

## ORIGINAL ARTICLE

# Intravenous antibiotics reduce the presence of *Aspergillus* in adult cystic fibrosis sputum

Caroline G Baxter,<sup>1,2</sup> Riina Rautemaa,<sup>1,2</sup> Andrew M Jones,<sup>3</sup> A Kevin Webb,<sup>3</sup> Matthew Bull,<sup>4</sup> Eshwar Mahenthalingam,<sup>4</sup> David W Denning<sup>1,2</sup>

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<sup>1</sup>The National Aspergillois Centre, University Hospital of South Manchester, Manchester, UK

<sup>2</sup>Manchester Academic Health Science Centre, University of Manchester, Manchester, UK

<sup>3</sup>Manchester Adult Cystic Fibrosis Centre, University Hospital of South Manchester, Manchester, UK

<sup>4</sup>Organisms and Environment Division, Cardiff School of Biosciences, Cardiff University, Cardiff, South Glamorgan, UK

## Correspondence to

Dr Caroline G Baxter, The National Aspergillois Centre 2nd Floor Education and Research Centre, University Hospital of South Manchester, Southmoor Road, Manchester M23 9LT, UK; [caroline.baxter@manchester.ac.uk](mailto:caroline.baxter@manchester.ac.uk)

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## ABSTRACT

**Background** *Pseudomonas aeruginosa* and *Aspergillus fumigatus* frequently co-colonise the airways of patients with cystic fibrosis (CF). This study aimed to assess the impact of short-term administration of intravenous antipseudomonal antibiotics during CF exacerbations on the presence of *Aspergillus*.

**Methods** Pre- and post-antibiotic sputum samples from 26 adult patients with CF and chronic *Pseudomonas* colonisation were analysed for the presence of *Aspergillus* by fungal culture, real-time PCR and galactomannan antigen (GM). Lung function (forced expiratory volume in 1 s and forced vital capacity % predicted) and blood levels of total IgE, specific *A fumigatus* IgE and specific *A fumigatus* IgG were measured at the start and end of antibiotics. Respiratory viral real-time PCR and bacterial community profiling using ribosomal intergenic spacer analysis (RISA) were performed to estimate concurrent changes in the lung microbiome.

**Results** *Aspergillus* PCR and GM were more sensitive than culture in detecting *Aspergillus* species (culture 8%, GM 31%, PCR 77%). There was a significant decline in the presence of *Aspergillus*, measured both by PCR and GM index, following antibacterial therapy (PCR: median increase in crossing threshold 1.7 (IQR 0.5–3.8),  $p < 0.001$ ; GM: median fall in GM index 0.7 (IQR 0.4–1.6),  $p = 0.016$ ). All patients improved clinically with a significant increase in lung function ( $p < 0.0001$ ). RISA community analysis showed large changes in bacterial community similarity in 67% of patients following antibiotics. Viral RT-PCR demonstrated the presence of a concurrent respiratory virus in 27% of patients.

**Conclusions** Intravenous antibiotics targeting *Pseudomonas* during CF pulmonary exacerbations have a negative impact on the presence of *Aspergillus* in sputum samples.

## BACKGROUND

Recurrent pulmonary exacerbations are a predominant feature for many patients with cystic fibrosis (CF). Traditionally, treatment is with oral or intravenous antibiotics targeting bacteria grown from sputum culture. However, the CF lung microbiome is a complex environment consisting of many different bacteria, viruses and fungi, many of which may be contributing to pulmonary exacerbations. Molecular techniques to identify these organisms have demonstrated a much wider diversity of organisms than described by standard culture.<sup>1,2</sup>

The most common bacterium isolated from the sputum of adult patients with CF is *Pseudomonas*

## Key messages

### What is the key question?

- Does the treatment of cystic fibrosis (CF) pulmonary exacerbations with antipseudomonal antibiotics affect the presence of *Aspergillus* in the sputum?

### What is the bottom line?

- Short-course intravenous antibiotics targeting *Pseudomonas aeruginosa* reduce the presence of *Aspergillus*.

### Why read on?

- An understanding of the interactions between these two important organisms within CF lungs could alter future therapeutic strategies and improve prognosis.

