

Factors Effecting Impact of *Aspergillus fumigatus* Sensitization in Cystic Fibrosis

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Summary. The clinical impact of *Aspergillus fumigatus* (Af) sensitization in cystic fibrosis (CF) is controversial. We examined the effect of Af sensitization (Afs) on pulmonary function and growth using a retrospective cohort analysis over two 5-year study periods: 1996–2000 (19 Afs cases and 19 controls) and 2001–2005 (24 Afs cases and 23 controls). Sensitization was defined as Af specific radioallergosorbent test (RAST) ≥ 17.5 iu/ml and total serum IgE level ≥ 150 iu/ml. We examined the impact of changing treatment schedules over these periods. Afs cases had lower median FEV₁ %predicted (%PR) compared to matched controls 1996: 67 versus 80, $P < 0.01$; 2001: 78 versus 93, $P < 0.01$. Afs cases in the 2001 cohort had a higher FEV₁ %PR compared to Afs cases in the 1996 cohort: 78 versus 67, $P < 0.01$. For the 1996 Afs cohort FEV₁ %PR fell significantly over 5 years but not for the 2001 Afs cohort. Af RAST and total IgE reflected the changes in pulmonary function. Children in the 2001 Afs cohort were prescribed significantly more oral antifungal treatment (odds ratio 4.3, 95%CI 1.2–15.7, $P = 0.03$). Afs children continue to have poorer lung function compared to controls but this observational, hypothesis generating study, suggests that the use of antifungal treatment is associated with better lung function. **Pediatr Pulmonol.** 2007; 42:785–793. © 2007 Wiley-Liss, Inc.

Key words: ABPA; allergic bronchopulmonary aspergillosis; cystic fibrosis.

INTRODUCTION

Cystic fibrosis (CF) is one of the commonest autosomal recessive disorders found in Caucasians. Patients with CF frequently have positive sputum cultures, including to *Aspergillus fumigatus* (Af), a ubiquitous fungal organism found commonly in house dust, water damaged walls or ceilings, and decomposing organic matter. Its spores are approximately 2–3.5 μm in diameter, which allows deposition in terminal airways and alveoli.¹ Normal individuals, when exposed to Af, rapidly clear it. However, Af may persist in patients with CF, chronically infecting the respiratory tract.²

A proportion of CF patients, who are exposed to Af will develop immunological sensitization to Af. A further proportion will go on to develop allergic bronchopulmonary aspergillosis (ABPA) which is a syndrome consisting of respiratory and systemic symptoms and signs; although its diagnosis remains difficult in the absence of any definite, internationally accepted set of clinical or laboratory criteria.³ It is generally accepted that treatment for ABPA should be with Prednisolone and oral antifungals. In CF, Af immune sensitization without or with minimal clinical features has been reported to occur in up to 59% of patients,^{3–6} but when and whether treatment should be instituted is much less clear. Serological and microbiological sensitization can be defined in different ways.³ It has been suggested that

sensitization to Af in children with CF, is associated with poorer lung function and a worse prognosis³; however other studies have failed to show any correlation.^{7,8} It is conceivable that these different outcomes might be related to differences in definitional criteria for Af sensitization. In our institution we tend to define the clinical syndrome of ABPA as respiratory exacerbation with decline in pulmonary function, which has not responded to conventional antibiotics, where there is evidence of Af immunological sensitization; in particular where the total IgE is ≥ 150 iu/ml, if there has been a greater than fourfold rise in total IgE and the Af specific radioallergosorbent test

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(RAST) ≥ 17.5 iu/ml.⁹ Individuals with these criteria are then defined as having ABPA and commenced on treatment with oral corticosteroids with or without oral antifungal treatment. We do not employ skin sensitivity testing and the presence of Af in the sputum is not a mandatory prerequisite nor are radiological and/or CT lung changes such as bronchiectasis essential. There are additional subjects with more minor symptoms who fulfill the immunological criteria and receive oral antifungal treatment with or without corticosteroid. Because of the difficulties defining the clinical syndrome of ABPA, we decided to include all CF cases that were sensitized to Af using simple measurable immunological criteria without specifically defining clinical features irrespective of their treatment status and to examine clinical outcome in terms of lung function and growth measures.

We hypothesized that with modern treatment, particularly with the increasing use of antifungal agents, Af sensitization would be reduced and its clinical consequences less significant.

