

# Bacterial colonisation in patients with bronchiectasis: microbiological pattern and risk factors

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**Background:** A study was undertaken to investigate the incidence, diagnostic yield of non-invasive and bronchoscopic techniques, and risk factors of airway colonisation in patients with bronchiectasis in a stable clinical situation.

**Methods:** A 2 year prospective study of 77 patients with bronchiectasis in a stable clinical condition was performed in an 800 bed tertiary university hospital. The interventions used were pharyngeal swabs, sputum cultures and quantitative protected specimen brush (PSB) bacterial cultures (cut off point  $\geq 10^2$  cfu/ml) and bronchoalveolar lavage (BAL) (cut off point  $\geq 10^3$  cfu/ml).

**Results:** The incidence of bronchial colonisation with potential pathogenic microorganisms (PPMs) was 64%. The most frequent PPMs isolated were *Haemophilus influenzae* (55%) and *Pseudomonas* spp (26%). Resistance to antibiotics was found in 30% of the isolated pathogens. When the sample was appropriate, the operative characteristics of the sputum cultures were similar to those obtained with the PSB taken as a gold standard. Risk factors associated with bronchial colonisation by PPMs in the multivariate analysis were: (1) diagnosis of bronchiectasis before the age of 14 years (odds ratio (OR)=3.92, 95% CI 1.29 to 11.95), (2) forced expiratory volume in 1 second (FEV<sub>1</sub>) <80% predicted (OR=3.91, 95% CI 1.30 to 11.78), and (3) presence of varicose or cystic bronchiectasis (OR=4.80, 95% CI 1.11 to 21.46).

**Conclusions:** Clinically stable patients with bronchiectasis have a high prevalence of bronchial colonisation by PPMs. Sputum culture is a good alternative to bronchoscopic procedures for evaluation of this colonisation. Early diagnosis of bronchiectasis, presence of varicose-cystic bronchiectasis, and FEV<sub>1</sub> <80% predicted appear to be risk factors for bronchial colonisation with PPMs.

The lower respiratory tract of healthy non-smokers is sterile.<sup>1,2</sup> By contrast, patients with bronchiectasis and chronic obstructive pulmonary disease (COPD) are often colonised with potentially pathogenic microorganisms (PPMs).<sup>3</sup> Scientific evidence suggests that colonisation of distal airways by PPMs may be harmful to patients with bronchiectasis. These microorganisms represent a potential risk for lung infections and may secrete several inflammatory mediators that cause progressive tissue damage and airway obstruction. The phenomenon of chronic colonisation, secondary inflammatory reaction, and progressive lung injury is a "vicious cycle" and is the reason why appropriate evaluation of distal airway colonisation is needed.<sup>4</sup> To break this vicious cycle it is necessary to identify the colonising bacteria and to know which antibiotic to administer. Although different studies have used sputum bacteriology as a diagnostic tool to evaluate the presence of bronchial infection during exacerbations in patients with bronchiectasis,<sup>5</sup> very few have evaluated the pattern of bronchial colonisation in clinically stable patients. Sputum culture is a simple non-invasive and inexpensive procedure, although it is limited by potential sample contamination by oropharyngeal flora. The use of fiberoptic bronchoscopy guided diagnostic techniques may avoid oropharyngeal flora and provide uncontaminated samples of the lower airways. However, very few studies have used bronchoscopic diagnostic techniques for microbiological evaluation of distal airways in patients with bronchiectasis in a stable clinical condition.<sup>6,7</sup> These studies have shown that PPMs, mainly *Haemophilus influenzae* and *Pseudomonas* spp, may be isolated from 60–90% of these patients. Microbiological evaluation of the distal airways in patients with stable bronchiectasis may aid in determining the role of colonisation in disease progression. The aims of the present study in clinically

stable patients with bronchiectasis were: (1) to analyse the bacterial flora that colonise the airways, (2) to compare non-invasive (pharyngeal swabs and sputum) and bronchoscopic (PSB and BAL) techniques, and (3) to analyse potential risk factors associated with the presence of PPMs in the airways.