*aeruginosa*, which chronically colonises CF airways in up to 75% of adult patients.<sup>3</sup> The propensity for chronic infection is aided by biofilm formation driven by quorum sensing.<sup>4</sup> Biofilm formation is also thought to be a feature of *Aspergillus fumigatus* colonisation, the most prevalent filamentous fungus causing disease in CF.<sup>5,6</sup> *A fumigatus* is cultured from the sputum of 12–57% of patients with CF, but significantly higher rates of detection have been demonstrated by real-time PCR (RT-PCR).<sup>7,8</sup> The interaction between these two common organisms and their biofilms is a recent topic of interest, with studies showing that *P aeruginosa* inhibits *A fumigatus* growth in vitro, possibly due to secretion of small diffusible molecules.<sup>9,10</sup>

The short-term use of intravenous antipseudomonal antibiotics to treat pulmonary exacerbations is known to reduce *P aeruginosa* biomass transiently, but the concurrent effect on the presence of *A fumigatus* has not been studied.<sup>11</sup> However, long-term antibiotics (both oral and nebulised) used routinely to reduce pulmonary exacerbations and improve lung function have been linked to greater rates of *A fumigatus* colonisation, the mechanism and clinical impact of which is unknown.<sup>12,13</sup> While bacterial diversity appears to correlate with the use of long-term antibiotics, short-term antibiotics used for pulmonary exacerbations for 14–21 days have relatively little impact on diversity but do reduce overall bacterial biomass.<sup>11</sup> Any additional effect that these antibiotics have on organisms other than bacteria is largely unknown.

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Furthermore, the agonist and antagonist relationships created between organisms during treatment are abundant but poorly understood.<sup>14 15</sup>

Determining the interactions between these two important organisms may result in new therapeutic strategies and improve prognosis for patients with CF. This study aimed to establish whether intravenous antibiotic therapy, targeting *P aeruginosa* during CF pulmonary exacerbations, affects the presence of *Aspergillus* determined by real-time PCR and galactomannan (GM) assay. Secondary aims were to estimate changing bacterial community profiles using ribosomal intergenic spacer analysis (RISA) and to assess the prevalence of concurrent viral respiratory infections.

## METHODS

### Study design and patient selection

This was a prospective observational cohort study. Patients were enrolled into the study from the Manchester Adult Cystic Fibrosis Centre, UK; all gave written informed consent to participate. Inclusion criteria included age  $\geq 18$  years, confirmed diagnosis of CF by genetic testing and/or sweat testing, chronic pulmonary *P aeruginosa* colonisation (determined by recurrent positive sputum cultures for  $> 1$  year) and recent onset pulmonary exacerbation leading to a decline in health status and lung function. The first 30 patients who met the inclusion criteria between November 2010 and March 2011 were recruited to the study. Patients were seen initially by CF specialist clinicians independent of the study and assessed with regard to their need for intravenous antibiotics and their suitability for outpatient treatment. Clinical samples were collected on day 1, immediately prior to antibiotic commencement (pre-antibiotics), and on day 14, following the last dose of antibiotics (post-antibiotics). Patients administered their own antibiotics at home for 14 days and then attended an end of treatment outpatient appointment. Tobramycin drug levels were monitored to assess toxicity and compliance.

### Demographics and lung function

Baseline data were collected from patient records at enrollment and included demographic details, CF comorbidities, prior sputum microbiology and inhaled/oral medical treatments. Lung function (forced expiratory volume in 1 s (FEV<sub>1</sub>) and forced vital capacity (FVC)) was performed pre- and post-antibiotics by experienced technicians according to European Respiratory Society guidelines.<sup>16</sup>

### Sputum collection

Patients produced two non-induced sputum samples pre-antibiotics and one sample post-antibiotics. Samples were refrigerated at  $\pm 4^{\circ}\text{C}$  and processed within 24 h of collection. One of the two pre-antibiotic sputum samples was used for fungal culture, *Aspergillus* PCR and GM detection and the other sample was used for respiratory viral RT-PCR.

### Sputum fungal culture

An equal volume of Sputasol (Oxoid, Basingstoke, UK) was added to the sputum sample and culture performed according to the Health Protection Agency National Standards Method BSOP 57 but modified to plate 10  $\mu\text{l}$  rather than 1  $\mu\text{l}$  of sputum onto three Sabouraud dextrose agar plates (SABC, Oxoid).<sup>17</sup> Sputum samples then underwent further homogenisation by sonication, as described previously, and culture was repeated.<sup>8</sup> Plates were incubated at 25°C, 37°C and 45°C for 72 h with

daily inspection to record growth and colony forming units (CFUs). Fungal colonies were identified by microscopy.

### Galactomannan

The Platelia *Aspergillus* enzyme immunoassay (Bio-Rad, Marnes-La-Coquette, France) was used to detect GM. Three hundred microlitres of homogenised sputum was processed in accordance with the manufacturer's instructions for serum samples and an optical density index of  $\geq 0.5$  was considered positive. Our group has demonstrated this assay to have an intra-assay coefficient of variation (CV) of 5% by simultaneously testing two aliquots of sputum from 20 patients with CF and an inter-assay CV of 9% when testing 12 samples each day over 5 days (C G Baxter, 2012, unpublished).

