Long term follow up of changes in FEV_1 and treatment intensity during *Pseudomonas aeruginosa* colonisation in patients with cystic fibrosis

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Abstract

***Background*—Colonisation with *Pseudomonas aeruginosa* (PA) is a striking feature of lung involvement in cystic fibrosis. To identify the clinical consequences of the different steps of colonisation with PA under a defined therapeutic regime (no prophylactic antibiotic treatment as long as patients had no severe pulmonary disease), their influence on pulmonary function and on therapeutic intensity was examined.**

***Methods*—Forty patients with cystic fibrosis were followed from first detection of PA (PA1), chronic PA colonisation (PAc), first mucoid PA detection (PAm), to chronic mucoid PA colonisation (PAcm). Percentage predicted forced expiratory volume in one second (FEV_1), the number of intravenous antibiotic treatment courses, and the percentage of patients on inhaled antibiotics were followed retrospectively and longitudinally in relation to the different steps of PA colonisation. The annual changes in FEV_1 and therapeutic intensity in the two years preceding each step were compared with the two years following each step. Changes in FEV_1 were related to therapeutic intensity.**

***Results*—The mean (SD) annual changes in FEV_1 (% predicted) worsened significantly only with the transition to the mucoid stages (PAm: 4.6 (13.2) versus –4.3 (8.1); PAcm: 7.3 (12.0) versus –4.8 (7.4)) with a mean difference (95% CI) between before and after the transition of 8.9 (2.6 to 15.2) for PAm and 12.1 (6.4 to 17.6) for PAcm. With non-mucoid PA stages the therapeutic intensity increased in the year of transition and with mucoid PA stages it increased in the years following transition. Therapeutic intensity was unrelated to changes in FEV_1.**

***Conclusion*—With the treatment regime used an accelerated decrease in FEV_1 was successfully prevented in the non-mucoid stages but not in the mucoid stages of PA colonisation.**

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Keywords: *Pseudomonas aeruginosa* colonisation; pulmonary function; antibiotic treatment; cystic fibrosis

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Cystic fibrosis is the most common life threatening inherited multiorgan disease in Caucasians.[1] The major cause of morbidity and mortality is lung involvement. A striking feature of lung involvement in cystic fibrosis is the colonisation with *Pseudomonas aeruginosa* (PA) in the lower respiratory tract. In patients with cystic fibrosis colonisation with PA in the lower respiratory tract is a multistep process. It starts with first detection of PA and finishes with chronic mucoid colonisation.[1] The time from first detection of PA to chronic mucoid colonisation is variable. Only a few patients never reach the end point with chronic mucoid PA colonisation. If a differentiation is made between PA strains during PA colonisation, only mucoid PA strains and not non-mucoid strains in respiratory tract cultures have a predictive effect on survival.[2] The significance of PA is not only under discussion with regard to survival, but also with respect to pulmonary function. A longitudinal follow up study starting two years before and finishing two years after PA colonisation observed no immediate or rapid reduction in lung function after pulmonary colonisation with PA.[3] A very recent longitudinal study focused on the acquisition of chronic mucoid PA. This step in the course of PA colonisation was associated with an accelerated decline in pulmonary function.[4] In a cross sectional study the FEV_1 of patients without PA was compared with that of patients with different strains of PA. Only patients with mucoid PA had significantly lower FEV_1 than those without PA colonisation.[5] In summary, these reports show that PA colonisation is a relevant factor in the clinical course of cystic fibrosis and that the stage of PA colonisation may be an important cofactor.

