Effect of respiratory *Achromobacter* spp. infection on pulmonary function in patients with cystic fibrosis

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**Abstract**

**Purpose.** Cystic fibrosis (CF) patients are susceptible to infection with *Achromobacter* spp., although its clinical significance remains controversial. The aim of this study was to investigate the clinical impact of infection with *Achromobacter* spp. in CF patients.

**Methods.** CF outpatients with multiple sputum cultures and follow-up lung function tests were assigned to the case group (infected with *Achromobacter* spp.) or the control group (never infected with *Achromobacter* spp.) according to the isolation of *Achromobacter* spp. The *Achromobacter* spp. group included two subgroups, taking into consideration whether the isolation of *Achromobacter* spp. was intermittent or chronic. Baseline lung function tests and longitudinal behaviour were examined in relation to *Achromobacter* spp. status.

**Results.** A total of 190 CF patients were treated from January 2003 to December 2015 in the CF unit and 21 (11 %) had at least one positive culture for *Achromobacter* spp. Of these, 11/21 (52.4 %) patients were chronically infected with *Achromobacter* spp. An analysis of changes during follow-up showed the annual rate of FEV1 decline: −2.3±1.6 % in the *Achromobacter* spp. group compared to −1.1±0.9 % (P=0.02) in the control group. The chronically infected group also had a significantly greater decline in FEV1 compared to the control group (−2.9±1.9 vs −1.1±0.9; P=0.04). The mean number of annual pulmonary exacerbations during the study period was significantly higher in the case group (1.9±0.9 vs 1.1±0.8; P=0.03).

**Conclusions.** The *Achromobacter* spp. status in CF shows a trend towards more severe airflow obstruction and an association with accelerated decline in lung function parameters.

**INTRODUCTION**

Cystic fibrosis (CF) patients are often infected with opportunistic bacteria that cause bronchopulmonary infections that lead to the deterioration of lung function and eventually premature death [1]. Although *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Haemophilus influenzae* have traditionally been the pathogens most commonly isolated in the airways of CF patients, with improved survival, other pathogens, such as *Burkholderia cenocepacia* complex, *Stenotrophomonas maltophilia* and *Achromobacter* spp. are frequently detected [2–4].

The genus *Achromobacter* contains genetically distinct species and subspecies [5]. Before the description of the new species, most *Achromobacter* spp. were referred to as *A. xylosoxidans* because conventional methods were unable to differentiate between the different species [6]. *Achromobacter* spp. is an aerobic, non-fermenting Gram-negative bacillus that is oxidase- and catalase-positive, and is widely distributed in the environment [7]. *Achromobacter* spp. is increasingly recognized as a significant nosocomial pathogen that usually affects immunocompromised patients, causing serious infections such as bacteraemia [8]. *Achromobacter* spp. have been isolated from various sources, such as blood, cerebrospinal fluid, stool, urine and sputum; and some authors have described nosocomial outbreaks produced by this micro-organism that have been attributed to disinfectant solutions [9].

The clinical impact of *Achromobacter* spp. infection in CF patients and the role of the micro-organism in declining lung function are still controversial. There are few published data on the natural course of lung disease in CF patients with *Achromobacter* spp. sputum cultures. It has been
suggested that the acquisition of chronic *A. xylosoxidans* infection is associated with more extensive lung disease and more severe airflow obstruction [10], although it is not clear whether *A. xylosoxidans* is simply a marker of severe disease due to some other cause or whether it contributes to disease progression. Somayaji *et al.* [11] observed that patients with CF and chronic *Achromobacter* infection were at increased risk of death or transplantation. A recent Danish national study demonstrated the marked effect of each Gram-negative CF pathogen, including *Achromobacter* and nontuberculous mycobacteria, on lung function [12]. By contrast, Edwards *et al.* [13] did not find evidence of lung function decline or risk of pulmonary exacerbation following persistent infection with *Achromobacter*. A previous study by our group was unable to establish a relationship between chronic *Achromobacter* infection and lung function, suggesting that the lack of relationship may have been due to the small sample size or short follow-up period [5]. The aim of this study was to further investigate the clinical repercussions of infection with *Achromobacter* spp. in CF patients.

