

# Effect of antibiotic treatment on inflammatory markers and lung function in cystic fibrosis patients with *Pseudomonas cepacia*

D Peckham, S Crouch, H Humphreys, B Lobo, A Tse, A J Knox

## Abstract

**Background** - The acquisition of *Pseudomonas cepacia* in patients with cystic fibrosis is associated with increasing deterioration in lung function and more frequent hospital admissions. *Pseudomonas cepacia* is usually resistant to several antibiotics in vitro, but the response of patients colonised with the organism has not been extensively studied in vivo.

**Methods** - A three month prospective study was performed to investigate the response of 14 *Ps cepacia* positive patients and 10 *Ps cepacia* negative patients to a two week course of intravenous antibiotics. All those who were *Ps cepacia* negative and six of the 14 *Ps cepacia* positive patients had *Ps aeruginosa* in their sputum which was sensitive to the prescribed therapy. The inflammatory markers C-reactive protein, white blood cell count, serum lactoferrin, neutrophil elastase/ $\alpha_1$ -antitrypsin complex, and tumour necrosis factor alpha were measured at the start and end of each antibiotic course.

**Results** - The median (range) % improvement in baseline FEV<sub>1</sub> and FVC following treatment in the group as a whole was 15.2% (-23.5% to 156.3%) and 23.9% (-36.8% to 232.7%) respectively. There was no statistical difference in improvement in lung function, body weight, or inflammatory markers between individuals who were *Ps cepacia* positive and those who were *Ps cepacia* negative.

**Conclusions** - Patients who are *Ps cepacia* positive appear to respond as well to intravenous antibiotics as those who are *Ps cepacia* negative, despite having lower lung function and a bacterium in their sputum which is resistant in vitro to the antibiotics used.

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In the UK the incidence and prevalence of *Pseudomonas cepacia* among patients with cystic fibrosis has significantly increased over the past few years.<sup>1-3</sup> This can partly be explained by improvement in microbiological isolation techniques and by an increase in both social and hospital contacts among patients with cystic fibrosis.<sup>2</sup> Despite the increased evidence for patient to patient transmission, it remains unclear whether *Ps cepacia* is a cause of, or marker of, disease severity.

In a small proportion of patients with *Ps*

*cepacia* rapid and unexpected lung deterioration develops despite intensive antibiotic treatment. The isolation of *Ps cepacia* from blood cultures accompanied by clinical evidence of systemic sepsis provides evidence of a possible pathogenic role for this organism.<sup>1,4,5</sup> In most patients, however, a gradual decline in lung function occurs following the isolation of the organism.<sup>6</sup>

*Pseudomonas cepacia* is resistant to most antipseudomonal antibiotics in vitro.<sup>7</sup> Our clinical impression is nevertheless that patients respond clinically to these antibiotics. Markers such as serum levels of neutrophil elastase/ $\alpha_1$ -antitrypsin complex, lactoferrin, tumour necrosis factor alpha (TNF $\alpha$ ), C-reactive protein, and white blood cell count have been shown previously to reflect the inflammatory process in patients with cystic fibrosis and are reduced following effective antipseudomonal treatment in patients colonised with *Ps aeruginosa*.<sup>8-10</sup> It is not known, however, whether similar changes are seen after treatment in patients colonised with *Ps cepacia*. We have therefore prospectively studied the effectiveness of intravenous antibiotic therapy in a group of adult patients with cystic fibrosis, with and without *Ps cepacia*, measuring treatment response as change in inflammatory markers, lung function, and body weight.

## Methods

### STUDY DESIGN

This was a prospective study which included all patients who required two weeks of intravenous antibiotics for infective exacerbations of cystic fibrosis over a three month period at Nottingham City Hospital. Patients were assessed both before and at the end of the two week course of antibiotics with measurements of body weight, lung functions, and venous blood samples for measurements of inflammatory markers.