Changes in pulmonary function in cystic fibrosis are influenced not only by the stage of PA colonisation but also by antibiotic treatment effective against PA. The question which arises is how to treat PA at the different stages of colonisation in order to minimise the decrease in pulmonary function. Antibiotic treatment for acute exacerbations[6–8] or maintenance therapy[9–13] improved pulmonary function with intravenous,[6 10 11] oral[7] or inhalation[8 9 12 13] application. Very little detailed information is available on the therapeutic condition in relation to described changes in pulmonary function with the transition to different stages of PA colonisation.[3 4] Only one recent study has described changes in pulmonary function in patients with cystic fibrosis in relation to different steps of PA colonisation and gave detailed information about the treatment regime which was used.[11] The patients with chronic PA colonisation were treated 3–4

times a year with intravenous antibiotics effective against PA. This treatment was independent of the actual clinical status and was unchanged over the whole observation period. With this treatment regime forced vital capacity (FVC) remained nearly constant over a period of 10 years in patients with mucoid PA at the first isolation of PA. No information was given on FEV_1 which is more related to airway obstruction than FVC. There is no general consensus on how to treat PA during the course of colonisation in patients with cystic fibrosis. Some treat PA at the first detection as described by the Danish cystic fibrosis centre[14 15] while others did not.[16]

No long term data are available on changes in pulmonary function with inhaled antibiotic maintenance treatment in relation to different steps in the course of PA colonisation. A recent report discussing the antibiotic prescribing practices in the UK and Eire showed that, for maintenance of lung function in patients with persistent PA, all physicians used nebulised antibiotics, but the indication for their use varied between units.[17] Indications for intravenous therapy (PA related) were routine use, irrespective of disease severity, in five out of 10 cystic fibrosis units and severe or rapidly deteriorating lung function in the other five.[17] In the USA there is no routine use of intravenous therapy, irrespective of disease severity, in the presence of PA.[16 18] These observations are supported by data from the Cystic Fibrosis Registries of the USA[19] and Europe[20] if one calculates the number of PA colonised patients and the number of annual intravenous treatment courses per patient and the percentage of patients on nebulised antibiotics. Studies comparing different treatment regimes—that is, maintenance nebulised antibiotics versus intravenous treatment given 3–4 times annually irrespective of disease severity—with respect to different steps in the course of PA colonisation are not available.[21] Our objective was to identify the clinical consequences of the different steps of PA colonisation under a defined therapeutic regime with no prophylactic antibiotic treatment as long as patients had no severe pulmonary disease. The influence of different stages of PA colonisation on changes in pulmonary function and on therapeutic intensity were longitudinally examined.

Methods

In a retrospective longitudinal study performed between 1983 and 1995 we analysed FEV_1 and therapeutic efforts in terms of antibiotic therapy effective against PA. The last entry into the study was on 30 September 1992. Data were taken from the data base of the Cystic Fibrosis Centre at the Medical School, Hannover. The criteria for entry into the study were as follows: (1) sputum or throat swab free of PA with a minimum of two cultures or the age of the patient less than one year at the date of first PA detection, (2) first detection of PA after 1 January 1985, (3) the patient was regularly followed in the Cystic Fibrosis Centre for at least three years after the first PA positive sputum or throat swab culture. Only data of patients for whom informed consent was received were used.

PATIENTS

Cystic fibrosis was diagnosed by increased chloride concentrations (>60 mmol/l) in a sweat test[22] and typical clinical symptoms of cystic fibrosis, or the detection of disease causing mutations in both cystic fibrosis transmembrane conductance regulator (CFTR) alleles. Patients were seen regularly one to four times a year.

DETECTION OF PA AND DEFINITION OF STEPS IN THE COURSE OF PA COLONISATION

At each visit a sputum sample or a deep throat swab was collected. The samples were cultured on standard media and PA was identified by a standard test, as has been described in detail for a subset of these patients.[23] Mucoid strains were identified visually by typical morphology (abundance of watery and viscous slime). The steps in the course of PA colonisation were defined as first PA detection (PA1), chronic PA colonisation (PAc), first detection of mucoid PA strains (PAm), and chronic mucoid PA colonisation (Pacm).[4] For transition to the chronic stages (PAc and PAcm) more than 50% of cultures in a 12 month period had to be positive for PA and the related phenotype (mucoid or non-mucoid). This is the same definition as that used by others[4] who longitudinally followed changes in pulmonary function in relation to chronic mucoid colonisation.