MATERIALS AND METHODS

Subject Population and Data Collection

All data for the study were extracted from the Royal Brompton Children's CF database. The data were derived from annual assessment blood tests, lung function tests, and growth measures. If we were unable to find all the information from the database, then patient medical notes including hospital computerized records were checked.

All the subjects selected for the study had been diagnosed with CF. The diagnosis was verified by a positive sweat test (chloride level >60 mmol/L from two sweat samples) and/or the presence of two recognizable CF disease causing mutations.¹⁰

Annual assessment data including microbiological and prescribed medication data were used in the study. The outcome measures were lung function and growth. Spirometry was obtained at the time of the annual assessment by lung function technicians, in the respiratory

laboratory of the Brompton Hospital, using pneumotachographs (Viasys Healthcare Jaeger UK). FEV₁ and FVC were measured and expressed as percentages of predicted normal values adjusted for height and sex.¹¹ Growth was assessed by measuring weight (minimal clothing) and height (Harpender stadiometer). Values were expressed as standard deviation scores (SDS).¹² Differences in prescribed medication between groups were also examined focusing particularly on the use of antifungal treatments.

Study Design

From all the CF children attending our clinic, the Af sensitization (Afs) cases selected were those children who had significant Afs at the beginning of a 5-year study period, were old enough to perform laboratory lung function tests and attended at least three out of the five annual assessments. Two time periods were examined. The first started in 1996 (defined as the 1996 cohort) and the second in 2001 (the 2001 cohort). The rationale for selecting these time periods was based on the fact that historically, clinical outcomes tend to improve over time. The criteria used to define Af sensitization, were a positive Af specific RAST ≥ 17.5 iu/ml and a positive total serum IgE level ≥ 150 iu/ml, measured in the first year of each 5-year period. The methods used for these immunological assays have in principle remained the same over the 10-year period. Each cohort was matched to control populations of CF children without significant Af sensitization. The control populations were matched for sex, age, genotype, pancreatic status, and the presence of chronic *Pseudomonas aeruginosa* (Pa) airway infection (confirmed from sputum or deep throat swabs from non-expectorating patients). All those with chronic Pa infection received long-term nebulised anti*Pseudomonas* antibiotic therapy. Each cohort was tracked for 5 years. Afs cases were compared with controls within each timeframe, that is, 1996–2000 and 2001–2005 (the first two years of each cohort were compared with the last two years, in order to generate sufficient numbers for statistical analysis) as well as between timeframes, that is, 1996–2000 versus 2001–2005.

Statistical Analysis

A pilot study of the data found a 10% difference in predicted FEV₁ (SD ± 18) between the 1996 Afs cases and non-sensitized control populations. This allowed calculation of the power and necessary sample size to define the number of cases necessary to show a significant difference in pulmonary function. To demonstrate a statistically significant difference, a sample size of 20 cases (with each subject having five measurements over the 5 years, i.e., 100 observations) was needed (for a 5% significance level at 80% power).¹³ This calculation assumed that the data approximated to a normal

ABBREVIATIONS

ABPA	Allergic bronchopulmonary aspergillosis
Af	<i>Aspergillus fumigatus</i>
Afs	<i>Aspergillus fumigatus</i> sensitized
CF	Cystic fibrosis
FEV ₁	Forced expiratory volume in 1 sec
FEV ₁ %PR	FEV ₁ as percent predicted for age and height
FVC	Forced vital capacity
FVC % PR	FVC as percent predicted for age and height
IQR	Interquartile range
Pa	<i>Pseudomonas aeruginosa</i>
RAST	Radioallergosorbent test
RhDNase	Recombinant human deoxyribonuclease
SD	Standard deviation
SDS	Standard deviation score

distribution. However, because the immunological and outcome data contained outliers, non-parametric Mann–Whitney testing was used to compare median values between groups. The statistical software package (SPSS version 6) was used. Differences between the matching criteria of cohorts were tested by Chi-squared analysis or Fisher's exact probability test (where a cell number was less than five). Outcome data were plotted on graphs using median values with bootstrap 95%CI error bars.¹⁴ The second part of the study investigating changes in treatments over time was not formally powered. Differences in treatments over time within the Afs cohorts and similarly the controls were examined using logistic regression. The cohorts formed the dichotomous dependent variables and specific medications the covariates where 0=no treatment during the 5 year period, 1=treatment one or more times. Each covariate was examined separately and then together by multiple regression. For all tests, a *P*-value of 0.05 was taken as the limit of statistical significance.

