

Aspergillus fumigatus colonization in cystic fibrosis: implications for lung function?

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Abstract

Aspergillus fumigatus is commonly found in the respiratory secretions of patients with cystic fibrosis (CF). Although allergic bronchopulmonary aspergillosis (ABPA) is associated with deterioration of lung function, the effects of *A. fumigatus* colonization on lung function in the absence of ABPA are not clear. This study was performed in 259 adults and children with CF, without ABPA. *A. fumigatus* colonization was defined as positivity of >50% of respiratory cultures in a given year. A cross-sectional analysis was performed to study clinical characteristics associated with *A. fumigatus* colonization. A retrospective cohort analysis was performed to study the effect of *A. fumigatus* colonization on lung function observed between 2002 and 2007. Longitudinal data were analysed with a linear mixed model. Sixty-one of 259 patients were at least intermittently colonized with *A. fumigatus*. An association was found between *A. fumigatus* colonization and increased age and use of inhaled antibiotics. In the longitudinal analysis, 163 patients were grouped according to duration of colonization. After adjustment for confounders, there was no significant difference in lung function between patients colonized for 0 or 1 year and patients with 2–3 or more than 3 years of colonization (p 0.40 and p 0.64) throughout the study. There was no significant difference in lung function decline between groups. Although colonization with *A. fumigatus* is more commonly found in patients with more severe lung disease and increased treatment burden, it is not independently associated with lower lung function or more severe lung function decline over a 5-year period.

Keywords: *Aspergillus fumigatus*, colonization, cystic fibrosis, lung disease, lung function

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Introduction

Cystic fibrosis (CF) is characterized by chronic infection of the airways, in which *Pseudomonas aeruginosa* is the most important pathogen. Chronic infection with *P. aeruginosa* is associated with increased morbidity and mortality [1]. Filamentous fungi such as *Aspergillus fumigatus* are also commonly found in respiratory secretions of patients. Reported prevalence rates of colonization in patients with CF vary, ranging from 6% to 58% [2–11]. The variation in

the reported prevalence has been hypothesized to be attributable to the age of patients and climatic conditions [8]. Colonization with *A. fumigatus* is defined as the presence of *A. fumigatus* in respiratory secretions, and is different from the main clinical manifestation of *A. fumigatus* in CF, allergic bronchopulmonary aspergillosis (ABPA). This condition is characterized by pulmonary infiltrates and the production of IgG and IgE antibodies specific for *A. fumigatus*, as well as a rise in total IgE [2]. The prevalence of ABPA varies between 2% and 8%, and lung function has been shown to deteriorate over time in relation to *A. fumigatus* sensitization and ABPA [12].

The effect of colonization with *A. fumigatus* on lung function in the absence of ABPA is not clear. Case reports of patients colonized with *A. fumigatus* whose respiratory deterioration did not respond to antibiotics, but was brought to a halt by antifungal therapy, suggest a direct negative effect

of *A. fumigatus* on pulmonary condition [13]. Recently, a longitudinal study has shown that chronic infection with *A. fumigatus* is associated with increased hospitalization [14]. However, in several cross-sectional studies, there was no independent relationship between colonization and lung disease [6,11].

We aimed to study whether colonization with *A. fumigatus* was associated with unfavourable clinical outcome, by evaluating factors independently associated with *A. fumigatus* colonization and by longitudinally studying the effect of the duration of *A. fumigatus* colonization on the course of lung function.

Methods

Patient selection

The CF centre in Utrecht currently provides care for approximately 350 patients. Patients undergo a routine yearly multidisciplinary examination, the results of which are prospectively recorded in a database. Demographics, CF genotype, body mass index (BMI) values and pulmonary function data were collected from the database, and were recorded during the yearly routine examination, which is considered to be a moment of stability in patients' clinical condition. BMI Z-scores were calculated on the basis of growth curves [15].

