

Eradication Therapy against *Pseudomonas aeruginosa* in Non-Cystic Fibrosis Bronchiectasis

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Key Words

Pseudomonas aeruginosa · Non-cystic fibrosis bronchiectasis · Eradication treatment · Nebulised tobramycin

Abstract

Background: No prospective study has assessed eradication treatment of early *Pseudomonas aeruginosa* colonisation in bronchiectasis not due to cystic fibrosis (CF). **Objectives:** To evaluate the efficacy of 3 months of nebulised tobramycin after a short course of intravenous antibiotics in the eradication of *P. aeruginosa* and its clinical consequences in non-CF bronchiectasis following initial *P. aeruginosa* infection. **Methods:** A 15-month, single-masked, randomised study including 35 patients was conducted in a tertiary university hospital. Following the isolation of *P. aeruginosa* and a 14-day intravenous treatment with ceftazidime and tobramycin, patients received 300 mg nebulised tobramycin twice daily or placebo during 3 months, and were followed up for 12 months thereafter. **Results:** The median time to recurrence of *P. aeruginosa* infection was higher in the tobramycin than in the placebo group ($p = 0.048$, log-rank test). At the end of the study 54.5% of the patients were free of *P. aerugi-*

nosa in the tobramycin group and 29.4% in the placebo group. The numbers of exacerbations ($p = 0.044$), hospital admissions ($p = 0.037$) and days of hospitalisation ($p = 0.034$) were lower in the tobramycin than in the placebo group. A global, non-significant trend to improvement in the tobramycin group was observed in most of the other studied parameters on comparing the two groups. Bronchospasm in the tobramycin group was remarkable. **Conclusions:** Our study shows that 3 months of nebulised tobramycin following a short course of intravenous antibiotics may prevent bronchial infection with *P. aeruginosa* and has a favourable clinical impact on non-CF bronchiectasis.

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Introduction

In patients with cystic fibrosis (CF), chronic bronchial *Pseudomonas aeruginosa* infection is associated with worsening lung function and an increase in mor-

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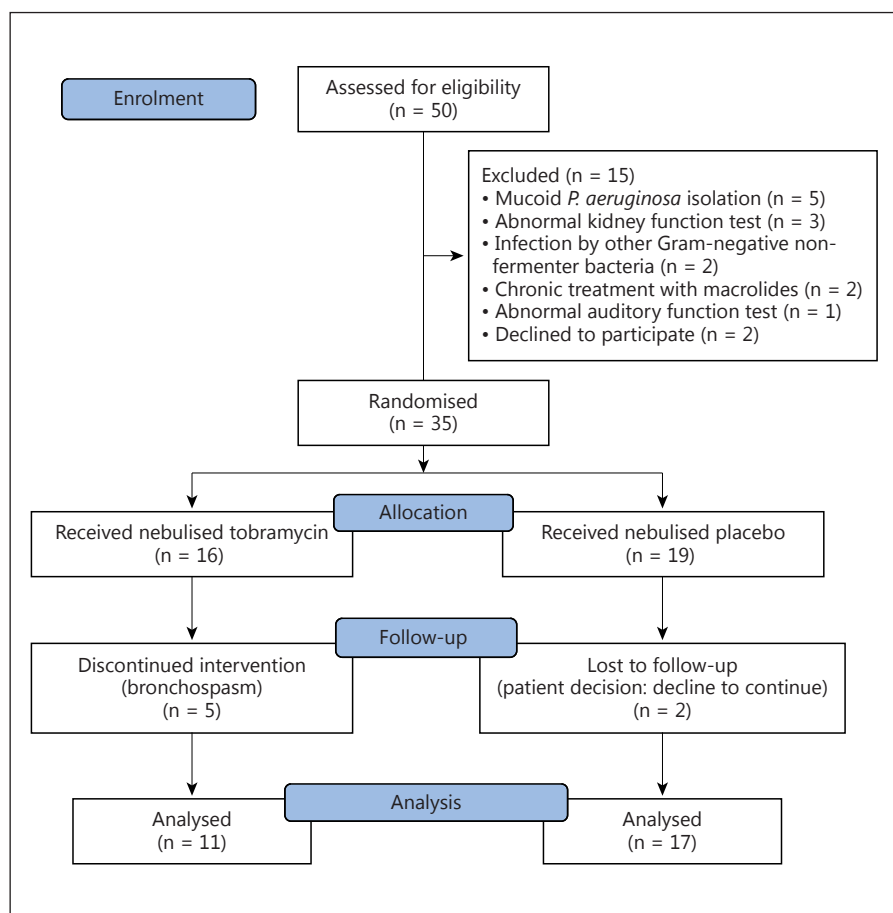