TREATMENT

The antibiotic treatment regime followed remained unchanged during the observation period. This was especially the case regarding the indication for antibiotic treatment. PA was treated on demand in slightly to moderately severely ill patients (judged by clinical symptoms and related to a clinical score published in 1979[24]) and routinely 3–4 times a year in severely ill patients with two week intravenous antibiotic courses in hospital. Severely ill patients received additional maintenance treatment with daily inhalation of antibiotics as first described in 1981.[9] Routine early antibiotic treatment of first PA colonisation was not performed before 1993 with either inhaled or systemic antibiotics. No patient in this study received early antibiotic treatment at first PA colonisation. Systemic anti-inflammatory treatment was not provided. Treatment guidelines were defined by one of us (HHvd) over the whole observation period.

From 1988 onwards patients were placed on separate floors in the ward according to PA status (free versus colonised).

DATA PROCESSING

To exclude patients with transient PA colonisation, only those patients who started with first PA detection and finished with chronic mucoid PA colonisation during the observation period were further investigated. Four separate subgroups (PA1, PAc, PAm and PAcm) of patients were created, each related to one single step in

the course of PA colonisation. For each of these four subgroups data regarding pulmonary function and treatment intensity were analysed longitudinally and separately. The investigation period began two calendar years before and ended two years after the year in which each single step in the course of PA colonisation took place. The annual changes in FEV_1 (% predicted), annual number of intravenous treatments, and the percentage of patients with daily inhaled antibiotics (tobramycin or colistin) were calculated. Equal time intervals before and after each single step in the course of PA colonisation were compared in terms of pulmonary function. The best annual FEV_1 (% predicted) was used to calculate the annual decrease in FEV_1 as a percentage of the baseline value from two years before transition to transition, and from transition to two years later. Data from the years before and after transition were compared with the year of transition with regard to therapeutic efforts.

PULMONARY FUNCTION TESTS

Until 1990 pulmonary function tests were performed by body plethysmography (Dr Fenyves & Gut, Basel, Switzerland). Since 1991 either a Compact Lab Transfer (Jäger, Würzburg, Germany) or, since 1992, a Bodyscope (Ganshorn, Niederlauer, Germany) was used. FEV_1 (% predicted) was calculated using Knudson's equilibrations.[25] Pulmonary function tests started at the age of six and were repeated if possible on every visit to the Cystic Fibrosis Centre.

STATISTICAL ANALYSIS

Non-parametric tests (Wilcoxon test for paired samples and McNemar test) were used for comparison within groups. The influence of antibiotic treatment on changes in annual FEV_1 (% predicted) in relation to each single step in the course of PA colonisation was tested with analysis of variances. These tests were done separately in each transition group. The difference in annual changes in FEV_1 (% predicted) from baseline before and after transition was used as a dependent variable. Therapeutic intensity during the two years after transition was classified into four groups according to the number of intravenous treatments and whether or not inhaled antibiotics were used (yes or no): group 1, two annual intravenous antibiotic treatments and no inhaled antibiotics; group 2, two annual intravenous antibiotic treatments and inhaled antibiotics; group 3, more than two annual intravenous antibiotic treatments and no inhaled antibiotics; and group 4, more than two annual intravenous antibiotic treatments and inhaled antibiotics. A p value of <0.05 was considered significant (SPSS statistics software).