The Royal Brompton, Harefield & NHLI Research Ethics Committee, approved the study.

RESULTS

Cohort Characteristics

In the 1996 cohort there were 19 Afs cases 9 of whom had ABPA treated with one or more courses of oral corticosteroid and 19 control subjects. In the 2001 cohort there were 24 Afs cases of whom 12 had ABPA treated with one or more courses of oral corticosteroid but only 23 controls could be found who matched the specified criteria (i.e., sex, age, genotype, pancreatic status, and presence of Pa). Five Afs cases and three controls from the 1996 cohort carried through into the 2001 cohort. Characteristics of the cases and control cohorts are detailed in Table 1. There were no significant differences between cases and controls within timeframes but subjects were older and contained more females in 2001 compared to 1996. Two children from the 1996 Afs cohort and one child from the 2001 Afs cohort have subsequently died. There have been no deaths in either of the control cohorts.

Figure 1 demonstrates that although there was an increase in the number of children attending the clinic between 1996 and 2005 there was no significant change in the overall prevalence of Afs over the 10-year time period of the study (median 8.1%, range 6–11%). Overall there was no significant difference between the 1996 and 2001 Afs cohorts for Af RAST (*P*=0.52) or total serum IgE (*P*=0.74), measured each year over the whole 5-year study periods (Table 1; Fig. 2a,b). Analysis within the timeframe of the 1996 cohort, comparing the first two years with the last two years (1996–1997 vs. 1999–2000 and 2001–2002 vs. 2004–2005), revealed no significant differences in median Af RAST or total serum

TABLE 1—Cohort Data

	1996 Afs cases n = 19		1996 controls n = 19		2001 Afs cases n = 24		2001 controls n = 23		1996 Afs cases vs. 2001 Afs cases vs. controls <i>P</i> -value		1996 vs. 2001 Afs cases <i>P</i> -value controls <i>P</i> -value	
Median age years at entry (IQR)	13.66 (9.78–15.99)		12.44 (9.02–14.96)		12.58 (9.61–16)		14.61 (12.68–15.29)		0.64	0.07	0.79	0.03
No. male:female ratio	16:3		16:3		13:11		12:11		1.00	0.89	0.04	0.03
Genotype DF508/DF508	10		13		17		14		0.40	0.45	0.81	0.45
No. pancreatic insufficient	19		19		23		23		1.00	1.00	1.00	1.00
Median age years at diagnosis (IQR)	0.17 (0.07–1.00)		0.42 (0.22–1.69)		0.50 (0.25–1.12)		0.33 (0.14–1.97)		0.09	0.52	0.10	0.45
Median age years at first referral (IQR)	5.21 (2.40–10.20)		4.56 (3.21–8.56)		3.19 (0.79–7.53)		3.21 (0.97–5.19)		0.83	0.73	0.20	0.10
No. established Pa infection	16		18		24		19		0.23	0.05	0.23	0.36
Median IgE iu/ml at entry (IQR)	1030 (388–1858)		30 (16–88)		1690 (497–2791)		79 (34–183)		<0.01	<0.01	0.37	0.16
Median Af RAST (iu/ml) at entry (IQR)	37.4 (24–79.1)		0.35 (0.35–0.35)		40 (28.25–66.25)		0.34 (0.34–0.98)		<0.01	<0.01	0.81	0.29

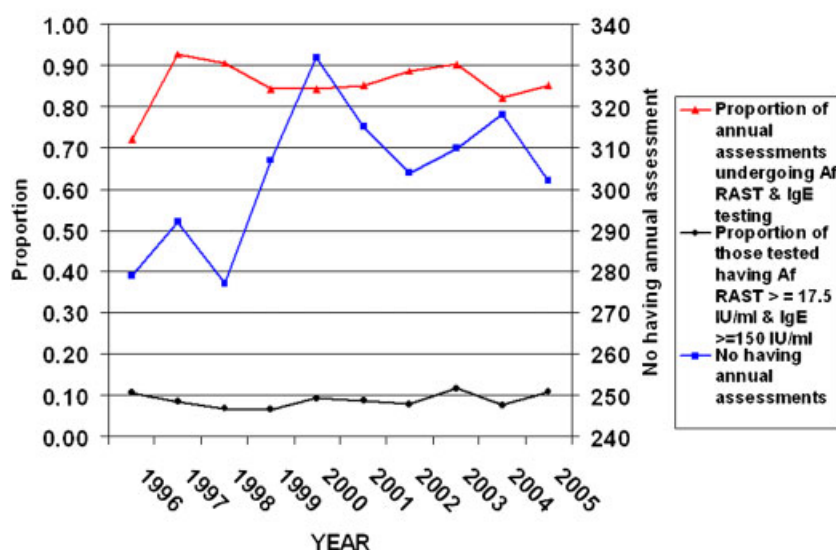
Prevalence of *Aspergillus fumigatus* sensitization

