Eur Respir J 2003; 22: 503–506
DOI: 10.1183/09031936.03.00004503
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European Respiratory Journal
ISSN 0903-1936

Inflammatory markers in cystic fibrosis patients with transmissible *Pseudomonas aeruginosa*

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Inflammatory markers in cystic fibrosis patients with transmissible Pseudomonas aeruginosa. *A.M. Jones, L. Martin, R.J. Bright-Thomas, M.E. Dodd, A. McDowell, K.L. Moffitt, J.S. Elborn, A.K. Webb. ©ERS Journals Ltd 2003.*

ABSTRACT: Chronic *Pseudomonas aeruginosa* infection in cystic fibrosis (CF) leads to a damaging host inflammatory response. There are an increasing number of reports of *P. aeruginosa* cross-infection at CF centres. The clinical significance of acquisition of a transmissible strain for patients who already harbour *P. aeruginosa* is unclear. In this study, levels of inflammatory markers in clinically stable adult CF patients who harbour transmissible and sporadic strains of *P. aeruginosa* have been compared.

Patients with CF and chronic *P. aeruginosa* infection were grouped into those who harbour a transmissible *P. aeruginosa* and those who harbour their own sporadic strains. Total white cell and differential counts, sputum neutrophil elastase (NE), interleukin (IL)-8, tumour necrosis factor (TNF)-α, plasma IL-6 and NE/α_1-antitrypsin complexes, serum C-reactive protein, and urine TNF receptor 1 were all measured in clinically stable patients 4–6 weeks following completion of intravenous antibiotic therapy.

The two groups (both n=20) were well matched for per cent predicted forced expiratory volume in one second, per cent predicted forced vital capacity and body mass index. There were no significant differences in levels of white cell counts or inflammatory markers between the two groups.

At times of clinical stability, cystic fibrosis patients infected with transmissible *Pseudomonas aeruginosa* do not have a heightened inflammatory response above that of those harbouring sporadic strains.

Eur Respir J 2003; 22: 503–506.

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Keywords: Cross-infection
cystic fibrosis
inflammation
Pseudomonas aeruginosa

Received: January 14 2003
Accepted after revision: May 8 2003

The major cause of morbidity and mortality for individuals with cystic fibrosis (CF) is pulmonary disease due to chronic infection and a damaging host inflammatory response. The major bacterial pathogen involved is *Pseudomonas aeruginosa* [1]. Recently, there have been a number of reports of *P. aeruginosa* cross-infection outbreaks at CF centres and holiday camps [2–8]. Isolates of many of the transmissible strains commonly exhibit unusual phenotypic features; morphotypes of the most prevalent transmissible strain at the Manchester Adult CF Centre, *P. aeruginosa* strain MA, are associated with an unusual pyocin type, are nonpigmented, nonmotile and are often resistant to the majority of usual anti-pseudomonal antibiotics [3]. The clinical significance of acquisition of a transmissible strain for patients who already harbour their own sporadic strain of *P. aeruginosa* is unclear, although some clinicians have reported an increase in morbidity [2, 8, 9] and mortality [2] among CF patients infected with a transmissible strain of *P. aeruginosa*.

Airway inflammation is a major factor in the pathogenesis of CF lung disease. Inflammatory mediators as markers of the host response to infection may reflect the intensity of lung injury and relate to changes in clinical status. When clinically stable, CF patients with chronic *P. aeruginosa* infection are known to have increased levels of inflammatory markers compared to the non-CF population [10, 11]. If transmissible strains of *P. aeruginosa* are more virulent they may provoke an enhanced inflammatory response. In this study, levels of inflammatory markers in clinically stable adult CF patients who harbour a transmissible *P. aeruginosa* have been compared with those harbouring sporadic strains of *P. aeruginosa*.

Methods

CF patients with chronic *P. aeruginosa* infection who attend the Manchester Adult CF Centre were invited to participate in the study if they were clinically stable (defined as an absence of new symptoms and stable pulmonary function) and had received a course of *i.v.* antibiotic treatment 4–6 weeks earlier. The course of *i.v.* antibiotic treatment was of at least 7 days duration and consisted of a combination of at least two different classes of intravenous anti-pseudomonal antibiotics. The *in vitro* sensitivity of the *P. aeruginosa* from the most recently available sputum isolate is used to guide antibiotic choice and adjusted where appropriate when a current culture is available. Approval for the study was given by South Manchester Research Ethics Committee and written informed consent was obtained from each patient.