## METHODS

Bronchiectasis was diagnosed by clinical symptoms and a high resolution CT (HRCT) scan. A stable clinical condition was defined as the absence of clinical criteria of an exacerbation (impairment of respiratory symptoms with an increase in the volume, change in the macroscopic characteristics of the sputum, or fever). Exclusion criteria were admission to hospital in the previous 2 months, use of antibiotics in the previous 4 weeks, or the presence of a serious concomitant illness.

## Study protocol

In all patients an HRCT scan was performed during the 3 month period prior to the bronchoscopic examination and the extent and type of bronchiectasis as well as the presence of infiltrates were evaluated. The presence and extent of bronchiectasis in each lobe was graded using a scale from 0 to 3 where 0=no bronchiectasis, 1=involvement of one segment, 2=more than one segment involved, and 3=gross cystic bronchiectasis involving the entire lobe. Since the lingula was considered an independent lobe, the maximum possible number of points was 18. The total final score was calculated as the total number of points divided by the maximum possible points multiplied by 100. In patients with a clinical suspicion of chronic sinusitis, a paranasal sinus CT scan was also performed.

On the day of the study a complete clinical evaluation and forced spirometric tests (Collins Survey III Plus, USA) were performed before the bronchoscopic examination. Clinical data included age at diagnosis of bronchiectasis (above or below 14 years of age), probable cause of bronchiectasis, smoking habit, volume and characteristics of expectoration (mucous, mucopurulent, purulent), prior or current episodes of haemoptysis, and current oral or inhaled corticosteroid treatment.

Samples of the upper and lower respiratory tract were obtained from all patients. Initially a pharyngeal swab (Eurotubo; Industrias Aulabor SA, Barcelona, Spain) and a sample of spontaneous sputum were taken. If the patient was unable to expectorate, a sample of induced sputum was obtained by inhalation of 3% hypertonic saline for 15 minutes with an ultrasonic nebuliser (Ultraneb 2000; DeVilbiss Healthcare Inc, Somerset, PA, USA). Upper airway anaesthesia for bronchoscopic examination was achieved by nebulisation of 8 ml lidocaine 5% through a buccal clip for 15 minutes. The bronchoscope (Olympus BF 30; Olympus, New Hyde Park, NY, USA) was passed transnasally or transorally into the trachea avoiding any suction through the inner channel. Using the technique described by Wimberley,<sup>8</sup> a protected specimen brush (PSB) sample (Microbiology Brush; Mill-Rose Laboratory Inc, Mentor, Ohio, USA) was obtained from the most affected lobe (based on the HRCT scan findings). Bronchoalveolar lavage (BAL) was carried out after PSB in the same lobe using 150 ml sterile saline in three 50 ml aliquots. The fluid recovered was pooled and mixed in a single container.

### Microbiological evaluation

Once homogenised, samples from the pharyngeal swab, sputum, PSB and BAL were Gram stained. Only sputum samples of Murray-Washington classification degrees IV or V were processed (degree IV=10–25 epithelial cells and >25 leucocytes; degree V=<10 epithelial cells and >25 leucocytes per field using a low magnification lens ( $\times 100$ )).<sup>9</sup> PSB and BAL samples were serially diluted (dilutions of 1:10, 1:100, and 1:1000). All microbiological samples were plated on blood, chocolate, Wilkins-Chalgren, and Sabouraud agar and Ziehl-Nielsen staining and Lowenstein culture were performed on all the samples. The cultures were evaluated for growth after 48 hours. Negative bacterial cultures were discarded after 5 days, negative cultures for fungi after 4 weeks, and Lowenstein cultures after 6 weeks. Susceptibility testing was performed using the broth microdilution or  $\beta$ -lactamase test and classified as sensitive, intermediate or resistant according to the criteria published by the National Committee for Clinical Laboratory Standards.<sup>10</sup> The results of the PSB and BAL were expressed quantitatively as colony forming units (cfu) per ml.