Patients were selected for two separate analyses. The first was a cross-sectional analysis of data from 2007, to study which factors were associated with *A. fumigatus* colonization, in order to generate a hypothesis on the effect of *A. fumigatus* colonization in our patient population. In this analysis, all patients who were under treatment at the centre and who had been seen for routine care in 2007 were included. Patients were excluded if they were chronically infected with *Burkholderia cepacia* complex, if they had a history of lung transplantation or ABPA (defined according to diagnostic criteria [16]) and if they did not have pulmonary function or microbiology data available for 2007. The second analysis aimed to investigate the longitudinal effect of *A. fumigatus* colonization on lung function, to further study the hypothesis generated by the cross-sectional analysis; a retrospective cohort analysis was performed, in which all patients who were regularly followed at the CF centre from 2002 to 2007 (a minimum of 4 years of lung function measurements and microbiology data) and who had a lung function measurement performed in 2002 (to ensure a baseline lung function value) were included. All patients gave written informed consent for storage and evaluation of their clinical data in the CF database for scientific purposes, and the use of this database is permitted by the ethical board.

Microbiology

Sputum or throat swab samples were taken at each visit at the CF centre from all patients. *A. fumigatus* and *P. aeruginosa* colonization status were investigated by evaluating cultures performed between 2002 and 2007. All samples were cultured according to the standard diagnostic laboratory protocol, which included testing for fungi. Colonization with *A. fumigatus* in a given calendar year was defined as the presence of *A. fumigatus* in >50% of respiratory cultures performed in that year. Patients were categorized according to the number of years during the observation period in which they met the criteria for *A. fumigatus* colonization.

Statistical analysis

For the cross-sectional data, simple logistic regression was performed to evaluate the association between the study variables and the dependent variable (*A. fumigatus* colonization). In order to study which variables were independently associated with the outcome variable, variables showing an association ($p < 0.10$) were entered into a multiple logistic regression model, using the 'enter' method. The number of variables in the model was limited to ensure at least ten outcome events per independent variable [17].

Patients were divided into categories according to the number of years during the observation period in which they met the criteria for *A. fumigatus* colonization (group 1, no colonization or 1 year, group 2, 2–3 years; and group 4, 4 years or more).

Differences among the three groups during the first year of observation were evaluated by the Kruskal–Wallis test (for non-normally distributed continuous variables), ANOVA (for normally distributed continuous variables) or the chi-square test (for dichotomous variables). A linear mixed model was used to evaluate the effect of *A. fumigatus* colonization on lung function during the 5-year study period. The model assumed a linear trend in forced expiratory volume in 1 s (FEV₁) as a percentage of predicted over time for each patient, and allowed for random patient-specific slope and intercept. First, a basic model was created, with *A. fumigatus* colonization group, and random slope for time. To this model, several possible confounders (variables associated with colonization in the cross-sectional analysis or those different for the three groups at baseline) were added, to check whether this significantly improved the model fit.

A difference in decline of FEV₁ among the three groups was tested by examining the improvement in model fit after addition of an interaction term to the model (time $\times$ group). This interaction term would allow for different slopes over

time for the three groups. SPSS version 15.0 for Windows was used for all statistical analyses.

Results

Cross-sectional analysis

In 2007, 335 patients visited the CF centre for an annual check-up. Of these patients, seven were excluded because they had a history of lung transplantation, and five were excluded because they were chronically infected with *B. cepacia* complex. Nine patients did not have samples for respiratory culture taken at the CF centre, 24 did not have lung function data available for 2007 (20 were too young for pulmonary function testing, and four did not have pulmonary function test data available), and 31 were excluded because of a history of ABPA. In total, 259 were available for analysis.

Simple logistic regression indicated a significant association between the presence of *A. fumigatus* colonization in 2007 and increased age, decreased FEV₁, chronic *P. aeruginosa* infection, increased number of hospitalizations, and increased use of inhaled antibiotics and recombinant human DNase (Table 1). In total, six variables with a p-value of <0.10 in the simple logistic regression were entered into the multiple logistic regression model: age, FEV₁, number of hospitalizations, inhaled antibiotic use, recombinant human DNase use and chronic *P. aeruginosa* infection. In the final model, *A. fumigatus* colonization was independently associated with increased age (p 0.02 and p 0.09 for age categories 13–24 and ≥25 years, respectively, vs. reference category 0–12 years) and the use of inhaled antibiotics (p 0.001).

Longitudinal analysis

Between 2002 and 2007, there were 225 patients who regularly visited the CF centre (at least 4 years of lung function and microbiology data available). Six patients were excluded because of a history of lung transplantation, and 30 patients were excluded because they had (developed) ABPA. Four patients were excluded because of chronic *B. cepacia* complex infection. Twenty-two patients did not have lung function data for 2002 available (20 because they were too young for spirometry, and two because they did not have their lung function measured at this CF centre). In total, 163 patients were included in the longitudinal analysis.

