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Chronic infection with *Achromobacter xylosoxidans* in cystic fibrosis patients; a retrospective case control study

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Abstract

Background: In cystic fibrosis (CF), chronic infection of the airways with *Achromobacter xylosoxidans* have become more frequent. The pathogenic role of this is yet unclear.
Methods: A retrospective case-control study of all patients chronically infected with *A. xylosoxidans* for at least 3 years. 15 patients (6 males) with chronic *A. xylosoxidans* infection were matched by age, FEV_1 and body mass index *z*-score to 15 controls (7 males) at the time of establishment of chronic infection. Clinical parameters of the groups were compared from the time of establishment of chronic infection until spring 2006, giving a follow-up time of 3–11 years. Chest X-rays taken 3 years prior to establishment of chronic infection and after 3 years of chronic infection were compared using a modified Brasfield score. Finally, strains from individual patients were analysed using PFGE to investigate possible cross-infection.
Results: The median slope of decline of FEV_1 in the case group changed from +3.1% to −0.5% predicted/year ($p<0.002$). In the control group, median slope of decline in FEV_1 changed from +1.5% to −0.4% predicted/year (n.s.). Median slope of decline in FVC in the case group changed from +3.5% to −0.5% predicted/year ($p<0.002$). In the control group, median slope of decline in FVC changed from +1.7% to +0.4% predicted/year (n.s.). No significant difference in the slopes of decline of FEV_1 or FVC was found between the case group and the control group at either time. Change in BMI *z*-score was calculated for each group before and during chronic infection. No difference was found between the groups at any time or within a group. Specific antibodies against *A. xylosoxidans* were measured in patients with chronic infection. Patients with rapidly increasing antibody levels showed significantly faster deterioration in FEV_1 ($p<0.05$) and FVC ($p<0.02$). Chest X-ray scores increased in 6 of 10 chronically infected patients and in 3 of 10 controls (n.s.).
Eight patients harboured a common *A. xylosoxidans* strain, indicating either cross-infection or a common source.
Conclusion: *A. xylosoxidans* may lead to a decline in lung function in a subgroup of chronically infected CF patients characterised by a rapid increase in specific precipitating antibodies. Cross-infection may possibly occur.

1. Background

Several studies have shown the ability of *Achromobacter xylosoxidans* to cause chronic colonization of airways of patients suffering from cystic fibrosis [1–8].

A. xylosoxidans is isolated with increasing frequency in CF patients all over the world. Identification of the microorganism can be difficult and the prevalence of respiratory tract infections caused by *A. xylosoxidans* might be underestimated. The U.S. Cystic Fibrosis Foundation's National Patient Registry reported an increasing rate of respiratory infection with *A. xylosoxidans.* Percentage of patients harbouring *A. xylosoxidans* increased from 0.5% to 1.9% and to 2.7% in the years 1995, 1996 and 1997, respectively, and further to 3.8% and 5.2% in 1999 and 2002 [9–13]. 8.7% of CF patients participating in a study of aerosolised tobramycin taking place in 1995–1996 were harbouring *A. xylosoxidans* [2]. *A. xylosoxidans* may possess the ability to cause cross-infection between patients [3,7,14], although some patients may be exposed to these micro-organisms for a very long time before achieving infection [4].

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In the light of our own observation of an increasing number of patients becoming chronically infected with *A. xylosoxidans*, the present study was carried out.

The aim of the present study is to describe the occurrence of chronic infection with *A. xylosoxidans* in our CF centre, to assess the importance of this infection on the progress of CF lung disease and to evaluate the possibility of cross-infection with this microorganism among CF patients.

2. Materials and methods

2.1. Study design

A retrospective case-control study of CF patients treated at the Copenhagen CF clinic and chronically infected with *A. xylosoxidans*. The patients were followed in a 16 years period, from 3 years before the first patient became chronically infected (1990) until end of study period in March 2006.

The diagnosis of CF was established on the basis of abnormal sweat electrolytes, characteristic clinical features and genotype.