Fig. 1. Prevalence of *Aspergillus fumigatus* (Af) sensitization 1996 to 2005—demonstrating relatively constant prevalence of 8.1%. Over the 10-year period.

IgE. However, analysis of the 2001 Af cohort revealed a significant fall in Af RAST from 37.5 to 28.3 iu/ml ($P=0.02$) and total serum IgE from 1550 to 704 iu/ml ($P=0.02$).

Longitudinal Analysis of Growth Outcome Data

There was no significant difference between 1996 and 2001 Afs case and control groups for weight. For 1996: median weight SDS -0.65 versus -0.45 ($P=0.58$). For 2001: median weight SDS -0.31 versus -0.51 ($P=0.54$). For height in 1996: median height SDS -0.45 versus -0.69 ($P=0.09$). In 2001: median height SDS -0.42 versus -0.39 ($P=0.71$).

Longitudinal Analysis of Lung Function

Figure 3a,b demonstrate the median values for FEV₁ percent predicted (FEV₁ %PR) in Afs case and control groups year by year from 1996 to 2000 and from 2001 to 2005, respectively. Afs cases had significantly poorer FEV₁ %PR compared to controls. For the 1996 cohort: median FEV₁ %PR = 67 versus 80 ($P<0.01$); and for the 2001 cohort median FEV₁ %PR = 78 versus 93 ($P<0.01$).

Figure 3c compares 1996 and 2001 Afs cohorts for median FEV₁ %PR. FEV₁ %PR in the 2001 cohort was significantly higher with median FEV₁ %PR = 78 versus 67 ($P<0.01$). Comparing the first two years of each Afs cohort with the last two years: for the 1996 cohort the median FEV₁ %PR = 69 versus 65 ($P=0.05$); for the 2001 cohort the median FEV₁ %PR = 80 versus 82 ($P=0.85$). Similarly for the controls (Fig. 3d) FEV₁ %PR was significantly greater for the 2001 cohort

compared to 1996, median FEV₁ %PR = 93 versus 80 ($P<0.01$).

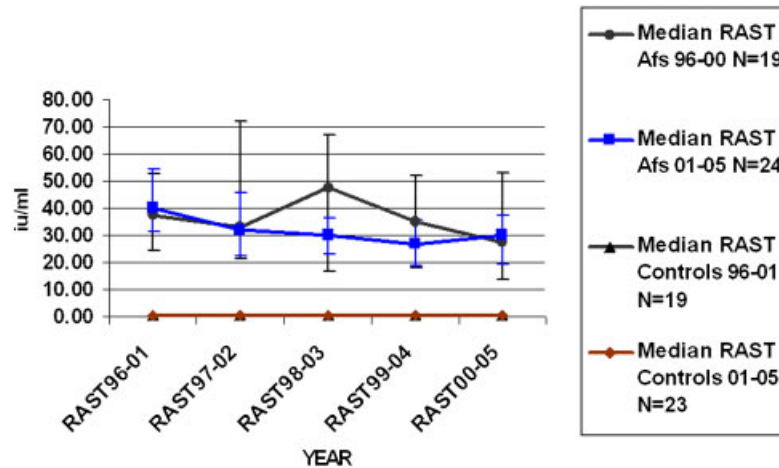
Similar findings were made when using FVC as the outcome measure. For 1996 Afs cases versus controls: median FVC %PR = 81 versus 91 ($P<0.01$). For 2001 Afs cases versus controls: median FVC %PR = 87 versus 97 ($P<0.01$). For 1996 Af cases versus 2001 Af cases: median FVC %PR = 81 versus 87 ($P=0.01$) and comparing the first and last two years in each cohort: 1996 median FVC %PR = 85 versus 76 ($P=0.04$) and 2001 median FVC %PR = 86 versus 90 ($P=0.81$). For controls an improvement in the median values for FVC %PR was seen; 1996 versus 2001: median FVC %PR = 91 versus 97 ($P=0.01$).