There were differences in possible confounding clinical and demographic characteristics between the three groups: patients with longer duration of colonization were older (p <0.001), were more likely to use inhaled antibiotics (p <0.001), had more hospitalizations (p 0.02), had lower FEV₁ values (p <0.001) and had lower BMI Z-scores (p 0.04) (Table 2).

With regard to the decline in FEV₁ in time, patterns were rather similar for the three groups (Fig. 1). The differences in FEV₁ between the groups were explained by the investigated possible confounders (the use of inhaled antibiotics, BMI Z-score, age at baseline and number of hospitalizations) rather than by the presence of *A. fumigatus* colonization. As shown in Table 3, the three groups had a significantly different estimate for FEV₁ in the unadjusted model, and this difference disappeared after adjustment for confounders. Adjustment for the number of cultures performed per year did not change the difference in estimates between the three groups, and this variable was therefore not included in the model. Including an interaction term between FEV₁ patterns

TABLE 1. Simple and multiple logistic regression analysis showing an independent association between *Aspergillus fumigatus* colonization and age and inhaled antibiotic use (n = 259 patients), with *A. fumigatus* colonization (defined as >50% of cultures positive in 2007) as dependent variable

	Colonized (n = 61)	Not colonized (n = 198)	Simple logistic regression		Multiple logistic regression	
			p	OR (95% CI)	p	OR (95% CI)
Age category, n (%)						
0–12 years (reference category) (n = 106)	10 (16.4)	96 (48.5)	<0.001		0.06	
13–24 years (n = 99)	33 (54.1)	66 (33.3)	<0.001	4.80 (2.2–10.4)	0.02	2.90 (1.2–7.0)
≥25 years (n = 54)	18 (29.5)	36 (18.2)	<0.001	4.80 (2.0–11.4)	0.09	2.39 (0.9–6.5)
Male, n (%)	27 (44.3)	112 (56.6)	0.09	0.61 (0.3–1.1)		
dF508 homozygosity, n (%)	38 (63.3)	116 (61.7)	0.82	1.07 (0.6–2.0)		
Pancreatic insufficiency, n (%)	52 (85.2)	170 (85.9)	0.91	0.95 (0.4–2.2)		
CF-related diabetes, n (%)	8 (13.1)	20 (10.1)	0.51	1.34 (0.6–3.2)		
FEV ₁ % predicted mean (SD)	63.6 (25.0)	81.5 (25.5)	<0.001	0.97 ^a (0.96–0.99)	0.31	0.99 ^a (0.98–1.01)
BMI Z-score, mean (SD)	−0.62 (1.00)	−0.38 (1.02)	0.11	0.80 ^a (0.6–1.1)		
Inhaled antibiotics, n (%)	40 (65.6)	49 (24.7)	<0.001	5.79 (3.1–10.8)	0.001	3.72 (1.7–8.2)
rhDNase, n (%)	34 (55.7)	66 (33.3)	0.002	2.52 (1.4–4.5)	0.58	1.21 (0.6–2.4)
Chronic <i>Pseudomonas aeruginosa</i> infection, n (%)	43 (70.5)	80 (40.4)	<0.001	3.52 (1.9–6.5)	0.99	0.99 (0.4–2.3)
Number of hospitalizations, mean (SD)	0.67 (SD 1.3)	0.29 (SD 0.7)	0.01	1.47 ^a (1.1–2.0)	0.27	1.20 ^a (0.9–1.7)

BMI, body mass index; CF, cystic fibrosis; FEV₁, forced expiratory volume in 1 s; rhDNase, recombinant human DNase; SD, standard deviation.

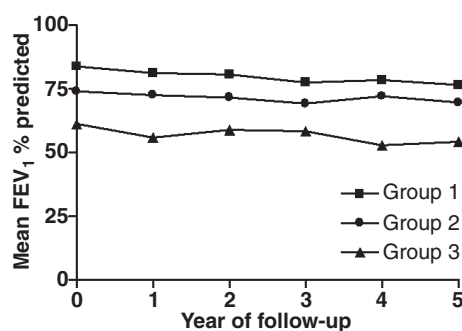
^aChange per unit of variable.