Results

A total of 40 patients with cystic fibrosis were followed from first detection of PA to chronic mucoid PA colonisation during the observation period. Thirty patients were ΔF508 homozygous, eight were ΔF508 compound heterozygous, and two had other CFTR mutations. On average, 8.6% (range 4.1–15.1) of all patients were seen less than three times a year during the observation period. All patients were pancreatic insufficient. The mean (SD) age at the appearance of each step in the course of PA colonisation for the whole group was: PA1 10.5 (6.9) years, PAc 11.7(6.8) years, PAm 12.5(6.5) years, and PAcm 13.5 (6.4) years. The sex ratio was 1:1. The number of patients fulfilling the criteria for a five year longitudinal follow up with respect to pulmonary function and therapeutic efforts at each single step in the course of PA colonisation was further reduced according to age dependence of pulmonary function tests and duration of observation period. Observation started two years before the year of transition and pulmonary function measurements also started at this time. Thus, only patients who were at least six years old at the beginning of observation and eight years old at the time of transition could be included. For example, in group PAcm seven out of 40 patients who were followed from PA1 to PAcm were omitted because they were aged less than eight years at transition and therefore had incomplete pulmonary function data, five had an observation period of less than two years in our Cystic Fibrosis centre before transition, one had missing data during the observation period, and five were followed up for less than two years after transition. Subgroups of patients were followed longitudinally and separately for each step (PA1, PAc, PAm, and PAcm) in the course of PA colonisation over a five year period (two years before, in the year of the event, and two years after). As a result there were different numbers of patients in each subgroup: 11 patients around PA1, 19 patients around PAc, 21 patients around PAm, and 22 patients around PAcm. All patients in the different subgroups had passed from first PA detection to chronic mucoid PA colonisation during the observation period. The mean (SD) observation time after first PA detection was 6.16 (2.6) years, (range 3.3–9.79) for PA1, 6.9 (2.6) years (range 3.3–10.3) for PAc, 6.7 (2.7) years (range 3.3–0) for PAm, and 7.4 (5.0) years (range 3.3–10.8) for PAcm.

PULMONARY FUNCTION

Table 1 shows the mean of the best annual FEV_1 (% predicted) for each independent subgroup of patients related to single steps in the course of PA colonisation. Data from two years before, the year of transition, and two years after transition are shown for each independent subgroup. FEV_1 (% predicted) remained unchanged in relation to PA1 and PAc over the whole period. PAm was associated with a significant decrease in mean (SD) FEV_1 (% predicted) from the year of PAm (67.3 (23.4)) to two years after PAm (61.8 (23.8)). From two years before to the year of PAcm mean (SD) FEV_1 (% predicted) increased from 67.1 (22.1) to 75.1(23.4); $p<0.05$. It then decreased from the year of PAcm to the point two years after PAcm (75.1 (23.4) versus 67.8 (22.0); $p<0.05$).

Table 1 Mean (SD) best annual FEV_1 (% predicted) two years before, in the year of transition, and two years after the year of transition for the four subgroups of patients with cystic fibrosis colonised with Pseudomonas aeruginosa (PA)

	Mean (SD) FEV_1 (% predicted) in the years		
Subgroup	*−2*	*0*	*2*
PA1 (n = 11)	64.3 (21.0)	65.0 (21.6)	63.6 (21.29
PAc (n = 19)	67.3 (22.7)	68.4 (24.6)	66.1 (30.5)
PAm (n = 21)	63.6 (22.3)	67.3 (23.4)	61.8 (23.8)*
PAcm (n = 22)	67.1 (22.1)*	75.1 (23.4)	67.8 (22.0)*

PA1 = first PA detection; PAc = chronic PA colonisation; PAm = first mucoid PA detection; PAcm = chronic mucoid PA colonisation.
*p<0.05.

The slope of changes in annual FEV_1 (% predicted) with respect to the different steps in the course of PA colonisation is shown in fig 1. The mean (SD) annual changes in FEV_1 (% predicted) ranged from an increase of 7.3 (12.0) of baseline in the two years preceding the year of PAcm to a decrease of 4.8 (7.4) in the two years succeeding transition to PAcm. They worsened significantly only with the transition to mucoid stages: PAm, 4.6 (13.2) versus –4.3 (8.1); PAcm, 7.3 (12.0) versus –4.8 (7.4) with a mean difference (95% CI) between before and after transition of 8.9 (2.6 to 15.2) for PAm and 12.1 (6.4 to 17.6) for PAcm. With regard to PA1 and PAc, there were no significant differences in annual FEV_1 (% predicted) changes in the two years before compared with the two years after transition. In the two years before transition to PAm and to PAcm an increase in FEV_1 (% predicted) was observed.