### PATIENTS

Twenty four adult patients were studied, 10 of whom were colonised with *Ps aeruginosa* without *Ps cepacia* (*Ps cepacia* negative) and 14 of whom were colonised with *Ps cepacia*, with or without *Ps aeruginosa* (*Ps cepacia* positive). Clinical details are outlined in table 1. All patients had been chronically infected with *Ps aeruginosa* for more than two years. Of the patients who were *Ps cepacia* positive, this bacterium had been isolated from one patient three weeks before antibiotic therapy while the remaining 13 had repeatedly been positive for

Division of  
Respiratory Medicine,  
Medical Research  
Centre, City Hospital,  
Nottingham NG5 1PB  
D Peckham  
S Crouch  
B Lobo  
A Tse  
A J Knox

Department of  
Microbiology, Queens  
Medical Centre,  
University Hospital,  
Nottingham NG7 2UH  
H Humphreys

Reprint requests to:  
Dr D Peckham.

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Table 1 Clinical details of 24 adult patients with cystic fibrosis

	<i>Ps cepacia</i> positive (n=14)	<i>Ps cepacia</i> negative (n=10)	p
Mean age (years)	22.8	22.2	
M:F	10:4	6:4	
Schwachman score	45 (25-70)	65 (30-80)	<0.05
Chrispin-Norman score	20 (16-32)	20 (9-32)	>0.05
Post-treatment FEV <sub>1</sub> (l/s)	1.17 (0.58-2.21)	1.66 (0.71-4.93)	0.05
% predicted FEV <sub>1</sub>	31 (14-64)	48 (17-88)	>0.05
Post-treatment FVC (l)	1.75 (0.76-3.5)	2.73 (1.66-5.64)	<0.001
% predicted FVC	46 (16-70)	65 (35-79)	<0.05

Values are median (range).

*Ps cepacia* for more than six months. Two patients who were *Ps cepacia* positive and one who was *Ps cepacia* negative were on low dose oral prednisolone prior to antibiotic therapy (10-20 mg/day). None of the patients were started on steroids over the three month study period. Four patients in both groups received their antibiotics at home while being reviewed weekly on the ward and the remainder were treated as inpatients. Pathogens isolated from sputum before the start of intravenous antibiotic therapy are summarised in table 2.

#### LUNG FUNCTION

Forced expiratory volume in one second (FEV<sub>1</sub>) and forced vital capacity (FVC) were measured as the highest of three blows on a Vitalograph  $\alpha$  spirometer (Buckingham, UK).

#### MICROBIOLOGY

Neat sputum and a 1:10 000 dilution of sputum from patients with cystic fibrosis was routinely cultured for *Haemophilus influenzae*, *Staphylococcus aureus*, and *Ps aeruginosa* following digestion with *N*-acetyl cysteine using cefsulodin chocolate agar and MacConkey agar. Investigation for the presence of mycobacteria and atypical respiratory pathogens was carried out when indicated. Sputum samples were also inoculated on to *Ps cepacia* selective agar medium (MAST, UK) incorporating ticarcillin (100 mg/l) and polymyxin B (30 000 units/l). Agar plates were incubated for 40 hours at 37°C and *Ps cepacia* was identified by colonial appearance, oxidase reaction, biochemical reaction, and resistance to polymyxin B. Antimicrobial susceptibility testing to gentamicin, tobramycin, ceftazidime, azlocillin, ciprofloxacin, aztreonam, imipenem, and polymyxin B was carried out using the disc diffusion method.<sup>11</sup>

#### ANTIBIOTIC TREATMENT

All patients who were *Ps cepacia* negative

Table 2 Organisms isolated from sputum of patients before treatment with intravenous antibiotics

	Frequency
<i>Ps cepacia</i> negative	
<i>Ps aeruginosa</i> alone	8
<i>Staph aureus</i> and <i>Ps aeruginosa</i>	1
<i>H influenzae</i> and <i>Ps aeruginosa</i>	1
<i>Ps cepacia</i> positive	
<i>Ps cepacia</i> alone	6
<i>Ps cepacia</i> and <i>Ps aeruginosa</i>	6
<i>Ps cepacia</i> , <i>Staph aureus</i> and <i>H influenzae</i>	1
<i>H influenzae</i> and <i>Ps cepacia</i>	1