Patients were grouped on the basis of results of prospective bacterial fingerprinting of *P. aeruginosa* isolates from sputa,

as previously reported [3]. Patients were grouped into those who have harboured a transmissible strain of *P. aeruginosa* (*P. aeruginosa* strain MA) for at $\geqslant$12 months and those who harbour their own sporadic *P. aeruginosa*. The two groups were prospectively matched by per cent predicted forced expiratory volume in one second (%FEV1). Patients co-infected with *Burkholderia cepacia* complex or other transmissible strains of *P. aeruginosa* were excluded from the study. Baseline patient demographics in the study were age, sex, CF genotype, presence of pancreatic insufficiency and/or diabetes mellitus, and use of nebulised antibiotics, deoxyribonuclease, long-term oral macrolide antibiotics, inhaled corticosteroids, and oral corticosteroids. %FEV1, per cent predicted forced vital capacity (%FVC) and body mass index (BMI) were measured. The total duration of the previous course of *i.v.* antibiotics and time from completion to the study visit were recorded. The *in vitro* phenotypic appearance (nonmucoid or mucoid) and antibiotic sensitivity patterns of the *P. aeruginosa* isolates were noted. For the purpose of this study, multiresistance was defined as resistance to two of the three major classes of usual antipseudomonal antibiotics (aminoglycosides, quinolones, β-lactams). Antibiotic resistance was assessed by the British Society for Antimicrobial Chemotherapy standardised disc testing method. Susceptibility and resistance of isolates was based on standard breakpoint values for each antibiotic (British Society for Antimicrobial Chemotherapy).

A sample of whole blood was analysed the same day for total white cell and differential cell counts. Samples of sputum, urine, plasma and serum were obtained for measurement of inflammatory markers. Sputum was diluted weight for weight with 0.9% (weight/volume) saline in a 1:5 ratio, centrifuged at 3000×*g* for 30 min and the supernatant collected. All samples were stored in aliquots at -80°C pending the measurement of inflammatory mediators.

Inflammatory markers were analysed as a single batch by a blinded investigator. The levels of the following markers were measured: sputum neutrophil elastase (NE), interleukin (IL)-8 and tumour necrosis factor (TNF)-α, plasma IL-6 and NE/α_1-antitrypsin complexes, serum C-reactive protein (CRP), and urine TNF receptor 1. NE was detected by spectrophotometric assay, utilising the substrate *N*-Methoxysuccinyl-Ala-Ala-Pro-Val-p-nitroanilide. All other parameters were measured by commercially available enzyme-linked immunosorbent assays (ELISA), with the exception of NE/α_1-antitrypsin complexes, which were detected by an ELISA developed within the authors' laboratory, and relevant performance and quality control checks were conducted.

Statistical analysis

The baseline demographics of the two groups were compared by Chi-squared and unpaired t-tests. The differences in total white cell and neutrophil counts were analysed using a two-sample t-test. Group differences in levels of inflammatory markers were assessed by the Mann-Whitney U-test.

Results

For the transmissible group there were 22 eligible patients (*i.e.* patients who had chronic infection with the transmissible *P. aeruginosa* strain MA for >12 months); 20 out of 22 patients were recruited. Two were not recruited, as they did not receive a course of *i.v.* antibiotics over the study period. The 20 patients with the transmissible strain were matched by %FEV1 with 20 CF patients harbouring sporadic *P. aeruginosa*. The two groups (sporadic *versus* transmissible *P. aeruginosa* strains) were well matched in terms of mean age, %FEV1, %FVC, BMI and demographic features (table 1). Patients received *i.v.* antibiotic therapy for a median (range) of 14 (9–37) and 14 (8–28) days (p=0.68) in the sporadic and transmissible *P. aeruginosa* groups, respectively. The study visit was a mean (SE) 35.8 (1.2) and 33.7 (0.9) days (p=0.20) after the final day of *i.v.* antibiotic treatment for the sporadic and transmissible *P. aeruginosa* groups, respectively. There were no differences between the sporadic and transmissible *P. aeruginosa* groups in median change in %FEV1 (-2.2 (-11.5–15.4) and -2.5 (-23.5–6.3); p=0.57), %FVC (-3.9 (-14.1–6.6) and -5.0 (-50.7–6.4); p=0.37) or BMI (0.15 (1.2–-1.3) and 0.15 (-0.8–1.2); p=0.36) from the end of the course of *i.v.* antibiotics to the study day.