### *Aspergillus* PCR

Fungal DNA was extracted from 3 ml of the remaining homogenised sputum sample using the commercial fungal DNA extraction kit MycXtra (Myconostica, UK) according to the manufacturer's instructions. This kit removes inhibiting substances such as dornase  $\alpha$  and inhaled antibiotics and then uses bead beating to release DNA. *Aspergillus* DNA was detected using the commercial RT-PCR assay MycAssay *Aspergillus* (Myconostica), targeting a portion of the 18S ribosomal gene, on a SmartCycler RT-PCR instrument (Cepheid, California, USA); 10  $\mu\text{l}$  of DNA template was used in 25  $\mu\text{l}$  reactions. The manufacturer's instructions were followed in the processing of all DNA extractions except that suggested cut-off values were disregarded and the limit of blank, a crossing threshold (Ct) value of  $< 38$ , was considered positive. This DNA extraction and RT-PCR assay has good reproducibility in CF sputum: our group has demonstrated an intra-assay CV of 1.5% when 10 homogenised sputum samples were split, DNA extracted and RT-PCR performed and an inter-assay CV of 1.1% when 40 extracted DNA samples were run through the RT-PCR assay on two occasions (C G Baxter, 2012, unpublished).

### Ribosomal intergenic spacer analysis (RISA)

Following *Aspergillus* PCR, the remainder of the extracted DNA was used for bacterial diversity profiling by RISA. Intergenic spacer sizes vary between different bacterial species. Briefly, 20 ng of total sputum DNA (quantified using a NanoDrop 1000 spectrophotometer) and the RISA primers (1406F, 5'-TGYACACACCGCCCGT-3' and 23R, 5'-GGGT TBCCCCATTCRG-3'; each at a final concentration of 10 pmol/ $\mu\text{l}$  in the reaction) were combined in a 25  $\mu\text{l}$  PCR reaction using standard reagents (Qiagen, Crawley, UK) and amplified as described elsewhere.<sup>18</sup> The amplified intergenic spacer regions were then separated on an Agilent 2100 BioAnalyser (Agilent, Woking, UK) using the DNA 7500 microfluidics kit as described elsewhere.<sup>19</sup> The resulting bacterial diversity profiles were then analysed using GelCompar II (Applied Maths, Gent, Belgium) and before/after percentage similarity was calculated.

### Viral PCR

The method and results of viral PCR can be found in the online data supplement (S1).

### Serology

A blood sample was taken from each patient pre- and post-antibiotics. Each sample was tested for total IgE (tIgE), specific *A fumigatus* IgE (sIgE) and specific *A fumigatus* IgG (sIgG) using the ImmunoCap assay (Phadia, Uppsala, Sweden).

## Statistics

SPSS V.16 (Chicago, USA) was used to analyse all results. The results were non-parametric and were compared using the Wilcoxon signed rank test and the Mann–Whitney U test.

## RESULTS

### Baseline demographics

Thirty patients gave consent to participate; 26 completed the study and four failed to attend their post-antibiotics appointment. The baseline demographic and clinical details are shown in table 1. The patients received dual intravenous antibiotics for 14 days; all patients received tobramycin and, in addition, 11 received ceftazidime, 11 meropenem, two piperacillin/tazobactam (Tazocin) and two aztreonam.

### Sputum culture and PCR

Fifty-two sputum samples (pre- and post-antibiotic samples from 26 patients) were cultured. Routine fungal culture following Sputasol homogenisation showed no growth in 24 samples and yeast in 28 samples, whereas culture after additional sonication showed no growth in 18 samples, yeast in 32 samples and *A fumigatus* in two samples. The two samples culturing *A fumigatus* were both pre-antibiotic samples. Yeast identification was not performed.

Yeast was present in 16 patient samples pre-antibiotics and 16 post-antibiotics, but four patients became negative for yeast while four became positive. The four patients who became negative had very low numbers of CFUs (<4) pre-antibiotics. For the 16 patients with yeast cultured post-antibiotics, there was a significant increase in CFUs (post-sonication counts) after antibiotics (median CFU pre-antibiotics 4 (IQR 5–66), median CFU post-antibiotics 16 (IQR 4–163), Wilcoxon signed rank test  $Z=-3.47$ ,  $p<0.001$ ,  $r=0.61$ ).