received an aminoglycoside (gentamicin or tobramycin) with 5 g three times daily azlocillin (one patient), or 2 g three times daily ceftazidime (seven patients), 2 g three times daily aztreonam (one patient) or 1 g three times daily imipenem alone (one patient). Patients who were *Ps cepacia* positive received a combination of an aminoglycoside with either ceftazidime (eight patients), azlocillin (five patients), or aztreonam (one patient) at the identical doses to patients who were *Ps cepacia* negative. Antibiotic regimens were selected according to sensitivity results of both *Ps aeruginosa* and *Ps cepacia*, and in patients who were *Ps cepacia* positive combination therapy was used. The choice of antibiotics was arbitrary when a multiresistant strain of *Ps cepacia* was isolated in the absence of *Ps aeruginosa*. At the start of treatment all 10 isolates of *Ps aeruginosa* among patients who were *Ps cepacia* negative and five isolates of *Ps aeruginosa* from patients who were *Ps cepacia* positive were fully sensitive to the antibiotics used. One patient who was *Ps cepacia* positive grew a *Ps aeruginosa* isolate which proved to be sensitive to tobramycin alone. Of the 14 patients who were *Ps cepacia* positive 12 had a multiresistant strain of *Ps cepacia* in their sputum while the isolates from two patients were sensitive to ceftazidime alone. Two patients in the *Ps cepacia* positive group and one in the *Ps cepacia* negative group received either high dose oral amoxicillin (3 g twice daily) or intravenous cefuroxime (1.5 g three times daily) to treat additional *H influenzae* (table 2), while one patient in each group was treated with oral flucloxacillin (1 g four times daily) for additional *Staphylococcus aureus* infection. Serum aminoglycoside levels were measured around the fourth dose and again on the third or fourth day thereafter, with dose adjustment as appropriate to maintain a serum peak concentration of 7-10 mg/l. Five patients who were *Ps cepacia* negative and five who were *Ps cepacia* positive were on long term nebulised antibiotic therapy which was discontinued during the study period. Of the patients who were *Ps cepacia* negative two were on colomycin (1 megaunit twice daily), two on gentamicin (80 mg twice daily) and one on tobramycin (80 mg twice daily), while amongst patients who were *Ps cepacia* positive four were on colomycin (1 megaunit twice daily) and one was on gentamicin (80 mg twice daily).

#### INFLAMMATORY MARKERS

Full blood count with differential counts was measured by conventional automated analysis, C-reactive protein by a nephelometry method,<sup>10</sup> and other inflammatory markers by enzyme linked immunosorbent assay (ELISA) following serum storage at -70°C. The lactoferrin ELISA used<sup>12</sup> had a lower detection limit of 0.005 nmol/l. Immunoreactive TNF was measured according to a previously described method<sup>13</sup> with a lower detection limit of 6.25 pg/ml. Elastase/ $\alpha_1$ -antitrypsin was measured using a modification of a previously described method<sup>14</sup> where the streptavidin horseradish peroxidase step was replaced with

Table 3 Median (range) results for inflammatory markers before and after treatment in the combined group of 24 adult patients with cystic fibrosis

Inflammatory markers	Before treatment	After treatment	p
White cell count ( $\times 10^9/l$ )	10.9 (2.4–21.5)	8.7 (2.0–18.3)	0.005
C-reactive protein (mg/l)	29 (<11–169)	<11 mg/l (<11–130)	0.001
Lactoferrin (nmol/l)	6.97 (3.87–8.99)	4.87 (3.29–7.87)	0.02
Tumour necrosis factor (pg/ml)	42.25 (0–1055)	27.2 (0–604.5)	>0.05
Elastase/ $\alpha_1$ -antitrypsin complex (ng/ml)	728 (277–1541)	407.5 (54–842)	<0.001

an alkaline phosphatase conjugate to streptavidin. An AMPAK amplification kit (Dako) was used to increase the sensitivity of the assay allowing detection of the complex to 10 ng/ml in the serum.

#### DATA ANALYSIS

Because the data were not normally distributed the results were expressed as medians and ranges. Changes in weight, lung function, and inflammatory markers were analysed in patients who were *Ps cepacia* positive, those who were *Ps cepacia* negative, and both groups combined using Wilcoxon and Mann–Whitney tests for paired and unpaired data respectively (Microsoft Corporation, Redmond, USA). Subgroup analysis comparing the same variables in the *Ps cepacia* positive group (six patients *Ps aeruginosa* positive, six patients *Ps aeruginosa* negative) was also carried out. A p value <0.05 was regarded as statistically significant.