Fig. 1. Flow diagram of participants through each stage of the randomised trial.

bidity and mortality [1]. Early detection of infection with *P. aeruginosa* in patients with CF may be important as certain antibiotic treatments have proven useful in preventing or delaying chronic bronchial infection with *P. aeruginosa* [2]. Although the most effective treatment has yet to be determined, eradication of *P. aeruginosa* seems more likely using strategies with nebulised antibiotics [3].

Extensive lung disease and severe bronchial obstruction appear to be present on the development of chronic bronchial infection with *P. aeruginosa* in patients with non-CF bronchiectasis [4]. More hospital admissions [5, 6], worse quality of life [5] and a rapid decline in lung function [7] are also more frequent among these patients compared to those with infections with other bacteria. Thus, as occurs in patients with CF, chronic bronchial infection with *P. aeruginosa* in non-CF is a severe and similar complication. Appropriate antibiotic inhalation therapy for chronic bronchial infection with *P. aeruginosa* results in a reduction in *P. aeruginosa* density in spu-

tum and a significant clinical improvement. However, eradication of this microorganism in chronic bronchial infection is not possible [8, 9] or very difficult to achieve [10–12]. Based on results from CF studies, the British Thoracic Society recommended attempting early eradication of *P. aeruginosa* with oral ciprofloxacin, and second-line treatment with intravenous or nebulised anti-pseudomonal antibiotics [13]. Nevertheless, to our knowledge no prospective studies have assessed the eradication of *P. aeruginosa* in non-CF patients after its first isolation in sputum. In fact, recent reviews on inhaled antibiotics for lower airway infections urge to evaluate early eradication of this microorganism in non-CF bronchiectasis [14, 15].

The aim of this study was to evaluate the efficacy of a 3-month treatment with nebulised tobramycin following a short course of intravenous antibiotics for the eradication of *P. aeruginosa* in non-CF bronchiectasis. This prospective study also examines the outcome of this eradication treatment.

Methods

A 15-month, single-masked, randomised study was conducted in a tertiary university hospital. Patients were included from March 2006 to March 2009. Thirty-five patients with non-CF bronchiectasis over 18 years of age were recruited after the first isolation of *P. aeruginosa* in sputum (fig. 1). The diagnosis of bronchiectasis was made by high-resolution chest CT. Sweat tests and blood analyses were negative for the most frequent mutations of CF in Spain [16]. The patients were followed up according to the standard clinical protocol of our institute with analysis of a sputum sample culture approximately every 3 months. The exclusion criteria were: infection with other Gram-negative non-fermenter bacteria, mucoid *P. aeruginosa* or microorganisms resistant to any of the antibiotics used in the study, concomitant use of quinolones, chronic treatment with macrolides, and abnormal kidney and auditory function test results.

When *P. aeruginosa* was detected, all patients were treated with intravenous ceftazidime and tobramycin for 14 days during the first 4 weeks from initial detection. Tolerance to nebulised tobramycin was tested by hospital pharmacy personnel prior to randomisation which was created by computer-generated random numbers. Treatment assignment was blinded until completion of the study. The patients were instructed as to the preparation and nebulisation technique by the hospital personnel. Following intravenous treatment, one group received 300 mg of nebulised tobramycin (Tobi[®]), twice daily, for 3 months, while the other group received placebo treatment consisting of a 0.9% sodium chloride solution. A short-acting bronchodilator was administered approximately 1 h before nebulised tobramycin. Inhalation treatment was administered with a jet nebuliser (Pari LC Plus[®]; Pari, UK) with a high-flow-rate compressor (CR 60; Medic-Aid, UK) which has been demonstrated to be useful for the inhalation of tobramycin (Tobi[®]) [17]. The guidelines as to the use of nebulisers established by the British Thoracic Society were also met [18]. Both groups were followed up over a 12-month treatment-free period.