**METHODS**

**Study design and population**

The study was performed in the Cystic Fibrosis reference unit at the University Hospital 12 de Octubre, a 1300-bed facility serving a population of 450,000 in south Madrid (Spain). This cystic fibrosis unit is also the reference centre for the South area of the Community of Madrid. All CF patients infected with *Achromobacter* spp. between January 2003 and December 2015 were analysed. Each patient was seen on a 3-monthly basis and the visits included the collection of sputum for culture, but this interval was shortened depending on clinical need. Patients were assigned to the case group (infected with *Achromobacter* spp.) or the control group (never infected with *Achromobacter* spp.), depending on their *Achromobacter* spp. infection status. To avoid possible bias due to the period of time or infections related to cystic fibrosis, according to clinical records, each patient was matched with a control CF patient with the same clinical characteristics with regard to age (+/−2 years), gender, cystic fibrosis transmembrane conductance regulator (CFTR) mutation, related diseases such as exocrine pancreatic sufficiency (EPS), CF-related diabetes, allergic bronchopulmonary aspergillosis and distal intestinal obstruction syndrome (DIOS), follow-up time and *Ps. aeruginosa* colonization, but without *Achromobacter* spp. isolates in their respiratory samples. The *Achromobacter* spp. group included two subgroups of patients who were used to consider whether the isolation of *Achromobacter* spp. during follow-up was intermittent or chronic. Patients were considered to be chronically colonized if at least three positive cultures were obtained in 1 year from the time of the initial culture, with a minimum 1-month interval between them, for at least 2 years [14]. Patients that did not comply with these culture requirements, but had some sporadic *Achromobacter*-positive cultures were considered to be intermittently colonized.

All clinical records were reviewed and the following data were collected for analysis: age, gender, CF transmembrane regulator (CFTR) mutations and presence of CF-related diseases such as exocrine pancreatic sufficiency, CF-related diabetes, allergic bronchopulmonary aspergillosis and distal intestinal obstruction syndrome. Co-colonization with other micro-organisms, such as *S. aureus*, *P. aeruginosa*, *B. cepacia*, *Stenotrophomonas maltophilia* and *Enterobacteriaceae*, was also recorded. The criterion for chronic colonization by *Ps. aeruginosa* was the same as that adopted for *Achromobacter* spp. During the study period, the number of annual pulmonary exacerbations was evaluated, according to the clinician, and defined as increased cough or sputum production, fever, weight loss, decreased exercise tolerance and absenteeism from school or work due to illness, with clinical findings including tachypnea, new crackles, decreased pulmonary function tests, reduced oxyhaemoglobin saturation and new findings on the chest radiograph [15].

**Microbiological studies**

The patients were regularly monitored in the CF unit for evaluation of clinical status. Sputum or nasopharyngeal aspirate samples were taken for microbiological examination. After previous treatment with dithiothreitol, the samples were inoculated onto Columbia 5 % blood agar, chocolate agar, MacConkey agar, mannitol salt agar and *Burkholderia cepacia* selective agar. During this period, phenotypic identification was performed using the Wider (Francisco Soria-Melguizo, Spain), the API 20 NE (bioMérieux, Marcy l’Etoile, France), the MicroScam system (Bechman, Coulter, Inc., CA, USA) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) (Bruker Daltonics, Bremen, Germany). 16S rRNA sequence analysis was used to confirm the phenotypic identification of *Achromobacter* spp.