Microorganisms were identified according to conventional methods and classified as PPMs and non-potentially pathogenic microorganisms (non-PPMs). The following microorganisms were considered as non-PPMs: *Streptococcus viridans*, *Neisseria* spp, *Corynebacterium* spp, *Candida* spp, *Enterococcus* spp, and coagulase negative staphylococcus. Both PPMs and non-PPMs were defined as significant when present as  $\geq 10^2$  cfu/ml in PSB specimens and  $10^3$  cfu/ml in BAL fluid, as previously described.<sup>6</sup>

The ethical committee of the Hospital Clinic approved the study protocol. Informed consent was obtained from all patients.

### Statistical analysis

Data were reported as mean (SD) values. Continuous variables were compared using a non-parametric test (Mann-Whitney U test) and categorical variables by the  $\chi^2$  or Fisher exact test when appropriate. All reported p values were two tailed and the level of significance was 5%. Sensitivity (SE), specificity

(SP), positive predictive value (PPV), negative predictive value (NPV), and kappa coefficient were used to measure the agreement between diagnostic tests using PSB as the gold standard.

A logistical regression model was performed evaluating risk factors associated with colonisation of the airways by PPMs. The dependent variable was the presence or absence of PPMs in the airways in counts higher than  $10^2$  cfu/ml in the PSB specimen. The independent variables were smoking habit (0=non-smoker; 1=active smoker or ex-smoker), expectoration (0=no; 1=yes), expectoration type (0=mucous; 1=mucopurulent or purulent), haemoptysis (0=no; 1=yes), age at diagnosis (0=under 14 years; 1=over 14 years), bronchiectasis (0=cylindrical; 1=cystic or varicose), CT scan score (0= $\leq 50\%$ ; 1= $> 50\%$ ), presence of inflammatory infiltrates in CT scan (0=no; 1=yes), previous corticosteroid treatment (0=no; 1=yes), sinusitis (0=no; 1=yes), and FEV<sub>1</sub> (0=<80%; 1= $> 80\%$ ). Variables with a p value of <0.10 were accepted for multivariate analysis. All data were analysed using the statistical package spss for Windows version 8.0 (SPSS, Chicago, IL, USA).

### RESULTS

Seventy seven consecutive clinically stable patients with bronchiectasis were studied over a 2 year period (January 1998 to January 2000). Table 1 shows the demographic data, characteristics of the bronchiectasis, and spirometric values of the patients studied. Women (66%) and non-smokers (79%) predominated. The most frequent cause of bronchiectasis was following an infection (65%). Thirty eight patients (49%) had airflow obstruction (defined as a forced expiratory volume in 1 second (FEV<sub>1</sub>) of <80% predicted associated with an FEV<sub>1</sub>/FVC index of <70%), and 30 (39%) had normal

**Table 1** Baseline characteristics of the 77 patients evaluated

Characteristics	Values	Range
Mean (SD) age (years)	58 (14)	16–76
Sex		
Women	51 (66%)	
Men	26 (34%)	
Smoking history		
Smoker	5 (6%)	
Ex-smoker	11 (14%)	
Non-smoker	61 (79%)	
Aetiology of bronchiectasis		
Post-infectious	52 (65%)	
Idiopathic	20 (25%)	
Ciliary dyskinesia	2 (3%)	
Young's syndrome	1 (1%)	
Rheumatoid arthritis	1 (1%)	
Immunological deficiency (IgG <sub>2</sub> deficiency)	1 (1%)	
Chronic expectoration	49 (64%)	
Haemoptysis	28 (36%)	
Age at diagnosis		
<14 years	42 (54%)	
>14 years	35 (46%)	
Mean (SD) HRCT score (%)	39 (21)	6–100
Type of bronchiectasis		
Cylindrical	56 (73%)	
Cystic-varicose	21 (27%)	
Inflammatory infiltrates (HRCT scan)	29 (38%)	
Sinusitis (CT scan)	28 (58%)	
Forced spirometry		
FEV <sub>1</sub> (l)	2.0 (1.0)	0.54–5.19
FEV <sub>1</sub> (%)	75 (23)	18–120
FVC (l)	2.9 (1.1)	1.32–6.15
FVC (%)	82 (18)	43–126
FEV <sub>1</sub> /FVC (%)	67 (13)	

Values are expressed as n (%) for qualitative variables and mean (SD) for quantitative variables.  
HRCT=high resolution CT scan; FEV<sub>1</sub>=forced expiratory volume in 1 second; FVC=forced vital capacity.