2.2. Patients

All CF patients in the center are seen on a regular monthly basis. At each visit, the clinical status of the patients is assessed by weight, height and lung function parameters (forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV_1), Pneumothac, Draeger). Lower respiratory tract secretions are obtained by coughing (sputum producers) or endo-laryngeal suction, and sputum microbiology is carried out. Infection is considered chronic, when an organism has been cultured for six consecutive sputum samples (monthly) or less if there is a simultaneous increase in precipitating antibodies against the specific microorganism above normal level (0–1) [15].

All patients are treated according to fixed guidelines, which include PEP mask, and, since 1994, daily administration of Pulmozyme. *A. xylosoxidans* is treated at the first isolate with 3 weeks of inhaled colistin and oral amoxicillin with clavulanic acid. If this treatment is insufficient, i.e. the microorganism is present in the next lower respiratory tract secretion, the treatment is repeated, this time for 3 months. If the infection fulfills the criteria for chronic infections, the patients are treated with continuous inhaled and oral antibiotics, depending on the resistance pattern of the microorganism, and with elective courses of i.v. antibiotics for 2 weeks every 3 months, similar to patients chronically infected with *Pseudomonas aeruginosa* [16]. This treatment strategy was not changed during the study period.

All patients chronically infected with *A. xylosoxidans* were evaluated for inclusion in a case-control study. Only patients chronically infected for at least 3 years were included. All cases and controls were matched on age (±3 years), gender, FEV_1 (±10% predicted) at the time of establishment of chronic *A. xylosoxidans* infection in the case patients, and by co-infection with *P. aeruginosa* or other gram-negative microorganisms. Patients chronically co-infected with *Burkholderia multivorans* or *Mycobacterium abscessus* were excluded from the study because of difficulties in finding control subjects.

2.3. Changes in clinical parameters

FEV_1 and FVC were expressed as slope of decline in a 3-year period prior to establishment of chronic infection and in the entire observation period (3–9 years) after establishment of chronic infection. Change in nutritional status was expressed as change in body mass index (BMI) standard deviation scores (*z*-scores) prior to and during chronic infection.

2.4. Chest X-rays

Two independent radiologists, blinded to status of individual patients, scored chest X-rays obtained 3 years before and 3 years after establishment of chronic infection. All chest X-rays were taken during a stable period. We used a modified Brasfield score [17], judging only the overall severity of changes on roentgenograms, since the quality of some of the old chest X-rays made it impossible to judge scores on air trapping, linear markings, nodular-cystic lesions and large lesions.

2.5. Cross-infection

Because of the rapidly increasing number of patients chronically infected with *A. xylosoxidans*, pulsed field gel electrophoresis (PFGE) [18] was done, if possible, on strains isolated from individual patients.

2.6. Measurement of specific anti-achromobacter antibodies

Specific IgG anti-achromobacter antibodies were measured at least once a year, using the method described for *P. aeruginosa* by crossed immunoelectrophoresis [19]. Normal values: 0–1 precipitins.

3. Statistical analysis

Gender and genotype in the case and control groups, and speed of increase in precipitating antibodies in the two groups colonized with unique and shared strains were compared using chi-squared test.

Calculation of slope of decline in lung function was done using linear regression analysis. All monthly performed lung functions measured were included for calculation of the slope of decline in the two time periods.

Change in body mass index (BMI) *z*-score was calculated using linear regression analysis. All monthly measure-

ments of height and weight were used to calculate the change in BMI *z*-score.

Lung function and BMI *z*-score values were compared using Student's *t*-test: either paired *t*-test when comparing data over time in a group or non-paired *t*-test when comparing different groups. Level of significance <0.05 (two-tailed).

Chest X-ray scores were compared using Mann–Whitney's test when comparing groups and Wilcoxon signed ranks test when comparing changes over time in a group.

Correlation between change in lung function and level of precipitating antibodies was calculated using Pearson's correlation.

