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

Caroline G Baxter,1,2 Riina Rautemaa,1,2 Andrew M Jones,3 A Kevin Webb,3 Matthew Bull,4 Eshwar Mahenthiralingam,4 David W Denning1,2

**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.1,2

The most common bacterium isolated from the sputum of adult patients with CF is *Pseudomonas aeruginosa*, which chronically colonises CF airways in up to 75% of adult patients.3 The propensity for chronic infection is aided by biofilm formation driven by quorum sensing.4 Biofilm formation is also thought to be a feature of *Aspergillus fumigatus* colonisation, the most prevalent filamentous fungus causing disease in CF.5,6 *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).7,8 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.9,10

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.11 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.12,13

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.11 Any additional effect that these antibiotics have on organisms other than bacteria is largely unknown.
Furthermore, the agonist and antagonist relationships created between organisms during treatment are abundant but poorly understood.14 15

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 ≥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 enrolment and included demographic details, CF comorbidities, prior sputum microbiology and inhaled/oral medical treatments. Lung function (forced expiratory volume in 1 s (FEV₁) and forced vital capacity (FVC)) was performed pre- and post-antibiotics by experienced technicians according to European Respiratory Society guidelines.16

Sputum collection

Patients produced two non-induced sputum samples pre-antibiotics and one sample post-antibiotics. Samples were refrigerated at ±4°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 μl rather than 1 μl of sputum onto three Sabouraud dextrose agar plates (SABC, Oxoid).17 Sputum samples then underwent further homogenisation by sonication, as described previously, and culture was repeated.8 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 ≥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 α 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 μl of DNA template was used in 25 μl 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’-TGYACACACCGCCGT-3’ and 23R, 5’-GGGTT TBCCCCATTCRG-3’; each at a final concentration of 10 pmol/μl in the reaction) were combined in a 25 μl PCR reaction using standard reagents (Qiagen, Crawley, UK) and amplified as described elsewhere.18 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.19 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 (sIgE), specific *A fumigatus* IgE (sIgE) and specific *A fumigatus* IgG (sIgG) using the ImmunoCap assay (Phadia, Uppsala, Sweden).


Thorax: first published as 10.1136/thoraxjnl-2012-202412 on 19 March 2013. Downloaded from http://thorax.bmj.com/ on April 5, 2024 by guest. Protected by copyright.
Cystic fibrosis

Statistics
SPSS V16 (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).20 This standard curve compares well with manufacturer data of extraction from spore solutions and with our data of extraction efficiency from CF sputum.

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 kU/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 tlgE or sIgE.

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

<table>
<thead>
<tr>
<th>Baseline clinical characteristics</th>
<th>n=26</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>25±8</td>
</tr>
<tr>
<td>Male</td>
<td>14 (54%)</td>
</tr>
<tr>
<td>F508 del homozygous</td>
<td>15 (58%)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21±3</td>
</tr>
<tr>
<td>CFRD</td>
<td>8 (31%)</td>
</tr>
<tr>
<td>Pancreatic insufficiency</td>
<td>25 (96%)</td>
</tr>
<tr>
<td>Inhaled steroids</td>
<td>21 (81%)</td>
</tr>
<tr>
<td>Long-term azithromycin</td>
<td>25 (96%)</td>
</tr>
<tr>
<td>Long-term nebulised antibiotics</td>
<td>25 (96%)</td>
</tr>
<tr>
<td>Triazole therapy &gt;3 months prior to enrolment</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>Chronic Pseudomonas</td>
<td>26 (100%)</td>
</tr>
<tr>
<td>Chronic Burkholderia spp</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Chronic MSSA</td>
<td>9 (35%)</td>
</tr>
<tr>
<td>Chronic MRSA</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>NTM</td>
<td>1 (4%)</td>
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<tr>
<td><strong>FEV₁% predicted</strong></td>
<td>42±15</td>
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<tr>
<td><strong>FVC% predicted</strong></td>
<td>59±17</td>
</tr>
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</table>

BMI, body mass index; CFRD, cystic fibrosis-related diabetes; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; MRSA, methicillin-resistant Staphylococcus aureus; MSSA, methicillin-sensitive Staphylococcus aureus; NTM, non-tuberculous mycobacteria.
Lung function

Patients receiving intravenous antibiotic therapy demonstrated an improvement in both FEV₁ (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 FEV₁ 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.15 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.11 22

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.12 13 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.10 23 Thus, it was expected that a reduction in P aeruginosa

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

<table>
<thead>
<tr>
<th>Patient</th>
<th>Aspergillus PCR Ct 1</th>
<th>Aspergillus PCR Ct 2</th>
<th>Change in Aspergillus PCR Ct</th>
<th>GM index 1</th>
<th>GM index 2</th>
<th>Change in GM index</th>
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<tr>
<td>1</td>
<td>36.3</td>
<td>37.8</td>
<td>1.5</td>
<td>7.13</td>
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<td>2</td>
<td>32.5</td>
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<td>3.7</td>
<td>9.38</td>
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<td>−3.34</td>
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<td>3</td>
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<td>2.92</td>
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<tr>
<td>26</td>
<td>38.0</td>
<td>38.0</td>
<td>0</td>
<td>0.00</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Ct, crossing threshold; GM, galactomannan.

![Figure 1](http://thorax.bmj.com/)  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%.

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.11 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 al10 prior to biofilm formation, in keeping with the incomplete inhibition seen during the in vitro studies by Kerr et al.9 *P. aeruginosa* biofilms offer some protection against antibacterial effects, as do *A. fumigatus* biofilms against antifungal susceptibility,24 25 but biofilms can be disrupted by antibiotics such as macrolides.26 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.