ANTIBIOTIC TREATMENT

Data on the antibiotic treatment in relation to the different steps were also taken from the cystic fibrosis data base. The therapeutic efforts increased in relation to the different steps in the course of PA colonisation. The mean (SE) number of annual intravenous antibiotic treatment courses increased from 0 (0) in the year prior to first PA detection to a maximum of 1.43 (0.30) two years after first mucoid PA detection. With first PA detection and chronic PA colonisation the mean (SE) annual number of intravenous treatments in the years of the event versus the previous years increased: PA1, 0 (0) versus 0.64 (0.15) with a mean difference (95% CI) of 0.64 (0.30 to 0.98), median 1; PAc, 0.21 (0.16) versus 0.84 (0.19) with a mean difference (95% CI) of 0.63 (0.20 to 1.06), median 1. After the event no further increase was observed. With first mucoid PA detection only an increase in the mean (SD) number of annual intravenous treatments from the year of the event to the time two years before the event was noted: PAm, 0.43 (0.20) versus 0.91 (0.18) with a mean difference (95% CI) of 0.48 (0.01 to 0.88), median 0. A further increase in the mean number of annual intravenous treatments from transition to the time two years later was noticed: PAm, 0.91 (0.18) versus 1.43 (0.30) with a mean difference (95% CI) of 0.52 (0.01 to 1.03), median 0. In the two years after chronic mucoid PA colonisation there was an increase in the mean (SD) number of annual intravenous treatment courses compared with the year of transition: PAcm, 0.61 (0.14) versus 1.3 (0.33) with a mean difference (95% CI) of 0.73 (0.15 to 1.31), median 0. With transition to PA1 and PAc the percentage of patients with ⩾1 intravenous antibiotic treatment courses per year increased as shown in fig 2A. After transition to PAm and PAcm the percentage of patients with ⩾2 annual intravenous treatments increased as shown in fig 2B.

The percentage of patients with daily antibiotic inhalation therapy increased from zero in the years before chronic PA colonisation to a maximum of 42.9% two years after first mucoid PA detection as shown in fig 3. The percentage of patients with daily antibiotic inhalation therapy increased only with the mucoid steps (PAm and PAcm) if the year of

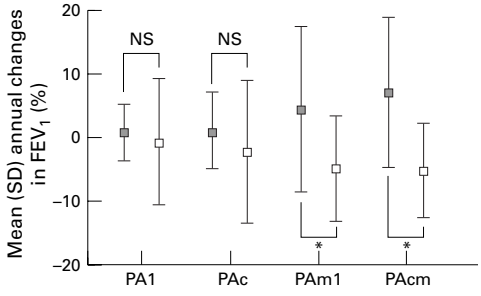

*Figure 1 Mean annual changes in FEV_1 (% predicted) from baseline in the two years preceding versus the two years following transition for the different stages of P aeruginosa (PA) colonisation. PA1 = first detection of PA; PAc = chronic PA colonisation; PAm = first mucoid PA detection; PAcm = chronic mucoid PA colonisation. *p <0.05; NS = not significant.*

