Eur Respir J 1999; 13: 338–342
Printed in UK – all rights reserved

European Respiratory Journal
ISSN 0903-1936

Risk factors for lower airway bacterial colonization in chronic bronchitis

E. Monsó, A. Rosell, G. Bonet, J. Manterola, P.J. Cardona, J. Ruiz, J. Morera

Risk factors for lower airway bacterial colonization in chronic bronchitis. E. Monsó, A. Rosell, G. Bonet, J. Manterola, P.J. Cardona, J. Ruiz, J. Morera. ©ERS Journals Ltd 1999.

ABSTRACT: The aim of this study was to determine the prevalence and risk factors for lower airway bacterial colonization (LABC) in stable chronic bronchitis (CB).

Forty-one outpatients with CB were enrolled in the study (age 63.8±9.1 yrs (mean± SD); forced expiratory volume in one second (FEV1)/forced vital capacity (FVC) 62.8±11.2; current/former smokers 24/17). All patients had normal chest radiographs and an indication for performing fibreoptic bronchoscopy (pulmonary nodule, remote haemoptysis). The protected specimen brush (PSB) was used for bacterial sampling, and concentrations ≥1,000 colony-forming units (cfu)·mL^{-1} were considered positive for LABC. The repeatability of the procedure in CB was assessed in a random subsample of 18 subjects.

A 72.2% quantitative agreement was found in the repeatability assessment of the PSB technique. Positive PSB cultures, obtained in 9 out of 41 (22%) patients, mainly yielded *Haemophilus influenzae*. The logistic regression model, used to determine which variables were related to colonization, showed that LABC was associated with current smoking (odds ratio (OR) 9.83, confidence interval (CI) 1.16–83.20) and low FVC (OR 0.73, CI 0.65–0.81). Age and FEV1 were not related to LABC.

It was concluded that the prevalence of LABC in stable CB is high (22%), and current smoking is an important risk factor.

Eur Respir J 1999; 13: 338–342.

Serveis de Pneumologia i Microbiologia, Hospital Germans Trias i Pujol, Badalona, Catalonia, Spain.

Correspondence: E. Monsó
Servei de Pneumologia
Hospital Germans Trias i Pujol
Ap correus 72
08916 Badalona
Catalonia
Spain
Fax: 34 934978843

Keywords: Chronic bronchitis
lower airway bacterial colonization
protected specimen brush
risk factors
repeatability
smoking

Received: October 27 1997
Accepted after revision July 13 1998

Funded by FISss 94/0608.

Nontypeable *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Moraxella catarrhalis* are commonly related to the exacerbations of chronic bronchitis (CB) [1]. In exacerbated CB, lower airway microbiological sampling, performed following procedures that preclude contamination from oropharyngeal flora, yields positive bacterial cultures in 50–75% of cases [2–5]. However, 25% of such patients also give positive cultures when sampled in a clinically stable situation, showing that lower airway bacterial colonization (LABC) is common in CB [4]. The causes of LABC are poorly understood.

A cross-sectional study of 41 outpatients with stable CB was conducted using the protected specimen brush (PSB) technique [6, 7], in order to determine the prevalence of LABC and the risk factors associated with such colonization in this population.

Materials and methods

Population

A series of 41 stable CB outpatients were defined as having chronic phlegm (>3 months·yr^{-1} for ≥2 yrs) and normal or near-normal (pulmonary nodule <3 cm diameter) chest radiographs, with no acute lung disease [8]. Only patients with no changes in the volume or appearance of sputum or level of dyspnoea in the previous 15 days were considered to have stable CB. Patients who had been admitted to hospital within the last 6 months, who had been treated with antibiotics during the month prior to microbiological sampling, with clinical or radiological signs suggestive of bronchiectasis or who had alveolar or interstitial opacities on a chest radiograph were not included. Patients with a history of diagnosed bronchial asthma or positive reversibility tests were also excluded. All patients meeting the inclusion criteria who had been examined with fibreoptic bronchoscopy (indication: pulmonary nodule <3 cm diameter or remote haemoptysis) over a 1-yr period were included in a cohort study on the effect of bronchial colonization on lung health (age 63.8±9.1 yrs (mean±SD), height 164.3±6.5 cm, all males, current/former smokers 24/17). This paper describes the findings of the initial cross-sectional phase of this cohort study.

Forced spirometry was performed in all patients using a dry spirometer (Micro Medical, Rochester, UK) 1 h before premedication and fibreoptic bronchoscopy. The highest values of the forced expiratory volume in one second (FEV1) and forced vital capacity (FVC) recorded from three technically acceptable manoeuvres with <5% variation between them were considered.