**Table 2** Microorganisms isolated in respiratory samples using different procedures

	PS (n=71)	S (n=42)	PSB (n=75)	BAL (n=59)
PPMs	13 (18)	22 (52)	46 (61)	33 (56)
<i>H influenzae</i>	3 (4)	11 (26)	24 (32)	19 (32)
<i>H parainfluenzae</i>	1 (1)	–	–	–
<i>M catarrhalis</i>	–	2 (5)	3 (4)	–
<i>S pneumoniae</i>	2 (3)	6 (14)	6 (8)	4 (7)
<i>S aureus</i>	2 (3)	–	2 (3)	2 (3)
<i>Nocardia</i> spp	–	–	1 (1)	1 (2)
<i>E coli</i>	–	1 (2)	1 (1)	1 (2)
<i>Proteus</i> spp	1 (1)	1 (2)	–	1 (2)
<i>Enterobacter</i> spp	–	–	–	1 (2)
<i>P aeruginosa</i>	3 (4)	1 (2)	8 (11)	5 (8)
<i>P aeruginosa mucoid strain</i>	–	3 (7)	3 (4)	1 (2)
<i>P fluorescens</i>	–	–	1 (1)	1 (2)
<i>K pneumoniae</i>	1 (1)	–	–	–
<i>A xylosoxidans</i>	–	–	1 (1)	1 (2)
<i>Aspergillus</i> spp	1 (1)	1 (2)	1 (1)	1 (2)
Non-PPMs				
<i>S viridans</i>	49 (69)	23 (55)	18 (24)	14 (24)
<i>Corynebacterium</i> spp	6 (8)	1 (2)	5 (7)	1 (2)
Coagulase negative staphylococcus	–	1 (2)	2 (3)	2 (3)
<i>Neisseria</i> spp	50 (70)	22 (52)	10 (13)	5 (8)
<i>Enterococcus</i> spp	–	–	1 (1)	1 (2)
<i>Candida</i> spp	1 (1)	1 (2)	–	2 (3)

Results are expressed as n (%). n=number of patients in whom the procedure was performed. PS=pharyngeal swabs; S=sputum; PSB=protected specimen brush; BAL=bronchoalveolar lavage; PPMs=potential pathogenic microorganisms; non-PPMs=non-potential pathogenic microorganisms.

spirometric values. Most of the patients (73%) presented with cylindrical bronchiectasis according to the HRCT scan. A paranasal sinus CT scan was performed in 48 patients and confirmed a diagnosis of sinusitis in 28 (58%). Twenty two patients (29%) were receiving inhaled corticosteroids (maximum 1600 µg/day) and only one patient was on chronic oral steroid treatment (10 mg/day).

Pharyngeal swabs were obtained from 71 patients, isolating 13 strains of PPMs in 13 patients (18%) and 106 strains of non-PPMs in 71 patients (100%) (table 2).

Sixty two patients underwent sputum analysis. In 45 patients (73%) the sample was obtained spontaneously although only 29 (64%) fulfilled the Murray-Washington criteria and were further processed for culture. In 17 patients (37%) who were unable to expectorate spontaneously, the sputum sample was obtained using the induction technique with 13 (76%) being valid for culture. Culture of the 42 good quality sputum samples (both spontaneous and induced) yielded 26 PPMs corresponding to 22 patients (52%) and 48 non-PPMs corresponding to 25 patients (60%). Protected specimen brush specimens were obtained from 75 patients and culture yielded 51 PPMs in 46 patients (61%) and 36 non-PPMs in 22 patients (29%). Culture of BAL fluid samples from 59 patients yielded 38 PPMs in 33 patients (56%) and 25 non-PPMs in 19 patients (32%).