4. Results

4.1. Patients

At the end of the study period, 22 of 284 CF patients (current number) treated at the Copenhagen CF center (8% of the entire population) had been chronically infected with *A. xylosoxidans* (10 males). In Fig. 1, the cumulative number of chronically infected patients in the entire study period is illustrated. Of all chronically infected patients, 15 patients (6 males) fulfilled the inclusion criteria. Two patients were not old enough to being able to perform lung function measurement prior to establishment of chronic infection, but were included for evaluation of nutritional status. Five patients had been chronically infected for less than 3 years and were not included. Two other patients were co-infected with either *B. multivorans*, or *M. abscessus*, which made it impossible to find controls of the same age, sex, lung-function and these specific co-infections. Age, FEV_1, gender and number of patients with diabetes at the time of inclusion in the study are shown in Table 1. All patients included in the study were pancreas insufficient and were carrying severe CF mutations. None of the patients chronically infected with *A. xylosoxidans* died during the study period, but two patients had lung transplantation done during the study period and were excluded from further evaluation at that moment.

Table 1
Gender, FEV_1 and age in the two groups

	Cases	Controls	*P*-value
Number (males)	15 (6)	15 (7)	n.s.
Diabetes	3	2	n.s.
Median FEV_1 (% predicted), no=13	87.8 (range 52.2–110.7)	90.8 (range 48.2–109.6)	n.s.
Median age, no=15	16.1 (range 6.6–27.8)	15.7 (range 7–35.9)	n.s.

Median follow-up time after establishment of chronic infection was 6 years (range 3–11).

Three of the 13 included patients had chronic co-infection with *P. aeruginosa*. In two of these patients, sputum cultures remained positive for *P. aeruginosa* after establishment of chronic infection with *A. xylosoxidans*, and one patient has only intermittently been carrying this microorganism after 3 years of chronic *A. xylosoxidans* infection.

4.2. Lung function

The changes in lung function in both case and control groups in the 3-year period prior to as well as in the years after establishment of chronic *A. xylosoxidans* are shown in Figs. 2a–b and 3a–b.

We found no significant difference in FEV_1, when comparing the groups of cases and controls neither prior to the study period, nor during the study period (Table 2).

However, FEV_1 changed significantly within the case group when comparing change in FEV_1 prior to chronic infection and during chronic infection ($p<0.002$). This was not the case in the control group (Table 2 and Fig. 2a–b).

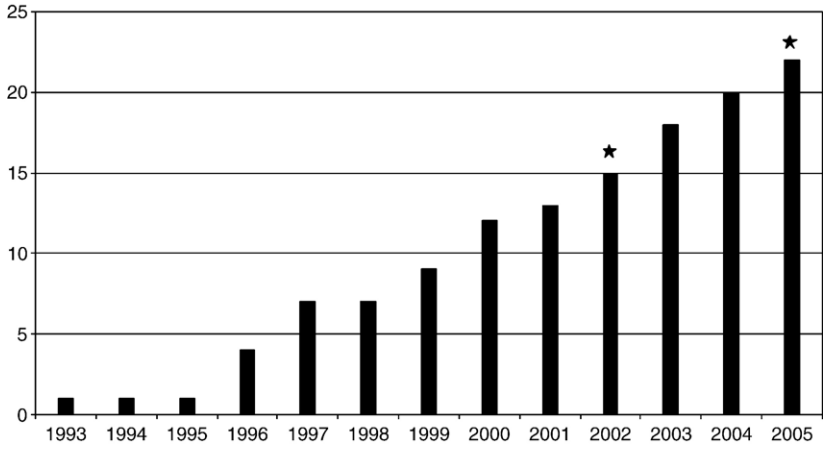

Fig. 1. Cumulative number of patients chronically infected with *A. xylosoxidans* in the Copenhagen Cystic Fibrosis Centre. (★) Two patients have got lung transplantation: one in 2002, after 6 years of chronic infection; one in 2005, after 12 years of chronic infection.

