CHEST

Original Research

GENETIC AND DEVELOPMENTAL DISORDERS

# IgE-Mediated Immune Responses and Airway Detection of *Aspergillus* and *Candida* in Adult Cystic Fibrosis

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***Background:*** **The recovery of *Aspergillus* and *Candida* from the respiratory secretions of patients with cystic fibrosis (CF) is common. Their relationship to the development of allergic sensitization and effect on lung function has not been established. Improved techniques to detect these organisms are needed to increase knowledge of these effects.**

***Methods:*** **A 2-year prospective observational cohort study was performed. Fifty-five adult patients with CF had sputum monitored for *Aspergillus* by culture and real-time polymerase chain reaction and *Candida* by CHROMagar and carbon assimilation profile (API/ID 32C). Skin prick tests and ImmunoCAP IgEs to a panel of common and fungal allergens were performed. Lung function and pulmonary exacerbation rates were monitored over 2 years.**

***Results:*** **Sixty-nine percent of patient sputum samples showed chronic colonization with *Candida* and 60% showed colonization with *Aspergillus*. There was no association between the recovery of either organism and the presence of specific IgE responses. There was no difference in lung function decline for patients with *Aspergillus* or *Candida* colonization compared with those without ($FEV_1$ percent predicted, $P = .41$ and $P = .90$, respectively; FVC % predicted, $P = .87$ and $P = .37$, respectively). However, there was a significantly greater decline in $FEV_1$ and increase in IV antibiotic days for those sensitized to *Aspergillus* ($FEV_1$ decline, $P = .03$; IV antibiotics days, $P = .03$).**

***Conclusions:*** **Allergic sensitization is not associated with recovery of *Candida* or *Aspergillus* from the sputum of patients with CF. *Aspergillus* but not *Candida* sensitization is associated with greater lung function decline and pulmonary exacerbations.** ***CHEST 2013; 143(5):1351–1357***

**Abbreviations:** CF = cystic fibrosis; ICS = inhaled corticosteroid; PCR = polymerase chain reaction; SABC = Sabouraud dextrose with chloramphenicol agar; sIgE = specific IgE; sIgG = specific IgG; SPT = skin prick test

The incidence and diversity of fungi isolated from the sputum of patients with cystic fibrosis (CF) is increasing. The most common filamentous fungus cultured from CF sputum is *Aspergillus fumigatus* and the most common yeast is *Candida albicans*.[1,2]

The prevalence of positive serologic IgE responses to *A fumigatus* in CF is high; up to 65% of patients have values ranging from low to those consistent with allergic bronchopulmonary aspergillosis.[3] No link has been found between culture-positive sputum and the development of *Aspergillus* IgE sensitization. However, culture may not be the method of choice to evaluate this relationship because the sensitivity and the repeatability of standard *Aspergillus* culture are low.[4] Although evidence supports reduced lung function with *Aspergillus* sensitization, the association between colonization and lung function has not yet been established and requires further prospective studies.[3,5,6]

Manuscript received May 31, 2012; revision accepted October 16, 2012.

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**Funding/Support:** This study was supported by the National Commissioning Group, National Aspergillosis Centre, University Hospital of South Manchester.

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**DOI: 10.1378/chest.12-1363**

The prevalence of *C albicans* IgE sensitization is lower than that of *Aspergillus* but is still high at a reported 27%.[3] *Candida* species is far easier and more reliably isolated by standard culture. However, compared with *Aspergillus*, there are fewer studies of the clinical impact of *Candida* colonization and IgE sensitization.[7,8]

The primary objective of the present study was to determine whether a relationship exists between the presence of *Candida* (determined by culture) or *Aspergillus* (determined by real-time polymerase chain reaction [PCR]) and the development of serologic IgE responses. Secondly, the study aimed to assess the clinical impact of *Aspergillus* and *Candida* colonization and sensitization on lung function and pulmonary exacerbation rates.