During the 3 first months, all patients were controlled monthly and 5, 7, 9, 12 and 15 months thereafter. The number of exacerbations, number of hospital admissions and days of hospitalisation were registered. Supplementary use of oral antibiotics was allowed on the development of an exacerbation, defined as more frequent coughing, greater dyspnoea and an increase in sputum volume and purulence [19]. The decision as to hospital admission was made by a physician from the emergency department who was unaware of the study. Blood and a sputum sample were collected before antibiotic nebulisation at each control as well as in cases of exacerbation or hospital admission. A quantitative bacteriological culture and determination of tobramycin susceptibility were performed. The sputum sample underwent Gram staining [20] and microbiological study. All samples were cultured conventionally in blood agar and McConkey and Sabouraud media and semiquantitatively cultured in chocolate agar with a calibrated loop, and more than 10⁶ colony-forming units per millilitre of sample were detected. The microorganisms isolated were identified by standardised or automated means, and the antibiogram was made according to the Kirby-Bauer technique [21]. Forced vital capacity (FVC), forced respiratory volume in the first second (FEV₁) – both performed according to the criteria of the American Thoracic Society [22] – PO₂ and PCO₂ were tested at the beginning and the end of the study. Auditory acuity measures in both ears at frequencies between 500 and 8,000 Hz [23] and St. George's Respiratory Questionnaire [24] were also performed.

Table 1. Baseline clinical characteristics of the study population

	Tobramycin group (n = 16)	Placebo group (n = 19)
Sex (M/F), n	10/6	9/10
Age, years	69.36 ± 2.10	70.11 ± 1.93
Tobacco use, n (%)		
Smokers	2 (12.50)	2 (10.52)
Ex-smokers	9 (56.25)	7 (36.84)
Non-smokers	5 (31.25)	10 (52.60)
FVC, % of predicted	63.58 ± 15.58	61.85 ± 21.45
FEV ₁ , % of predicted	56.81 ± 21.30	55.30 ± 30.33
PCO ₂ , mm Hg	42.35 ± 2.57	43 ± 7.11
PO ₂ , mm Hg	72.91 ± 10.60	74.75 ± 11.87
ESR, mm/h	19.64 ± 10.56	25.55 ± 19.57
CRP, mg/dl	1.36 ± 2.90	0.43 ± 0.50
Leucocytes, ×10 ⁹ /l	8,440 ± 2,950	7,777.77 ± 3,493

Values are given as means ± SD unless specified otherwise. ESR = Erythrocyte sedimentation rate; CRP = C-reactive protein.

Statistical Analysis

For demographic purposes, the results are expressed as means and standard deviations. The paired Student t test was used to compare means (Mann-Whitney U test, when necessary). The χ^2 test (Fisher's exact test, when appropriate) was used to compare proportions. Statistical analysis was performed using the SPSS software package version 18.0 (SPSS Inc., USA). For single-outcome comparisons, the treatment effect was considered significant with p values ≤ 0.05.

The study was approved by the institutional Ethics Committee of the Hospital Universitari Vall d'Hebron (Comitè Ètic d'Investigació Clínica; No. 156/2005). Written informed consent was obtained from all the patients included in the study.

Results

The 35 patients included in the study were distributed into two groups, without any statistical difference between them in any of clinical characteristics at the beginning of the study and after the intravenous and before the nebulisation treatment (table 1). Five patients withdrew due to bronchospasm during the first month of the inhaled tobramycin treatment. These patients had a worse – albeit not statistically significant (p = 0.052) – FEV₁ than the remaining 30 study patients (table 2). Two other patients from the placebo group abandoned the study during the first month.

The proportion of patients free of *P. aeruginosa* in the first month was 90.9% in the tobramycin group and 76.5% in the placebo group; these values were 54.5 and

Table 2. Characteristics at enrolment (baseline period) of the patients who withdrew because of bronchospasm as well as of the rest of the patients included in the study

	Patients who withdrew because of bronchospasm (n = 5)	Other patients (n = 30)	p
Age, years	74.4 (69.0–79.0)	69.1 (53.0–83.0)	0.19
FVC, % predicted	57.7 (38.6–75.0)	63.9 (35.0–99.0)	0.46
FEV ₁ , % predicted	34.8 (21.4–44.6)	58.0 (21.5–116.0)	0.052

Values are given as medians (ranges).