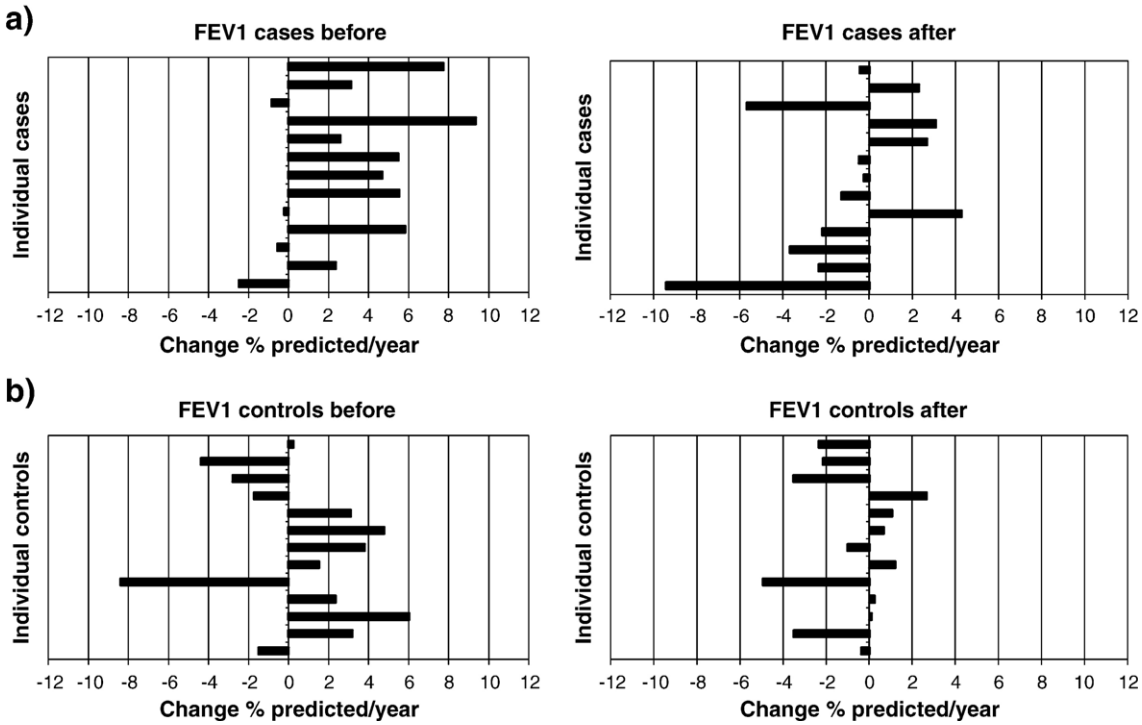

Fig. 2. Change in FEV1 before and during chronic infection in case group (a) and control group (b).

The difference in change in FEV_1 in the periods before and during chronic infection in the case-group was −4.9% predicted/year (range −8.2 to +4.5) and in the control-group −2% predicted/year (range −6.7 to +4.4) ($p<0.05$).

Likewise, we found no significant difference in FVC when comparing the two groups neither prior to the study period nor during the study period (Table 3).

However, the slope of FVC changed significantly within the case group when comparing change in FVC