## Materials and Methods

### *Study Design*

This prospective observational cohort study was carried out between October 2008 and February 2011. Approval was obtained from the South Manchester research ethics committee (07/Q1403/70). Patients were invited to participate between October 2008 and February 2009 if they were aged ≥ 18 years and given a diagnosis of CF confirmed by genetic or sweat testing. Patients were enrolled during routine outpatient appointments at the Manchester Adult Cystic Fibrosis Centre, and all gave written informed consent. Patients were excluded at enrollment if they were unable to produce a ≥ 2-mL sputum sample spontaneously or had an exacerbation of pulmonary symptoms requiring additional therapy.

### *Lung Function and Clinical Data Collection*

Baseline demographic and clinical details were collected from medical case records. Lung function ($FEV_1$ and FVC % predicted) at enrollment and 2 years after enrollment was obtained by documenting the patient's best lung function achieved within that year. This method was chosen to minimize the wide variability in lung function measurements observed in patients with CF. All lung function was performed postbronchodilator by experienced clinical staff according to European Respiratory Society guidelines.[9] Total days of IV antibiotics were prospectively monitored over 2 years to examine exacerbation rates.

### *Sputum Processing and DNA Extraction*

Each patient was given 10 mL of sterile water and asked to rinse his or her mouth for 30 s and return the water to a sterile universal container. A sputum sample was then collected without sputum induction. This was done to differentiate oral cavity and lower respiratory tract colonization. Patients provided two sputum samples within 1 year. Sputum samples were homogenized with Sputasol (Oxoid Limited), and culture was performed according to the UK Health Protection Agency National Standards Method BSOP 57 but modified to plate 10 μL rather than 1 μL of sputum.[10] Ten microliters of homogenized sputum was inoculated onto each of three Sabouraud dextrose with chloramphenicol agar (SABC) (Oxoid Limited) plates and one CHROMagar *Candida* plate (M-Tech Diagnostics Ltd). SABC plates were incubated at 30°C, 37°C, and 45°C for 72 h. CHROMagar plates were incubated at 37°C for 72 h. This culture method was repeated for the oral rinse sample but with no homogenization.

Following culture, the remaining sputum sample underwent additional homogenization using sonication (Sonics VC505; Sonics & Materials, Inc).[11] Fungal DNA was extracted using the MycXtra DNA extraction kit (Myconostica).

### *Real-Time PCR*

Real-time PCR to detect *Aspergillus* DNA was performed on a SmartCycler (Cepheid) using a validated commercial PCR kit targeting the 18S ribosomal gene of *Aspergillus,* MycAssay *Aspergillus* (Myconostica).[12] The assay limit of blank was a crossing threshold of 38 cycles.

### *Immunology*

A 7-mL clotted blood sample was taken from each patient for fungal serology. ImmunoCAP (Phadia AB) total IgE, specific IgG (sIgG) to *A fumigatus*, and specific IgEs (sIgEs) to *A fumigatus, C albicans, Alternaria alternata, Penicillium chrysogenum (notatum)*, and *Cladosporium herbarum* were measured. A cutoff level of ≥ 0.4 International Units/mL was considered positive for sIgE assays, and a cutoff of > 40 mg/L was considered positive for sIgG. Skin prick tests (SPTs) were performed by standard methods to a panel of 10 allergens, using the following antigens (Allergopharma): grass pollen mix, tree pollen mix, *Dermatophagoides pteronyssinus* (house dust mite), cat and dog dander, *A fumigatus, C albicans, A alternata, P chrysogenum, and C herbarum*.[13]

### Candida *Species Identification*

*Candida* species identification was performed using three methods for the first positive sputum sample from each patient. First, the color and appearance of the yeast colonies growing on CHROMagar were recorded. This was then followed by a germ tube test. Using a sterile loop, a pure colony of yeast cells was inoculated into sterile tubes containing 0.5 mL horse serum. The tube was incubated for 2 h at 37°C, then 10 μL was examined microscopically for the presence of germ tubes. Finally, carbon assimilation profile was performed using the commercial API/ID 32C kit (bioMérieux SA) according to manufacturer instructions.[14]