Table 1. – Demographics of cystic fibrosis (CF) groups

	Sporadic *P. aeruginosa* group	Transmissible *P. aeruginosa* group	p-value
Age yrs	27.5±9.4	28.0±7.5	0.82
%FEV1	44.4±21.2	45.7±17.8	0.84
%FVC	60.1±19.9	67.0±19.2	0.27
BMI	20.8±3.2	20.5±2.7	0.74
CF genotype			
ΔF508/ΔF508	8	15	
ΔF508/other	11	3	
other/other	1	2	
Male sex	10	10	1.00
Diabetes mellitus	8	9	0.75
Pancreatic insufficiency	20	20	1.00
Nebulised antibiotic	14	14	1.00
DNAse	15	12	0.50
Oral macrolide	12	12	1.00
Inhaled steroid	18	19	0.55
Oral steroid	3	2	0.63
Mucoid *P. aeruginosa*	15	11	0.19
Multi-resistant *P. aeruginosa*	3	16	<0.001

Data are presented as mean±SD group values or n unless otherwise stated. *P. aeruginosa*: *Pseudomonas aeruginosa*; FEV1: forced expiratory volume in one second; FVC: forced vital capacity; BMI: body mass index; DNAse: deoxyribonuclease. n=22 in both groups.

Whole blood, plasma and serum samples were obtained from all patients. Two patients in the transmissible *P. aeruginosa* group were unable to produce a sputum sample. Two patients in the sporadic *P. aeruginosa* group were unable to produce a urine sample because of time restraints on the study day. The results of whole blood white cell and neutrophil counts and the levels of inflammatory markers are given in table 2. There was no difference between the two groups for the levels of white cell counts and the inflammatory markers.

Discussion

This study examines differences in levels of inflammatory markers between two groups of clinically stable CF patients with chronic *P. aeruginosa* infection; those who harbour their own sporadic *P. aeruginosa* and those who harbour a transmissible strain (*P. aeruginosa* strain MA). There were no differences in levels of inflammatory markers between the two groups.

Table 2. – White cell counts (WCC) and inflammatory markers in cystic fibrosis patients with chronic *Pseudomonas aeruginosa* (*P. aeruginosa*) infection

	Sporadic *P. aeruginosa* group	Transmissible *P. aeruginosa* group	p-value
Total WCC $\times 10^9 \cdot L^{-1}$	10.3±0.6	10.5±0.7	0.81
Neutrophil count $\times 10^9 \cdot L^{-1}$	7.4±0.6	7.6±0.7	0.87
Plasma IL-6 $pg \cdot mL^{-1}$	5.8 (1.3–25.7)	6.4 (1.2–38.8)	0.91
Plasma NE/α_1-antitrypsin complexes $ng \cdot mL^{-1}$	246 (109.5–2114.8)	229 (118.3–533.3)	0.57
Serum CRP $mg \cdot L^{-1}$	7.5 (1–29)	8 (1–71)	0.61
Sputum IL-8 $ng \cdot mL^{-1}$	13.5 (5.5–56.5)	10.0 (3.8–15.6)	0.24
Sputum TNFα $pg \cdot mL^{-1}$	11 (0–169.3)	9 (0–278.5)	0.78
Sputum NE $\mu g \cdot mL^{-1}$	22.0 (10.5–38.6)	17.3 (7.1–173.5)	0.67
Urine TNFr1 $pg \cdot mL^{-1}$	1167 (286.9–3531)	988 (286.9–5082.6)	0.55

Data are presented as mean±SE or median (range). IL: interleukin; NE: neutrophil elastase; CRP: C-reactive protein; TNF: tumour necrosis factor; TNFr1: tumour necrosis factor receptor 1. n=20 for each group, except sputum samples n=18 for sporadic *P. aeruginosa* group and urine samples n=18 for epidemic *P. aeruginosa* group.