**Lung function**

Lung function parameters, forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV1) were performed on a Jaeger spirometer MasterScope and the lung function calculation used in this study was the one proposed by the European Society of Clinical Respiratory and the World Health Organization, Europe Branch [16]. FEV1 was reported as a percentage of predicted value. Decline in lung function was defined as the annual rate of loss of FEV1% ([initial FEV1 %−actual FEV1 %]/initial FEV1 %×100)/follow-up in years) [17]. Normalized values for pulmonary function testing were validated in the study by Crapo [18]. The pulmonary function parameters were not considered in exacerbations to avoid the confounding factor.

The primary outcome of this cross-sectional study was the lung function decline between two time points: at the first positive culture of *Achromobacter* spp. (baseline moment) and the last positive culture (present moment). Patients who died or were transplanted during the study period were excluded.
Statistical analysis

Categorical variables were reported using frequencies and percentages. Continuous variables were described in terms of means (±SD) and range (min–max). Group comparisons were made using non-paired t-testing for continuous variables. Paired t-tests were in group comparisons of serial lung function trends. Categorical variables were compared using the chi-squared test or Fisher’s exact test. To determine whether there were significant variations in FEV1 and FVC values between the first and last isolation, the Wilcoxon signed rank test was used. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS 18.0 for Windows; SPSS, Inc., Chicago, IL, USA). P values of ≤0.05 were considered statistically significant.

RESULTS

Patients

A total of 190 CF patients were treated in the CF unit, and 25 were infected with *Achromobacter* spp. Of these, four patients were excluded because of death (one) and lung transplantation (three), during the study period. Finally, 21 (11 %) patients were infected with *Achromobacter* spp. at the clinical follow-up (71.4 % females, mean age 27.8 ±12.7 years, mean colonization 8.5 years). Of these, 11/21 (52.4 %) were chronically infected with *Achromobacter* spp. (72.7 % females, mean age 28.5±11.7 years, mean infection period 8.1 years) and 10/21 (47.6 %) were considered to be intermittently infected with *Achromobacter* spp. (70 % females, mean age 27.9±12.3 years, mean infection period 7.9 years). The control group consisted of 21 CF patients who had no positive cultures for *Achromobacter* spp. during clinical follow-up (71.4 % females, mean age 27.5 ±11.8 years). Table 1 shows the clinical characteristics of the case and control groups.

Lung function

A comparison between the two groups of patients showed that the baseline FEV1 and FVC levels were 69.8±12.8 and 79.6±12.1 for the case group, and 78.8±17.4 and 89.9±16.1 for the control group, respectively (P=0.20 for both comparisons) (Table 2). We also compared the baseline FEV1 and FVC levels between the chronically infected and the control groups; although these values were lower for the case group, the differences were not statistically significant (P=0.18 and P=0.17, respectively) (Table 2). Although a decline in the percentage of predicted FEV1 and FVC over time was observed in all of the CF patients included in the study, the lung function decline, measured as the change in annual FEV1, was higher in the *Achromobacter* infection group (−2.3±1.6) than in the control group (−1.1±0.9; P=0.02). Moreover, the chronically colonized group showed a significantly greater decline in FEV1 compared to the control group (−2.9±1.9 vs −1.1±0.9; P=0.01). No significant changes in these parameters were observed in the intermittently colonized and control groups (−1.6±0.8 vs −1.1±0.9; P=0.14).

Pulmonary exacerbations

The mean number of annual pulmonary exacerbations during the study period was significantly higher in the case group (1.9±0.9 vs 1.1±0.8; P=0.03). There were more annual pulmonary exacerbations in the chronically colonized (2.1 ±1.1) and intermittently colonized groups (1.7±0.9) than in the control group (1.1±0.8).