### *Antifungal Resistance Testing*

Each *Candida* colony was tested for resistance to fluconazole using the European Committee on Antimicrobial Susceptibility Testing microdilution method.[15,16] A fluconazole minimum inhibitory concentration of ≤ 2 mg/L was considered sensitive, and > 4 mg/L was considered resistant.[16]

### *Statistical Analysis*

Statistical analysis was performed using SPSS version 16 (International Business Machines Corp) software. Parametric data were expressed as mean ± SD, and nonparametric data were expressed as medians with interquartile ranges. After natural log transformation, simple logistic regression was performed to evaluate the associations between lung function decline and clinical data. Between-group comparisons were analyzed using $\chi^2$ tests and Mann-Whitney *U* tests with Bonferonni correction to assess lung function decline. Immunologic results were compared using Spearman correlation coefficient and global levels of agreement.

## Results

All patients were able to produce sputum samples of ≥ 2 mL, but three patients were excluded because of concurrent pulmonary exacerbation. Fifty-five patients completed the study.

### Candida

*Candida* was isolated on CHROMagar from at least one of the two sputum samples in 43 patients (78%). Thirty-eight patients (69%) had *Candida* growth from both sputum samples and were deemed colonized. Five oral rinse samples (9%) grew *Candida*. There was no association with any baseline clinical characteristics and the presence of *Candida* colonization (Table 1).

Fifteen of the 43 first positive samples (35%) grew more than one species of *Candida* on CHROMagar. The maximum number of species from any one sample was three. The number of colony-forming units per species ranged from one to 47. A total of 66 yeasts were identified from the 43 samples. Based on CHROMagar appearance, these were provisionally identified as follows: 44 *C albicans/Candida dubliniensis*, 13 *Candida glabrata*, four *Candida tropicalis*, 1 *Candida parapsilosis*, and four unidentified. The germ tube test revealed that 38 of the 66 yeasts had evidence of germ tube formation. API/ID 32C testing confirmed the following speciation: 42 *C albicans*, three *C dubliniensis*, 12 *C glabrata*, four *C tropicalis*, two *C parapsilosis* complex, one *Candida guilliermondii*, one *Trichosporan mucoides*, and one unidentified. When results were compared with CHROMagar, there was 93% concordance. Ten yeasts demonstrated fluconazole resistance, including one *C albicans*, eight *C glabrata*, and one *T mucoides*.

### Aspergillus

Eleven patients (20%) grew *A fumigatus* from at least one sample. One patient also grew *Aspergillus flavus*. However, 33 patients (60%) had positive PCR findings from both samples, and one patient had positive PCR on just one sample. Twenty-one patients had neither positive culture nor positive PCR results. There was no association between baseline clinical characteristics and *Aspergillus* colonization (Table 1). There was no association between *Aspergillus* recovery and *Candida* growth ($\chi^2 = 0.23$, $P = .63$).

### *Immunology*

Fifty-one patients had SPTs to common allergens. Thirty-five percent had positive SPT results to mixed grass pollen, 24% to house dust mite, 18% to mixed tree pollen, 12% to cat dander, and 4% to dog dander. Eighteen patients (35%) had allergy to two or more common allergens. There was no correlation between presence of atopy to common allergens and colonization with either *A fumigatus* or *Candida*.