Microbiological sampling

Premedication, with 0.01 mg·kg^{-1} intramuscular atropine, was administered and topical anaesthesia was achieved by nebulization of 5 mL 4% lidocaine through a

For editorial comments see page 233

mouthpiece over 15 min. No topical anaesthetics were injected through the inner channel of the fibreoptic bronchoscope and suction was not used before PSB sampling. Intranasal anaesthesia was achieved by instillation of 2 mL 4% lidocaine. A bronchoscope was inserted through a nasal fossa, the tip was positioned in the trachea and microbiological sampling was performed as described elsewhere [4]. In brief, a PSB (Mill-Rose Laboratories, Mentor, OH, USA) was inserted through the inner suction channel and advanced under direct vision to the level of a right lower lobe segmentary/subsegmentary bronchus, where the sample was obtained.

Microbiological processing

Specimens were transported to the laboratory within 15 min of collection and 0.1-mL aliquots of the original suspension were placed on agar plates for aerobic and anaerobic culture (MacConkey agar; 5% defibrinated sheep blood in Columbia base; 5% defibrinated sheep blood in Columbia base, colistin and nalidixic acid; chocolate agar; alpha-charcoal yeast extract (CYE) selective legionella medium; Saboraud medium; Wilkins–Chalgren laked blood agar; and Wilkins–Chalgren laked blood agar, vancomycin and kanamycin). Accepted laboratory methods were used for bacterial identification and susceptibility testing [9]. A bacterial count ≥1,000 colony-forming units (cfu)·mL^{-1} was considered positive.

Repeatability of the protected specimen brush technique in chronic bronchitis

To assess, in CB, the repeatability of the PSB technique, two PSB samples were obtained from the same lung area in 18 patients selected at random. Quantitative and qualitative agreement of the paired samples were assessed. Results obtained from the second PSB sample were only considered for the repeatability analysis.

Statistical analysis

All the data were analysed using the SAS 6.04 statistical package (SAS Institute, Cary, NC, USA). Results are expressed as means and standard deviations unless otherwise stated. A logistic regression model was constructed in order to assess the risk factors for LABC in CB, taking into account age, smoking and lung function (FEV_1, FVC, FEV_1/FVC) as independent variables. For this analysis, a unit of variation in a lung function parameter was defined as a 10% change in the value of that parameter. Results were considered statistically significant when $p<0.05$.

Results

Prevalence of lower airway bacterial colonization

The 41 patients with stable CB had, in most cases, normal lung function (11 (26.8%) patients) or light/moderate obstructive ventilatory patterns (FEV_1 ≥50% pred, 25 patients (61.0%)), as revealed by forced spirometry (FVC 91.0±18.9% predicted; FEV_1 74.6±23.7 % pred; FEV_1/FVC 62.8±11.2). Positive PSB cultures were obtained in nine (22.0%) of the 41 patients. Nontypeable *H. influenzae* was cultured from five samples, *Streptococcus viridans* group from two, *Proteus mirabilis* from one and *Neisseria* spp. from one. Four samples were positive for a second bacterium (*S. pneumoniae* in two cases, *S. viridans* group in one and *Corynebacterium* spp. in one case). In three cases, the PSB culture yielded low concentrations (<1,000 cfu·mL^{-1}) of *S. viridans* group and *Neisseria* spp. and these results were considered negative (table 1).

Repeatability of the protected specimen brush technique

Using the 1,000 cfu·mL^{-1} threshold recommended to identify a positive bacteriological result, quantitative agreement was found in 13/18 (72.2%) cases, a level similar to that described for pneumonia. Qualitative agreement was lower, however, as only 9/19 (47.4%) bacterial species recovered grew in the two consecutive samples (table 2). From these results, it was considered that the quantitative repeatability of the PSB technique in CB is equivalent to the repeatability of the procedure in pneumonia, although the qualitative repeatability of the procedure is lower.

Risk factors for lower airway bacterial colonization

The logistic regression model used to determine which variables are predictors of LABC in stable CB showed that current smoking, as opposed to former smoking, was associated with LABC (odds ratio (OR) 9.83, confidence interval (CI) 1.16–83.20). A slight but statistically significant association between low FVC, and LABC was also found (OR 0.73, CI 0.65–0.81). None of the other dependent variables included in the model (age, FEV_1 or FEV_1/FVC) showed any additional association with LABC (table 3).

