.7%) over the seven-year period. *Aspergillus* was isolated an average of 5.49 times after first positive culture in those with persistent *Aspergillus*.

3.3. Baseline characteristics

Baseline characteristics of subjects who developed persistent *Aspergillus* isolation were compared to those subjects who did not (Table 1). Among the 1541 individuals who developed persistent *Aspergillus* isolation, 1162 (75.4%) were <20 years old and 379 (24.6%) were 20 or older at baseline. Median age of first acquisition of persistent *Aspergillus* was 16.4 years [IQR 12.2, 22.0].

3.4. Characteristics associated with incident persistent *Aspergillus* infection

Univariate associations between host factors and persistent *Aspergillus* isolation are represented in Table 2. The adjusted model reflects the independent associations of the demographic and clinical factors with acquisition of persistent *Aspergillus*

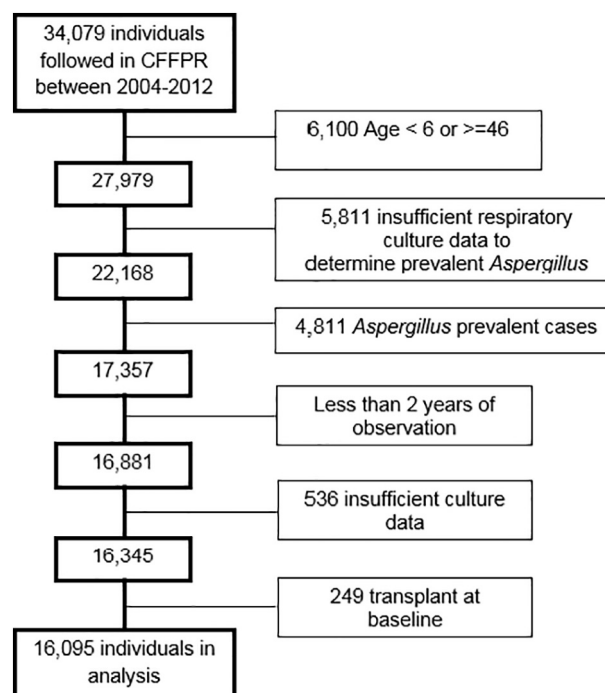


Fig. 1. Flowsheet of population in analysis.

Table 1

Baseline characteristics comparing subjects who developed persistent *Aspergillus* and those without persistent *Aspergillus*.

Baseline characteristic	Persistent <i>Aspergillus</i> ^a	No persistent <i>Aspergillus</i>	p-Value
	n (%)	n (%)	
Total	1541 (9.6)	14,554 (90.4)	
<i>Demographics</i>			
Age, years (mean ± sd)	15.8 ± 8.49	15.2 ± 9.66	0.02
Female sex	813 (52.8)	7048 (48.4)	0.001
White	1481 (96.1)	13,489 (92.7)	<0.001
F508del allele	1356 (88.0)	11,869 (82.6)	<0.001
BMI percentile in children ^b , % (mean ± sd)	45.3 ± 25.2	50.9 ± 26.6	<0.001
BMI in adults ^b , kg/m ² (mean ± sd)	21.8 ± 3.21	22.5 ± 3.99	0.002
<i>CF disease characteristics</i>			
FEV ₁ % predicted (mean ± sd)	80.3 ± 23.0	82.6 ± 25.4	0.001
Pancreatic insufficiency	1463 (94.9)	13,003 (89.3)	<0.001
CFRD	211 (13.7)	1846 (12.7)	0.23
ABPA	83 (5.4)	501 (3.4)	<0.001
<i>Treatments</i>			
Inhaled antibiotic	942 (61.1)	6779 (46.6)	<0.001
Chronic macrolide	716 (46.5)	5256 (36.1)	<0.001
Oral antibiotic	226 (14.7)	1595 (11.0)	<0.001
IV antibiotic courses in baseline year (mean ± sd)	0.89 ± 1.34	0.73 ± 1.29	<0.001
Inhaled corticosteroid	909 (59.0)	7153 (49.2)	<0.001
Oral corticosteroid	149 (9.7)	910 (6.3)	<0.001
<i>Microorganisms</i>			
<i>Pseudomonas aeruginosa</i>	915 (59.4)	7143 (49.1)	<0.001
MRSA	389 (25.2)	3059 (21.0)	<0.001
<i>B. cepacia</i>	23 (1.5)	446 (3.1)	<0.001

