Pediatric Pulmonology 25:88–92 (1998)

Placebo-Controlled, Double-Blind, Randomized Study of Aerosolized Tobramycin for Early Treatment of *Pseudomonas aeruginosa* Colonization in Cystic Fibrosis

Heinz Georg Wiesemann, MD,[1]* Gratiana Steinkamp, MD,[2] Felix Ratjen, MD,[1] Adolf Bauernfeind, PhD,[3] Barbara Przyklenk, MD, PhD,[3] Gerd Döring, PhD,[4] and Horst von der Hardt, MD[2]

Summary. In chronic *Pseudomonas aeruginosa* pulmonary infection of patients with cystic fibrosis (CF), antibiotic therapy generally fails to eradicate the bacterial pathogen. The mucoid bacterial phenotype, high sputum production by the host, and low airway levels of antibiotics seem to be responsible for the observed decrease in antibiotic efficacy. We hypothesized that early antibiotic treatment by inhalation in CF patients may be able to prevent or at least delay airway infection. In a prospective placebo-controlled, double-blind, randomized multicenter study, 22 CF patients received either 80 mg b.i.d. of aerosolized tobramycin or placebo for a period of 12 months shortly after the onset of *P. aeruginosa* pulmonary colonization.

Two patients in the tobramycin and six patients in the placebo group stopped inhalation before the 12 month treatment period. Using life table analysis, the time to conversion from a *P. aeruginosa*-positive to a *P. aeruginosa*-negative respiratory culture was significantly shorter in the tobramycin-treated group than in the placebo group ($P < 0.05$, log rank test). Lung function parameters and markers of inflammation did not change in either group during treatment. The results of this study suggest that early tobramycin inhalation may prevent and/or delay *P. aeruginosa* pulmonary infection in CF patients. **Pediatr. Pulmonol. 1998; 25:88–92.**

Key words: cystic fibrosis; *Pseudomonas aeruginosa;* early tobramycin inhalation therapy; randomized clinical trial.

INTRODUCTION

The majority of patients with cystic fibrosis (CF) suffer from chronic pulmonary infection with *Pseudomonas aeruginosa*.[1–3] Once the infection is established, even aggressive antibiotic treatment strategies can only temporarily reduce the number of *P. aeruginosa* organisms in the bronchial tree.[4,10,11] In most patients chronic pulmonary infection with *P. aeruginosa* is associated with worsening of the clinical status and an increase in sputum volume. The phenotypic switch from non-mucoid to mucoid types of *P. aeruginosa* imposes a barrier to antibiotics and reduces the bioactivity of aminoglycosides.[14,15] In contrast, non-mucoid bacteria and low amounts of sputum are generally present when *P. aeruginosa* colonizes the CF airways initially.

Previous studies using short courses of intravenous antibiotics in patients with recent infection have only resulted in temporary elimination of *P. aeruginosa* from the bronchial tree.[11] Similar results were obtained with a combination of oral ciprofloxacin and inhaled colistin.[6,16] Considering these findings and the proven efficacy of inhaled antibiotics in patients with chronic

[1]Department of Pediatrics, University Hospital, Essen, Germany.

[2]Department of Pediatrics, University Hospital, Hannover, Germany.

[3]Max von Pettenkofer Institute, University of Munich, Munich, Germany.

[4]Department of General and Environmental Hygiene, Hygiene-Institute, University Hospital, Tübingen, Germany.

Contract grant sponsor: Bundesminister für Forschung und Technologie, Germany.

The study was performed in cooperation with H. Bärmeier and B. Böwing, University Hospital, Erlangen, M. Ballmann and F. Rohde-Brand, University Hospital, Hannover, R.M. Bertele-Harms, K. Harms, and J. Rosenecker, University Hospital, Munich, K. Hummel, M. Pohl, and H.G. Posselt, University Hospital, Frankfurt, and U. Stephan, University Hospital, Essen, Germany.

*Correspondence to: Dr. H.G. Wiesemann, Department of Pediatrics, University Hospital, D-45122 Essen, Germany.

Received 30 November 1996; accepted 10 October 1997.

P. aeruginosa infection,[4,5,7,8,17] we hypothesized that aerosolized tobramycin immediately after the onset of *P. aeruginosa* pulmonary colonization in CF may prevent or delay infection. Therefore, we carried out a prospective placebo-controlled, double-blind, randomized multicenter study of tobramycin inhalation in 22 CF patients.