Table 1. – Results of protected specimen brush culture in chronic bronchitis (n=41)

Case No.	Bacteria	cfu·mL^{-1}
Positive (>1000 cfu·mL^{-1})		
1	*Haemophilus influenzae*	2000
2	*H. influenzae*	20000
	Streptococcus pneumoniae	1000
3	*H. influenzae*	10000
4	*Neisseria s*pp.	4000
5	*H. influenzae*	50000
	S. pneumoniae	5000
6	*Streptococcus viridans* group	2000
7	*Proteus mirabilis*	4000
	S. viridans group	5000
8	*H. influenzae*	8000
9	*S. viridans* group	3000
	Corynebacterium spp.	1000
Negative (<1000 cfu·mL^{-1}) (n=32)		
10	*S. viridans* group	<100
	Neisseria spp.	<100
11	*S. viridans* group	400
12	*S. viridans* group	<100
13–41	Sterile	–

cfu: colony-forming units.

Table 2. – Repeatability of protected specimen brush cul-ture in chronic bronchitis (n=18)

Case No.	Bacteria	Sample culture cfu·mL^{-1}	
		1	2
1	*Haemophilus influenzae*	2000	3500
	Moraxella catarrhalis	500	0
	Streptococcus pneumoniae	500	3500
2	*H. influenzae*	20000	12500
	S. pneumoniae	1000	0
3	*H. influenzae*	10000	3000
4	*Neisseria* spp.	4000	0
5	*H. influenzae*	50000	0
	S. pneumoniae	5000	0
6	*Streptococcus viridans* group	2000	5000
8	*H. influenzae*	8000	3000
10	*S. viridans* group	<100	<100
	Neisseria spp.	<100	<100
	Staphylococcus aureus	0	<100
11	*S. viridans* group	400	100
12	*S. viridans* group	<100	0
13	*S. pneumoniae*	0	5000
14	Sterile	0	0
15	Sterile	0	0
16	*H. influenzae*	0	100,000
17	Sterile	0	0
18	*S. viridans* group	0	8000
19	Sterile	0	0
20	Sterile	0	0

cfu: colony-forming units.

Discussion

This PSB study found LABC in 22% of clinically stable CB outpatients, mainly attributable to nontypeable *H. influenzae*, and demonstrated that current smoking is a risk factor for LABC.

Nontypeable *H. influenzae*, *S. pneumoniae* and *M. catarrhalis* are commonly found in sputum cultures from patients with CB [10–14]. However, as sputum samples can be contaminated with oropharyngeal secretions, they are of limited value in discriminating between infection, colonization and oropharyngeal contamination [15, 16]. The PSB sampling technique described by WIMBERLEY and coworkers [6, 7] permits the culture of micro-organisms, in lower respiratory secretions without contamination by upper respiratory airway flora. This technique has been shown to be highly accurate for the bacteriological diagnosis of pneumonia in patients who have not received antibiotics in the period prior to sampling [17, 18]. In such patients, repeated PSB samples show quantitative agreement when a threshold of 1,000 cfu·mL^{-1} is considered to indicate a positive result. The distinction between the presence or absence of infection based on this diagnostic threshold is only moderately affected by the variability in the measurement, as shown by the 80–90% quantitative repeatability of PSB sampling reported in several studies [19–21]. Because the repeatability of this technique in stable CB has not been previously assessed, to the authors' knowledge, paired PSB samples were obtained from the same lung area in a subsample of 18 patients, using the 1,000 cfu·mL^{-1} threshold to identify a positive bacteriological result. Quantitative agreement was found in 72.2% of the paired cases, from which it can be considered that the quantitative repeatability of PSB in CB is equivalent to that in pneumonia.

Table 3. – Predictors of lower airway bronchial colonization

Dependent variable	SE	OR	CI
Age	–	–	NS
Current smoking	0.63	9.83	1.16–83.2
FVC % pred	-0.33	0.73	0.65–0.81
FEV_1 % pred	–	–	NS
FEV_1/FVC	–	–	NS

SE: standardized estimate; OR: odds ratio; CI: confidence interval; FVC: forced vital capacity; FEV_1: forced expiratory volume in one second.

In the present study nontypeable *H. influenzae* was the most common pathogen identified in the colonized lower airways of stable CB outpatients. *H. influenzae* is often found in the respiratory secretions of CB patients, and can persist in lower airway secretions in spite of serum antibodies against the bacterium [22]. Furthermore, *H. influenzae* strains cultured from lower airway secretions during an exacerbation are often found in the respiratory secretions when the disease is stable, regardless of the antibiotic treatments prescribed [23, 24]. Microbial persistence of *S. pneumoniae* in lower airway secretions has also been related to the clinical exacerbations that appear when CB patients are followed over time [25].