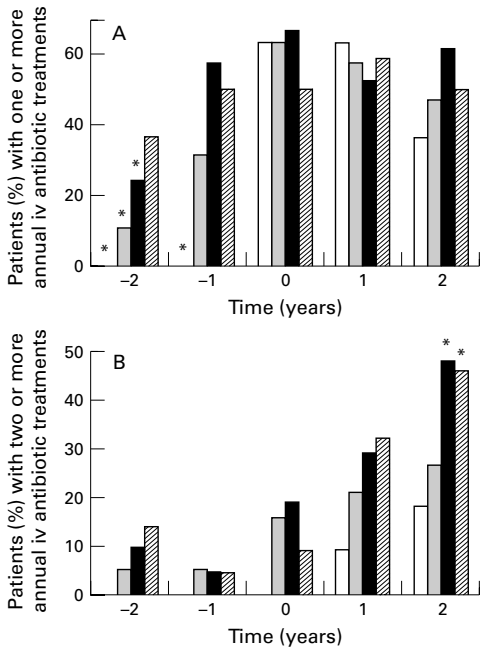

*Figure 2 Percentage of patients with (A) one or more annual intravenous antibiotic treatments and (B) two or more annual intravenous antibiotic treatments in relation to different steps in the course of P aeruginosa (PA) colonisation in patients with cystic fibrosis (white bars = PA1, light grey bars = PAc, black bars = PAm, hatched bars = PAcm). Years before and years after each step were compared with the year in which the step took place. *p <0.05.*

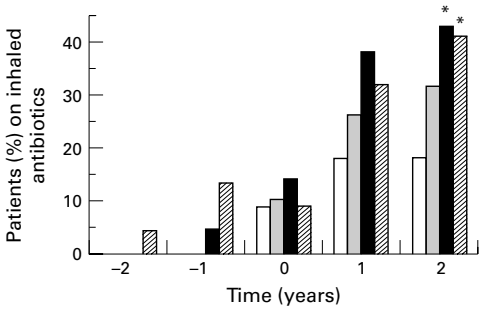

*Figure 3 Percentage of patients on inhalation antibiotics in relation to different steps in the course of P aeruginosa (PA) colonisation in patients with cystic fibrosis (white bars = PA1, light grey bars = PAc, black bars = PAm, hatched bars = PAcm). Years before and years after each step were compared with the year in which the step took place. *p <0.05.*

transition to PAm or PAcm was compared with the time two years later. The changes in the groups PA1 and PAc were not significant.

CHANGES IN THERAPEUTIC INTENSITY AND ANNUAL FEV_1

Neither the number of intravenous treatment courses nor continuous antibiotic inhalation therapy, nor both of these together, had a significant influence on changes in pulmonary function when multifactorial ANOVA was applied. There was a weak trend (p = 0.17) towards a more highly intensified therapy in those patients with the most distinct decrease in pulmonary function after chronic mucoid PA colonisation. The therapeutic intensity was classified into four groups (see Methods). For PAcm the mean (SE) difference in annual change in FEV_1 (% predicted) was –9.02 (2.82) in group 1 (n = 11), –8.59 (4.37) in group 2 (n = 4), –10.86 (5.58) in group 3 (n = 2), and –22.02 (8.77) in group 4 (n = 5). The differences between the groups were not significant.

Discussion

The natural clinical course of PA colonisation in patients with cystic fibrosis is superimposed by treatment. The intensity of treatment might often change in the course of PA colonisation from first PA detection to chronic mucoid PA colonisation. We therefore included data on treatment intensity in terms of antibiotic therapy effective against PA in our study. As we followed patients with the same criteria through the different steps which constitute the course of PA, we were able to compare different steps. Our results indicated that the events in the course of PA infection had a different influence on the annual changes in pulmonary function. This might help to explain the somewhat conflicting results of earlier studies which demonstrated no[3] or a significant[4] decrease in pulmonary function with PA colonisation in the lower respiratory tract. One study[3] investigated a group of patients with a mixed state of PA infection. Differentiation between mucoid and non-mucoid PA was not undertaken. In the other study[4] only data regarding chronic mucoid PA infection were reported. In that study an accelerated decrease in FEV_1 (% predicted) after chronic mucoid PA colonisation was described. Our results indicate that the early steps (PA1 and PAc) in the course of PA colonisation were not accompanied by dramatic changes in the annual decrease in FEV_1 (% predicted). At the same time an increase in the number of annual intravenous antibiotic treatment courses was noted. The percentage of patients with inhaled antibiotic treatment also increased with time but did not reach significance when the years before and after the event were compared with the year of the event (PA1 and PAc). The question as to whether the natural course of PA colonisation or the therapeutic interventions during the early steps resulted in the stable course of pulmonary function remains open. Even if first detection of PA is not related to an accelerated decrease in FEV_1, it marks the starting point of a series of events for most patients who will reach the stage of chronic mucoid PA colonisation within the next few years.[14 15] Vaccination[26] to prevent PA colonisation or early aggressive antibiotic treatment of PA whenever PA was detected[14 15] to prevent chronic colonisation seem therefore to be important treatment options. First results with a therapeutic regime that treated every PA detection with antibiotics (oral and inhaled) demonstrated a delay in chronic PA colonisation.[14 15]