TABLE 2. Group differences in possible confounding factors during first year of the observation period

	Group 1 (n = 115)	Group 2 (n = 29)	Group 3 (n = 19)	P
Age (years), median (IQR)	11 (7–18)	14 (10–19)	21 (13–29)	<0.001
Male, n (%)	63 (54.8)	15 (51.7)	9 (47.4)	0.82
dF508 homozygosity, n (%)	77 (68.8)	16 (57.1)	14 (73.7)	0.41
Pancreatic insufficiency, n (%)	98 (85.2)	21 (75.0)	17 (89.5)	0.33
CF-related diabetes, n (%)	15 (13.0)	3 (10.3)	5 (26.3)	0.25
Inhaled antibiotics, n (%)	10 (8.7)	7 (24.1)	11 (57.9)	<0.001
rhDNase, n (%)	21 (18.3)	5 (17.2)	5 (26.3)	0.68
>50% cultures positive for <i>Pseudomonas aeruginosa</i> , n (%)	37 (39.4)	13 (56.5)	11 (61.1)	0.12
FEV ₁ predicted mean (SD)	83.9 (22.8)	73.3 (20.4)	61.3 (22.7)	<0.001
Number of hospitalizations, median (IQR)	0 (0–0)	0 (0–1)	0 (0–0)	0.02
BMI Z-score, mean (SD)	−0.46 (1.01)	−0.57 (1.08)	−1.10 (1.07)	0.04

BMI, body mass index; CF, cystic fibrosis; FEV₁, forced expiratory volume in 1 s; IQR, interquartile range; rhDNase, recombinant human DNase; SD, standard deviation.

Patients were grouped according to duration of *Aspergillus fumigatus* colonization (>50% of cultures positive for *A. fumigatus* in a given year) during the observation period (from 2002 to 2007): group 1, 0–1 years of *A. fumigatus* colonization; group 2, 2–3 years; and group 4, ≥4 years. Significant differences between three groups were tested with the chi-square test for dichotomous variables, with the Kruskal–Wallis test for non-normally distributed continuous variables, or with ANOVA for normally distributed continuous variables.

**FIG. 1.** Mean forced expiratory volume in 1 s (FEV₁) per group over the study period: group 1 (n = 115), 0–1 years of *Aspergillus fumigatus* colonization; group 2, (n = 29), 2–3 years; and group 4 (n = 19), ≥4 years.**TABLE 3.** Lung function estimates for the three groups based on the mixed model analysis, showing no statistically significant difference in lung function between the three groups after adjustment for covariates

Variable	Unadjusted		Adjusted ^a	
	Estimate	95% CI	Estimate	95% CI
Intercept	84.73	80.73 to –88.73	104.85	99.55 to –110.15
Time	–1.30	–1.75 to –0.86	–1.32	–1.75 to –0.88
<i>Aspergillus fumigatus</i> colonization				
Group 1 (0–1 year)	0		0	
Group 2 (2–3 years)	–9.93	–18.70 to –1.16	–3.08	–10.20 to 4.04
Group 3 (≥4 years)	–23.41	–33.85 to –12.96	–2.25	–11.64 to –7.13

^aAdjusted for baseline body mass index Z-score, age at baseline, number of hospitalizations, and years of inhaled antibiotic use.

and time, in both the unadjusted and adjusted model, failed to improve model fit significantly. This further confirms that there was no significant difference in the slope of lung function between the three groups. The overall decline in FEV₁ was 1.30% points per year (Table 3).

Discussion

The results presented here suggest that colonization with *A. fumigatus*, in the absence of ABPA, does not have a negative effect on the course of lung function on a population level over a 5-year period.

Several other studies have investigated the association between *A. fumigatus* colonization and lung function cross-sectionally. The results from the cross-sectional analysis performed in the present study are similar to those reported by Milla *et al.* An unadjusted association between lung function and *A. fumigatus* colonization was found that was no longer significant after adjustment for covariates and appeared to be explained by age and gender [6], indicating that the increased risk of advanced pulmonary disease was associated with demographic factors that have been reported to be associated with severity of lung disease [18]. Another cross-sectional study by Bargon *et al.* also found no significant association between *A. fumigatus* colonization (defined as at least two cultures positive out of a minimum of four) and lung function, in a population of adult CF patients. Similar to our findings, a significant association with inhaled antibiotics was found [11]. The authors suggest that the use of prophylactic antibiotics may have predisposed the patients to *A. fumigatus* colonization. This has also been suggested from a study on the use of inhaled tobramycin, in which the isolation of *A. fumigatus* was increased in the treatment group, at the end of the study period [19]. On the basis of our findings, it is difficult to say whether there is a causal relationship between the use of inhaled antibiotics and *A. fumigatus* colonization, as the association was already present at the start of the observation period. However, as logistic regression analysis of the cross-sectional results showed an independent association between the presence of *A. fumigatus* (even after correction for severity of lung disease by correcting for FEV₁) and the use of inhaled antibiotics, a causal relationship seems possible.