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All patients (n=26)</th>
<th>Wilcoxon signed rank test</th>
<th>Values are shown as median (IQR).</th>
<th>GM, galactomannan.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total IgE (kUI/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>78 (28–230)</td>
<td>Z=−0.47 p=0.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>62 (25–220)</td>
<td></td>
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<tr>
<td><strong>A fumigatus specific IgE (kUa/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>1.5 (0.0–8.9)</td>
<td>Z=−0.73 p=0.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>1.3 (0.7–7.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>A fumigatus specific IgG (mg/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>73 (50–112)</td>
<td>Z=−1.82 p=0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>63 (51–100)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>IgG (mg/l)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Pre</td>
<td>78 (65–113)</td>
<td>Z=−1.50 p=0.13</td>
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<tr>
<td>Post</td>
<td>75 (55–102)</td>
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<tr>
<td><strong>Total bacterial diversity</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Pre</td>
<td>104 (14–293)</td>
<td>Z=−1.68 p=0.09</td>
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</tr>
<tr>
<td>Post</td>
<td>61 (21–253)</td>
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Table 4 Changes in lung function pre- and post-antibiotics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All patients (n=26)</th>
<th>Positive Aspergillus PCR (n=20)</th>
<th>Negative Aspergillus PCR (n=6)</th>
<th>Values are shown as median (IQR).</th>
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</thead>
<tbody>
<tr>
<td><strong>FEV1 % predicted</strong></td>
<td>30 (22–36)</td>
<td>33 (25–38)</td>
<td>22 (21–33)</td>
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<tr>
<td>Pre-antibiotics</td>
<td>36 (26–50)</td>
<td>39 (30–51)</td>
<td>24 (22–43)</td>
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<tr>
<td>Post-antibiotics</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.07</td>
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<tr>
<td>p Values</td>
<td></td>
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<tr>
<td><strong>FVC % predicted</strong></td>
<td>49 (35–59)</td>
<td>52 (36–60)</td>
<td>35 (31–47)</td>
<td>0.03</td>
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<tr>
<td>Pre-antibiotics</td>
<td>59 (44–69)</td>
<td>63 (49–70)</td>
<td>41 (36–64)</td>
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<tr>
<td>Post-antibiotics</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.03</td>
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<tr>
<td>p Values</td>
<td></td>
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</table>

Changes in lung function were measured using Wilcoxon signed rank tests. FEV1, 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. 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 exacerbation periods. However, intravenous antibiotics may indicate that the prevalence of both A fumigatus and Candida species from oral rinse samples are very low during non-exacerbation periods. However, intravenous antibiotics may predispose to oral Candida infections which could potentially influence GM results post-antibiotics.

Acknowledgements We would like to thank all the staff at the Manchester Adult Cystic Fibrosis Centre for their assistance in conducting this study, particularly William Flight for all his help with patient coordination.

Contributors CGB is guarantor of the study. CGB, RR, AMJ and DWD contributed to the study conception and design; CGB and EM contributed to the acquisition of data; CGB, MB, EM and DWD contributed to the interpretation and analysis of data; CGB drafted the manuscript and all authors revised the manuscript critically for important intellectual content and provided final approval of the version to be published.

Funding This study was supported by the National Commissioning Group, National Aspergillosis Centre, University Hospital of South Manchester, UK. MB is supported by a BBfS Doctoral Training Grant (BB/F016557/1).

Competing interests No support was received from any organisation for the submitted work. Relevant financial activities outside the submitted work in the last 3 years include: CGB has received travel grants from Merck and P&G 3 years include: CGB has received travel grants from Merck and P&G; RR, AMJ and DWD contributed to the study conception and design; CGB and EM contributed to the acquisition of data; CGB, MB, EM and DWD contributed to the interpretation and analysis of data; CGB drafted the manuscript and all authors revised the manuscript critically for important intellectual content and provided final approval of the version to be published.

Provenance and peer review Not commissioned; internally peer reviewed.

REFERENCES


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

*Caroline G. Baxter¹, ³, Riina Rautemaa¹, ³, Andrew M. Jones², Kevin Webb², Matthew Bull⁴, Eshwar Mahenthiralingam⁴, David W. Denning¹, ³

ONLINE REPOSITORY MATERIAL

S1 Viral PCR method and results

**Viral PCR method**

Three hundred microlitres of neat pre-antibiotic sputum was added to 300 µL of Qiagen AL buffer and heated to 80°C for 20 minutes. One millilitre of phosphate buffered solution was then added to the sample and mixed. RNA extraction was automated using the Qiagen BioRobot MDx workstation. Samples were tested for a panel of respiratory viruses: Influenza A and B, Rhinovirus, Adenovirus, Respiratory syncytial virus (RSV), Parainfluenza types 1-3, and Metapneumovirus (MPV). Respiratory viral strains were obtained from the National Institute for Biological Standards and Control (NIBSC, Hertfordshire, UK), except for Rhinovirus and MPV which were obtained from previously positive in-house samples. Viral detection was performed by multiplex real-time (TaqMan) one-step RT-PCR on an ABI 7500 real-time PCR platform (Applied Biosystems, California, USA). A Ct value of <45 was considered positive.

**Viral PCR results**

Seven of the 26 patients (27%) were found to have positive viral PCR from sputum pre-antibiotics. Viruses identified were: 3 Rhinovirus, 2 Adenovirus, 1 Influenza B, and 1 RSV. There was no correlation between *Aspergillus* PCR and viral PCR (Table S1).
<table>
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<tr>
<th>Patient</th>
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<th>Aspergillus PCR 2</th>
<th>GM 1</th>
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