Fifty-one patients had both SPTs and sIgE against the fungal allergens. Combining IgE and SPT results, *A fumigatus* sensitization was most prevalent at 61%, *P chrysogenum* at 50%, *A alternata* at 28%, *C albicans* at 18%, and *C herbarum* at 17%. Twenty-four patients (47%) demonstrated allergy to two or more fungal allergens. ImmunoCAP IgE results were compared with SPTs: The level of agreement and sensitivity was highest for *A fumigatus*, whereas sensitivity was low for *Cladosporium* and *Candida* (Table 2). Spearman correlation coefficient comparing levels of sIgE with size of SPT wheals showed the strongest correlation for *A fumigatus* ($r = 0.798$, $P < .005$). Correlation coefficients for the remaining fungal allergens were as

**Table 1—*Patient Demographics and Baseline Clinical Characteristics***

| Characteristic | All Patients (N = 55) | Persistent *Candida* | | | Persistent *Aspergillus* | | |
|---|---|---|---|---|---|---|---|
| | | Yes (n = 38) | No (n = 17) | *P* Value[a] | Yes (n = 33) | No (n = 22) | *P* Value[a] |
| Age, y | 29 ± 10 | 29 ± 10 | 30 ± 9 | .84 | 28 ± 8 | 32 ± 12 | .10 |
| Male sex | 27 (49) | 22 (58) | 5 (19) | .08 | 16 (49) | 11 (50) | 1.00 |
| BMI, kg/m$^2$ | 21.2 ± 3.7 | 21.1 ± 3.9 | 21.2 ± 3.5 | .91 | 21 ± 3 | 21 ± 4 | .90 |
| ΔF508 homozygous | 32 (58) | 24 (63) | 8 (47) | .38 | 22 (67) | 10 (46) | .17 |
| CFRD | 21 (38) | 14 (37) | 7 (41) | .77 | 10 (30) | 11 (50) | .17 |
| HbA1c, % | 6.2 ± 1.4 | 6.1 ± 1.5 | 6.2 ± 1.3 | .89 | 6.2 ± 1.6 | 6.2 ± 1.3 | .98 |
| Pancreatic insufficiency | 50 (91) | 36 (95) | 14 (82) | .17 | 30 (91) | 20 (91) | 1.00 |
| Inhaled corticosteroids | 45 (80) | 33 (87) | 12 (71) | .26 | 25 (76) | 20 (91) | .28 |
| Azithromycin | 52 (95) | 36 (95) | 16 (94) | 1.00 | 32 (97) | 20 (91) | .56 |
| Nebulized antibiotic | 46 (84) | 33 (87) | 13 (77) | .44 | 28 (85) | 18 (82) | 1.00 |
| Triazole antifungal | 6 (11) | 4 (11) | 2 (12) | 1.00 | 4 (12) | 2 (9) | 1.00 |
| Chronic *Pseudomonas* | 51 (93) | 35 (92) | 16 (94) | 1.00 | 31 (94) | 20 (91) | 1.00 |
| Chronic *Burkholderia* species | 2 (4) | 1 (3) | 1 (6) | .53 | 0 (0) | 2 (9) | .16 |
| Chronic MSSA or MRSA | 20 (36) | 15 (40) | 5 (30) | .56 | 13 (39) | 7 (32) | .78 |
| $FEV_1$, % predicted | 58 ± 22 | 58 ± 22 | 59 ± 23 | .87 | 59 ± 22 | 57 ± 23 | .76 |
| FVC, % predicted | 74 ± 19 | 74 ± 20 | 74 ± 19 | .76 | 76 ± 19 | 71 ± 21 | .45 |

Data are presented as No. (%) or mean ± SD. CFRD = cystic fibrosis-related diabetes; HbA1c = hemoglobin A1c; MRSA = methicillin-resistant *Staphylococcus aureus*; MSSA = methicillin sensitive *S aureus*.

[a]*P* values were calculated using an independent Student *t* test and Fisher exact test.