As a causal relationship between colonization and deterioration of lung function cannot be inferred from a cross-sectional analysis, we also performed a longitudinal study. Patients who were chronically colonized throughout the study period had lower lung function than patients who were colonized for <4 years during the study period. However,

this difference was no longer statistically significant after adjustment for confounders. Most importantly, there was no statistically significant difference in decline in lung function between the three groups of patients, suggesting that, within a time frame of 5 years, *A. fumigatus* colonization does not determine the decline in lung function. These findings differ from a recent longitudinal analysis in children with CF. Amin et al. [14] also found that patients with persistent *A. fumigatus* infection had lower lung function than patients who were not persistently infected (defined as two or more positive cultures per year). However, it was not reported whether there was a difference in decline between these patients.

The possibility of detection bias is a limitation of our study. More severely affected patients are more likely to visit the CF centre, more likely to produce sputum and, therefore, more likely to be sampled. This was partly accounted for by defining *A. fumigatus* colonization as a proportion of all cultures performed in a year (>50% positive). Furthermore, the mean number of cultures performed per year was corrected for in the model, but because this did not change the difference in lung function estimate between the three colonization groups, this factor was removed. Another limitation is the possibility of incomplete collection of respiratory culture data, as some patients are not exclusively under treatment at the CF centre and are sometimes seen in the outpatient departments of non-CF centre hospitals. Furthermore, in the present study we excluded patients with ABPA, because the aim of the study was to look at the effect of *A. fumigatus* colonization in the absence of ABPA. However, we did not include measurements of specific antibodies to *A. fumigatus* in our analysis, which means that there could have been patients who did not fulfil the criteria of ABPA, but who were sensitized to *A. fumigatus*. As sensitization to *A. fumigatus* has been shown to be associated with deterioration in lung function [12], this could have influenced the data.

The difficulty in studying the effect of *A. fumigatus* colonization in the present study lies in the fact that, from the start of the observation period, the three groups differed in many respects: patients had worse lung function, higher age and decreased nutritional status with increased duration of colonization, similar to what was found in the cross-sectional analysis. It is not clear whether these patients had decreased lung function because they had been colonized before the start of the observation period, or whether they were colonized because of more severely damaged lungs. The latter explanation would be in accordance with the finding that colonization with *A. fumigatus* is uncommon in young children, and initial colonization occurs at a later age than colonization with bacterial pathogens such as *Staphylococcus aureus* and *P. aeruginosa* [20]. In the present study, more insights into

the causality of *A. fumigatus* colonization have been obtained by analysing longitudinal follow-up data and evaluating potential confounders. The results suggest that, as there is no effect of prolonged colonization on the course of lung function during the observation period, the decreased pulmonary function at baseline is not likely to have been caused by *A. fumigatus* colonization. However, as in some other studies, *A. fumigatus* was more frequently found in patients with more severe lung disease and with increased treatment burden. Therefore, although an effect on decline in lung function in this study could not be found, further prospective studies are needed to definitively study causal relationships between *A. fumigatus* colonization and treatment burden and pulmonary exacerbations, and the effect of antifungal treatment on clinical outcome in colonized patients.

Conclusion

This study shows that patients with *A. fumigatus* colonization have less favourable clinical status and have a greater burden of treatment. However, over a 5-year period, colonization with *A. fumigatus* in the absence of ABPA is not independently associated with a more severe decline in lung function.

Author Contributors

A. M. M. de Vrankrijker, C. K van der Ent, R. J. L. Willems, M. J. M. Bonten and T. F. W. Wolfs were involved in conception and design; A. M. M. de Vrankrijker, C. K van der Ent and F. T. van Berkhout were involved in the acquisition of data; and A. M. M. de Vrankrijker, C. K van der Ent, R. K. Stellato, R. J. L. Willems, M. J. M. Bonten and T. F. W. Wolfs were involved in analysis and interpretation of data. All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors were involved in final approval of the version to be published.