Twenty of the 26 patients (77%) had a positive *Aspergillus* PCR pre-antibiotics, of which 15 remained positive post-antibiotics. Six patients were PCR negative on both samples.

There was a statistically significant increase in the PCR Ct value (indicating less DNA) between pre- and post-antibiotic sputum samples (median increase in Ct 1.7 (IQR 0.5–3.8), Wilcoxon signed rank test  $Z=3.8$ ,  $p<0.001$ ,  $r=0.52$ ; table 2). Applying the standard curve of genomic concentrations against Ct values developed from serum samples, there was a median reduction in *Aspergillus* genomes of 91 (IQR 26–460) post-antibiotics (Wilcoxon signed rank test  $Z=-3.12$ ,  $p=0.002$ ).<sup>20</sup> This standard curve compares well with manufacturer data of extraction from spore solutions and with our data of extraction efficiency from CF sputum.<sup>8 21</sup> There were no correlations between antibiotic regime and PCR results.

### Galactomannan

GM was positive in eight patients (31%) pre-antibiotics and in six patients (23%) post-antibiotics. Three patients converted from positive to negative while one converted from negative to positive. Seventeen patients remained GM negative in both sputum samples (table 2). There was a significant fall in GM index for the eight pre-antibiotic positive patients (median fall in GM index 0.7 (IQR 0.4–1.6)), (Wilcoxon signed rank test  $Z=-2.42$ ,  $p=0.016$ ,  $r=0.5$ ).

A comparison of *Aspergillus* PCR with GM index showed that 15 patients were PCR positive on both samples, five of whom remained GM positive while 10 remained GM negative; five patients changed from PCR positive to negative, three of whom became GM negative and two were GM negative on both samples; six patients were PCR negative on both samples, five were also GM negative on both samples while one was GM positive after antibiotic therapy (table 2).

### Ribosomal intergenic spacer analysis (RISA)

Total bacterial community profiling by RISA was only performed for the first six patients enrolled due to financial and time constraints (figure 1). A large change in community similarity following intravenous antibiotics was seen for four of the six patients. Pre-antibiotic and post-antibiotic profile similarities were 90%, 89%, 76% and 59%. Two patients showed little alteration in response to treatment (95% similarities). There was no correlation between degree of change in community similarity and changes in *Aspergillus* PCR. Although community similarity changed, actual diversity of bacteria did not alter.

### Serology

Three patients had a tIgE >500 kIU/l, 14 patients had a sIgE ≥class 2 (0.7 kUa/l) and 20 patients had a sIgG >40 mg/l. Although all parameters fell with treatment, there was no statistically significant change in any parameter (table 3). However, patient numbers were small, meaning that the study was underpowered to detect small differences. Subanalysis of patients showed that those with a fall in *Aspergillus* DNA (rise in Ct value) or a fall in both *Aspergillus* DNA and GM index similarly had no significant changes in immunological parameters (table 3). However, there was a significant difference between baseline sIgG levels in those with positive PCR and GM pre-antibiotics ( $n=8$ ) compared with those with negative PCR and GM ( $n=6$ ) (median 118 mg/l (IQR 84–145) and 59 mg/l (IQR 43–83), respectively). Similarly, the fall in sIgG was significantly greater in those with positive PCR and GM pre-antibiotics (median fall 15 mg/l (IQR 7–23) and  $-4$  mg/l (IQR 10–11.5), respectively; Mann–Whitney U test  $Z=-2.39$ ,  $p=0.013$ ). These differences were not seen for tIgE or sIgE.

**Table 1** Patient baseline clinical and demographic details: data are expressed and number (%) or mean±SD

Baseline clinical characteristics	n=26
Age (years)	25±8
Male	14 (54%)
F508 del homozygous	15 (58%)
BMI (kg/m <sup>2</sup> )	21±3
CFRD	8 (31%)
Pancreatic insufficiency	25 (96%)
Inhaled steroids	21 (81%)
Long-term azithromycin	25 (96%)
Long-term nebulised antibiotics	25 (96%)
Triazole therapy >3 months prior to enrolment	2 (8%)
Chronic <i>Pseudomonas</i>	26 (100%)
Chronic <i>Burkholderia</i> spp	1 (4%)
Chronic MSSA	9 (35%)
Chronic MRSA	0 (0%)
NTM	1 (4%)
FEV <sub>1</sub> % predicted	42±15
FVC % predicted	59±17

BMI, body mass index; CFRD, cystic fibrosis-related diabetes; FEV<sub>1</sub>, forced expiratory volume in 1 s; FVC, forced vital capacity; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*; NTM, non-tuberculous mycobacteria.