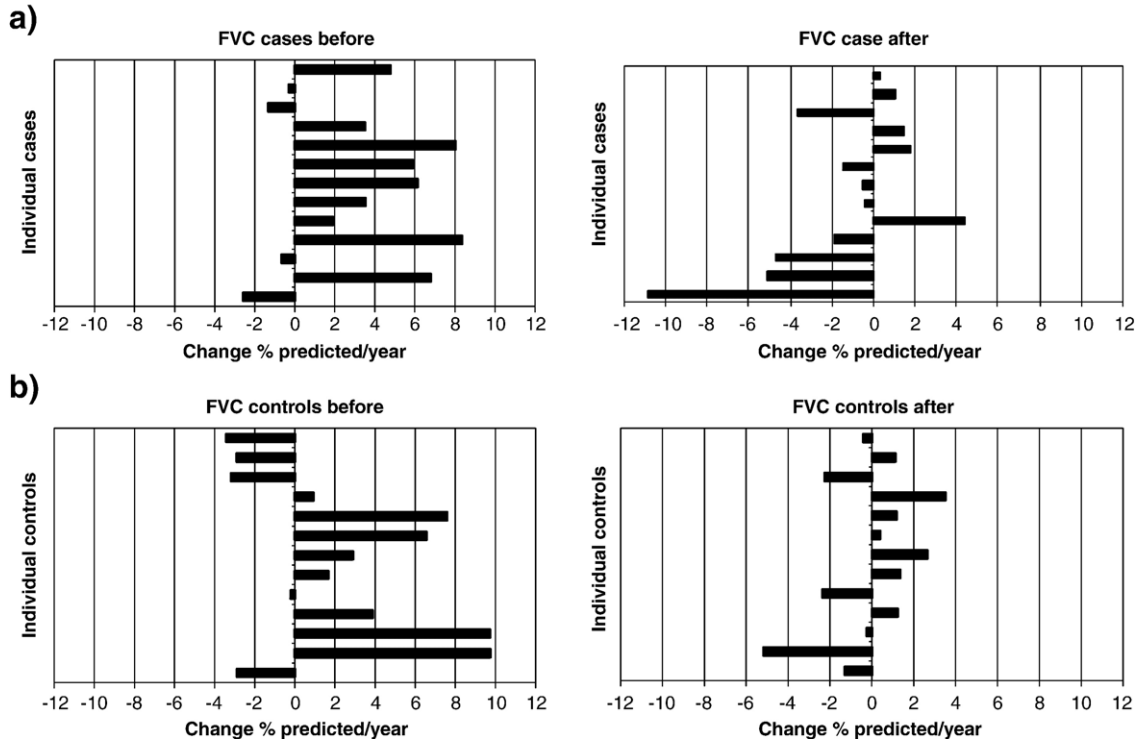

Fig. 3. Change in FVC before and during chronic infection in case group (a) and control group (b).

Table 2
Median change in FEV_1 (% predicted/year) before and during chronic infection

	3 years before	During chronic infection	*P*-value
Cases	+3.1 (range −2.5 to +9.4)	−0.5 (range −9.4 to +4.3)	<0.002
Controls	+1.5 (range −8.4 to +6)	−0.4 (range −4.9 to +2.7)	n.s.
P-value	n.s.	n.s.	

prior to chronic infection and during chronic infection ($p<0.002$). This was not the case in the control group (Table 3 and Fig. 3a–b).

The difference in change in FVC in the periods before and during chronic infection in the case group was −4.5% predicted/year (range −11.9 to +2.4) and −0.3% predicted/year (range −14.9 to +4) in the control group (n.s.).

In the case group, the number of patients who had decreasing levels of FEV_1 and FVC before the chronic infection was established was 4, and this number increased to 9 and 8, respectively. In the control group, the number of patients with decreasing levels of FEV_1 and FVC before the study period was 5 in both groups, and during the study period 7 and 6, respectively.

4.3. Nutritional status expressed as BMI z-score

Median change in BMI *z*-score was −0.11/year (range −1.24 to +0.34) in the case group and −0.05/year (range −0.3 to +0.24) in the control group prior to the observation period. During the study period, the nutritional status of the patients improved both in case and control groups. Median change in BMI *z*-score in the case group was +0.01/year (range −0.27 to +0.27) and +0.02/year in the control group (range −0.36 to +0.25). We found no significant difference between or within the groups.

4.4. Chest X-ray scores

Pairs of chest X-rays were found on 20 of 30 patients: 10 in the case group and 10 in the control group. Possible range of scores was 0–5. We found no patients with a score of 5. 3 years prior to chronic infection, the group of cases had a median score of 2 (range 0–4) and the group of controls had a median score of 1 (range 0–3) (n.s.). 3 years after chronic infection, the case group had a median score of 3 (range 1–4) and the group of controls had a median score of 1 (range 0–4) (n.s.). We found no difference in scores within groups over time. However, in the case group, six patients had increasing scores over time, compared to three patients in the control group.

Table 3
Median change in FVC (% predicted/year) before and during chronic infection

	Before	During chronic infection	*P*-value
Cases	+3.5 (range −2.6 to +8.4)	−0.5 (range −10.9 to +4.3)	<0.002
Controls	+1.7 (range −3.4 to +9.7)	+0.4 (range −5.2 to +3.5)	n.s.
P-value	n.s.	n.s.	

4.5. Specific precipitating antibodies

The level of specific precipitating antibodies against *A. xylosoxidans* increased during the first 3 years of chronic infection (Table 4). The antibody levels increased to a median of 7 precipitins (range 1–21) in the first year of chronic infection and further to 14 (1–31) and 17 (1–34) in the second and third years of chronic infection, respectively. In the control group, the antibody level remained low (0–1 precipitins).