Two different PPMs were found in the sputum samples from three patients, in the PSB samples from five patients, and in the BAL fluid samples from three patients. On pooling the results obtained with the different techniques used to assess bronchial colonisation, 49 of the 77 patients had significant PPM counts in the respiratory tract, yielding an overall colonisation rate of 64%. Community acquired pathogens accounted for 71% of these isolates (40/56), with *Pseudomonas* spp and *Alcaligenes xylosoxidans* representing 25% of the cases (14/56) and unusual microorganisms such as *Nocardia* spp and *Aspergillus* spp representing 3% (2/56). The most frequent microorganisms isolated were *H influenzae* from 27 patients (55%), different strains of *Pseudomonas* spp from 13 (26%), and *S pneumoniae* from six (12%).

The results of PSB and sputum analysis agreed in 75% of the patients in whom both techniques were used (both positive for PPMs in 18/40 patients and both negative for PPMs in 12/40).

PPMs were isolated from PSB specimens in eight patients in whom sputum culture was negative and from sputum samples of two patients in whom the culture of PSB specimens was negative. In 80% of the patients in whom both techniques were used, the results of PSB and BAL agreed (both positive for PPMs in 30/60 and both negative for PPMs in 18/60). PPMs were isolated from PSB specimens in nine patients (15%) in whom the BAL fluid was negative, and from the BAL fluid in three patients (5%) in whom the PSB specimen was negative.

Using 10<sup>2</sup> cfu/ml in the PSB as the gold standard, the operative characteristics for the different techniques used were as follows: pharyngeal swabs, SE 24%, SP 89%, PPV 77%, NPV 44%, κ=0.11, p=0.15; sputum (spontaneous or induced), SE 69%, SP 86%, PPV 90%, NPV 60%, κ=0.50, p=0.001; spontaneous sputum, SE 84%, SP 75%, PPV 89%, NPV 67%, κ=0.57, p=0.006; induced sputum, SE 57%, SP 100%, PPV 100%, NPV 67%, κ=0.55, p=0.026; BAL fluid, SE 77%, SP 86%, PPV 91%, NPV 67%, κ=0.59, p=0.

Interestingly, we found that patients colonised with PPMs had worse lung function than those not-colonised with PPMs (table 3). Although patients colonised with *Pseudomonas* spp had the most severe airway obstruction, the differences were not statistically significant from those colonised with other PPMs (data not shown).

Susceptibility tests to antibiotics for PPMs isolated by PSB were performed. Of 24 *H influenzae* strains isolated by PSB, seven (29%) were β-lactamase positive and, of the three strains of *M catarrhalis* isolated, one was β-lactamase positive.

**Table 3** Forced spirometric measurements and HRCT score in patients with and without PPM airway colonisation

	PPMs	Non-PPMs	p Value
FEV <sub>1</sub> (%)	70 (23)	85 (22)	0.005
FVC (%)	78 (18)	88 (18)	0.02
FEV <sub>1</sub> /FVC (%)	65 (12)	70 (14)	NS
HRCT score	41 (19)	38 (23)	NS

NS=not significant.  
For other abbreviations see Tables 1 and 2.

**Table 4** Risk factors associated with airway colonisation by PPMs

	p Value	OR (CI)
Univariate analysis		
Smoking status	NS	
Chronic expectoration	0.006	3.92 (1.44 to 10.62)
Expectoration	NS	
Haemoptysis	NS	
Early diagnosis	0.002	4.40 (1.66 to 11.65)
Type of bronchiectasis	0.007	5.57 (1.43 to 21.14)
HRCT score (%)	NS	
Inflammatory infiltrates (CT scan)	NS	
Sinusitis (CT scan)	0.025	4.40 (1.15 to 16.81)
FEV <sub>1</sub> <80% predicted	0.001	5.47 (1.97 to 15.12)
Corticosteroid treatment*	NS	
Multivariate analysis		
Diagnosis (before and after 14 years)		3.92 (1.29 to 11.95)
FEV <sub>1</sub> <80% predicted		3.91 (1.30 to 11.78)
Type of bronchiectasis		4.80 (1.11 to 21.46)

OR=odds ratio; CI=confidence interval. OR is shown only in variables which were significant on univariate analysis.