MATERIALS AND METHODS

Patients and Study Design

The study was performed between October 1991 and May 1994 in the CF centers of the children's hospitals of Erlangen, Essen, Frankfurt, Hannover, and Munich, Germany. CF patients older than 4 years of age, with *P. aeruginosa*-negative throat swabs or sputum cultures for more than 1 year and negative serum antibody titers were eligible for the study. Patients less than 4 years of age were excluded because we expected them not to execute the inhalation procedure correctly. Once *P. aeruginosa* was detected for the first time, three additional samples were taken at weekly intervals and were sent to the reference center (Max v. Pettenkofer Institute, Munich). When two of three samples were positive, patients were screened for serum antibodies to *P. aeruginosa.* If antibodies to the three exoenzymes exotoxin A, alkaline phosphatase, and elastase of *P. aeruginosa* were negative, patients were randomly assigned to either 80 mg tobramycin (Eli Lilly, Bad Homburg, Germany) or placebo containing the same preservatives as the conventional drug preparation (phenol 10 mg, sodium disulfite 2.88 mg, sodium EDTA 0.2 mg, and 2 ml distilled water of pH between 6 and 8) mixed with 2 mL of isotonic saline. Randomization was performed in groups of two patients by flipping a coin. The taste was dominated by phenol as part of the preservatives; there was no taste difference between the two solutions. The study medication was inhaled twice daily for a period of 12 months.

Inhalation was performed with a jet nebulizer (Pari Boy, Pari-Werke, Paul Ritzau, Starnberg, Germany). The output of the compressor was 0.25 mL/min, and the mass median diameter of the aerosol was 3.5 μ determined with a Malvern Master Sizer at 23°C. Patients were instructed to maintain regular tidal breathing. The patients needed approximately 10 min for inhalation of the solution. *N*-acetylcysteine inhalation, pancreatic enzymes, vitamins, and inhalation therapy with saline and/or bronchodilators were continued. When the patients inhaled saline and/or bronchodilators, the study medication was given separately. During the study period sputum samples or deep throat swabs were obtained at monthly intervals. Serum antibodies against elastase, exotoxin A, and alkaline protease were determined at the end of a 3 month interval.

The study was approved by the ethics committee of all participating centers; written informed consent was obtained from all patients or their parents.

Abbreviations

CF	Cystic fibrosis
cpm	Counts per minute
ESR	Erythrocyte sedimentation rate
FEV_1	Forced expiratory volume in 1 s
FVC	Forced vital capacity
P. aeruginosa	*Pseudomonas aeruginosa*

Clinical and Laboratory Investigations

Chest radiographs were taken once a year on a routine basis; the severity of x-ray changes was assessed using the Chrispin-Norman score.[19] Vital capacity (VC) and forced expiratory volumes in 1 s (FEV_1) were obtained with a water-sealed bell spirometer (Exspirograph, Fa Godard).[20] Before the study, sputum or deep throat swabs were screened monthly for *P. aeruginosa* in the CF centers. When positive, the diagnosis was confirmed in the bacteriological reference laboratory of the Max von Pettenkofer Institute, Munich, with three specimens collected within 3 weeks. Specimens were cultured on charcoal agar for *P. aeruginosa* and other microorganisms (Transgerm GO, Merck, Darmstadt, Germany). Microorganisms were identified and semiquantified by routine methods.[1] Serum antibodies against elastase, exotoxin A, and alkaline protease were determined using sensitive radioimmunoassays.[21–23] Briefly, microtiter plates coated with a complex of rabbit IgG specific for elastase and the respective antigens were filled with 100 μl of 10-fold dilutions of patient serum samples and incubated at 4°C overnight. After washing, the plates were incubated with 100 μL ^{125}I-labeled rabbit IgG specific for elastase (200,000 cpm) at 4°C overnight. After washing, the bound radioactivity was determined in a gamma-counter (Berthold, Wildbad, Germany). Similar radioimmunoassays were carried out for the determination of serum antibody titers against exotoxin A and alkaline phosphatase. Total IgG, IgE, and erythrocyte sedimentation rate (ESR) were determined by routine laboratory methods. Tobramycin levels in patients' serum were determined approximately 1 h after inhalation by a fluorescence-polarization immunoassay (Abbott, Wiesbaden, Germany) every 3 months. The lower detection limit of the assay was 0.2 μg/mL.