4.6. Correlation of levels of specific anti-achromobacter antibodies and clinical condition of the patients

Patients with established chronic infection with *A. xylosoxidans* could be subdivided into two groups: one with a rapid increase in precipitating antibodies against *A. xylosoxidans* to at least 15 during the first 3 years of infection ($N=7$) and one with a slower increase in precipitating antibodies ($N=6$). Median change in FEV_1 in the group with rapidly increasing antibodies was +2.4% predicted/year (range −2.5 to +9.4) before chronic infection and decreased to a median of −2.3% predicted (range −9.4 to +3.1) during chronic infection ($p<0.02$). Median change in FEV_1 in the other group was +3.9% predicted/year (range −0.2 to +7.7) before chronic infection and changed to a median of +0.9% predicted/year (range −1.3 to +4.3) during chronic infection (n.s.). We found no difference between the two groups prior to chronic infection, but during chronic infection the difference was significant ($p<0.05$). Correlation between level of precipitating antibodies and change in FEV_1 was highly significant ($p<0.005$) (Fig. 4).

Median change in FVC in the group with rapidly increasing precipitating antibodies changed from +3.5% predicted/year (range −2.6 to +8.4) prior to chronic infection to −3.7% predicted/year (range −10.9 to +1.4) during chronic infection ($p<0.005$). Median change in FVC in the group with slowly increasing precipitating antibodies changed from +4.2% predicted/year (range −0.3 to +8) before chronic infection to +0.7% predicted/year (range −0.5 to +4.3) (n.s.). There was no difference between the

Table 4
Level of specific anti-achromobacter precipitating antibodies in the years after establishment of chronic infection

Year	Median level (range)	Number of patients
1	7 (1–21)	14
2	14 (1–31)	14
3	17 (1–34)	15

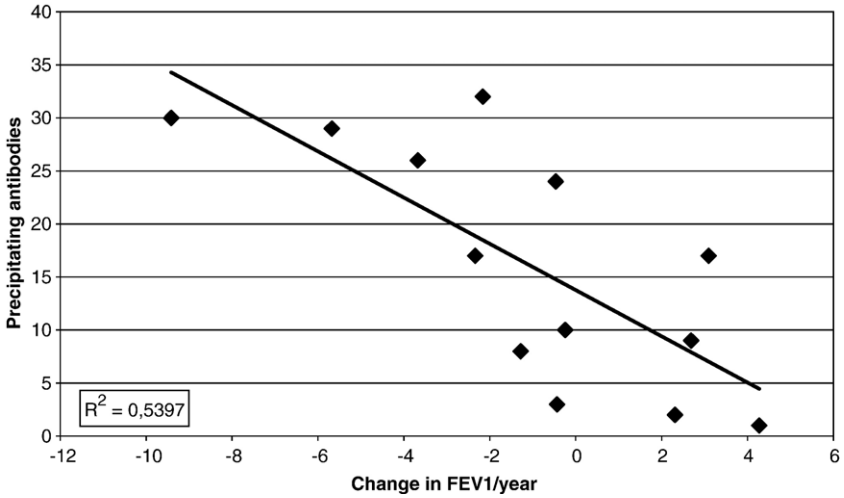

Fig. 4. Correlation between FEV1 and level of precipitating antibodies in the third year of chronic infection.

subgroups prior to chronic infection, but during chronic infection the difference was significant ($p < 0.02$).

We found no difference between the two subgroups when comparing change in BMI *z*-score.

4.7. PFGE

Isolates from 18 of 23 chronically infected patients (13 of 15 patients included in this study) were collected and typed using pulsed field gel electrophoresis. We found eight patients sharing the same strain, while 10 patients had individual genotypes. One of the eight patients carrying the same genotype is the index patient, chronically infected since 1993.

Of the eight patients carrying the cluster genotype, six had a rapid increase in precipitating antibodies. Of the 10 patients carrying individual genotypes, four had a rapid increase in precipitating antibodies (n.s.).

5. Discussion

The present study calls attention to a new emerging chronic infection, which affects an increasing number of CF patients and which might have a negative influence on the clinical course of disease.