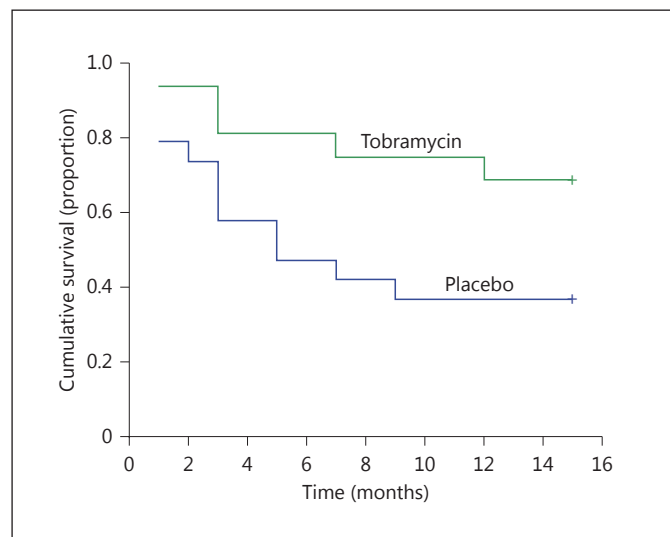


Fig. 2. Kaplan-Meier plot of time to recurrence of *P. aeruginosa* in non-CF bronchiectasis patients treated with tobramycin inhalation solution or placebo. The median time to recurrence was significantly higher in the tobramycin than in the placebo group ($p = 0.048$, log-rank test).

29.4%, respectively, at the end of the study. Figure 2 shows the statistically significant cumulative rate of colonisation with *P. aeruginosa* in the two groups ($p = 0.048$).

The number of exacerbations and the number and days of hospital admissions were significantly lower in the tobramycin than in the placebo group. The period of antibiotic use (oral, parenteral) in days was longer in the placebo group, but without statistical significance ($p = 0.052$). A global – albeit not statistically significant – trend to improvement was shown in most of the other parameters studied in the tobramycin inhalation group on comparison with the placebo group. Thus, no significant differences were achieved in the mean change from enrolment to the end of the study period in white blood

Table 3. Effect of treatment with the tobramycin nebulisation solution versus placebo

	Tobramycin group (n = 16)	Placebo group (n = 19)	p
Exacerbations, n	1.27 ± 1.62	2.5 ± 1.63	0.04
Admissions, n	0.06 ± 0.25	0.47 ± 0.51	0.04
Days of admission	0.90 ± 3.01	13.56 ± 22.64	0.03
Days of antibiotic use	8.4 ± 11.2	19.1 ± 24.4	0.052
ESR change, mm/h	-3.25 ± 16.76	10.72 ± 18.47	0.11
CRP change, mg/dl	-0.67 ± 2.01	2.04 ± 4.24	0.09
Leucocyte change, ×10 ⁹ /l	1,436 ± 3,119	785.71 ± 4,544	0.69
FVC change, %	-2.55 ± 6.26	1.35 ± 12.17	0.36
FEV ₁ change, %	-0.78 ± 5.12	1.46 ± 6.23	0.36
PO ₂ change, mm Hg	-10.97 ± 13.19	0.25 ± 19.37	0.18
PCO ₂ change, mm Hg	5.72 ± 14.84	-5.77 ± 21.11	0.20

Values are given as means ± SD. ESR = Erythrocyte sedimentation rate; CRP = C-reactive protein.

cell count, erythrocyte sedimentation rate, C-reactive protein level, FEV₁, FVC, PO₂ and PCO₂ (table 3) or in the scores on the four scales of the quality-of-life questionnaire (table 4).

Tobramycin-resistant *P. aeruginosa* was not detected in sputum during the study. However, other opportunistic organisms were identified in sputum cultures of 2 patients in the tobramycin group [*Stenotrophomonas maltophilia* (1 patient); *Achromobacter xylooxidans* and *Pseudomonas putida* (1 patient)] and in sputum cultures of 6 patients in the placebo group [*Pseudomonas fluorescens* (1 patient); *S. maltophilia* (2 patients); *Aspergillus* spp. and *Nocardia* spp. (1 patient); *S. maltophilia*, *Aspergillus* spp. and *Comamonas* spp. (1 patient); *Achromobacter anthropi* and *S. maltophilia* (1 patient)].