Statistical Analysis

The difference in the time to the conversion of respiratory cultures to *P. aeruginosa* negative was tested in a ''time to event'' analysis using the log rank test (SAS life test procedure). Pulmonary function tests were compared

TABLE 1—Characteristics of Subjects at Study Entry

	Placebo (mean ± SD)	Tobramycin (mean ± SD)
No.	11	11
Age (yr)	11.4 ± 10.1	9.8 ± 8.3
Gender (F/M)	5/6	4/7
Height (cm)	126.9 ± 31.9	140 ± 28.1
Weight for height (%)	83 ± 31.2	99.7 ± 9.5
FVC (% predicted)	100.4 ± 7.9	86.8 ± 19.4
FEV_1 (% predicted)	83 ± 31.8	78.6 ± 33.2
Shwachman score[1]	68.6 ± 9.5	68.0 ± 11.4
Chrispin Norman score[19]	6.2 ± 4.8	7.2 ± 4.9

[1]Without x-ray rating (maximum 75).

using Student's t-test. An error probability of <5% was regarded as significant.

RESULTS

Twenty-two patients were included in the study. Their clinical data are summarized in Table 1. There was no difference between the placebo and tobramycin group with respect to age, relative weight for height, VC, FEV_1, gender distribution, or Shwachman or Chrispin-Norman scores. Eleven patients were allocated to the tobramycin and 11 to the placebo group.

All but one of the *P. aeruginosa* species were nonmucoid before and after termination of the study. The different pyocin and serotypes were nearly equally distributed. Other *Pseudomonas* species in these groups were *P. stutzeri* (one patient), *P. putida* (one patient), and *P. fluorescens* (one patient). Concomitant respiratory isolates were *Stenotrophomonas maltophilia* in four patients (two in each group), *Staphylococcus aureus*, *Haemophilus influenzae*, and species of Enterobacteriaceae. One hundred and thirty-one (83 tobramycin group, 48 placebo group) of the microbiologic samples were throat swabs, and 27 (16 tobramycin group, 11 placebo group) were sputa. After the first detection of *P. aeruginosa* in sputum or the deep throat swabs, and after the confirmation of the presence of *Pseudomonas* organisms by two more throat or sputum cultures, aerosol treatment was started within 7–12 weeks (mean 11.3 ± 3.7 weeks). As in previous studies,[4,8] aerosolized tobramycin was well tolerated, and no severe adverse effects were reported. Tobramycin levels in the serum did not exceed 0.2 mg/L. Nine patients in the tobramycin group and five patients in the placebo group completed the study (Table 2). The reasons for premature withdrawal were:

—unwillingness to do daily inhalation (two patients in each group);
—need for intravenous treatment (two patients in the placebo group);
—cough during inhalation (one patient in the placebo group);
—lack of the expected effect on cough and sputum production (one patient in the placebo group).

Analysis of the data of all patients in a ''time to event'' analysis (the event being the first conversion of the sputum culture from *P. aeruginosa* positive to *P. aeruginosa* negative) yielded a significant difference between the two groups (Fig. 1; $P < 0.05$, log rank test). Due to the low rate of conversion from *P. aeruginosa* positive to *P. aeruginosa* negative in the placebo group, the mean time of conversion could only be assessed in tobramycin-treated patients. In the tobramycin group the time to conversion to *P. aeruginosa*-negative cultures was 1.89 months (Fig. 1).

Specific serum antibody titers against *P. aeruginosa* exotoxin A, elastase, and alkaline phosphatase during the study period were available in eight patients of the tobramycin group and in five patients of the placebo group. In the placebo group, antibody titers increased steadily during the study period, whereas they remained low in the tobramycin group (Fig. 2). In all but one patient in each group the results of the antibody determination and the sputum culture were concordant at the end of study.

There was no significant difference between the groups in lung function parameters, IgG, IgE, and ESR during or after the treatment period (data not shown).