Definition of abbreviations: BMI = body mass index, MRSA = methicillin resistant *Staphylococcus aureus*, *B.cepacia* = *Burkholderia cepacia*, FEV₁ = Forced expiratory volume in 1 s, CFRD = Cystic fibrosis related diabetes, ABPA = Allergic bronchopulmonary aspergillosis.

^a Subjects who develop persistent *Aspergillus* isolation defined as two or more positive cultures during 12 month period after first isolation.

^b age stratified- BMI percentile for children only, BMI for adults only.

isolation, accounting for number of cultures performed per year (Table 3). White race (OR 1.74, 95% CI 1.23, 2.48, $p = 0.002$) and pancreatic insufficiency (OR 1.48, 95% CI 1.08, 2.04, $p = 0.02$) were independently associated with greater odds of persistent *Aspergillus* isolation. Inhaled antibiotics (OR 1.33, 95% CI 1.22, 1.46, $p < 0.001$), macrolides (OR 1.23, 95% CI 1.14, 1.32, $p < 0.001$), and inhaled corticosteroids (OR 1.14, 95% CI 1.09, 1.20, $p = 0.001$) were notable chronic therapies associated with increased risk for persistent *Aspergillus* isolation. Individuals with *Pa* infection were at 12% increased odds for persistent *Aspergillus*. The interaction between inhaled antibiotics and *Pa* was examined and included in the multivariable model (p for interaction 0.04). However, the difference in effect estimate for inhaled antibiotics in *Pa*-colonized individuals was small and of unclear clinical significance (OR 1.34, 95% CI 1.23, 1.47, $p < 0.001$).

Sensitivity analyses were conducted to further explore the associations between host factors and any *Aspergillus* isolation

Table 2

Univariate associations between factors and persistent *Aspergillus* isolation.

	Odds ratio	95% confidence interval	p-Value
<i>Demographics</i>			
Baseline age, 10 year increments	1.02	0.96, 1.09	0.51
Female	1.17	1.02, 1.34	0.03
White versus non-white	1.90	1.34, 2.67	<0.001
F508del	1.63	1.28, 2.07	<0.001
<i>Disease characteristics</i>			
Pancreatic insufficiency	2.20	1.61, 3.02	<0.001
Annual FEV ₁ % predicted, 10% increments	0.96	0.94, 0.98	<0.001
CFRD	1.17	1.07, 1.28	<0.001
<i>Treatments</i>			
Inhaled antibiotics	1.45	1.35, 1.55	<0.001
Macrolide	1.39	1.30, 1.49	<0.001
Oral antibiotics (non-macrolide)	1.29	1.19, 1.39	<0.001
IV antibiotic courses	1.10	1.08, 1.12	<0.001
Inhaled corticosteroid	1.25	1.17, 1.34	<0.001
Oral steroids	1.32	1.20, 1.45	<0.001
<i>Infections</i>			
<i>Pseudomonas aeruginosa</i>	1.20	1.13, 1.27	<0.001
MRSA	1.08	1.01, 1.15	0.02
<i>Burkholderia cepacia</i>	1.20	0.98, 1.49	0.08

Definition of abbreviations: FEV₁ = Forced expiratory volume in 1 s, CFRD = Cystic fibrosis related diabetes, IV = intravenous, MRSA = methicillin resistant *Staphylococcus aureus*.