**Table 2** *Aspergillus* PCR and GM pre- and post-antibiotics

Patient	<i>Aspergillus</i> PCR Ct 1	<i>Aspergillus</i> PCR Ct 2	Change in <i>Aspergillus</i> PCR Ct	GM index 1	GM index 2	Change in GM index
1	36.3	37.8	1.5	7.13	2.48	-4.65
2	32.5	36.2	3.7	9.38	6.04	-3.34
3	32.4	32.9	0.5	2.97	0.86	-2.11
4	33.5	33.9	0.4	8.83	4.14	-4.69
5	35.0	36.3	1.3	0.73	0.70	-0.03
6	32.3	32.7	0.4	0.22	0.00	-0.22
7	33.1	36.2	3.1	0.00	0.12	0.12
8	30.4	33.3	2.9	0.00	0.00	0
9	31.3	33.3	2.0	0.19	0.16	-0.03
10	31.1	33.1	2.0	0.03	0.00	-0.03
11	31.3	33.6	2.3	0.30	0.19	-0.11
12	34.3	33.7	-0.6	0.00	0.00	0
13	30.8	34.9	4.1	0.00	0.00	0
14	35.4	36.6	1.2	0.06	0.00	-0.06
15	33.5	35.4	2.2	0.29	0.25	-0.04
16	30.4	38.0	7.6	2.92	0.27	-2.65
17	32.4	38.0	5.6	9.20	0.00	-9.20
18	33.8	38.0	4.2	1.28	0.12	-1.16
19	31.7	38.0	6.3	0.00	0.02	0.02
20	33.4	38.0	4.6	0.02	0.00	-0.02
21	38.0	38.0	0	0.45	10.53	10.08
22	38.0	38.0	0	0.00	0.00	0
23	38.0	38.0	0	0.25	0.39	0.13
24	38.0	38.0	0	0.49	0.39	-0.10
25	38.0	38.0	0	0.01	0.00	-0.01
26	38.0	38.0	0	0.00	0.01	0.01

Ct, crossing threshold; GM, galactomannan.

### Lung function

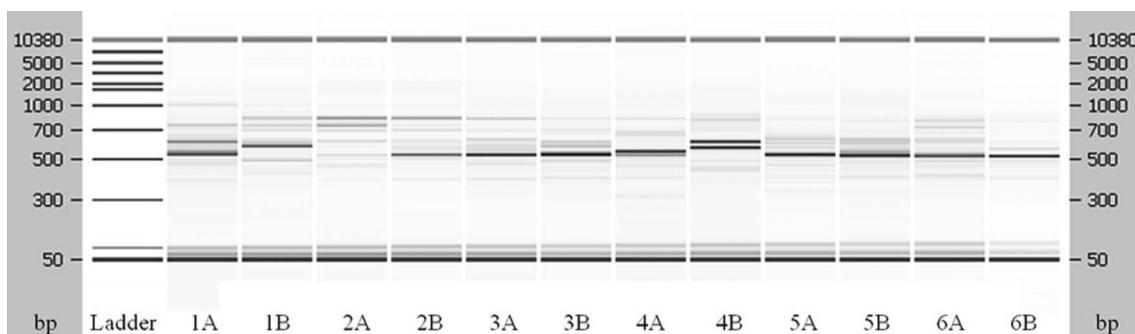
Patients receiving intravenous antibiotic therapy demonstrated an improvement in both FEV<sub>1</sub> ( $Z=-4.29$ ,  $p<0.001$ ,  $r=0.60$ ) and FVC ( $Z=-4.46$ ,  $p<0.001$ ,  $r=0.62$ ; table 4). The rise in FEV<sub>1</sub> was greater for patients with positive *Aspergillus* PCR at the start of treatment than for those with negative PCR (Mann-Whitney U test  $Z=-2.02$ ,  $p=0.046$ ,  $r=0.40$ ).

### DISCUSSION

There is growing evidence for the wide microbial diversity within CF airways, but the dynamic interspecies communications within communities has only just begun to be investigated.<sup>15</sup> This study has shown a significant reduction in

*Aspergillus* species, measured both by PCR and GM index, following antibacterial therapy targeting *P aeruginosa* colonisation. *P aeruginosa* colony counts were not performed in this study but previous studies have confirmed a significant reduction in colony counts with antipseudomonal antibiotics.<sup>11 22</sup>

The observed reduction in *Aspergillus* species with *Pseudomonas* treatment was unexpected as previous studies have suggested an increased prevalence of *A fumigatus* colonisation with the use of both oral and nebulised antibiotics.<sup>12 13</sup> Furthermore, in vitro studies have suggested that *P aeruginosa* inhibits *A fumigatus* growth and biofilm formation by secretion of small carbon chain molecules and the phenazine pyocyanin.<sup>10 23</sup> Thus, it was expected that a reduction in *P aeruginosa*