#### Results

Patients who were *Ps cepacia* positive had significantly lower median FEV<sub>1</sub>, FVC, and Schwachman scores after treatment than patients who were *Ps cepacia* negative, although Chrispin–Norman scores were similar (table 1).

#### SPIROMETRY

When patients who were *Ps cepacia* positive and *Ps cepacia* negative were combined lung function improved significantly following antibiotic treatment from a median (range) % predicted FEV<sub>1</sub> and FVC before therapy of 28.5% (11–67%) and 38% (9.4–80%) respectively to a median (range) FEV<sub>1</sub> of 34.5% (14–88%, p<0.05) and FVC of 53% (16–79%, p<0.05). The median % improvement of FEV<sub>1</sub> and FVC following antibiotics was 15.23% (–23.46% to 156.25%) and 23.99% (–36.8% to 232.7%) respectively.

There was no difference in % improvement of FEV<sub>1</sub> and FVC before and after treatment when comparing patients who were *Ps cepacia* positive and negative. The median (range) % improvement of FEV<sub>1</sub> and FVC from baseline in patients who were *Ps cepacia* positive was 19.49% (–23.46% to 156.25%, p>0.05) and 23.35% (–36.8% to 232.65%, p<0.05) re-

spectively, and 8.075% (–7.73% to 99.6%, p>0.05) and 23.99% (–3.9% to 145.9%, p>0.05) in patients who were *Ps cepacia* negative.

There was no difference between the % improvement in both FEV<sub>1</sub> and FVC among the six patients who were *Ps aeruginosa* and *Ps cepacia* positive, and the six patients who were *Ps cepacia* positive but *Ps aeruginosa* negative.

#### WEIGHT

The median (range) weight of all patients was 53.2 (39.1–72.5) kg before treatment and 54.2 (40.6–76.6) kg (p=0.001) after treatment. The median improvement in weight was 0.5 (–1.2 to 4.7) kg.

The median weight of patients who were *Ps cepacia* positive was 53.2 (39.1–66.3) kg before and 54.1 (40.6–68.8) kg after treatment whereas in patients who were *Ps cepacia* negative the corresponding results were 53.4 (44.6–72.5) kg and 55.3 (45–76.6) kg respectively. The difference in change in weight between patients who were *Ps cepacia* positive and negative was not significant. There was no difference in the change in weight during treatment when the six patients with *Ps aeruginosa* and *Ps cepacia* were compared with the six patients with *Ps cepacia* alone. Seven of the patients who were *Ps cepacia* positive were on long term nasogastric feeding compared with only two of the patients who were *Ps cepacia* negative.

#### INFLAMMATORY MARKERS

The results before and after treatment for the combined group are outlined in table 3. There was a significant fall in white blood cell count and serum levels of C-reactive protein, lactoferrin and  $\alpha_1$ -antitrypsin following treatment for the combined group. No differences were detected in the parameters studied between patients who were *Ps cepacia* positive and those who were *Ps cepacia* negative or between the two *Ps cepacia* subgroups (six *Ps aeruginosa* positive, six *Ps aeruginosa* negative). No significant difference was seen in the pretreatment, post-treatment, or in the change in any of the measured parameters. The median changes following treatment in the two groups are outlined in table 4.

Table 4 Median (range) change in inflammatory markers following intravenous antibiotic therapy in 14 patients who were *Ps cepacia* positive and 10 patients who were *Ps cepacia* negative

Change in inflammatory markers following treatment	<i>Ps cepacia</i> positive	<i>Ps cepacia</i> negative	p
White cell count ( $\times 10^9/l$ )	–1.1 (2.9 to –9.2)	–1.6 (1.2 to –8.0)	>0.5
C-reactive protein (mg/l)	–32 (5 to –144)	–14 (21 to –169)	>0.5
Lactoferrin (nmol/l)	–0.03 (1.93 to –0.56)	–0.12 (0.80 to –0.56)	>0.5
Tumour necrosis factor (pg/ml)	–38.75 (53 to –235)	0.2 (196.6 to –450.5)	>0.5
Elastase/ $\alpha_1$ -antitrypsin complex (ng/ml)	–350 (168 to –752)	–407 (100 to –836)	>0.5

The p value shown is for the difference in the change in each variable between patients who were *Ps cepacia* positive and those who were *Ps cepacia* negative.