\*Corticosteroid treatment included patients with both inhaled or oral corticosteroid treatment. NS=not significant.

Two strains of *S pneumoniae* showed high resistance to penicillin (MIC  $\geq 2$   $\mu\text{g/ml}$ ), three strains had intermediate resistance (MIC 0.1–1.0  $\mu\text{g/ml}$ ), and only one strain was susceptible to ampicillin (MIC  $\leq 0.06$   $\mu\text{g/ml}$ ). Overall, resistance to  $\beta$ -lactams was found in 30% of the patients colonised with *H influenzae*, *S pneumoniae*, or *M catarrhalis*. Similarly, of the two strains of *Staphylococcus aureus* isolated, one was resistant to oxacillin (MRSA), and of the 11 strains of *Pseudomonas aeruginosa*, two were resistant to ciprofloxacin (both *P aeruginosa* mucoid strain) and one showed intermediate susceptibility.

#### Risk factors associated with airway colonisation by PPMs

The results of the univariate and multivariate analysis are summarised in table 4. Univariate analysis showed factors associated with the presence of PPMs in the airways to be the presence of chronic expectoration, evidence of varicose or cystic bronchiectasis in the HRCT scan, diagnosis of bronchiectasis before the age of 14 years, presence of sinusitis, and lung function impairment (FEV<sub>1</sub> <80% predicted based on ROC curve analysis). Factors not related to the presence of PPMs were smoking habit, macroscopic characteristics of expectoration, haemoptysis, HRCT scan score, inflammatory infiltrates in the HRCT scan, and previous corticosteroid treatment. Multivariate analysis showed that age at diagnosis (below 14 years), type of bronchiectasis (cystic-varicose), and FEV<sub>1</sub> <80% predicted were independently associated with the presence of PPMs in the airways.

#### DISCUSSION

This study shows that (1) bronchial colonisation by PPMs is common in patients with bronchiectasis in a stable clinical situation. In more than 60% of these patients the distal airways were colonised, with *H influenzae* and *Pseudomonas* spp being the most frequent microorganisms isolated; (2) sputum culture is an adequate tool for the evaluation of bronchial colonisation in these patients; and (3) early diagnosis of bronchiectasis (before the age of 14), evidence of varicose-cystic bronchiectasis, and an FEV<sub>1</sub> of <80% predicted are risk factors for the presence of PPMs in the airways.

The use of bronchoscopic techniques for the evaluation of bacterial colonisation allowed collection of uncontaminated samples of the distal airways. Specifically, we used quantitative cultures of PSB samples as the gold standard and compared the results with those obtained in BAL fluid and sputum. Using these techniques we observed that 64% of the

patients with bronchiectasis in a stable clinical condition were colonised with PPMs, *H influenzae* being the most common microorganism isolated followed by both *Pseudomonas* spp and *S pneumoniae*. Two other reports have investigated airway colonisation flora using the PSB method in clinically stable patients with bronchiectasis. Pang *et al*<sup>7</sup> found that 81% of the patients evaluated had PPMs, *Pseudomonas* spp being the most frequent microorganism isolated. In a small series of 17 patients with bronchiectasis we found PPMs in 82% of the patients.<sup>6</sup> Antibiotics received before inclusion in the study, the prevalence of smoking habit, and the severity of airflow obstruction may explain the differences observed in the prevalence of colonisation in the present series and the previously reported studies.<sup>6 11–13</sup> It is known that airway colonisation is sometimes produced by microorganisms that require special considerations to their pathogenic role. In this sense, we isolated *Nocardia asteroides* and *Aspergillus* spp in two different clinically stable patients with bronchiectasis. In the present series neither of the two patients colonised with *S aureus* had allergic bronchopulmonary aspergillosis or cystic fibrosis.<sup>14 15</sup>

Interestingly, we observed that 33% of the *S pneumoniae* strains isolated were resistant to penicillin, 29% of the *H influenzae* were  $\beta$ -lactamase positive, and 33% of the *Pseudomonas* spp were resistant to quinolones. This pattern of resistance is in accordance with previous studies on respiratory infections recently carried out in Spain.<sup>16</sup> The potential virulence of some of the PPMs isolated and the pattern of resistance to antibiotics reinforce the importance of periodical assessment of the airway flora of patients with bronchiectasis.