Changes in Pharmacological Management Between 1996 and 2001 Cohorts

Afs Cases

Over each 5-year period (1996–2000 and 2001–2005) medication and frequencies of administration were recorded. Using logistic regression, differences between cohorts were examined. Eight different treatment schedules were analyzed (Table 2). For the purposes of the analyses each treatment was examined separately and then altogether with multiple regression. Odds ratios were calculated by using the 2×2 table and calculating relative risks. In the 2001 Afs cohort there was a trend towards more frequent use of all treatment schedules except for inhaled steroids. However, the only statistically significant treatment difference was the use of itraconazole and/or

a Af RAST longitudinal change in AF sensitized and control cases
1996 vs 2001 cohorts



b Total IgE longitudinal change in AF sensitized and control cases
1996 vs 2001 cohorts

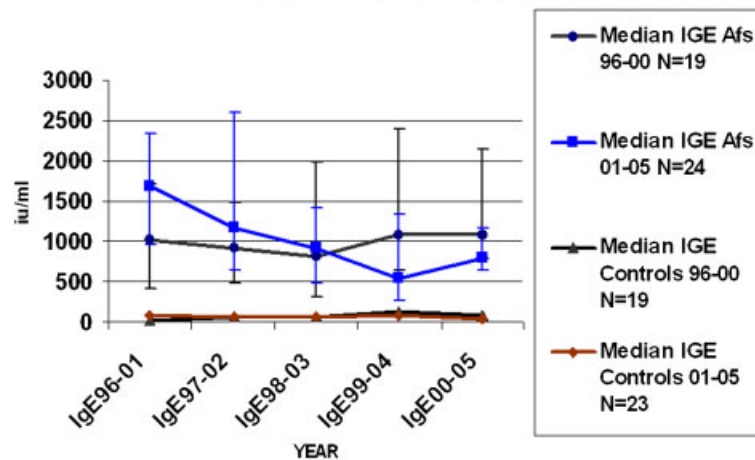


Fig. 2. a: Longitudinal tracking of median *Aspergillus* specific RAST for Afs cases and control subjects—demonstrating a fall in the level for the Afs cohort. **b:** Longitudinal tracking of median Total serum IgE for Afs cases and control subjects—demonstrating a fall for the Afs cohort.

voriconazole ($P = 0.03$), which was more than four times more likely to have been prescribed in the 2001 cohort in comparison to the 1996 cohort (16 out of 24 cases vs. 6 out of 19). Multiple regression analysis revealed that this was independent of other treatment schedules and may be regarded as a significant change in the management of Afs cases ($P = 0.05$).

Control Children

Six different types of treatment and schedules were analyzed (Table 2). In the 2001 control cohort there was a trend towards more frequent use of all treatment

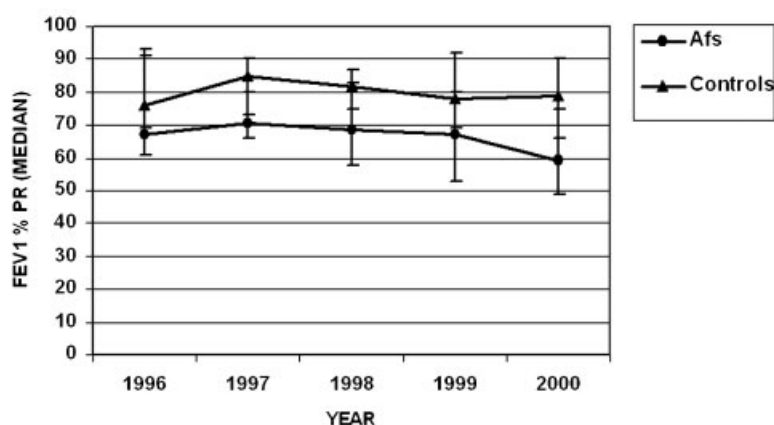
schedules except for inhaled steroids, but the only statistically significant treatment difference was the use of azithromycin ($P = 0.01$). Controls in the 2001 cohort were more than four times likely to have received treatment with azithromycin in comparison to those in the 1996 cohort (16 out of 23 cases vs. 6 out of 19). The results of multiple regression analysis suggest that the increased use of azithromycin may be regarded as a significant treatment change in the controls ($P = 0.02$). Azithromycin was used more in Afs cases in the 2001 cohort compared to 1996, but the difference was not statistically significant (2001: 13 out of 24 cases vs. 1996: 7 out of 19).