(Supplemental Table 1). Similar relationships were observed with the addition of an increased risk for any *Aspergillus* isolation for older baseline age. Analyses stratified by baseline age <20 and greater than or equal to 20 showed similar results (Supplemental Table 2). For children, the observed associations in the main model between individual level factors and persistent *Aspergillus* remained with the exception of oral steroids. Whereas, in the adult population, only oral steroids posed an increased risk for persistent *Aspergillus* (OR 1.28, 95% CI 1.11, 1.46, $p = 0.001$) and interestingly, higher annual FEV₁% predicted was associated with increased incident persistent *Aspergillus* (OR 1.09, 95% CI 1.05, 1.14, $p < 0.001$).

Additional sensitivity analyses were explored: Model A) persistent *Aspergillus* isolation defined as three or more occurrences of *Aspergillus* over the study period, Model B) persistent *Aspergillus* isolation defined as 50% or greater positive *Aspergillus* cultures from first isolation until end of the study period, and Model C) model inclusive of prevalent cases of *Aspergillus*. Models A and C did not produce substantially different results (data not shown). Model B utilizes a more strict definition of persistent *Aspergillus* isolation, resulting in 879 cases (5.5% prevalence), lower than that shown in other studies [2,4,16,20,34,35]. However, chronic macrolides (OR 1.20, 95% CI 1.06, 1.35, $p = 0.003$), inhaled antibiotics (OR 1.53, 95% CI 1.29, 1.82, $p < 0.001$), and IV antibiotic courses per year (OR 1.05, 95% CI 1.01, 1.08, $p = 0.02$) remained as significant risk factors (Supplemental Table 3).

Table 3

Risk factors for persistent *Aspergillus* isolation: adjusted multivariable model for frequency of cultures and interaction between *Pseudomonas aeruginosa* and inhaled antibiotics.^a

	Odds ratio	95% confidence interval	p-Value
<i>Demographics</i>			
Baseline age, 10 year increments	0.97	0.89, 1.05	0.43
Female	1.07	0.93, 1.23	0.36
White versus non-white	1.74	1.23, 2.48	0.002
<i>Disease characteristics</i>			
Pancreatic insufficiency	1.48	1.08, 2.04	0.02
Annual FEV ₁ % predicted, 10% increments	1.00	0.97, 1.02	0.70
CFRD	1.05	0.96, 1.15	0.33
<i>Treatments</i>			
Macrolide	1.23	1.14, 1.32	<0.001
Inhaled corticosteroid	1.12	1.04, 1.20	0.001
Oral steroids	1.13	1.04, 1.23	0.006
Oral antibiotics (non-macrolide)	1.16	1.08, 1.25	<0.001
Inhaled antibiotics			
Without Pa	1.33	1.22, 1.46	<0.001
With Pa	1.34	1.23, 1.47	<0.001
IV antibiotic courses	1.02	1.00, 1.05	0.03
<i>Infections</i>			
<i>Pseudomonas aeruginosa</i>	1.12	1.03, 1.22	0.01
MRSA	0.98	0.92, 1.05	0.61
<i>Burkholderia cepacia</i>	1.10	0.89, 1.35	0.38

Definition of abbreviations: FEV₁ = Forced expiratory volume in 1 s, CFRD = Cystic fibrosis related diabetes, IV = intravenous, Pa = *Pseudomonas aeruginosa*, MRSA = methicillin resistant *Staphylococcus aureus*.

^a Interaction term for inhaled antibiotics and *Pseudomonas aeruginosa* 0.90, *p* = 0.04.

4. Discussion

To our knowledge, this is the first study to examine the clinical characteristics and exposures associated with acquisition of *Aspergillus* respiratory isolation in a large multicenter cohort of CF patients. Previous studies exploring a similar relationship have been limited to specific populations, including single-centers, children, and those with ABPA [25,27,36–38]. White race and pancreatic insufficiency, were associated with higher risk of acquisition of persistent *Aspergillus*. More importantly, this study suggests that chronic medications used to maintain CF lung health and treat pulmonary exacerbations may be independently associated with increased risk for incident persistent *Aspergillus* isolation. While the associations with macrolides, inhaled and oral corticosteroids, oral non-macrolide antibiotics, and/or inhaled antibiotics with *Aspergillus* could just reflect confounding by disease severity, the use of these therapies also could be causal. Our findings warrant further understanding of the mechanism behind how the microbial environment of the CF respiratory tract may evolve in the presence of these medication exposures throughout one's life.