**Table 2—*Performance of ImmunoCAP sIgEs Compared With SPTs for Five Fungal Allergens***

| Fungal Allergen | Total Agreement, % | Sensitivity, % | Specificity, % | PPV, % | NPV, % | Gwet $AC_1$ |
|---|---|---|---|---|---|---|
| *Aspergillus* | 91 | 88 | 96 | 97 | 84 | 0.81 |
| *Alternaria* | 87 | 70 | 92 | 70 | 92 | 0.62 |
| *Cladosporium* | 92 | 57 | 98 | 80 | 93 | 0.62 |
| *Candida* | 92 | 56 | 100 | 100 | 91 | 0.67 |
| *Penicillium* | 81 | 74 | 86 | 78 | 83 | 0.60 |

Comparison of the ImmunoCAP assay to SPTs using global levels of agreement and Gwet $AC_1$, which gives more accurate percentages of agreement than Cohen κ coefficient when one agreed category has a small percentage. NPV = negative predictive value; PPV = positive predictive value; sIgE = specific IgE; SPT = skin prick test.

follows: *A alternata,* $r = 0.665$; *C albicans,* $r = 0.682$; *P chrysogenum,* $r = 0.689$; and *C herbarum,* $r = 0.667$.

A comparison of *C albicans* and *A fumigatus* colonization with SPTs and sIgEs is presented in Table 3. There was no correlation between *A fumigatus* colonization and presence of sensitization ($\chi^2 = 0.45$, $P = .50$) or *C albicans* colonization and sensitization ($\chi^2 = 0.002$, $P = .96$).

There was a strong correlation between presence of allergy to two or more common allergens and allergy to two or more fungal allergens ($\chi^2 = 15.3$, $P < .001$). Eight patients met minimum immunologic criteria for allergic bronchopulmonary aspergillosis (positive sIgE *A fumigatus*, sIgG *A fumigatus*, and total IgE >500 International Units/mL), all were *Aspergillus* PCR positive, and five of eight were *Candida* culture positive.[17]

### *Lung Function and Exacerbation Rates*

Simple linear regression analysis showed an independent association between not being on inhaled steroids and having a greater decline in $FEV_1$ ($P = .011$, $R^2 = 0.12$, $F\ (1, 52) = 6.9$). There were no associations with age, sex, ΔF508 homozygosity, BMI, CF-related diabetes, pancreatic insufficiency, azithromycin, nebulized antibiotics, or coinfection with *Pseudomonas aeruginosa*, *Burkholderia* species, or *Staphylococcus aureus*.

There was no statistical difference in lung function decline or IV antibiotics days between patients with *Aspergillus* or *Candida* colonization and those without (Table 4). There was also no difference in lung function decline between patients with both *Aspergillus* and *Candida* colonization and those with neither.

There was no difference in lung function decline or IV antibiotics days for patients sensitized to *Candida* (Table 5). However, patients with *Aspergillus* sensitization had a greater decline in $FEV_1$ percent predicted ($Z = -2.2$, $P = .027$, $r = 0.3$) and a higher number of IV antibiotics days ($Z = -2.1$, $P = .034$, $r = 0.3$). There were no significant differences in the use of inhaled corticosteroids (ICSs) between patients with and without *Aspergillus* sensitization ($\chi^2 = 0.61$, $P = .44$).

## Discussion

Both *Candida* and *Aspergillus* are commonly isolated from the sputum of adult patients with CF, with this study finding prevalences of 78% and 62%, respectively, similar to previous studies.[18] Colonization was chronic over 12 months in 88% of patients with *Candida* cultured from their sputum and in 97% of patients with *Aspergillus* PCR-positive sputum. PCR was more sensitive than culture in detecting *Aspergillus* from sputum, even after modifying the UK standards method to use a larger sample volume. This is in keeping with previous studies.[19] However, optimal culture methods are yet to be defined, and methods such as using undiluted sputum plugs have not yet been compared with PCR.[4,20] A number of risk factors for fungal colonization have been described, such as use of oral and inhaled antibiotics, ICSs, and *P aeruginosa* colonization.[5,21,22] The present study did not find differences in any baseline clinical characteristics, which is likely due to the small study numbers and an adult cohort, meaning that most patients