There are an increasing number of reports of *P. aeruginosa* cross-infection outbreaks among individuals with CF [2–7, 12]. The significance of *P. aeruginosa* cross-infection and the provision of resources for microbiological surveillance and implementation of infection control measures remains controversial [13–16]. Whilst new acquisition of *P. aeruginosa* by previously "*Pseudomonas*-naïve" CF patients is associated with a worsening of their clinical status [1, 17–21], the effect of acquisition of a different strain for patients who already harbour *P. aeruginosa* is unclear. However, there are reasons for CF physicians to be concerned about transmissible strains of *P. aeruginosa*. Isolates belonging to transmissible strains of *P. aeruginosa* often exhibit unusual phenotypic characteristics [3], including *in vitro* antibiotic resistance [2, 3, 6]. In the present study, a higher number (16 out of 20) of patients in the transmissible *P. aeruginosa* group were found to harbour a multi-resistant *P. aeruginosa* isolate than patients with sporadic strains (three out of 20) ($p<0.001$). Transmissible strains have also been associated with super-infection of CF patients who already harbour other *P. aeruginosa* [4]. The authors have previously documented an increase in treatment requirements for adult CF patients who harbour a transmissible strain of *P. aeruginosa* [9]. More worrying is a report from NIXON *et al.* [2] of an increased morbidity and mortality for CF children in association with *P. aeruginosa* cross-infection. A recent publication by ARMSTRONG *et al.* [8] observed a lower %FEV1 among patients at a paediatric CF centre who harboured a transmissible *P. aeruginosa*; however, the two groups were not prospectively matched.

There is a current lack of published data as to whether transmissible *P. aeruginosa* are more virulent than sporadic strains, but such information is needed by CF clinicians. The current study was designed to investigate whether adult CF patients who harbour transmissible *P. aeruginosa* strain MA demonstrate a more florid host inflammatory response than those who harbour sporadic strains of *P. aeruginosa*. Patients with chronic *P. aeruginosa* infection experience episodes of respiratory exacerbations that are associated with a decrease in lung function and a systemic inflammatory response, with increased levels of inflammatory markers, including TNF-α, NE/α_1-antitrypsin complexes, IL-6, IL-8 and CRP [11, 22–24]. *I.v.* antibiotic therapy leads to a reduction in host inflammatory responses [11, 23]. However, even at times of apparent clinical stability, CF patients with chronic *P. aeruginosa* infection still demonstrate increased levels of inflammatory markers indicating chronic inflammatory activity and accompanying lung injury [11, 24–26]. The persistent inflammatory response to chronic pulmonary infection also leads to an increased resting energy expenditure and patient metabolism [23], adversely affecting nutritional status. If some strains of *P. aeruginosa* are more virulent they should be more likely to provoke an enhanced host inflammatory response. However, in this study, no differences in levels of inflammatory markers were shown between CF patients with infection by transmissible and sporadic strains of *P. aeruginosa* at times of clinical stability.

Although these findings provide a degree of reassurance for clinicians that care for CF patients who harbour transmissible *P. aeruginosa*, the study has a number of drawbacks. First, as the number of CF patients identified as harbouring transmissible *P. aeruginosa* is small, subjects for such studies are limited and it is, therefore, difficult to fully exclude a type-2 error. Secondly, some patients who harbour the transmissible strain are co-infected with a sporadic *P. aeruginosa* strain. However, although inclusion of these patients might bias results, particularly if a transmissible strain was less virulent, these subjects make the group more representative of the CF patients in clinical practice. Thirdly, in addition to bacterial virulence factors, DNA polymorphisms in host genes regulating inflammatory responses to respiratory pathogens may influence levels of inflammatory markers [27]. It is difficult both to assess and make allowances for such a potential confounding variable in an *in-vivo* study. Finally, measuring levels of host inflammatory markers may not provide an accurate prediction of future clinical outcome. A previous study did not find a difference in levels of blood cytokines between CF patients with *P. aeruginosa* and *B. cepacia* complex [22], despite a well-recognised increased pathogenicity of *B. cepacia* complex in clinical practice [28, 29]. However, that study had smaller numbers of subjects and measured circulating inflammatory markers, which may not be as representative of inflammatory activity within the lung as those in bronchoalveolar lavage fluid or sputa. The present study recruited a greater number of patients and examined inflammatory markers in sputa and the circulation.

In conclusion, at times of clinical stability, cystic fibrosis patients infected with transmissible *Pseudomonas aeruginosa* do not have a heightened inflammatory response above those who harbour sporadic strains. Further prospective studies are needed to assess the long-term clinical effects of spread of transmissible *Pseudomonas aeruginosa* strains among individuals with cystic fibrosis.

Acknowledgements. The authors would like to gratefully acknowledge the assistance of J.R.W. Govan, C.J. Doherty and the staff of Medical Microbiology, University of Edinburgh, UK, in typing of the *P. aeruginosa* isolates.

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