DISCUSSION

Comparison of Afs cases with non-sensitized controls, in both 1996 and 2001 cohorts, revealed significantly poorer lung function in Afs cases. Our findings are consistent with Wojnarowski et al.³ who also demonstrated significantly poorer FEV₁ in Af sensitized CF children with elevated serum IgE levels. However, these findings refute those of other studies, such as by Maiz et al.,⁷ who demonstrated a trend towards poorer lung function in their sensitized population, but not a statistically significant difference. The authors attribute this discrepancy to a higher number of lung function tests, per person, in the Wojnarowski et al.³ study. It is clear

however that individual studies have used different immunological criteria to define Af sensitization. For example, in the Wojnarowski series³ only subjects with total IgE >400 kU/L (>400 iu/ml) plus specific Af IgE $\geq$ class 2 (RAST $\geq$ 3.5 iu/ml) were found to have poor pulmonary outcome. In the Maiz et al.⁷ study the immunological criteria for Af sensitization included a total IgE >400 kU/L (>400 iu/ml) and specific Af IgE $\geq$ class 1 (RAST $\geq$ 0.35 iu/ml). This study clearly included a much more heterogeneous population possibly explaining the lack of correlation between pulmonary outcome and sensitization. Brueton et al.⁸ also failed to find a consistent pattern between adverse pulmonary outcome and sensitization in their small series. In this

a FEV₁ %PR longitudinal change in sensitized and control cases
1996 cohort



b FEV₁ %PR longitudinal change in Af sensitized and control cases
2001 cohort

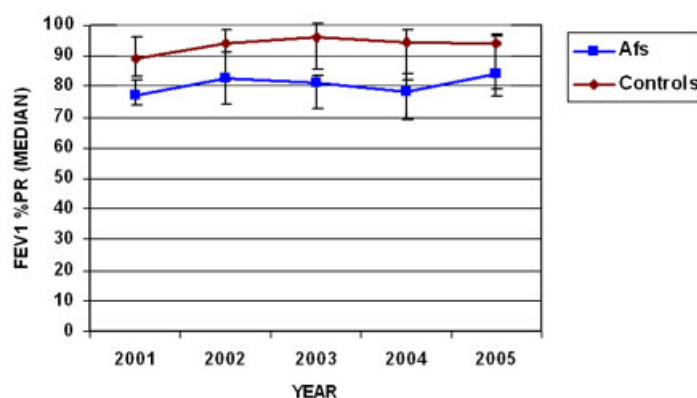
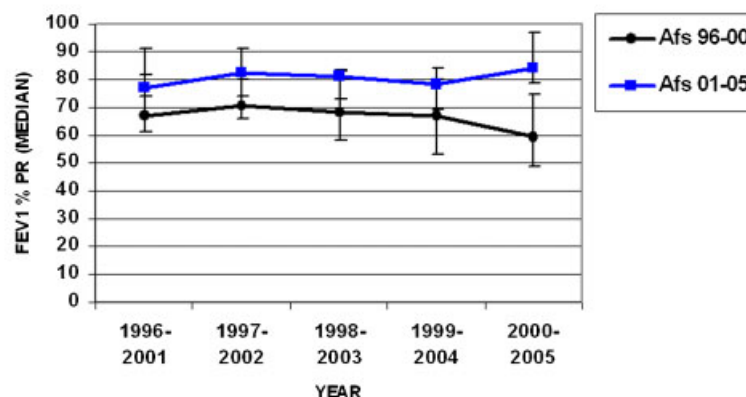


Fig. 3. a: Longitudinal tracking of median FEV₁ percent predicted (%PR) for Afs cases and control subjects (1996 cohorts)—demonstrating lower lung function values in Afs cases. b: Longitudinal tracking of median FEV₁ percent predicted (%PR) for Afs cases and control subjects (2001 cohorts)—demonstrating lower lung function values in Afs cases. c: Longitudinal tracking of median FEV₁ percent predicted (%PR) for Afs cases (1996 vs. 2001 cohorts)—demonstrating higher and maintained lung function in the 2001 Afs cohort. d: Longitudinal tracking of median FEV₁ percent predicted (%PR) for control subjects (1996 vs. 2001 cohorts)—demonstrating higher lung function values in the 2001 control cohort.