**Table 3—*Comparison of sIgE and SPT Results to Culture and PCR***

| Result | Culture Positive | Culture Negative | PCR Positive | PCR Negative |
|---|---|---|---|---|
| *Aspergillus* IgE positive | 6/11 (55) | 23/44 (52) | 16/33 (48) | 13/22 (59) |
| *Aspergillus* SPT positive | 7/11 (64) | 26/44 (59) | 19/33 (58) | 14/22 (64) |
| *Candida albicans* IgE positive | 3/34 (9) | 2/17 (12) | … | … |
| *C albicans* SPT positive | 6/34 (18) | 3/17 (18) | … | … |

Data are presented as No./total No. (%). Although 38 patients had sputum samples colonized with *Candida* species; only 34 of the 38 were colonized with *C albicans.* Because this was the species that the sIgE tests and SPTs were directed toward, only these 34 patients were included in the analysis. sIgEs and SPTs were not associated with colonization. PCR = polymerase chain reaction. See Table 2 legend for expansion of other abbreviations.

**Table 4—*Lung Function and Antibiotics Days With Colonization***

| Characteristic | All Patients (N = 55) | *Candida* Colonization | | | *Aspergillus* Colonization | | |
|---|---|---|---|---|---|---|---|
| | | Yes (n = 38) | No (n = 17) | *P* Value[a] | Yes (n = 33) | No (n = 22) | *P* Value[a] |
| 2-y IV antibiotics days | 42 (14-96) | 67 (16-108) | 29 (9-80) | .17 | 38 (15-93) | 42 (14-96) | .70 |
| 2-y change $FEV_1$ % predicted | 3.0 (3.0-8.0) | 2.5 (0.0-11.3) | 3.0 (1.5-6.0) | .90 | 3.0 (1.0-10.0) | 2.0 (0.0-6.5) | .41 |
| 2-y change FVC % predicted | 2.0 (2.0-8.0) | 2.0 (2.0-9.0) | 3.0 (0.0-5.5) | .37 | 3.0 (0.0-8.5) | 2.0 (0.0-5.0) | .87 |

Data are presented as median (interquartile range).
[a]*P* values were calculated using Mann-Whitney *U* tests.

were colonized with *P aeruginosa* (93%) and were taking oral and inhaled antibiotics and ICSs.

The diversity of *Candida* species isolated was wide, and a high proportion of patients had more than one *Candida* species coexisting. CHROMagar performs as well as SABC to isolate *Candida*, but CHROMagar allows concurrent speciation, identifying 93% of species in this study compared with assimilation profiling.[23] Although assimilation profiling is more accurate than CHROMagar identification, it is significantly more time consuming and expensive. The identification of germ tubes detected 84% of *C albicans and C dubliniensis* isolates. This method is rapid and inexpensive but only identifies these particular species. Very few sputum samples from the oral cavity grew *Candida*, suggesting that *Candida* grown from sputum samples represents true colonization of the bronchial tree. Fluconazole resistance was detected in one of 42 *C albicans* isolates and eight of 12 *C glabrata* isolates. This may be due to the frequent use of fluconazole in this group of patients receiving multiple courses of antibiotics for bacterial pulmonary exacerbations.

Sensitivity of IgE detection by ImmunoCAP was less than standard SPTs, marginally for *Aspergillus* but markedly for *Candida* and *Cladosporium*. The cause for this is not known but has previously been reported in the literature.[24-27] Alternative mechanisms of skin reactions, such as complement activation and IgG activation, do not explain the differences. It may be due to very low circulating levels of sIgE or differences in the allergen extract. ImmunoCAP has known advantages in terms of reproducibility, quantitation, and efficiency, making its use routine in many laboratories. However, if clinical allergy is suspected, SPTs should be used.

