Lower airway bacterial colonization in asymptomatic smokers and smokers with chronic bronchitis and recurrent exacerbations

I. Qvarfordt*, G. C. Riise*, B. A. Andersson† and S. Larsson*

*Department of Respiratory Medicine and Allergology, Sahlgrenska University Hospital, Göteborg and †Department of Clinical Immunology, Sahlgrenska University Hospital, Göteborg, Sweden

Bacterial colonization of the lower airways in patients with chronic bronchitis (CB) has been described mainly in patients with co-existing chronic obstructive pulmonary disease (COPD). Although smoking has been identified as a risk factor for bacterial colonization it is not known whether asymptomatic smokers (AS) can be colonized. The aim of this study was to study lower airway bacterial colonization in smokers with stable CB and recurrent exacerbations and compare with AS and healthy never-smokers (NS).

Thirty-nine smokers with CB and recurrent exacerbations (median FEV1 85% of predicted normal), 10 AS and 10 NS, underwent bronchoscopy and a two-step bronchoalveolar lavage (BAL) procedure where the first portion (20 ml, ‘pre-BAL’) was recovered separately from the rest (140 ml, ‘BAL’). The degree of oropharyngeal contamination of pre-BAL and BAL samples was evaluated by cytology. Semiquantitative bacterial cultures were performed on all samples.

Higher bacterial numbers than 10^3 colony-forming units (cfu) x ml^{-1} in BAL were found only in the two smoking groups. Using 10^3 cfu x ml^{-1} as cut-off, 6/10 (60%) in the AS- and 7/35 (20%) in the CB-group were colonized in the lower airways. In all, 29% of all smokers had bacterial colonization. Only bacteria belonging to the normal oropharyngeal flora were found. The proportion of samples with oropharyngeal contamination was significantly lower in BAL than in pre-BAL (5% vs. 21%, \( P=0.039 \)). The proportion of sterile samples was significantly higher in BAL than in pre-BAL (49% vs. 26%, \( P=0.002 \)).

Lower airway bacterial colonization was found both in asymptomatic smokers and in patients with CB. Colonization with potential respiratory pathogens is uncommon in patients with CB and recurrent exacerbations without severe airflow obstruction. The two-step BAL procedure seems to decrease oropharyngeal contamination.

Key words: bacterial colonization; chronic bronchitis; exacerbations; BAL; smoking.

Introduction

In smokers with chronic bronchitis (CB) with or without chronic airflow obstruction, recurrent exacerbations are common. Acute exacerbations in patients with severe obstructive airway disease often require hospitalization (1,2), and are responsible for substantial healthcare costs in many countries (3).

Acute exacerbations are in most cases associated with viral or bacterial infection (4,5). In approximately 50% of acute exacerbations high numbers of bacteria can be isolated from the lower airways (6,7). The bacterial species most frequently associated with these exacerbations are the upper respiratory tract commensals and potential pathogens \textit{Haemophilus influenzae} (HI), \textit{Moraxella catarrhalis} (MC) and \textit{Streptococcus pneumoniae} (SP) (7,8).

The presence and role of bacterial colonization in the lower airways in-between acute exacerbations has been a focus of research interest in recent years (7,9–12). Using invasive sampling methods and careful precautions to avoid oropharyngeal contamination, these studies have indicated colonization rates in patients with CB varying from 22% up to 80%, depending on how colonization was defined. Colonizing bacteria were found to be not solely potential pathogens (HI, MC and SP), but also bacteria belonging to the normal oropharyngeal flora (\( \alpha \)-haemolytic streptococi, \textit{Neisseria} spp., \textit{Corynebacterium} spp.).

In these studies, patients with rather advanced obstructive airway disease were investigated, and the bacteriological sampling was performed with a protected specimen brush (PSB) and limited to the large airways in all but one (10). In two of these studies (11,12), current smoking was...
identified as a risk factor for colonization in CB patients. However, asymptomatic smoking controls were not included in any of these studies and it is therefore unknown whether bacterial colonization of the lower airways is linked to the disease process or if it is a phenomenon associated with smoking as such also in the absence of CB. In addition, whether bacterial colonization extends beyond the large airways is not clear, although indications of this was recently presented (10).