In contrast to previous studies, our analysis did not show an association of older age with persistent *Aspergillus* [29,36,39]. However, the median age of persistent *Aspergillus* development was 16.4 years, suggesting that *Aspergillus* is most commonly

acquired during adolescence. Prior studies suggesting greater *Aspergillus* isolation with increasing age may have been subject to sampling bias since children have microbiologic surveillance by oropharyngeal swab, which may affect the detection of pathologic microbes in the lower respiratory tract, resulting in an older mean age of the *Aspergillus* diagnoses. In the AREST CF cohort, bronchoalveolar lavage (BAL) of infants demonstrated *Aspergillus* in 12.5% [40]. As the median predicted survival of individuals with CF continues to rise, further study of age-specific incidence and prevalence of *Aspergillus* is needed.

Among the treatment factors, inhaled antibiotic exposure had the strongest association with the acquisition of persistent *Aspergillus* isolation. It is possible that the chronic antibacterial killing of host commensal organisms fosters a hospitable environment for fungi to readily grow. Our findings are consistent with previous studies. Clinical trial data of intermittent inhaled tobramycin demonstrated greater isolation of *Aspergillus* in the tobramycin group compared to the placebo group [30,41]. Additional retrospective studies have supported a positive association between inhaled antibiotics and *Aspergillus* colonization [24,27,28]. Despite this, aerosolized antibiotics have clearly shown clinical benefit in CF [41,42] and we do not suggest that inhaled antibiotics use should be curtailed. Instead, these findings suggest the possible interaction between chronic antimicrobial exposure and its effect on the CF airway microenvironment, warranting further study.

Pa has known inhibitory effects in-vitro on *A. fumigatus* growth and biofilms through pyocyanin pathways [43,44]. However, our data suggests *Pa* isolation poses increased risk for development of persistent *Aspergillus*. Furthermore, the aforementioned association between inhaled antibiotics and persistent *Aspergillus* was marginally increased in *Pa*-positive compared to *Pa*-negative individuals. This may be due to unmeasured confounding or could be explained by complex microbial interactions. Further investigation is warranted, particularly because poor clinical outcomes have been previously described in individuals with co-infection of *Pa* and *A. fumigatus* [20,45].

Macrolide use was also a risk factor for persistence of *Aspergillus*. Long-term azithromycin therapy has previously been positively associated with *Aspergillus* colonization in children [25]. Although the exact mechanism is unclear, macrolides may inhibit neutrophil infiltration into the respiratory tract, resulting in altered innate immune response to face germination of *Aspergillus* conidia [46]. Furthermore, chronic oral antibiotics targeted at *Staphylococcus aureus* in an adult CF population have been previously associated with *Aspergillus* infection, [24] which is consistent with our current findings. However, the CFFPR does not differentiate if the non-macrolide oral antibiotic use was chronic or targeted at *S. aureus* or another pathogen. Our study identified inhaled and oral corticosteroids as risk factors for persistent *Aspergillus*. The data on the relationship between inhaled corticosteroids and development of *Aspergillus* isolation have been mixed in previous studies [25,37,38]. This potential relationship warrants further investigation as inhaled corticosteroids are commonly prescribed for chronic maintenance therapy in the CF population despite little data supporting use in the absence of asthma [34]. Finally, there have been mixed data describing the

relationship between IV antibiotic exposure and *Aspergillus* isolation; however, this may be due to short-term nature of IV antibiotics, rather than the chronic therapies [25,47].

Pancreatic insufficiency, which often represents greater CFTR dysfunction, was associated with increased odds of developing persistent *Aspergillus*. In the era of CFTR modulators and correctors, this relationship between genotype and phenotypic outcomes, such as chronic infection, require prospective study. Lower FEV₁ percent predicted, a marker of disease severity, was not a predictor of development of persistent *Aspergillus* isolation. Several retrospective studies have demonstrated lower baseline FEV₁ in patients with persistent *A. fumigatus* compared to those who never isolated *Aspergillus* [20,27]. Similar to our findings, others did not demonstrate an independent relationship between lung function and persistent isolation of *Aspergillus* [25].