DISCUSSION

This study was based on the assumption that chronic bronchial infection in CF patients is preceded by a period of colonization and acute infection in which *P. aeruginosa* may be eliminated by appropriate antibiotic treatment.[17,18] Bacterial colonization is generally defined as a state in which specific antibodies against the pathogen are missing, yet the organism is already intermittently detectable in respiratory secretions,[2,24] whereas in acute infection low antibody titers may be present.[25] Chronic infection has been defined as *P. aeruginosa* presence in monthly sputum specimens for 6 consecutive months in association with a rapid increase in specific serum antibodies.[26]

Accordingly, we *excluded* in the present study CF patients with pre-existing serum antibodies against *P. aeruginosa*- and *P. aeruginosa*-positive respiratory cultures and *included* patients seronegative for *P. aeruginosa* antibodies, but with positive cultures for *P. aeruginosa* in at least two of three sputum or cough swab specimens.[11,19] Antibiotic treatment was started as soon as possible after the first cultivation of *P. aeruginosa* with a mean interval between *Pseudomonas* detection and start of therapy of 11.3 weeks. Using a ''time to event'' model, we have shown that there was a significantly lower rate of conversion to positive respiratory cultures in the tobramycin than the placebo-treated group. This finding suggests that

TABLE 2—Time Course of Conversion of Respiratory Cultures From *P. aeruginosa* Positive to *P. aeruginosa* Negative During the Study Period

	Tobramycin			Placebo		
Months	No.[1]	*P. aeruginosa* +	Withdrawn	No.[1]	*P. aeruginosa* +	Withdrawn
0	11	9	2[2]	11	10	1[2]
1	9	6	0	10	8	2[3,4]
2	9	2	0	8	6	0
3	9	1	0	8	6	1*
4	9	1	0	7	5	0
5	9	1	0	7	5	1[4]
6	9	1	0	6	4	0
7	9	1	0	6	4	1[5]
8	9	1	0	5	4	0
9	9	1	0	5	4	0
10	9	1	0	5	4	0
11	9	1	0	5	4	0
12	9	1	0	5	4	0

[1]Refers to the number of patients under observation.
[2]Unwillingness to perform inhalation.
[3]Cough.
[4]Intravenous treatment.
[5]No effect.

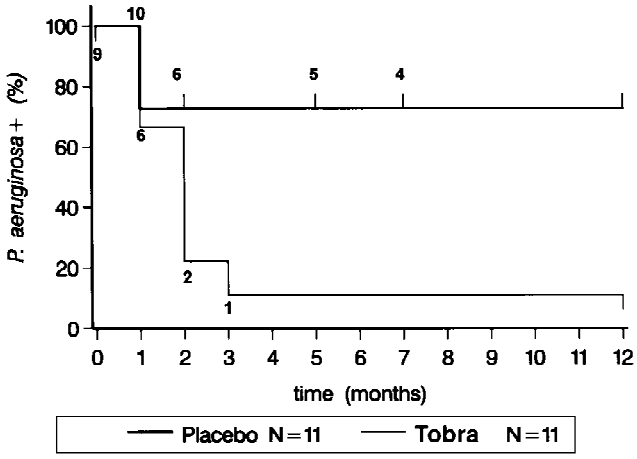

Fig. 1. Percentage of *P. aeruginosa*-positive patients in a Kaplan-Meier plot for both treatment groups over the 12 months study period. The tic marks signify when patients dropped out and also indicate the total number of *P. aeruginosa*-positive patients remaining in study. Significantly more patients in the tobramycin treated group became *P. aeruginosa* negative during the study period ($\beta < 0.05$, log rank test).

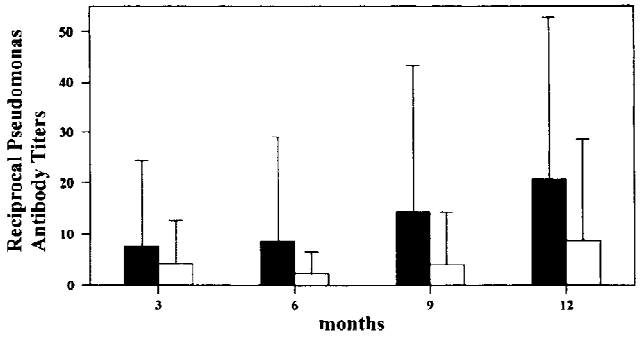

Fig. 2. Reciprocal antibody titers against *P. aeruginosa* alkaline phosphatase, elastase, and exotoxin A in cystic fibrosis patients receiving placebo (black bars) or tobramycin by inhalation (white bars) for a period of up to 12 months. Antibody titers reflect the sum against the three epitopes determined by three different radioimmunoassays. For details see the Materials and Methods section. Bars represent means ± SD of antibody titers against the three *P. aeruginosa* antigens of 12–24 single values.

inhalation of tobramycin in a dose of 80 mg twice daily is capable of eliminating *P. aeruginosa* from the bronchial tree of recently infected CF patients. The elimination is associated with the disappearance of or failure to develop *P. aeruginosa* antibodies.