c FEV1 %PR longitudinal change in Af sensitized cases
1996 vs 2001 cohort



d FEV1 %PR longitudinal change in control cases
1996 vs 2001 cohort

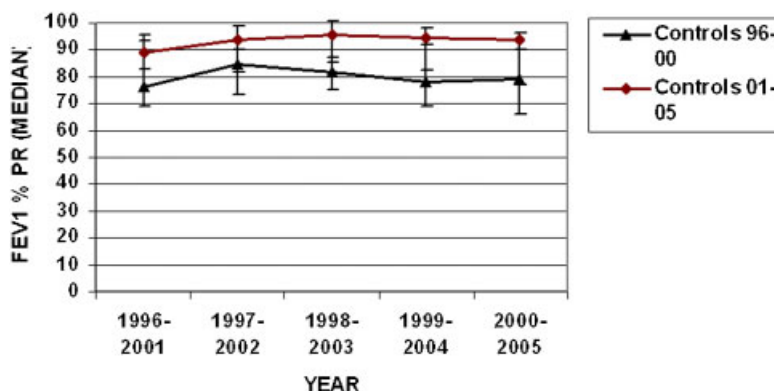


Fig. 3. (Continued)

study however the immunological criteria for sensitization included only the presence of blood eosinophilia and positive skin prick test to Af. The difference with our study is that it confirms there is a population of CF subjects with clearly defined immunological criteria, for sensitization to Af, who have poorer pulmonary function outcome.

Over the course of 5 years we demonstrated a marginal improvement in lung function observed for the 2001 Afs cohort, which coincided with a significant fall in median Af RAST and serum total IgE (Fig. 2a,b). These findings were not observed in the earlier 1996 cohort when lung function deteriorated significantly over the 5 years, although Af markers were not observed to change significantly. The implication is that a reduction in markers of sensitization to Af is associated with better pulmonary function. The findings also imply that in contrast to the 1996 cohort, some factor or intervention may have resulted in a fall in both Af markers and stabilization of lung function during the 5 year follow up period of the 2001 Afs cohort.

Comparing the 2001 with 1996 cohorts there was an improvement in lung function for both Afs and control groups. Analysis of medication data revealed Afs cases in the 2001 cohort were more than four times more likely to receive treatment with either itraconazole and/or voriconazole, one or more times during the 5 year period, in comparison to Afs cases in the 1996 cohort. This was independent of all other treatment schedules. This group demonstrated better pulmonary function. In our center children treated with antifungal agents often remain on them for a minimum period of 2 months but sometimes for much longer periods. Such children are often started in combination with systemic corticosteroids; but the study showed that it was not the greater use of corticosteroids in combination with an antifungal agent but the greater use of antifungal agents themselves that was significant. These findings may suggest more liberal use of treatment with antifungal agents, such as itraconazole or voriconazole, improves pulmonary function in sensitized CF children. This could imply earlier intervention with antifungal

TABLE 2—Pharmacological Management Data

Differences in pharmacological management between 1996 and 2001 cohorts	1996 (n)	2001 (n)	Odds ratio (95% CI)	P-value	P-value (MLR)
Afs cases	19	24			
Prednisolone	9	12	1.11 (0.33 to 3.70)	0.86	0.96
Itraconazole and/or voriconazole	6	16	4.33 (1.19 to 15.69)	0.03	0.05
Prednisolone and itraconazole and/or voriconazole	5	10	2.00 (0.54 to 7.37)	0.29	0.43
Nebulised rhDNase	11	15	1.21 (0.35 to 4.15)	0.76	0.86
Azithromycin	7	13	2.03 (0.59 to 6.93)	0.26	0.60
Inhaled steroids	18	20	0.28 (0.03 to 2.72)	0.25	0.08
IV antibiotics at least once in 5-year period	15	21	1.87 (0.36 to 9.59)	0.45	0.15
IV antibiotics at least five times or more in 5-year period	4	10	2.68 (0.68 to 10.53)	0.15	0.32
Controls	19	23			
Prednisolone	3	7	2.33 (0.51 to 10.67)	0.27	0.97
Nebulised rhDNase	3	10	4.10 (0.93 to 18.08)	0.06	0.07
Azithromycin	6	16	4.95 (1.33 to 18.41)	0.01	0.02
Inhaled steroids	16	18	0.68 (0.14 to 3.28)	0.63	0.13
IV antibiotics at least once	10	16	2.06 (0.58 to 7.29)	0.26	0.77
IV antibiotics at least five times or more in 5 years	2	3	1.28 (0.19 to 8.55)	0.80	0.63

CI, confidence interval; MLR, multiple logistic regression.

therapy in this group of children, might result in improved pulmonary function outcome. We acknowledge however, that because the study was retrospective, it should be considered as hypothesis generating, and that the findings need to be confirmed prospectively.