**Figure 1** Ribosomal intergenic spacer analysis (RISA) profiles comparing community similarity of sputum samples from six patients (A) pre- and (B) post-intravenous antibiotics. The ladder provides a reference to estimate the size of RISA PCR products. Each lane shows the total bacterial population from each sputum sample. The change in community similarity for the six paired profiles shows: 1A-B=76%, 2A-B=90%, 3A-B=95%, 4A-B=59%, 5A-B=95% and 6A-B=89%.

**Table 3** Changes in immunological parameters pre- and post-intravenous antibiotics

	Total IgE (kU/l)		Wilcoxon signed rank test	<i>A fumigatus</i> specific IgE (kUa/l)		Wilcoxon signed rank test	<i>A fumigatus</i> specific IgG (mg/l)		Wilcoxon signed rank test
	Pre	Post		Pre	Post		Pre	Post	
All patients (n=26)	78 (28–230)	62 (25–220)	Z=−0.47 p=0.64	1.5 (0.0–8.9)	1.3 (0–7.7)	Z=−0.73 p=0.47	73 (50–112)	63 (51–100)	Z=−1.82 p=0.07
Patients with a fall in <i>Aspergillus</i> DNA (n=19)	110 (28–250)	79 (31–290)	Z=−1.19 p=0.24	7.8 (0.0–10.6)	7.0 (0.0–8.1)	Z=−0.93 p=0.35	78 (65–113)	75 (55–102)	Z=−1.50 p=0.13
Patients with a fall in <i>Aspergillus</i> DNA and GM index (n=8)	104 (14–293)	61 (21–253)	Z=−1.68 p=0.09	0.6	0.6	Z=0.00 p=1.00	118 (84–145)	103 (72–143)	Z=−1.12 p=0.26

Values are shown as median (IQR).  
GM, galactomannan.

may lead to an increase in *A fumigatus*. The reasons for the observed fall in *Aspergillus* are unclear. Unlike short-term treatment, long-term antibiotics reduce bacterial diversity which may select more favourable growth conditions and host immunological responses for *Aspergillus* colonisation.<sup>11</sup> The effect of short-term intravenous antibiotics on biofilms must also be considered. It is important to note that the inhibitory effects of *P aeruginosa* on *A fumigatus* were only found to be significant by Mowat *et al*<sup>10</sup> prior to biofilm formation, in keeping with the incomplete inhibition seen during the in vitro studies by Kerr *et al*.<sup>9</sup> *P aeruginosa* biofilms offer some protection against antibacterial effects, as do *A fumigatus* biofilms against antifungal susceptibility,<sup>24, 25</sup> but biofilms can be disrupted by antibiotics such as macrolides.<sup>26</sup> Biofilm disruption may allow *P aeruginosa* to re-exert its inhibitory effects on *A fumigatus* growth. The interdependence between organisms for survival within the lung has not been studied, but it is also possible that *A fumigatus* may use *P aeruginosa* biofilms for host immune protection and favourable growth conditions meaning disruption would inhibit the presence of both organisms. These concepts are speculative and further research could have significant implications for CF therapeutic strategies and prognosis.

Total bacterial diversity and community profiling was only performed in this study for a very limited number of patients, which is a significant limitation of this aspect of the work. However, large changes in community similarity were seen in four of six patients. Total bacterial diversity may not change significantly, but the abundance of particular bacteria in each community does change as represented by the altering intensities of PCR bands (figure 1). This is supported by a recent study by Tunney *et al* which showed that changes in bacterial abundance for aerobes was greater than for anaerobes, with *P aeruginosa* being affected most when using antipseudomonal antibiotics.<sup>11</sup> These changes in community profile may also impact on the presence and growth of *Aspergillus* as bacteria other than *P aeruginosa* can inhibit fungal growth.<sup>27</sup> Future studies with greater

patient numbers would clearly benefit from full bacteriological culture and more robust molecular analysis of changing bacterial populations during antibiotic therapy to improve our understanding of the dynamic parallel changes in fungal populations within this context.