Table 1. Characteristics of the study population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Case patients (n=21)</th>
<th>Control patients (n=21)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) Mean±SD</td>
<td>27.8±12.7</td>
<td>27.5±11.8</td>
<td>0.75</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>6/21 (28.6 %)</td>
<td>6/21 (28.6 %)</td>
<td>1.00</td>
</tr>
<tr>
<td>Female</td>
<td>15/21 (71.4 %)</td>
<td>15/21 (71.4 %)</td>
<td>0.69</td>
</tr>
<tr>
<td>CFTR mutations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F508del/F508del</td>
<td>9/21 (42.9 %)</td>
<td>8/21 (38.1 %)</td>
<td>0.80</td>
</tr>
<tr>
<td>F508del/other</td>
<td>8/21 (38.1 %)</td>
<td>9/21 (42.9 %)</td>
<td>0.80</td>
</tr>
<tr>
<td>Other</td>
<td>4/21 (19 %)</td>
<td>4/21 (19 %)</td>
<td>0.42</td>
</tr>
<tr>
<td>Related disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPS</td>
<td>20/21 (95.2 %)</td>
<td>20/21 (95.2 %)</td>
<td>1.00</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>7/21 (33.3 %)</td>
<td>5/21 (23.8 %)</td>
<td>0.65</td>
</tr>
<tr>
<td>DIOS</td>
<td>3/21 (14.3 %)</td>
<td>3/21 (14.3 %)</td>
<td>1.00</td>
</tr>
<tr>
<td>ABPA</td>
<td>5/21 (23.8 %)</td>
<td>2/21 (9.5 %)</td>
<td>0.10</td>
</tr>
<tr>
<td>Follow-up time (years) Mean±SD</td>
<td>12±2.49</td>
<td>11±2.57</td>
<td>0.20</td>
</tr>
<tr>
<td>Annual exacerbations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean±SD</td>
<td>1.9±0.9</td>
<td>1.1±0.8</td>
<td>0.03</td>
</tr>
<tr>
<td>Co-infections</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>19/21 (90.5 %)</td>
<td>20/21 (95.2 %)</td>
<td>0.16</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>10/21 (47.6 %)</td>
<td>12/21 (57.1 %)</td>
<td>0.97</td>
</tr>
<tr>
<td>B. cepacia</td>
<td>3/21 (14.3 %)</td>
<td>0/21 (0 %)</td>
<td>–</td>
</tr>
<tr>
<td>S. maltophilia</td>
<td>4/21 (19 %)</td>
<td>6/21 (28.6 %)</td>
<td>1.00</td>
</tr>
<tr>
<td>Enterobactericeae</td>
<td>6/21 (28.6 %)</td>
<td>6/21 (28.6 %)</td>
<td>0.69</td>
</tr>
</tbody>
</table>

CFTR, cystic fibrosis transmembrane regulator; EPS, pancreatic insufficiency; DIOS, distal intestinal obstruction syndrome; ABPA, allergic broncho-pulmonary aspergillosis.
Co-infections

The most frequent concomitant species detected in the case patients were *S. aureus* (19/21, 90.5%), with 5 isolates being methicillin-resistant *S. aureus* (MRSA) (4 in intermittent case patients and 1 in chronic case patients), and *P. aeruginosa* (10/21, 47.6%), [5 in chronic case patients (2 with chronic colonization) and 5 in intermittent case patients (3 with chronic colonization)], with 3 of them having the mucoid morphotype. *B. cepacia* was found in three patients, two intermittent case patients and one chronic case patient. Among the control patients, 57.1% (12/21) were co-infected with *P. aeruginosa*, and of these, five were considered to be intermittently colonized and seven were considered to be chronically colonized. One control patient was chronically co-infected with MRSA. *P. aeruginosa* leads to a decrease in lung function and could be a source of bias in the analysis of results, we stratified the chronically *Achromobacter* group in those with (*n*=5) or without (*n*=6) *P. aeruginosa* co-infection. The comparison of the FEV1 values did not show statistical differences (53.40±44.7 ml/year vs 94.00±38.9 ml/year, *P*=0.145).