A. xylosoxidans is a multi-resistant microorganism, which makes antibiotic treatment difficult. In spite of early and aggressive treatment, patients rapidly developed chronic infection with *A. xylosoxidans*; in many patients in the case group, the level of specific precipitating antibodies defined chronic infection even after only a few positive sputum cultures. Although we only have a small number of patients in either group, we have followed the patients for a long time after establishment of chronic infection.

Prior to establishment of chronic infection, the groups of cases and controls in our study were in stable clinical condition with stable lung function parameters. After establishment of the chronic infection with *A. xylosoxidans*, the case group showed disease progression, particularly in a subgroup of chronically infected patients. This group is characterised by a more rapidly increasing level of specific precipitating antibodies, which might be caused by either more invasive infection due to an infection with a more virulent strain, leading to a greater inflammatory response, or a greater inflammatory response in general in this group of patients. PFGE-typing of 18 strains found in our population showed a cluster of eight patients harbouring the same strain, indicating either that cross-infection had occurred, or a common source of the infection. Transmissible *P. aeruginosa* strains associated with more aggressive infection have been reported [20].

In chronic infection caused by *P. aeruginosa* rapidly increasing number of precipitins to *P. aeruginosa* has been shown to be associated with poor prognosis [19]. The level of specific, precipitating anti-achromobacter antibodies increased much faster in our study group during chronic infection when compared to the level of anti-pseudomonas antibodies in the first years of chronic infection with *P. aeruginosa* [21] despite similar intensive antibiotic treatment. No significant correlation between occurrence of similar strains and either deterioration in lung function or levels of specific antibodies were found in the present study. The small number of patients can be a possible explanation.

Our observations can indicate that chronic infection with *A. xylosoxidans* may have a clinically relevant impact on the clinical condition of CF patients, especially when the infection results in a pronounced antibody response, and is caused by a transmissible strain.

Scoring of chest X-rays using a modified Brasfield score showed no difference between the groups. There was a tendency, however, that more patients in the case group had an increase in score over time. The number of pairs of chest X-rays scored were very small—only 10 pairs in each group, and this might be the reason why the difference was not significant.

We found no difference in increase in precipitating antibodies between patients carrying unique genotypes and patients carrying shared genotype, but again the number of patients in each investigated group was small.

Only a few other studies have been published on the effect of establishment of chronic infection with *A. xylosoxidans* on the course of CF disease [1,3–7,14,22,23]. Tan et al. [6] observed a greater decline in FVC than FEV1 and, for both parameters, a greater decline in the case group than in the control group during 4 years of observation, even though this difference was not statistically significant. Preliminary results from a study by Romano et al. [5] likewise showed no significant change in lung function in 15 patients chronically infected with *A. xylosoxidans*. Dunne and Maisch [22] found *A. xylosoxidans* in sputum from CF patients with exacerbations, but data on lung functions of these patients are not shown and sputum samples from CF patients with no respiratory symptoms were not investigated. Similar cases with clinical symptoms in patients harbouring *A. xylosoxidans* have been described by Romano et al. and by Abdul Wahab et al. [1,5]. In a study by Burns et al. [2], the patients included in a study of aerosolised tobramycin, where inclusion criteria were chronic infection with *P. aeruginosa* and FEV1 between 25% and 75% predicted, had a higher percentage of sputum cultures positive for *A. xylosoxidans* (8.7%) when compared to the Patient Registry Annual Report of the Cystic Fibrosis Foundation from the same time period (0.5% and 1.9%, respectively) [9,10]. One possible explanation can be that patients with a preserved normal lung function have lower occurrence of *A. xylosoxidans*. There are other case reports on patients with rapid decline in lung function and deterioration of the clinical status after acquiring chronic *A. xylosoxidans* infection, even leading to death in less than a year [5]. Interpretation of these data is difficult, because most patients had chronic co-infection with *P. aeruginosa*.

6. Conclusion

A. xylosoxidans may be responsible for greater decline in lung function, particularly in a subgroup of CF patients, characterized by a rapid increase in precipitating antibodies. Cross-infection may possibly occur. Study of disease progression in a larger group of CF patients infected with this microorganism is desirable to substantiate our observation.

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