Previous studies^{15,16} have highlighted the efficacy of triazole antifungals, such as itraconazole and voriconazole, in improving lung function and support their use in CF subjects who are strongly sensitized to Af. However, the studies described have looked at CF patients with ABPA. Because of the difficulties in defining individuals with ABPA, as discussed before, our study included a wider range of patients satisfying just two immunological criteria: Af RAST ≥ 17.5 iu/ml and a positive total serum IgE level ≥ 150 iu/ml. The symptoms with which these patients presented were very heterogeneous. In some cases there was a worsening cough or increased wheeze and shortness of breath. In others, the symptoms were minimal but radiological changes on annual assessment chest X-rays together with rising immunological *Aspergillus* markers prompted therapeutic intervention. However, using the above immunological criteria to define sensitization to Af, we have clearly defined a population of young CF patients who have poorer lung function. Shoseyov et al.¹⁷ demonstrated clinical improvement using antifungal therapy without steroids in a small study of six patients who similarly did not satisfy clinical criteria for ABPA but all of whom grew Af in sputum.

Analysis of our controls in the 2001 cohort revealed they were nearly five times more likely to receive treatment with azithromycin, on one or more occasions during the 5 year period, when compared with the 1996 cohort. This was independent of all other treatment schedules. The duration of azithromycin treatment was

quite variable but many subjects would receive a course for between 4 and 6 months. Controls in the 2001 cohort like their Afs counterparts also demonstrated better pulmonary function. These findings act as a positive control in our study, suggesting that the more liberal use of azithromycin has improved pulmonary function in non-Afs subjects. Previous studies^{18,19} support the argument that increased prescription of azithromycin improves pulmonary function in this group.

Critique of Techniques

A possible shortcoming of the pharmacological aspect of our study could be the failure to correct for multiple subgroup analyses. The study was powered to show a difference in lung function between two groups: Af sensitized and non-sensitized. Subdividing data into subgroups increases the number of statistical tests required to test for an interaction, which in turn raises the probability of false positive results. The way to correct for this is either by applying a stricter criterion for judging statistical significance in terms of a reduced P -value²⁰ or by increasing the number of subjects in the study. For our eight-treatment subgroup analysis however this would have required in excess of 2,560 Afs cases plus controls to provide a large enough sample size. It is doubtful whether such a study could ever be undertaken given the limited number of patients. Also, multicenter recruitment of defined subgroups requires adherence to consistent treatment protocols, something that has not yet been achieved in the UK. An extensive search of the literature revealed no prospective, interventional studies designed to test the efficacy of triazole antifungal agents in Afs cases. In such instances, retrospective studies alone may provide the best

evidence when informing treatment strategies and heightening preventative approaches.

Another shortcoming is our assumption that the 1996 cohorts were adequately matched to 2001 cohorts. In fact, there were significantly more Afs girls than boys in 2001 and controls were significantly older. However, these two factors would, if anything, mitigate against the improvement seen in lung function. The traditional understanding is that lung function of patients deteriorates with age and deteriorates faster in females, although recent evidence suggests this female disadvantage may not necessarily exist.²¹

There was a trend towards cases and controls being referred at an earlier age in the 2001 cohort compared to the 1996 cohort (Table 1). Earlier referral might have resulted in the preservation of lung function by the use of earlier treatment strategies, resulting in improved pulmonary function by the time subjects entered the study in 2001. We also failed to look at the time lag between the diagnosis of Afs and institution of treatment. However, the differences in age at referral were not statistically significant and we predict there would not have been a considerable difference in the onset of treatment between our cohorts. Further possible confounding factors might be ethnic and socioeconomic differences,^{22,23} which were not controlled for in this study.

CONCLUSION

We have shown that children with CF who are sensitized to Af have poorer lung function. Over a 10-year period however lung function improved, during an era when the prescribing of anti-fungal treatment was more common. Previous studies have examined lung function changes in Afs children^{3,7} however none, thus far, have investigated the impact of therapeutic intervention on this group. This novel association suggests that there is now a pressing need for randomized, controlled interventional studies to assess the indications for these agents.

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