Table 4. St. George Respiratory Questionnaire scale scores

	Baseline			Change		
	tobramycin	placebo	p	tobramycin	placebo	p
Symptoms	40.8±26.0	32.3±24.7	0.34	-18.9±32.2	-10.5±5.4	0.48
Activity	48.4±24.0	33.9±31.6	0.19	-15.2±41.3	-1.2±8.1	0.31
Impact	30.8±15.6	18.2±16.6	0.08	-12.9±22.3	-3.3±18.4	0.19
Total	37.8±18.3	26.5±22.2	0.16	-14.6±27.9	-4.9±26.4	0.31

Change refers to the difference between the beginning and the end of each study period. Values are given as means ± SD.

No auditory acuity changes were found in either group. Serum creatinine concentrations remained within the normal range throughout the study period in all patients.

Discussion

This study was based on the assumption that chronic bronchial infection with *P. aeruginosa* in non-CF bronchiectasis is preceded by a period of first and intermittent infection similar to what occurs in CF [2], and that this microorganism might be eliminated by appropriate antibiotic treatment. Thus, chronic bronchial infection with *P. aeruginosa*, one of the most unfavourable events in non-CF bronchiectasis patients [5–7], might be avoided or, at least, delayed. To our knowledge, this is the first randomised, placebo-controlled study to provide evidence that early eradication therapy of *P. aeruginosa* in non-CF bronchiectasis may be effective.

Many therapeutic options in non-CF bronchiectasis have arisen from the management of patients with CF. However, evidence of benefit in the CF population [25] does not always translate into equivalent benefits in the different non-CF bronchiectasis populations [26]. In this study we have shown that 3 months of nebulised tobramycin after 15 days of intravenous tobramycin and ceftazidime is effective in eradicating *P. aeruginosa* in non-CF bronchiectasis patients after the first isolation of this microorganism in sputum. We did not include patients with mucoid *P. aeruginosa*, since, in comparison with non-mucoid *P. aeruginosa*, this phenotype imposes a barrier to antibiotics, reduces the bioactivity of aminoglycosides [27] and is, consequently, associated with a lower percentage of success in eradication treatment [28]. Antibiotic therapy in the present study led to eradication of *P. aeruginosa* in 90.9% of the patients at the first month,

with clearance being maintained in 54.5% of the cases at 15 months. These eradication rates are similar to the 80% initially observed and the 54.2% at 14.3 months in a recent retrospective study with different antibiotic treatment regimes combined with 3 months of nebulised colistin in non-CF bronchiectatic patients [29]. Both in the previous [29] and in our study, numbers dropped off quite quickly at early stages, and then a certain plateau was reached. We do not know if a longer nebulised antibiotic treatment might be helpful to keep the *P. aeruginosa* eradication reached just after this therapy. Studies on the treatment of initial infection with *P. aeruginosa* in CF have described differences in the duration of eradication according to the patient selection criteria, treatment regimens and outcome measures [2]. However, long-term eradication rates in this disease [30–34] seem to be greater than those obtained in the patients with non-CF bronchiectasis in our study. Some potential factors may explain the differences in the efficacy of eradicating *P. aeruginosa* from the airways in these two populations. In non-CF bronchiectatic patients, infection with *P. aeruginosa* occurs in those with more severe lung disease and bronchial obstruction [4]. Thus, on one hand, greater lung inflammation and/or lung tissue injury may limit the ability to eradicate *P. aeruginosa*, while, on the other hand, with the presence of greater airway obstruction, the distribution of an aerosolised antibiotic may be reduced and the drug may not be able to reach the site of infection. Moreover, it has been shown that the clearance of tobramycin from the lower airways of younger CF patients may be slower, which could further favour the eradication of *P. aeruginosa* in this population [35].

Our study demonstrates that antibiotic eradication treatment has a notable clinical impact, with a significant reduction in exacerbations and days and numbers of admissions as well as a trend to improvement in all the oth-

er parameters studied being observed in patients receiving this therapy. In contrast, compelling evidence of a clinical benefit in CF with early intervention is very scarce [3], and it has been suggested that young CF patients with early infection with *P. aeruginosa* often have minimal symptoms and mild lung disease, which may lead to insensitive outcome measures [35].