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References

1. Kerem E, Corey M, Gold R, Levison H. Pulmonary function and clinical course in patients with cystic fibrosis after pulmonary colonization with *Pseudomonas aeruginosa*. *J Pediatr* 1990; 116: 714–719.
2. Nelson LA, Callera ML, Schwartz RH. Aspergillosis and atopy in cystic fibrosis. *Am Rev Respir Dis* 1979; 120: 863–873.
3. Bauernfeind A, Bertele RM, Harms K et al. Qualitative and quantitative microbiological analysis of sputa of 102 patients with cystic fibrosis. *Infection* 1987; 15: 270–277.
4. Mroueh S, Spock A. Allergic bronchopulmonary aspergillosis in patients with cystic fibrosis. *Chest* 1994; 105: 32–36.
5. Becker JW, Burke W, McDonald G et al. Prevalence of allergic bronchopulmonary aspergillosis and atopy in adult patients with cystic fibrosis. *Chest* 1996; 109: 1536–1540.
6. Milla CE, Wielinski CL, Regelman WE. Clinical significance of the recovery of *Aspergillus* species from the respiratory secretions of cystic fibrosis patients. *Pediatr Pulmonol* 1996; 21: 6–10.
7. Skov M, Koch C, Reimert CM, Poulsen LK. Diagnosis of allergic bronchopulmonary aspergillosis (ABPA) in cystic fibrosis. *Allergy* 2000; 55: 50–58.
8. Bakare N, Rickerts V, Bargon J, Just-Nubling G. Prevalence of *Aspergillus fumigatus* and other fungal species in the sputum of adult patients with cystic fibrosis. *Mycoses* 2003; 46: 19–23.
9. Skov M, McKay K, Koch C, Cooper PJ. Prevalence of allergic bronchopulmonary aspergillosis in cystic fibrosis in an area with a high frequency of atopy. *Respir Med* 2005; 99: 887–893.
10. Valenza G, Tappe D, Turnwald D et al. Prevalence and antimicrobial susceptibility of microorganisms isolated from sputa of patients with cystic fibrosis. *J Cyst Fibros* 2008; 7: 123–127.
11. Bargon J, Dauletbaev N, Kohler B et al. Prophylactic antibiotic therapy is associated with an increased prevalence of *Aspergillus* colonization in adult cystic fibrosis patients. *Respir Med* 1999; 93: 835–838.
12. Kraemer R, Delosea N, Ballinari P, Gallati S, Cramer R. Effect of allergic bronchopulmonary aspergillosis on lung function in children with cystic fibrosis. *Am J Respir Crit Care Med* 2006; 174: 1211–1220.
13. Shoseyov D, Brownlee KG, Conway SP, Kerem E. *Aspergillus* bronchitis in cystic fibrosis. *Chest* 2006; 130: 222–226.
14. Amin R, Dupuis A, Aaron SD, Ratjen F. The effect of chronic infection with *Aspergillus fumigatus* on lung function and hospitalization in patients with cystic fibrosis. *Chest* 2010; 137: 171–176.
15. TNO Prevention and Health Leiden University Medical Centre. *Growth diagrams* 1997. Houten, the Netherlands: Bohn Stafleu Van Loghum, 1998.
16. Stevens DA, Moss RB, Kurup VP et al. Allergic bronchopulmonary aspergillosis in cystic fibrosis—state of the art: Cystic Fibrosis Foundation Consensus Conference. *Clin Infect Dis* 2003; 37 (suppl 3): S225–S264.
17. Concato J, Feinstein AR, Holford TR. The risk of determining risk with multivariable models. *Ann Intern Med* 1993; 118: 201–210.
18. FitzSimmons SC. The changing epidemiology of cystic fibrosis. *J Pediatr* 1993; 122: 1–9.
19. Burns JL, Van Dalfsen JM, Shawar RM et al. Effect of chronic intermittent administration of inhaled tobramycin on respiratory microbial flora in patients with cystic fibrosis. *J Infect Dis* 1999; 179: 1190–1196.
20. Pihet M, Carrere J, Cimon B et al. Occurrence and relevance of filamentous fungi in respiratory secretions of patients with cystic fibrosis—a review. *Med Mycol* 2009; 47: 387–397.