### DISCUSSION

The results of this study suggest that persistent infection of CF patients with *Achromobacter* spp. could be a marker of airflow obstruction associated with an accelerated loss of lung function. Over the study period (13 years), 11% of our patient population had at least one positive culture for *Achromobacter* spp. This frequency was similar to those reported by Edwards et al. and Magni et al., with percentages of 11 and 8.9%, respectively [13, 19], but higher than the 5.3% reported by Baets et al. [20].

The colonizing ability of this micro-organism is known, although the clinical significance of this infection has been difficult to establish. Raso et al. [21] found an association between *Achromobacter* spp. infection and more severe disease in CF patients. Our study showed that CF patients infected with *Achromobacter* spp. had worse lung function and more severe airway obstruction than those not infected with this micro-organism. It was also shown that patients infected with *Achromobacter* spp. had an increased rate of lung function decline compared to the non-infected. The decline in lung function observed in our cohort was similar to that in previous observations [17, 22]. It was also found that patients infected with *Achromobacter* spp. had poorer lung function at the time of initial infection compared to those without *Achromobacter* spp.

It has been suggested that the acquisition of chronic infection with *Achromobacter* spp. is associated with a poor pulmonary status, as measured by FEV1 levels at the time that chronic colonization is established [23]. Our results suggested an association between chronic *Achromobacter* spp. infection and more severe airflow obstruction, which was present in patients who acquired *Achromobacter* spp. during the study period and in those who were chronically infected, but not in the group with intermittent isolation. The association with more severe disease was also true for predicted FVC. Some authors have reported that *Achromobacter* spp. has a high affinity for mucus and it is possible that the increased mucus viscosity and loss of cilia predisposes patients to colonization [19]. Another possible factor is antibiotic treatment, which may be given more frequently in cases of CF and drives airway bacterial flora towards the more antibiotic-resistant *Achromobacter* spp. [24]. These authors previously hypothesized that patients with chronic *Achromobacter* spp. infection had a poorer quality of life, in part because they had more severe disease, but also because they were given more medication and required more hospital admissions. Nevertheless, it is equally possible that without this extra treatment, patients may have experienced an accelerated decline following infection with *Achromobacter* spp. [25].

Compared with the control group, there were a higher number of pulmonary exacerbations and hospitalizations in the chronically infected group. This finding may be explained by the fact that the condition of this group of patients was more severe, or by other conditions contributing to the outcome, as previously reported [5, 22]. Half of the chronically infected *Achromobacter* spp. patients were co-infected with *P. aeruginosa*, and according to the results obtained in this study, *Achromobacter* spp. seems to have an independent
effect on lung function, although it is important to bear in
mind the multiple contributing factors that have not been
addressed here.

Several limitations of this study must be noted. First, the
small sample size reduced the statistical power of this study
considerably. Second, although we have matched every case
patient with a control patient with similar characteristics,
multiple other confounding factors and missing data could
have impacted on the results, taking into account the long
period of time over which the study was conducted. Third,
although we studied the lung function for all patients in
similar conditions, we only had information on two time
points (the initial and end time points) with which to ana-
lyse the annual lung function decline, so we were not able
to examine the lung function in a longitudinal manner. Fourth,
it is possible that other micro-organisms that were not
detected in this study could have influenced the outcomes.
In general, it is plausible that some patients had other fac-
tors that may have contributed to lung function decline and
reduced the observed differences between groups. A larger
multicentre study is needed to see whether our findings can
be confirmed.

In conclusion, the *Achromobacter* spp. status in CF shows a
trend towards more severe airflow obstruction and it seems
to be associated with an accelerated decline in pulmonary
function parameters. Further larger-scale studies are needed
to demonstrate the source and route of acquisition of *Achro-
mobacter* spp., and to correlate these with the outcomes for
CF patients infected by this micro-organism.

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**Conflicts of interest**
The authors declare that there are no conflicts of interest.

**Ethical statement**
This study received ethical approval from the Investigation Com-
mmittee of the Hospital Universitario 12 de Octubre (TP16/0065).

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