The clinical significance of the presence of non-PPMs in the airways is controversial. We have previously shown that patients with stable COPD colonised with PPMs have a more intense local and systemic inflammatory response than those colonised with non-PPMs.<sup>17</sup> This finding indicates that the potential lung damage of PPMs may be greater than that caused by non-PPMs, and suggests that the use of antibiotics to eradicate non-PPMs from the airways is probably not justified.

The comparison of the operative characteristics between the different sampling methods evaluated showed that sputum—a simple, non-invasive and inexpensive procedure—may be very useful to obtain reliable microbiological data in clinically stable patients with bronchiectasis. Two considerations deserve further explanation regarding the use of sputum: firstly, although the diagnostic yield of sputum was acceptable, the NPV was 66%. Thus, 34% of the patients with negative sputum cultures may have lower airway colonisation suggesting that, in some cases, sputum does not reflect events in the distant airways. Secondly, the use of the induction technique allowed us to obtain good quality sputum samples in 13 out of 17 patients who were previously unable to expectorate spontaneously, suggesting that induced sputum may be a good method for assessing bronchial colonisation. The good results obtained with sputum culture suggest that PSB may be reserved for patients unable to expectorate (spontaneously or induced) or those with a negative sputum culture and unfavourable clinical outcome. BAL does not seem to provide additional information to the results obtained by PSB.

In these patients the presence of associated sinusitis was a risk factor for airway colonisation by univariate analysis. Other authors have previously observed an association between bronchiectasis and sinusitis, which suggests that both disorders may share a common pathogenesis.<sup>18</sup> Chronic expectoration was also a risk factor for bacterial colonisation on univariate analysis. Interestingly, an association between chronic expectoration, colonisation by PPMs, and the existence of an intense inflammatory response in the airways has recently been reported.<sup>19</sup> This inflammatory reaction, characterised by neutrophil migration within the airways and secondary secretion of a variety of tissue damaging mediators,

seems to be of importance in the pathogenesis of bronchiectasis. Clinical variables associated with bronchial colonisation, as well as simple procedures to evaluate both the inflammation and colonisation of the airways appropriately, are thus needed for optimal management of patients with bronchiectasis.

Three variables were associated with airway colonisation with PPMs on multivariate analysis: (1) confirmation of diagnosis before the age of 14 years. Since the mean (SD) age of the population evaluated was 57 (14) years, the association observed could be explained by a longer evolution of the disease in patients diagnosed at an early age and thus with a greater risk of recurrent respiratory infections and progressive bronchial damage; (2) the presence of varicose-cystic bronchiectasis on the HRCT scan which, as with the age of diagnosis, may also reflect more advanced lung disease justifying the presence of PPMs in the airways, and (3) FEV<sub>1</sub> lower than 80% predicted. Other authors have observed a correlation between the presence of bacteria in the airways (particularly *P aeruginosa*) and lung function impairment.<sup>20–22</sup> In our series patients colonised with *P aeruginosa* also had a lower FEV<sub>1</sub> than other colonised patients, although the differences did not reach statistical significance probably because of the small number of patients colonised with this bacteria.

In summary, this study has shown the high prevalence of bronchial colonisation by PPMs in patients with bronchiectasis in a stable clinical condition, together with the usefulness of sputum culture as a first alternative for the proper evaluation of this colonisation. Variables indirectly related to long term evolution of the disease—such as an early diagnosis of bronchiectasis, the existence of cystic-varicose bronchiectasis on the HRCT scan, and FEV<sub>1</sub> below 80% predicted—are associated with the presence of airway colonisation by PPMs.

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