If changes in pulmonary function in relation to the later events (PAm and PAcm) in the course of PA infection were analysed we could demonstrate an accelerated decrease in annual FEV_1 (% predicted) changes in relation to PAm and PAcm. This confirmed recently published longitudinal data for PAcm alone.[4] Surprisingly, we and others[4] have observed an increase in FEV_1 in the two years before PAcm. The increase in FEV_1 in the years before PAcm might reflect the increased therapeutic efforts (number of intravenous treatment courses and percentage of patients on inhaled antibiotics) in the years previous to this step as shown by our results. Additional therapeutic efforts were undertaken in the years after PAcm in terms of annual intravenous antibiotic treatment courses and daily inhalation of antibiotics.

Maintenance therapy of chronic PA colonisation is very controversial. There are no controlled studies comparing different treatment regimes. However, our study seemed to demonstrate that our treatment of PA, indicated only by clinical symptoms, is too late and/or not intensive enough to stop deterioration of pulmonary function. Only one Cystic Fibrosis Centre reported data regarding changes in pulmonary function and therapeutic intensity related to different stages of PA colonisation.[11] In a group of patients with mucoid PA at the first isolation of PA the FVC remained practically unchanged over a period of 10 years after the first detection of PA.[11] Since the follow up study was not strictly longitudinal, the relevance of this observation is somewhat reduced. The number of patients decreased during the observation period. Nevertheless, the main difference was the treatment intensity, which was much higher in the study of the previous group[11] with three or

four annual intravenous antibiotic treatment courses compared with less than two annual intravenous antibiotic courses in our group. Maintenance antibiotic treatment effective against PA was started independent of clinical status, with the transition to chronic PA colonisation.[11] This was much earlier than in the treatment regime we used where only severely ill patients were treated with regular intravenous antibiotic courses 3–4 times per year and daily antibiotic inhalation. Even if antibiotic treatment of PA is not the only aspect of cystic fibrosis treatment, one might remember that the same group reported the best survival data to date for patients with cystic fibrosis.[27] Earlier and increased treatment intensity might therefore modulate the outcome even in relation to mucoid stages of PA colonisation. An indication for PA treatment based only on clinical symptoms seems to be insufficient to stop pulmonary deterioration.

There are some new therapeutic strategies[14 26] related to PA colonisation and might have a higher chance[18] of becoming part of routine treatment in many cystic fibrosis centres than the described approach[11] which is very expensive.

In conclusion, prophylactic treatment of PA colonisation by vaccination is presently being studied and may be available in the near future. Early treatment of first PA detection is now virtually accepted[14 18] but the optimal way of carrying it out—for example, how long treatment courses should be[14 15]—has still to be evaluated. Our data indicated that maintenance treatment of chronic PA colonisation should be carried out on a regular basis irrespective of clinical symptoms. It remains to be shown whether regular intravenous treatment (which seems to be highly effective[27] but is a burden for the patient and costly[18]) or, for example, nebulised antibiotics[9] is the optimal maintenance treatment of chronic PA colonisation.

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