The timing of the administration of antimicrobial therapy may be critical as there may be a window of opportunity after which antibiotic eradication treatment is no longer successful. Although patients in our study received therapy after the detection of the first positive culture, they may have already been infected prior to the visits carried out every 3 months, thereby delaying the initiation of treatment. Chronic bronchial infection in CF and the presence of chronic *P. aeruginosa* in sputum specimens have been defined as an increase in specific serum antibodies [36]. The presence of these antibodies has been used to identify individuals with CF who fail to clear *P. aeruginosa* [37] and even to exclude patients from antibiotic eradication treatment [31]. In non-CF bronchiectasis specific serum antibodies to *P. aeruginosa* are present, similar to what occurs in patients with CF [6]. Thus, it is reasonable to assume that these antibodies may also be used to better select non-CF bronchiectatic patients who may be candidates for receiving this treatment.

Nebulised tobramycin therapy had to be discontinued due to bronchospasm in 5 patients in whom FEV₁ was especially poor, suggesting that the risk of this complication should be considered in patients with low FEV₁ values [9]. No abnormalities in serum creatinine or audiometry were observed in our study, which supports the high safety margin and absence of nephrotoxicity and ototoxicity in the inhaled antibiotic [7–9]. The emergence of tobramycin-resistant *P. aeruginosa* was not observed,

neither was a higher rate of other opportunistic microorganisms in patients treated with tobramycin inhalation solution compared to those receiving placebo. Moreover, our results suggest that nebulised tobramycin could even reduce other opportunistic organisms, since they were detected less often in patients in the tobramycin group than in the placebo group. In fact, Murray et al. [12] showed that long-term nebulised gentamicin in patients chronically infected with *P. aeruginosa* or other pathogens could be useful for eradicating not only *P. aeruginosa* but also these other microorganisms.

According to the overall results of our study, and taking in account the significantly lower costs for this treatment compared to those needed to treat chronically infected CF patients [38], eradication treatment for *P. aeruginosa* in patients with non-CF bronchiectasis may be recommended. The use of additional systemic therapy, as performed in our study, might be justified, since more severe airway obstruction and disease related to non-CF bronchiectasis may not allow inhaled antibiotics to reach all areas, thereby reducing the probability of eradication. Moreover, monotherapy with a nebulised antibiotic has demonstrated a success rate similar to that seen with combination therapy in CF [2], and a 3-month treatment period has also been shown to provide additional benefit to other shorter regimens [39] and may, thus, be suitable for patients with non-CF bronchiectasis. Further studies including a larger number of patients and longer follow-up would most likely improve the results of our study.

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References

- 1 Emerson J, Rosenfeld M, McNamara S, Ramsey B, Gibson RL: *Pseudomonas aeruginosa* and other predictors of mortality and morbidity in young children with cystic fibrosis. *Pediatr Pulmonol* 2002;34:91–100.
- 2 Döring G, Flume P, Heijerman H, Elborn JS; Consensus Study Group: Treatment of lung infection in patients with cystic fibrosis: current and future strategies. *J Cyst Fibros* 2012; 11:461–479.
- 3 Langton Hewer SC, Smith AR: Antibiotic strategies for eradicating *Pseudomonas aeruginosa* in people with cystic fibrosis. *Cochrane Database Syst Rev* 2009;4:CD004197.
- 4 Wells A, Desai S, Whetton C, Wilson R, Cole P: The isolation of *Pseudomonas aeruginosa* from sputum in idiopathic bronchiectasis: an association with extensive disease and severe airflow obstruction. *Am Rev Respir Dis* 1993; 147:A645.
- 5 Wilson CB, Jones PW, O'Leary CJ, Hansell DM, Cole PJ, Wilson R: Effect of sputum bacteriology on the quality of life of patients with bronchiectasis. *Eur Respir J* 1997;10:1754–1760.
- 6 Caballero E, Drobnic ME, Pérez MT, Manresa JM, Ferrer A, Orriols R: Anti-*Pseudomonas aeruginosa* antibody detection in patients with bronchiectasis without cystic fibrosis. *Thorax* 2001;56:669–674.
- 7 Evans SA, Turner SM, Bosch BJ, Hardy CC, Woodhead MA: Lung function in bronchiectasis: the influence of *Pseudomonas aeruginosa*. *Eur Respir J* 1996;9:1601–1604.
- 8 Orriols R, Roig J, Ferrer J, Sampol G, Rosell A, Ferrer A, Vallano A: Inhaled antibiotic therapy in non-cystic fibrosis patients with bronchiectasis and chronic bronchial infection by *Pseudomonas aeruginosa*. *Respir Med* 1999; 93:476–480.
- 9 Drobnic ME, Suñé P, Montoro JB, Ferrer A, Orriols R: Inhaled tobramycin in non-cystic fibrosis patients with bronchiectasis and chronic bronchial infection with *Pseudomonas aeruginosa*. *Ann Pharmacother* 2005;39:39–44.