There are limitations to our study, including the inability to infer causality, risk of information bias, possibility of residual confounding, and misclassification. As sicker individuals have more clinical encounters with more sputum samples, the detection of *Aspergillus* could be biased by the severity of illness. We tried to address this by adjusting the analysis for disease characteristics and the number of cultures performed per year for each subject. We focused on incident *Aspergillus* acquisition to avoid the bias of a prevalent population. Finally, there is no validated, uniform definition of persistent *Aspergillus* infection/colonization. We used the most common definition established by other investigators [20,22,24–27,38] and performed sensitivity analyses with other definitions and showed similar and consistent findings. Moreover, the frequency of fungal surveillance and laboratory culturing methods used to isolate and identify fungi in CF patients are not uniform, resulting in variability across U.S. centers.

5. Conclusions

In summary, several individual-level factors were identified that may place individuals with CF at greater risk for developing persistent *Aspergillus* isolation, particularly inhaled and oral (macrolide and non-macrolide) antibiotics and inhaled corticosteroid use. These exposures may contribute to the changing CF airway microenvironment resulting in emergence of atypical organisms, such as filamentous fungi. However, our findings should not suggest the avoidance of antibiotic or inhaled corticosteroid use in the routine clinical care of individuals with CF. Further prospective study to evaluate these relationships are needed.

Conflict of interest

There is no conflict of interest.

Author contributions

Contributions: GH and NL had access to the full data in the study and take responsibility for the integrity of the data and data analysis. GH, MPB, CAM, MTJ, KJP, and NL contributed to the study design, data analysis, 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.2018.01.008>.

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Supplemental Table 1: Risk factors for any *Aspergillus* isolation (transient or persistent), adjusted for frequency of cultures and interaction between *Pseudomonas aeruginosa* and inhaled antibiotics*

	Odds ratio	95 % confidence interval	p-value
Demographics			
Baseline age, 10 year increments	1.14	1.08, 1.20	<0.001
Female	0.99	0.91, 1.08	0.83
White versus non-white	1.48	1.23, 1.79	<0.001
Disease characteristics			
Pancreatic insufficiency	1.92	1.61, 2.30	<0.001
Annual FEV ₁ % predicted, 10% increments	1.00	0.98, 1.01	0.81
CFRD	1.02	0.96, 1.09	0.46
Treatments			
Macrolide	1.22	1.16, 1.28	<0.001
Inhaled corticosteroid	1.15	1.09, 1.20	<0.001
Oral steroids	1.14	1.07, 1.21	<0.001
Oral antibiotics (non-macrolide)	1.13	1.08, 1.19	<0.001
Inhaled antibiotics*	1.40	1.32, 1.50	<0.001
IV antibiotic courses, number per year	1.03	1.02, 1.05	<0.001
Infections			
<i>Pseudomonas aeruginosa</i>	1.23	1.15, 1.30	<0.001
MRSA	1.03	0.98, 1.07	0.28
<i>Burkholderia cepacia</i>	1.04	0.90, 1.20	0.58
Definition of abbreviations: FEV ₁ = Forced expiratory volume in 1 second, CFRD= Cystic fibrosis related diabetes, IV= intravenous, MRSA=methicillin resistant <i>Staphylococcus aureus</i>			
* Interaction term for inhaled antibiotics and <i>Pseudomonas aeruginosa</i> 0.80, p<0.001			

Supplemental Table 2: Age-stratified subgroup analyses, adjusted for frequency of cultures and interaction between *Pseudomonas aeruginosa* and inhaled antibiotics