When serum lactoferrin levels are corrected for the number of circulating neutrophils the median (range) for the 24 patients before treatment was 0.885 (0.503–1.479) nmol/10<sup>6</sup> neutrophils and 0.947 (0.368–2.636) nmol/10<sup>6</sup> neutrophils following treatment ( $p > 0.05$ ). There were no significant differences between corrected values of serum lactoferrin and TNF levels before and after treatment when patients who were *Ps cepacia* positive and negative were compared ( $p > 0.05$ ).

### Discussion

*Pseudomonas cepacia* has been isolated with increasing frequency in specialist centres within the UK.<sup>12</sup> Isolation of *Ps cepacia* from patients with cystic fibrosis is associated with poor lung function, increasing age, recent hospitalisation, and close hospital or social contact with other patients who are *Ps cepacia* positive and siblings.<sup>15</sup> Despite some evidence to suggest that colonisation with *Ps cepacia* heralds a poorer prognosis, it is unclear whether this is because the organism is pathogenic or because it is a marker for increased disease severity due to other factors.<sup>15</sup> *Pseudomonas cepacia* is non-pathogenic in healthy individuals and in animal models it has been found to be relatively avirulent compared with *Ps aeruginosa*.<sup>16,17</sup> It is possible that *Ps cepacia* acts synergistically with other bacteria to exacerbate chest disease in cystic fibrosis.

In this study we have investigated the effect of intravenous antipseudomonal antibiotic treatment in patients with *Ps cepacia* infection and have compared this with patients colonised by *Ps aeruginosa*. Several parameters were used to assess response including lung function and various inflammatory markers. In our group of 24 patients studied prospectively we found that lung function and Schwachman scores were lower in patients colonised with *Ps cepacia* than in those with *Ps aeruginosa* alone. This is consistent with the results of other studies and suggests an association between colonisation with *Ps cepacia* and poor clinical status.<sup>4,18</sup> Despite the fact that patients who are *Ps cepacia* positive had worse lung function and Schwachman scores, antibiotic treatment was equally effective in improving lung function and weight in both groups. Both these parameters improved significantly over the two weeks of treatment in both groups of patients and was similar to that reported in previous studies of patients colonised with *Ps aeruginosa*.<sup>8,19,20</sup> The fact that weight improved equally in patients who were *Ps cepacia* positive despite their generally poorer clinical status may partially reflect the fact that more of them were on nasogastric nutritional supplementation.

Several inflammatory markers were measured including white blood cell count and serum levels of C-reactive protein, lactoferrin, neutrophil elastase/ $\alpha_1$ -antitrypsin complex, and TNF. These have all been shown previously to reflect the inflammatory process in patients with cystic fibrosis. Previous studies of patients colonised with *Ps aeruginosa* have shown that values of these markers fall following

treatment with intravenous antipseudomonal antibiotics.<sup>8–10</sup>

The inflammatory markers elastase/ $\alpha_1$ -antitrypsin, lactoferrin, and C-reactive protein fell in response to intravenous antibiotic therapy in both patients who were *Ps cepacia* positive and those who were *Ps cepacia* negative. The changes seen were similar to those found previously in studies of patients colonised with *Ps aeruginosa*.<sup>8,10</sup> No significant change in TNF levels occurred following antibiotic therapy in either group, but many patients had undetectable levels of TNF both before and after treatment. Previous studies have expressed lactoferrin levels as absolute values. When we did the same we found a significant reduction in lactoferrin levels with antibiotic treatment. The sole source of lactoferrin is the neutrophil, however, and neutrophil values also fell.<sup>21,22</sup> When the lactoferrin level was adjusted for the neutrophil count no significant change was seen. This suggests that the fall in lactoferrin levels is largely due to the fall in neutrophil count. As with the lung function results, there was no significant difference in improvement in any of the inflammatory markers between patients who were *Ps cepacia* positive and those who were *Ps cepacia* negative.