The importance of sensitization has been debated in years past. One difficulty is that the definition of sensitization differs between studies, with variable sIgE cutoff levels being proposed.[27-31] The present study defined patients with any rise in sIgE as sensitized and showed a greater $FEV_1$ decline and increased pulmonary exacerbation rates for those sensitized to *Aspergillus* but not for those sensitized to *Candida*. However, it must be noted that this study was not powered to detect changes in lung function, and overall differences were small. The body of evidence appears to support reduced lung function with *Aspergillus* sensitization in both children and adults, with a suggestion that antifungal therapy may be beneficial.[28-31] This is also true of patients who have asthma but do not have CF and has been linked to asthma control (severe asthma with fungal sensitization).[32-34] The picture is less clear for *Candida* sensitization because study numbers have been small, but sensitization has not correlated to lung function decline.[3,8] It is, therefore, important that further prospective studies, with the primary aim of analyzing lung function decline, are performed using multiple measures of lung function.

**Table 5—*Lung Function and Antibiotics Days With Sensitization***

| Characteristic | All Patients (N = 55) | *Candida* Sensitization | | | *Aspergillus* Sensitization | | |
|---|---|---|---|---|---|---|---|
| | | Yes (n = 9) | No (n = 46) | *P* Value[a] | Yes (n = 33) | No (n = 22) | *P* Value[a] |
| Baseline $FEV_1$ % predicted | 58 (37-75) | 50 (34-76) | 61 (40-76) | .408 | 50 (35-72) | 62 (41-79) | .196 |
| Baseline FVC % predicted | 75 (59-89) | 65 (56-80) | 77 (59-88) | .224 | 70 (57-87) | 76 (61-91) | .555 |
| 2-y change $FEV_1$ % predicted | 3.0 (3.0-8.0) | 2.0 (2.0-10.5) | 3.0 (1.0-8.8) | .517 | 4.0 (3.0-12.0) | 2.0 (2.0-6.0) | .027[b] |
| 2-y change FVC % predicted | 2.0 (2.0-8.0) | 2.0 (2.0-6.5) | 3.0 (0.0-8.8) | .366 | 3.5 (1.5-9.0) | 2.0 (2.0-6.5) | .086 |
| 2-y change in $FEV_1$, mL | 100 (50-300) | 100 (0-350) | 100 (50-300) | .638 | 175 (100-462) | 100 (0-175) | .019[b] |
| 2-y change in FVC, mL | 100 (0-350) | 50 (0-320) | 100 (0-300) | .468 | 187 (100-425) | 50 (0-295) | .028[b] |
| 2-y IV antibiotics days | 42 (14-96) | 38 (15-89) | 49 (23-104) | .567 | 63 (28-109) | 34 (14-93) | .034[b] |

Data are expressed as median (interquartile range). Sensitization was defined as any positive sIgE or SPT result. If an sIgE level of ≥ 0.7 kilo-Units/L (class 2 or greater) was used to define sensitization, lung function for those with and without *Candida* remained nonsignificant, but those with *Aspergillus* showed a greater $FEV_1$ decline ($P = .022$) and greater FVC decline ($P = .037$). There was no difference in lung function decline for sensitization to other fungi or common allergens. See Table 2 legend for expansion of abbreviations.
[a]*P* values were calculate using Mann-Whitney *U* tests.
[b]Significant at $P \leq .05$.

There was no difference in lung function deterioration with either *Aspergillus* or *Candida* colonization in this study. Some studies support these findings,[3,6,35] whereas others have found a positive association with lower lung function.[5,7] The largest study over 10 years by Chotirmall et al[7] found a greater decline in $FEV_1$ with *Candida* colonization. The use of azithromycin, antibiotics, and ICSs are noteworthy confounding factors between the previous studies because of their effects on bacterial-fungal interactions and airway inflammation. Furthermore, not all patients with positive *Aspergillus* PCR or culture have active fungal growth in the airways. PCR can detect both live and dead organisms as well as inert spores. It is probable that only actively growing organisms will contribute to the inflammatory response, and this has been demonstrated in vitro.[36] Therefore, other methods to distinguish colonization from infection in the airways, such as detection of *Aspergillus* galactomannan,[37] may lead to more-definitive answers regarding the effect of colonization on lung function.