	CHILDREN (n=11895)			ADULTS (n=4200)		
	Odds ratio	95 % confidence interval	p-value	Odds ratio	95 % confidence interval	p-value
Demographics						
Female	0.96	0.82, 1.14	0.67	1.08	0.82, 1.43	0.57
White versus non-white	1.73	1.17, 2.57	0.01	2.46	0.95, 6.38	0.07
BMI percentile/BMI*	1.00	0.97, 1.02	0.70	0.96	0.93, 0.98	0.001
Disease characteristics						
Pancreatic insufficiency	1.50	1.02, 2.23	0.04	1.35	0.82, 2.22	0.24
Annual FEV ₁ % predicted, 10% increments	0.97	0.94, 0.99	0.02	1.09	1.05, 1.14	<0.001
CFRD	1.00	0.88, 1.15	0.95	1.15	1.00, 1.32	0.05
Treatments						
Macrolide	1.31	1.20, 1.44	<0.001	1.10	0.98, 1.25	0.11
Inhaled corticosteroid	1.20	1.09, 1.31	<0.001	1.12	0.99, 1.28	0.07
Oral steroids	1.11	0.99, 1.25	0.08	1.28	1.11, 1.46	0.001
Oral antibiotics (non- macrolide)	1.18	1.07, 1.29	0.001	1.06	0.94, 1.20	0.35
Inhaled antibiotics†	1.43	1.28, 1.60	<0.001	0.99	0.79, 1.23	0.90
IV antibiotic courses	1.04	1.01, 1.07	0.01	1.00	0.97, 1.03	0.85
Infections						
<i>Pseudomonas aeruginosa</i> †	1.14	1.02, 1.28	0.03	1.01	0.83, 1.22	0.95
MRSA	1.01	0.93, 1.10	0.80	0.94	0.84, 1.06	0.33
<i>Burkholderia cepacia</i>	1.23	0.95, 1.60	0.11	0.90	0.61, 1.33	0.60
Definition of abbreviations: BMI= body mass index, FEV ₁ = Forced expiratory volume in 1 second, CFRD= Cystic fibrosis related diabetes, IV= intravenous, MRSA=methicillin resistant <i>Staphylococcus aureus</i> * BMI percentile units are for every 10% change and BMI units are for every 1 kg/m ² change † P for interaction between Pa and inhaled antibiotics was 0.05						

Supplemental Table 3: Adjusted model for risk factors for persistent *Aspergillus* isolation (strict definition) [†], adjusted for frequency of cultures and interaction between *Pseudomonas aeruginosa* and inhaled antibiotics

	Odds ratio	95 % confidence interval	p-value
Demographics			
Baseline age, 10 year increments	1.13	1.02, 1.24	0.01
Female	0.94	0.80, 1.11	0.49
White versus non-white	1.12	0.80, 1.57	0.51
Disease characteristics			
Pancreatic insufficiency	1.40	0.99, 1.98	0.06
Annual FEV ₁ % predicted, 10% increments	1.01	0.98, 1.04	0.58
CFRD	0.89	0.77, 1.03	0.89
Treatments			
Macrolide	1.20	1.06, 1.35	0.003
Inhaled corticosteroid	1.12	0.99, 1.26	0.07
Oral steroids	1.11	0.96, 1.29	0.14
Oral antibiotics (non-macrolide)	1.19	1.05, 1.35	0.005
Inhaled antibiotics*	1.53	1.29, 1.82	<0.001
IV antibiotic courses, number per year	1.05	1.01, 1.08	0.02
Infections			
<i>Pseudomonas aeruginosa</i> *	1.43	1.20, 1.70	<0.001
MRSA	0.94	0.84, 1.06	0.31
<i>Burkholderia cepacia</i>	0.97	0.71, 1.32	0.85
Definition of abbreviations: FEV ₁ = Forced expiratory volume in 1 second, CFRD= Cystic fibrosis related diabetes, IV= intravenous, MRSA=methicillin resistant <i>Staphylococcus aureus</i> [†] Persistent <i>Aspergillus</i> isolation defined as 50% or greater positive <i>Aspergillus</i> cultures from first isolation until end of the study period * Interaction term for inhaled antibiotics and <i>Pseudomonas aeruginosa</i> 0.80, p<0.001			