The power of this study is limited by the small number of patients and the high rate of dropouts, especially in the placebo group. The high dropout rate in the placebo group may raise concern about the adequacy of blinding. As the patients had no experience with inhalation of antibiotics before and the taste of both solutions was identical, it is unlikely that they may have recognized whether the blended solution contained the antibiotic or not. The high dropout rate in the placebo group may, however, have been influenced by the lack of subjective improvement or the lack of clearance of *P. aeruginosa* from respiratory cultures, as this information was not blinded throughout the study period.

The majority of patients in this study did not produce sputum, so microbiologic assessments had to be based on throat swabs. Throat swabs have a lower sensitivity than sputum samples for the detection of *P. aeruginosa*, although their specificity is adequate.[27] *P. aeruginosa*-specific serum antibodies appeared to correlate well with respiratory cultures in this study, suggesting that serum

antibodies may play a role as surrogate markers of early respiratory infection with *P. aeruginosa.*

Tobramycin inhalation did not affect the values of FVC or FEV_1, as in previous studies.[4,7,9] This was probably due to the small number of patients who completed the 12 month study period and the observation that the deleterious effects of *P. aeruginosa* on lung function do not occur in the early stages of colonization.[28] We conclude that long-term inhalation treatment with tobramycin 80 mg b.i.d. is capable of significantly reducing and/or delaying *P. aeruginosa* infection of the bronchial system in CF patients as long as inhalation therapy begins in the early stages of colonization. Further studies will disclose whether the colonization of *P. aeruginosa* persists after discontinuation of treatment.

ACKNOWLEDGMENTS

The authors are indebted to Thomas Pieperhoff for the randomization and the distribution of the study medication.