The observation that $FEV_1$ decline was less for patients taking ICSs is also interesting. The value of inhaled steroids is a subject of debate, with limited evidence in CF.[38] One randomized controlled trial of the effect of withdrawing ICSs found no difference in lung function or time to exacerbation over 6 months, whereas a pediatric study using data from the Epidemiologic Study of Cystic Fibrosis found a significant reduction in $FEV_1$ decline.[39,40]

In summary, real-time PCR significantly increased the detection of *Aspergillus* over standard culture. No association was found between the presence of either *Aspergillus* or *Candida* in sputum and IgE sensitization. There was no association between the isolation of either organism from sputum and lung function decline, but *Aspergillus* sensitization was associated with greater $FEV_1$ decline and increased pulmonary exacerbations.

## Acknowledgments

**Author contributions:** Dr Baxter had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
*Dr Baxter:* contributed to the study conception and design; data acquisition, interpretation, and analysis; drafting and critical review of the manuscript for important intellectual content; and final approval of the manuscript.
*Dr Moore:* contributed to the study conception and design, data acquisition, critical review of the manuscript for important intellectual content, and final approval of the manuscript.
*Dr Jones:* contributed to the study conception and design, critical review of the manuscript for important intellectual content, and final approval of the manuscript.
*Dr Webb:* contributed to the critical review of the manuscript for important intellectual content and final approval of the manuscript.
*Dr Denning:* contributed to the study conception and design, data interpretation and analysis, critical review of the manuscript for important intellectual content, and final approval of the manuscript.
**Financial/nonfinancial disclosures:** The authors have reported to *CHEST* the following conflicts of interest: Dr Baxter has received travel grants from Schering-Plough Ltd (now Merck & Co, Inc) and Pfizer, Inc, and has been paid for talks on behalf of Astellas Pharma Inc. Dr Moore has received a travel grant from Astellas Pharma Inc; has been paid for talks on behalf of Pfizer, Inc; and has received grant support from Pfizer, Inc. Dr Denning holds founder shares in F2G Limited, a University of Manchester spin-out company, and has received grant support from F2G Limited as well as from the Fungal Infection Trust (formerly the Fungal Research Trust); the Wellcome Trust, the Moulton Trust; the Medical Research Council; The Chronic Granulomatous Disease Society; the National Institute of Allergy and Infectious Diseases, National Institutes of Health; the European Union; and AstraZeneca. He acts as an advisor and consultant to F2G Limited and Myconostica (now part of Lab21 Ltd) as well as other companies over the past 5 years, including Pfizer, Inc; Schering-Plough Ltd (now Merck & Co, Inc); Nektar; Astellas Pharma Inc; and Gilead. He has been paid for talks on behalf of Merck & Co, Inc; Astellas Pharma Inc; Novartis AG; Dainippon Sumitomo Pharma Co, Ltd; and Pfizer, Inc. Drs Jones and Webb have reported that no potential conflicts of interest exist with any companies/organizations whose products or services may be discussed in this article.
**Role of sponsors:** The sponsors had no role in the design of the study, the collection and analysis of the data, or in the preparation of the manuscript.
**Other contributions:** This work was carried out at the National Aspergillosis Centre, University Hospital of South Manchester The authors thank all the laboratory staff members at the Mycology Reference Centre, University Hospital of South Manchester, for their assistance in sample processing and reporting. They also thank Julie Morris, PhD, Head of Medical Statistics, University Hospital of South Manchester, for her support in statistical analysis.

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