- 10 Haworth CS, Foweraker JE, Wilkinson P, Kenyon RF, Bilton D: Inhaled colistin in patients with bronchiectasis and chronic *Pseudomonas aeruginosa* infection. *Am J Respir Crit Care Med* 2014;189:975–982.
- 11 Barker AF, Couch L, Fiel SB, Gotfried MH, Illowhite J, Meyer KC, O'Donnell A, Sahn SA, Smith LJ, Stewart JO, Abuan T, Tully H, Van Dalmsen JV, Well CD, Quan J: Tobramycin solution for inhalation reduces sputum *Pseudomonas aeruginosa* density in bronchiectasis. *Am J Respir Crit Care Med* 2000;162:481–485.
- 12 Murray MP, Govan JR, Doherty CJ, Simpson AJ, Wilkinson TS, Chalmers JD, Greening AP, Haslett C, Hill AT: A randomized controlled trial of nebulized gentamicin in non-cystic fibrosis bronchiectasis. *Am J Respir Crit Care Med* 2011;183:491–499.
- 13 Pasteur MC, Bilton D, Hill AT: British Thoracic Society guideline for non-CF bronchiectasis. *Thorax* 2010;65(suppl 1):i1–i58.
- 14 Quon BS, Goss HC, Ramsey BW: Inhaled antibiotics for lower airways infections. *Ann Am Thorac Soc* 2014;11:425–434.
- 15 Vendrell M, Muñoz G, de Gracia J: Evidence of inhaled tobramycin in non-cystic fibrosis bronchiectasis. *Open Respir Med J* 2015;9:30–36.
- 16 Casals T, Nunes V, Palacio A, Giménez J, Gona A, Ibáñez N, Morral N, Estivill X: Cystic fibrosis in Spain: high frequency of mutation G542X in the Mediterranean coastal area. *Hum Genet* 1993;91:66–70.
- 17 Westerman EM, Boer AH, Touw DJ, Brun PP, Roldaan AC, Frijlink HW, Heijerman HG: Aerosolization of tobramycin (TOBI) with the PARI LC PLUS reusable nebulizer: which compressor to use? Comparison of the CR60 to the PortaNeb compressor. *J Aerosol Med Pulm Drug Deliv* 2008;21:269–280.
- 18 Nebulizer therapy. Guidelines. British Thoracic Society Nebulizer Project Group. *Thorax* 1997;52(suppl 2):S4–S24.
- 19 Murphy TF, Sethi S: Bacterial infection in chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1992;146:1067–1083.
- 20 Murray PR, Washington JA: Microscopic and bacteriologic analysis of expectorates sputum. *Mayo Clin Proc* 1975;50:339–344.
- 21 Bauer AW, Kirby WM, Sherris JC, Turck M: Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 1996;45:493–496.
- 22 Gardner RM, Hankinson JL: Standardization of spirometry – 1987 ATS update (American Thoracic Society). *J Occup Med* 1988;30:272–273.
- 23 Moore RD, Smith CR, Lietman PS: Risk factors for the development of auditory toxicity in patients receiving aminoglycosides. *J Infect Dis* 1984;149:23–30.
- 24 Jones PW, Quirk FH, Baveystock CM, Littlejohns P: A self-complete measure of health status for chronic airflow limitation. The St. George's Respiratory Questionnaire. *Am Rev Respir Dis* 1992;145:1321–1327.
- 25 Fuchs HJ, Borowitz DS, Christiansen DH, Morris EM, Nash ML, Ramsey BW, Rosenstein BJ, Smith AL, Wohl ME: Effect of aerosolized recombinant human DNase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. The Pulmozyme Study Group. *N Engl J Med* 1994;331:637–642.
- 26 O'Donnell AE, Barker AF, Ilowite JS, Fick RB: Treatment of idiopathic bronchiectasis with aerosolized recombinant human DNase I. rhDNase Study Group. *Chest* 1998;113:1329–1334.