The good clinical response to intravenous antibiotic therapy among patients who were *Ps cepacia* positive is surprising in the light of the in vitro sensitivity pattern seen with *Ps cepacia*. The organism is resistant to most commonly used antipseudomonal agents,<sup>7</sup> but combinations of antibiotics – for example, aminoglycosides such as gentamicin and a  $\beta$ -lactam agent such as azlocillin – may, however, inhibit the growth of *Ps cepacia* synergistically. In vitro combinations of other antibiotics have been shown to be synergistic against *Ps cepacia*.<sup>23–25</sup> Synergy between three antibiotics (rifampicin, imipenem, and ciprofloxacin) has previously been demonstrated and therefore two or more antimicrobial agents may be needed.<sup>25</sup> Many patients with cystic fibrosis who are colonised with *Ps cepacia* also carry *Ps aeruginosa* and exacerbation may be caused by *Ps aeruginosa* with or without *H influenzae* or *Staph aureus*. Our clinical experience would seem to indicate that combination chemotherapy results in in vivo activity against *Ps cepacia* despite resistance in vitro. Nottingham isolates of *Ps cepacia* are resistant to the aminoglycosides, azlocillin, ciprofloxacin, imipenem, aztreonam, and polymyxin B, but some are moderately sensitive to ceftazidime and most adult patients with cystic fibrosis show the same strain (unpublished observation).

We have considered several explanations for the clinical improvement of patients who were *Ps cepacia* positive despite in vitro antimicrobial resistance. Firstly, it is possible that in vitro antibiotic sensitivities do not reflect the in vivo situation within the lung due to the milieu of the inflammatory response. Alternatively the in vivo response may be due to synergism between antibiotics. Current methodology used in antibiotic susceptibility testing of *Ps cepacia* may be less appropriate for this organism which is slower growing than *Ps aeruginosa* and grows

preferentially at 30°C. It is also possible that a heavy growth of *Ps cepacia* may inhibit the recognition of other organisms such as *Staph aureus* and *Haemophilus*. Whilst this is a possibility it would not explain the results of treatment directed towards *Ps aeruginosa* in our study. An alternative explanation is that the response in both patient groups is due solely to effective physiotherapy which is causing improvement by enhancing sputum expectoration and reducing the inflammatory stimulus, thus reducing levels of inflammatory markers. This latter possibility would seem unlikely as previous studies have shown that intravenous antibiotics alone are more effective than physiotherapy alone at reducing *Ps aeruginosa* counts in sputum.<sup>20</sup> Antibiotics are also known to have other effects which may modify the response to infection including the release of endotoxin and the inhibition of the cytokine cascade.<sup>26</sup> The possible immunomodulatory effect combined with some in vivo antibacterial activity, physiotherapy, and nutritional support may explain the clinical improvement. Consequently, beneficial effects might be explained despite persistence of the organism. Lastly, we considered whether the response to treatment in patients colonised with *Ps cepacia* may reflect treatment of *Ps aeruginosa* rather than *Ps cepacia*. For this reason we have compared the two subgroups of patients who were *Ps cepacia* positive (with and without *Ps aeruginosa*) and have found no differences in either lung function or inflammatory markers between patients colonised with *Ps aeruginosa* and *Ps cepacia* and those colonised with *Ps cepacia* alone. This suggests that treatment was as effective when only *Ps cepacia* was present. Alternatively, the clinical response to antibiotic treatment among patients who appeared to be colonised by *Ps cepacia* alone may simply reflect the treatment of underlying *Ps aeruginosa* as the inability to isolate this organism from the sputum does not exclude its presence within the lower respiratory tract.

The fact that we found that patients who were *Ps cepacia* positive responded well to antibiotics suggests that the reason for the greater decline in their lung function in some studies may be the result of lung damage occurring between antibiotic courses.<sup>6</sup> It is interesting that our patients who were *Ps cepacia* positive required more courses of intravenous antibiotics over the previous 12 months than those patients who were *Ps cepacia* negative.

We conclude, therefore, that antibiotic treatment of patients who are *Ps cepacia* positive is often effective in vivo despite the multiresistant nature of this organism in vitro. Further work is required to determine the relation between in vitro sensitivity results and the response in vivo and the pattern of inflammatory response over a prolonged period in patients who are *Ps cepacia* positive. From a practical point of view anti-

biotics should not be withheld from patients because of in vitro resistant patterns.

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