The present study demonstrates that current smoking is a predictor of LABC in stable CB (OR 9.83). An association between current smoking and LABC was first suggested by IRWIN *et al.* [26] in a study of tracheobronchial colonization, using transtracheal aspiration. They found

positive results in 50% of the studied smokers, mainly due to the *S. viridans* group. This effect of current smoking on LABC may be related to the impairment of lower airway clearance described in smokers. Current smoking slows the mucociliary clearance rate in asymptomatic smokers [27] and this effect increases when CB appears [28, 29]. LABC is related not only to tracheobronchial clearance, but also to the appearance of changes in bronchial mucosa that encourage bacterial colonization [30]. RIISE *et al.* [31], in a study of the association between bronchial inflammation and LABC using bronchoalveolar lavage and the PSB technique, showed that the recruitment and activation of neutrophils and eosinophils is a characteristic of CB and is associated with *S. pneumoniae* colonization of the lower airway [32]. In the same study RIISE *et al.* [32] also found no differences in the LABC pattern of patients with chronic obstructive pulmonary disease (COPD) and patients with nonobstructive CB.

No association was found between airflow obstruction and LABC in stable CB patients in the present study, despite the demonstration of a minimal association between FVC and colonization (OR 0.73). Patients with a low FVC seem to have a higher probability of being colonized, but this effect is much less important than the effect of current smoking. The lack of an association between FEV_1 and colonization in this study confirms that COPD is not a major determinant of LABC when the obstructive pattern is light-to-moderate and the patient does not require exacerbation-related hospital admissions.

In conclusion, lower airway bacterial colonization is present in about one-quarter of stable chronic bronchitis outpatients and current smoking is a risk factor for such colonization. Lower airway bacterial colonization may play a role in the course of CB in smokers, but this hypothesis must be assessed through longitudinal studies.

Acknowledgements. The authors thank M.E. Kerans and S. Alonso for help in preparing the manuscript, and M.T. Artés for statistical advice.

References

1. Tager I, Speizer FE. Role of infection in chronic bronchitis. *New Engl J Med* 1975; 292: 563–571.
2. Fagon JY, Chastre J, Trouillet JL, *et al*. Characterization of distal bronchial microflora during acute exacerbation of chronic bronchitis. Use of the protected specimen brush technique in 54 mechanically ventilated patients. *Am Rev Respir Dis* 1990; 142: 1004–1008.
3. Martinez JA, Rodriguez E, Bastida T, Bugés J, Torres M. Quantitative study of the bronchial bacterial flora in acute exacerbations of chronic bronchitis. *Chest* 1994; 105: 976.
4. Monsó E, Ruiz J, Rosell A, *et al*. Bacterial infection in chronic obstructive pulmonary disease. A study of stable and exacerbated outpatients using the protected specimen brush. *Am J Respir Crit Care Med* 1995; 152: 1316–1320.
5. Riise GC, Larsson P, Jeansson S, Andersson BA. The intrabronchial microbial flora in chronic bronchitis patients: a target for *N*-acetylcysteine therapy? *Eur Respir J* 1994; 7: 94–101.
6. Wimberley NW, Bass JB, Boyd BW, Kirkpatrick MB, Serio RA, Pollock HM. Use of a bronchoscopic protected brush for the diagnosis of pulmonary infections. *Chest* 1982; 81: 556–562.
7. Wimberley N, Faling LJ, Bartlett JG. A fiberoptic bronchoscopy technique to obtain uncontaminated lower airway secretions for bacterial culture. *Am Rev Respir Dis* 1979; 119: 337–343.
8. Definition and classification of chronic bronchitis for clinical and epidemiological purposes. *Lancet* 1965; ii: 775–785.
9. Balows A, Hausler WJ, Herrmann KL, Isenberg HD, Shadomy HJ. Manual of Clinical Microbiology, 5th Edn. Washington DC, American Society of Microbiology, 1991.
10. Murphy TF, Sethi S. Bacterial infection in chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1992; 146: 1067–1083.
11. Murphy TF, Apicella MA. Nontypable *Haemophilus influenzae*: a review of clinical aspects, surface antigens, and the human immune response to infection. *Rev Infect Dis* 1987; 9: 1–15.
12. Feldman C, Smith C, Kaka S, DeJong P, Promnitz DA. The clinical significance of *Haemophilus influenzae* and *H. parainfluenzae* isolated from the sputum of adult patients at an urban general hospital. *S Afr Med J* 1992; 81: 495–498.
13. Phillips H, VanHook CJ, Butler T, Mark Tood W. A comparison of cefpodoxime proxetil and cefaclor in the treatment of acute exacerbation of COPD adults. *Chest* 1993; 104: 1387–1392.
14. Rosen MJ. Treatment of exacerbations of COPD. *Am Fam Phys* 1992; 45: 693–697.
15. Lentino JR, Lucks DA. Nonvalue of sputum culture in the management of lower respiratory tract infections. *J Clin Microbiol* 1987; 25: 758–762.
16. Lode H, Schaberg M, Raffenberg M, Mauch H. Diagnostic problems in lower respiratory tract infections. *J Antimicrob Chemother* 1993; 32: 29–37.
17. Chaste J, Viau F, Brun P, *et al*. Prospective evaluation of the protected specimen brush for the diagnosis of pulmonary infections in ventilated patients. *Am Rev Respir Dis* 1984; 130: 924–929.
18. Chastre J, Fagon JY, Bornet-Lecso M, *et al*. Evaluation of bronchoscopic techniques for the diagnosis of nosocomial pneumonia. *Am J Respir Crit Care Med* 1995; 152: 231–240.
19. Marquette CH, Herengt F, Mathieu D, Saulnier F, Courcol R, Ramon P. Diagnosis of pneumonia in mechanically ventilated patients. *Am Rev Respir Dis* 1993; 147: 211–214.
20. Marquette CH, Herengt F, Saulnier F, *et al*. Protected specimen brush in the assessment of ventilator-associated pneumonia. *Chest* 1993; 103: 243–247.
21. Timsit JF, Misset B, Francoual S, Goldstein FW, Vaury P, Carlet J. Is protected specimen brush a reproducible method to diagnose ICU-acquired pneumonia? *Chest* 1993; 104: 104–108.
22. Groeneveld K, Eijk PP, Van Alphen L, Jansen HM, Zanen HC. *Haemophilus influenzae* infections in patients with chronic obstructive pulmonary disease despite specific antibodies in serum and sputum. *Am Rev Respir Dis* 1990; 141: 1316–1321.
23. Groeneveld K, Van Alphen L, Eijk PP, Visschers G, Jansen HM, Zanen HC. Endogenous and exogenous reinfections by *Haemophilus influenzae* in patients with chronic obstructive pulmonary disease: the effect of