- 27 Levy J: Antibiotic activity in sputum. *J Pediatr* 1986;108(5 pt 2):841–846.
- 28 Nixon GM, Armstrong DS, Carzino R, Carlin JB, Olinsky A, Robertson CF, Grimwood K: Clinical outcome after early *Pseudomonas aeruginosa* infection in cystic fibrosis. *J Pediatr* 2001;138:699–704.
- 29 White L, Mirrani G, Grover M, Rollason J, Malin A, Suntharalingam J: Outcomes of *Pseudomonas* eradication in patients with non-cystic fibrosis bronchiectasis. *Respir Med* 2012;106:356–360.
- 30 Valerius NH, Koch C, Høiby N: Prevention of chronic *Pseudomonas aeruginosa* colonisation in chronic fibrosis by early treatment. *Lancet* 1991;338:725–726.
- 31 Wiesemann HG, Steinkamp G, Ratjen F, Baurerfeind A, Przyklenk B, Döring G, von der Hardt H: Placebo-controlled, double-blind, randomized study of aerosolized tobramycin for early treatment of *Pseudomonas aeruginosa* colonization in cystic fibrosis. *Pediatr Pulmonol* 1998;25:88–92.
- 32 Ratjen F, Döring G, Nikolaizik WH: Effect of inhaled tobramycin on early *Pseudomonas aeruginosa* colonisation in patients with cystic fibrosis. *Lancet* 2001;358:983–984.
- 33 Ratjen F, Munck A, Kho P, Angyalosi G; ELITE Study Group: Treatment of early *Pseudomonas aeruginosa* infection in patients with cystic fibrosis: the ELITE trial. *Thorax* 2010;65:286–291.
- 34 Treggiari MM, Retsch-Bogart G, Mayer-Hamblett N, Khan U, Kulich M, Kronmal R, Williams J, Hiatt P, Gibson RL, Spencer T, Orenstein D, Chatfield BA, Froh DK, Burns JL, Rosenfeld M, Ramsey BW; Early *Pseudomonas* Infection Control (EPIC) Investigators: Comparative efficacy and safety of 4 randomized regimens to treat early *Pseudomonas aeruginosa* infection in children with cystic fibrosis. *Arch Pediatr Adolesc Med* 2011;165:847–856.
- 35 Gibson RL, Emerson J, McNamara S, Burns JL, Rosenfeld M, Yunker A, Hamblett N, Accurso F, Dovey M, Hiatt P, Konstan MW, Moss R, Retsch-Bogart G, Wagener J, Waltz D, Wilmott R, Zeitlin PL, Ramsey B; Cystic Fibrosis Therapeutics Development Network Study Group: Significant microbiological effect of inhaled tobramycin in young children with cystic fibrosis. *Am J Respir Crit Care Med* 2003;167:841–849.
- 36 Döring G, Høiby N: Longitudinal study of immune response to *Pseudomonas aeruginosa* antigens in cystic fibrosis. *Infect Immun* 1983;42:197–201.
- 37 Anstead M, Heltshe SL, Khan U, Barbieri JT, Langkamp M, Döring G, Dharia S, Gibson RL, Treggiari MM, Lymp J, Rosenfeld M, Ramsey B: *Pseudomonas aeruginosa* serology and risk for re-isolation in the EPIC trial. *J Cyst Fibros* 2013;12:147–153.
- 38 Baumann U, Stoklossa C, Greiner W, von der Schulenburg JM, von der Hardt H: Cost of care and clinical condition in paediatric cystic fibrosis patients. *J Cyst Fibros* 2003;2:84–90.
- 39 Frederiksen B, Koch C, Høiby N: Antibiotic treatment of initial colonization with *Pseudomonas aeruginosa* postpones chronic infection and prevents deterioration of pulmonary function in cystic fibrosis. *Pediatr Pulmonol* 1997;23:330–335.