Both *Aspergillus* PCR and GM were more sensitive than culture for the detection of *Aspergillus* species (culture 8%, GM 31%, PCR 77%). PCR detects both live and dead organisms along with dormant spores whereas GM is predominantly produced by hyphae in the logarithmic phase of growth and is a major component of the biofilm.<sup>6, 28</sup> This may account for the differences in pre-antibiotic detection rates between these two tests as a large number of patients with CF are thought to have simple colonisation with inert or dead *A fumigatus* spores while fewer have active hyphal growth. This is also supported by the significant difference observed for specific *A fumigatus* IgG levels between patients with positive PCR and GM pre-antibiotics (n=8) and those without (n=6) (median sIgG 118 mg/l and 59 mg/l, respectively). The patients with positive PCR and GM pre-antibiotics also showed a trend towards a reduction in tIgE, sIgG, *Aspergillus* DNA and GM index post-antibiotics. It is not known how long antibody concentrations take to change significantly, but this study was conducted over a short time period and patient numbers were not powered to detect these serological changes.

One patient receiving piperacillin/tazobactam showed an increase in GM after antibiotics while remaining PCR negative. Intravenous antibiotics including piperacillin/tazobactam have been reported to lead to false positive results from serum GM analysis.<sup>29</sup> This is thought to be due to assay cross-reactivity with non-*Aspergillus* carbohydrate chains rather than contamination of products, and GM index levels are usually not very high. The rise in GM index in this case was high at 10.5 and no other samples suggested cross-reactivity. However, *Aspergillus* PCR was negative, in keeping with an alternative source of GM. GM is not specific to *Aspergillus* and can be found in other

**Table 4** Changes in lung function pre- and post-antibiotics

	All patients (n=26)			Positive <i>Aspergillus</i> PCR (n=20)			Negative <i>Aspergillus</i> PCR (n=6)		
	Pre-antibiotics	Post-antibiotics	p Values	Pre-antibiotics	Post-antibiotics	p Values	Pre-antibiotics	Post-antibiotics	p Values
FEV <sub>1</sub> % predicted	30 (22–36)	36 (26–50)	<0.001	33 (25–38)	39 (30–51)	<0.001	22 (21–33)	24 (22–43)	0.07
FVC % predicted	49 (35–59)	59 (44–69)	<0.001	52 (36–60)	63 (49–70)	<0.001	35 (31–47)	41 (36–64)	0.03

Values are shown as median (IQR).  
Changes in lung function were measured using Wilcoxon signed rank tests.  
FEV<sub>1</sub>, forced expiratory volume in 1 s; FVC, forced vital capacity.

fungi and *Candida* cell walls. This patient did not grow any yeast or other fungi pre- or post-antibiotics, but the influence of oropharyngeal flora is a possible caveat in this study as separate oral sampling was not performed. Our previous research has indicated that the prevalence of both *A fumigatus* and *Candida* species from oral rinse samples are very low during non-exacerbation periods.<sup>30</sup> However, intravenous antibiotics may predispose to oral *Candida* infections which could potentially influence GM results post-antibiotics.

In summary, this study has shown value for both PCR and GM in monitoring *Aspergillus* concentrations and growth in CF sputum. Short-term intravenous antibiotics targeting *P aeruginosa* appear to have a negative impact on the presence of *Aspergillus*. The clinical impact of this observation requires further research.