REFERENCES

1. Bauernfeind A, Bertele RM, Harms HK, Hörl G, Jungwirth R, Petermüller C, Przyklenk B, Weisslein-Pfister C. Qualitative and quantitative microbiological analysis of sputa of 102 patients with cystic fibrosis. Infection. 1987; 15:270–277.
2. Hoiby N. Microbiology of lung infections in cystic fibrosis patients. Acta Paediatr Scand Suppl. 1982; 301:33–54.
3. FitzSimmons FC. The changing epidemiology of cystic fibrosis. J Pediatr. 1993; 122:1–9.
4. Steinkamp G, Tümmler B, Gappa M, Albus A, Potel J, Döring G, von der Hardt H. Long-term tobramycin therapy in cystic fibrosis. Pediatr Pulmonol. 1989; 6:91–98.
5. Hodson ME, Penketh ARL, Battern JC. Aerosol carbenicillin and gentamycin treatment of *Pseudomonas aeruginosa* infection in patients with cystic fibrosis. Lancet. 1981; 2:1137–1139.
6. Jensen T, Pedersen SS, Garne S, Heilmann C, Hoiby N, Koch C. Colistin inhalation therapy in cystic fibrosis patients with chronic *Pseudomonas aeruginosa* infection. J Antimicrob Chemother. 1987; 19:831–838.
7. MacLusky IB, Gold R, Corey M, Levison H. Long-Term effect of inhaled tobramycin in patients with cystic fibrosis colonised with *Pseudomonas aeruginosa.* Pediatr Pulmonol. 1989; 7:42–48.
8. Ramsey BW, Dorkin HL, Eisenberg JD, Gibson RL, Harwood IR, Kravitz RM, Schidlow DV, Wilmott RW, Astely SJ, McBurnie MA, Wentz K, Smith AL. Efficacy of aerosolised tobramycin in patients with cystic fibrosis. N Engl J Med 1993; 328:1740–1746.
9. Schaad UB, Wedgwood-Krucko J, Suter S, Kraemer R. Efficacy of inhaled amikacin as adjunct to intravenous combination therapy (ceftazidime and amikacin) in cystic fibrosis. J Pediatr. 1987; 111:599–605.
10. Stead RJ, Hodson ME, Batten JC. Inhaled ceftazidime compared with gentamycin and carbenicillin in older patients with cystic fibrosis infected with *Pseudomonas aeruginosa.* Br J Dis Chest. 1987; 81:272–279.
11. Steinkamp G, Tümmler GB, Malottke R, von der Hardt H. Treatment of *Pseudomonas aeruginosa* colonisation in cystic fibrosis. Arch Dis Child. 1988; 64:1022–1028.
12. Lam J, Chan R, Lam K, Costerton JW. Production of mucoid microcolonies by *Pseudomonas aeruginosa* within infected lungs in cystic fibrosis. Infect Immun. 1980; 28:546–556.
13. Nickel JC, Ruseska I, Wright JB, Costerton JW. Tobramycin resistance of *Pseudomonas aeruginosa* cells growing as a biofilm on urinary catheter material. Antimicrob Agents Chemother. 1985; 27:619–624.
14. Levy J, Smith AL, Kenny MA, Ramsey BW, Schoenknecht FD. Bioactivity of gentamicin in purulent sputum from patients with cystic fibrosis or bronchiectasis: Comparison with activity in sputum. J Infect Dis. 1983; 148:1069–1076.
15. Levy J. Antibiotic activity in sputum. J Pediatr. 1986; 108:841–846.
16. Valerius NH, Koch C, Hoiby N. Prevention of chronic *Pseudomonas aeruginosa* colonisation in cystic fibrosis by early treatment. Lancet. 1991; 338:725–726.
17. Littlewood JM, Miller MG, Ghoneim AT, Ramsden CH. Nebulised colomycin for early *Pseudomonas* colonisation in cystic fibrosis. Lancet. 1985; 1:865.
18. Vazquez C, Municio M, Corera M, Gaztelurrutia L, Sojo A, Vitorio JC. Early treatment of *Pseudomonas aeruginosa* colonization in cystic fibrosis. Acta Pediatr. 1993; 82:308–309.
19. Chrispin AR, Norman AP. The systematic evaluation of the chest radiograph in cystic fibrosis. Pediatr Radiol. 1974; 2:101–106.
20. American Thoracic society. Standardisation of spirometry: 1987 update. Am Rev Respir Dis. 1987; 136:1285–1298.
21. Döring G, Obernesser H-J, Botzenhart K. Extracellular toxins of *Pseudomonas aeruginosa.* III. Radioimmunoassay for detection of alkaline protease. Zentralbl Bakteriol Mikrobiol Hyg (A). 1982; 252:239–247.
22. Obernesser H-J, Döring G. Extracellular toxins of *Pseudomonas aeruginosa.* IV. Radioimmunoassay for detection of elastase. Zentralbl Bakteriol Mikrobiol Hyg (A). 1982; 252:248–256.
23. Döring G, Goldstein W, Schiotz PO, Hoiby N, Botzenhart K. Role of *Pseudomonas aeruginosa* exoenzymes in lung infections of patients with cystic fibrosis. Infect Immun. 1985; 49:557–562.
24. Döring G, Obernesser H-J, Botzenhart K, Flehmig B, Hoiby N, Hofmann A. Proteases of *Pseudomonas aeruginosa* in cystic fibrosis. J Infect Dis. 1983; 147:744–750.
25. Brett MM, Simmonds EJ, Ghoneim ATM, Littlewood JM. The value of serum IgG titres against *Pseudomonas aeruginosa* in the management of early pseudomonal infection in cystic fibrosis. Arch Dis Child. 1992; 67:1086–1088.
26. Döring G, Hoiby N. Longitudinal study of immune response to *Pseudomonas aeruginosa* antigens in cystic fibrosis. Infect Immun. 1983; 42:197–201.
27. Ramsey BW, Wentz KR, Smith AL, Richardson M, Williamson-Warren J, Hedges DL, Gibson R, Redding GJ, Lent K, Harris K. Predictive value of oro-pharyngeal cultures for identifying lower airway bacteria in cystic fibrosis patients. Am Rev Respir Dis. 1991; 144:331–337.
28. Kerem E, Corey M, Gold R, Levison H. Pulmonary function and clinical course in patients with cystic fibrosis after pulmonary colonisation with *Pseudomonas aeruginosa.* J Pediatr. 1990; 116: 714–719.