antibiotic treatment on persistence. *J Infect Dis* 1990; 161: 512–517.
24. Lomholt H, Van Alphen L, Kilian M. Antigenic variation of immunoglobulin A_1 proteases among sequential isolates of *Haemophilus influenzae* from healthy children and patients with chronic obstructive pulmonary disease. *Infect Immun* 1993; 61: 4575–4581.
25. Van Alphen L, Jansen HM, Dankert J. Virulence factors in the colonization and persistence of bacteria in the airways. *Am J Respir Crit Care Med* 1995; 151: 2094–2099.
26. Irwin RS, Erickson AD, Pratter MR, *et al*. Prediction of tracheobronchial colonization in current cigarette smokers with chronic obstructive bronchitis. *J Infect Dis* 1982; 145: 234–241.
27. Del Donno M, Pavia D, Agnew JE, Lopez-Vidriero MT, Clarke SW. Variability and reproducibility in the measurement of tracheobronchial clearance in healthy subjects and patients with different obstructive lung diseases. *Eur Respir J* 1988; 1: 613–620.
28. Vastag E, Matthys H, Köhler D, Gronbeck L, Daikeler G. Mucociliary clearance and airways obstruction in smokers, ex-smokers and normal subjects who never smoked. *Eur J Respir Dis* 1985; 66: Suppl. 139, 93–100.
29. Goodman RM, Yewrgin BM, Landa JF, Golinvaux MH, Sackner MA. Relationship of smoking history and pulmonary function tests to tracheal mucous velocity in nonsmokers, young smokers, ex-smokers, and patients with chronic bronchitis. *Am Rev Respir Dis* 1978; 117: 205–214.
30. Widdicombe J. Relationships among the composition of mucus, epithelial lining liquid, and adhesion of microorganisms. *Am J Respir Crit Care Med* 1995; 151: 2088–2093.
31. Riise GC, Larsson S, Lofdahl CG, Andeersson BA. Circulating cell adhesion molecules in bronchial lavage and serum in COPD patients with chronic bronchitis. *Eur Respir J* 1994; 7: 1673–1677.
32. Riise GC, Ahlstedt S, Larsson S, *et al*. Bronchial inflammation in chronic bronchitis assessed by measurement of cell products in bronchoalveolar lavage fluid. *Thorax* 1995; 50: 360–365.