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## REFERENCES

- Nagano Y, Elborn JS, Millar BC, *et al.* Comparison of techniques to examine the diversity of fungi in adult patients with cystic fibrosis. *Med Mycol* 2010;48:166–76 e1.
- Bittar F, Rolain JM. Detection and accurate identification of new or emerging bacteria in cystic fibrosis patients. *Clin Microbiol Infect* 2010;16:809–20.
- 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;7:123–7.
- Singh PK, Schaefer AL, Parsek MR, *et al.* Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. *Nature* 2000;407:762–4.
- Muller FM, Seidler M, Beauvais A. *Aspergillus fumigatus* biofilms in the clinical setting. *Med Mycol* 2011;49(Suppl 1):S96–S100.
- Loussert C, Schmitt C, Prevost MC, *et al.* In vivo biofilm composition of *Aspergillus fumigatus*. *Cell Microbiol* 2009;12:405–10.
- Bakare N, Rickerts V, Bargon J, *et al.* Prevalence of *Aspergillus fumigatus* and other fungal species in the sputum of adult patients with cystic fibrosis. *Mycoses* 2003;46:19–23.
- Baxter CG, Jones AM, Webb K, *et al.* Homogenisation of cystic fibrosis sputum by sonication—an essential step for *Aspergillus* PCR. *J Microbiol Methods* 2011;85:75–81.
- Kerr JR. Suppression of fungal growth exhibited by *Pseudomonas aeruginosa*. *J Clin Microbiol* 1994;32:525–7.
- Mowat E, Rajendran R, Williams C, *et al.* *Pseudomonas aeruginosa* and their small diffusible extracellular molecules inhibit *Aspergillus fumigatus* biofilm formation. *FEMS Microbiol Lett* 2010;313:96–102.
- Tunney MM, Klem ER, Fodor AA, *et al.* Use of culture and molecular analysis to determine the effect of antibiotic treatment on microbial community diversity and abundance during exacerbation in patients with cystic fibrosis. *Thorax* 2011;66:579–84.
- Jubin V, Ranque S, Stremier Le Bel N, *et al.* Risk factors for *Aspergillus* colonization and allergic bronchopulmonary aspergillosis in children with cystic fibrosis. *Pediatr Pulmonol* 2010;45:764–71.
- 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;93:835–8.
- Han MK, Huang YJ, Lipuma JJ, *et al.* Significance of the microbiome in obstructive lung disease. *Thorax* 2012;67:456–63.
- Harrison F. Microbial ecology of the cystic fibrosis lung. *Microbiology* 2007;153:917–23.
- Miller MR, Hankinson J, Brusasco V, *et al.* Standardisation of spirometry. *Eur Respir J* 2005;26:319–38.
- Health Protection Agency. UK Standards for Microbiology Investigations. Investigation of bronchoalveolar lavage, sputum and associated specimens; BSOP 57, 2008. [http://www.hpa.org.uk/web/HPAwebFile/HPAweb\\_C/1317132860548](http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1317132860548) (accessed 24 Nov 2012).
- Borneman J, Triplett EW. Molecular microbial diversity in soils from eastern Amazonia: evidence for unusual microorganisms and microbial population shifts associated with deforestation. *Appl Environ Microbiol* 1997;63:2647–53.
- White J, Gilbert J, Hill G, *et al.* Culture-independent analysis of bacterial fuel contamination provides insight into the level of concordance with the standard industry practice of aerobic cultivation. *Appl Environ Microbiol* 2011;77:4527–38.
- White PL, Perry MD, Moody A, *et al.* Evaluation of analytical and preliminary clinical performance of Myconostica MycAssay *Aspergillus* when testing serum specimens for diagnosis of invasive Aspergillosis. *J Clin Microbiol* 2011;49:2169–74.
- Myconostica. MycXtra DNA extraction—effective extraction of fungal DNA from clinical respiratory samples. <http://myconostica.co.uk/mycextra> (accessed 24 Nov 2012).
- Regelmann WE, Elliott GR, Warwick WJ, *et al.* Reduction of sputum *Pseudomonas aeruginosa* density by antibiotics improves lung function in cystic fibrosis more than do bronchodilators and chest physiotherapy alone. *Am Rev Respir Dis* 1990;141:914–21.
- Kerr JR, Taylor GW, Rutman A, *et al.* *Pseudomonas aeruginosa* pyocyanin and 1-hydroxyphenazine inhibit fungal growth. *J Clin Pathol* 1999;52:385–7.
- Kobayashi H. Airway biofilms: implications for pathogenesis and therapy of respiratory tract infections. *Treat Respir Med* 2005;4:241–53.
- Seidler MJ, Salvenmoser S, Muller FM. *Aspergillus fumigatus* forms biofilms with reduced antifungal drug susceptibility on bronchial epithelial cells. *Antimicrob Agents Chemother* 2008;52:4130–6.
- Lutz L, Pereira DC, Paiva RM, *et al.* Macrolides decrease the minimal inhibitory concentration of anti-pseudomonal agents against *Pseudomonas aeruginosa* from cystic fibrosis patients in biofilm. *BMC Microbiol* 2012;12:196.
- Yadav V, Gupta J, Mandhan R, *et al.* Investigations on anti-*Aspergillus* properties of bacterial products. *Lett Appl Microbiol* 2005;41:309–14.
- Hope WW, Petraitis V, Petraitis R, *et al.* The initial 96 hours of invasive pulmonary aspergillosis: histopathology, comparative kinetics of galactomannan and (1->3) beta-d-glucan and consequences of delayed antifungal therapy. *Antimicrob Agents Chemother* 2010;54:4879–86.
- Boonsarngsuk V, Niyompattama A, Teosirimongkol C, *et al.* False-positive serum and bronchoalveolar lavage *Aspergillus* galactomannan assays caused by different antibiotics. *Scand J Infect Dis* 2010;42:461–8.
- Baxter CG, Moore CB, Jones AM, *et al.* IgE mediated immune responses and airway detection of *Aspergillus* and *Candida* in adult cystic fibrosis. *Chest*. Published Online First: 8 November 2012. doi:10.1378/chest.12–1363