We decided to study bacterial colonization of the lower airways in patients with CB and a history of recurrent exacerbations and compare with smokers without symptoms of inflammatory airways disease and healthy never-smokers. The aims were two-fold, (i) to investigate the possible association of smoking with presence of bacterial colonization and, (ii) to evaluate a two-step BAL technique for bacteriological sampling of the lower airways.

Materials and methods

DESIGN

The study was a bronchoscopic investigation of lower airway bacterial colonization in clinically well-defined subject groups. All subjects were evaluated for inclusion at a preliminary visit during which a medical examination, a test of ventilatory lung function and a pulmonary radiograph was performed. Eligible subjects underwent fibreoptic bronchoscopy 0–12 weeks later. All bronchoscopies were performed in an infection-free interval.

The study was performed at the Department of Respiratory Medicine at Sahlgrenska University Hospital, Göteborg, Sweden, and subjects were recruited from patient files and by advertisement in a daily newspaper. The study was approved by the Ethics Committee at the Göteborg University. The subjects gave their written consent after receiving both written and verbal information.

STUDY POPULATION

Three different subject groups were studied. The first was composed of healthy never-smokers (NS) with normal ventilatory lung function defined as forced expiratory volume in 1 sec (FEV1) > 80% of predicted normal based on age, sex and height (pred). Asymptomatic smokers (AS) with normal ventilatory lung function as defined above made up the second control group. All were to be current smokers, having smoked at least 10 cigarettes per day for more than 10 years without fulfilling the American Thoracic Society (ATS) criteria of chronic bronchitis (13).

The third group were to have symptoms of CB as defined by ATS (13), i.e. chronic or recurrent productive cough on most days for a minimum of 3 months per year during the past 2 years. Co-existing chronic airway obstruction defined as FEV1 < 80% pred was allowed. All were to be current smokers, having smoked at least 10 cigarettes per day for more than 10 years, and to have a history of two or more acute exacerbations during the past 12 months as defined by Boman et al. (14). The total number of exacerbations during the past 2 years was recorded.

For safety reasons all subjects were to be < 70 years old. Criteria for exclusion were: a baseline FEV1 < 40% pred, a post-bronchodilator increase in FEV1 > 15% pred, an abnormal chest radiograph, bronchial hypersecretion caused by other active pulmonary diseases such as sarcoidosis or cystic fibrosis, known immunodeficiency, α1-antitrypsin deficiency, a history of asthma, or subjects with concurrent severe diseases.

Bronchoscopy was performed at least 4 weeks after resolution of any symptoms of infectious respiratory disease or any other infection with systemic symptoms (fever, malaise etc.), after cessation of treatment with N-acetylcysteine or antibiotics and at least 8 weeks after any glucocorticosteroid treatment, whether local or systemic.

VENTILATORY LUNG FUNCTION

Ventilatory lung function (FEV1 % pred) was measured with a Vitalograph Alpha (Vitalograph Ltd, Buckingham, U.K.) in a standardized manner according to the directions of the European Respiratory Society (15).

CLINICAL CHEMISTRY AND HAEMATOLOGY

Immediately preceding bronchoscopy, 5 ml of venous blood was drawn for analysis of haemoglobin, platelet count, leucocytes (total and differential count) and C-reactive protein (CRP). The Sahlgrenska University Hospital laboratory’s instructions for handling of samples and its reference ranges for normality were used.

FIBRE-OPTIC BRONCHOSCOPY

Premedication was given as oral diazepam (5–10 mg) together with 0·5–1·0 ml morphine–scopolamine (10 mg · ml⁻¹+0·4 mg · ml⁻¹) i.m. 45 min before bronchoscopy. Local anaesthesia of the oropharynx was given as 1% tetracaine sprayed by a deVilbiss (deVilbiss Ltd., Heston, UK) aerosol device (~4 ml), followed by additional (2–3 ml) instillation of tetracaine into the larynx. The tetracaine solution was prepared without any preservatives by the hospital pharmacy and has no significant antibacterial activity (9). All bronchoscopies were performed transorally by one of two experienced bronchoscopists, and with the subject in the supine position. Olympus flexible fibreoptic bronchoscopes (Olympus Optical Co., Tokyo, Japan) of several models were used.

COLLECTION OF SAMPLES

The bronchoscope was introduced into the trachea and advanced into the right middle lobe bronchus where 1–2 ml of 1% tetracaine was given to anaesthetize the middle lobe bronchus. No suction was applied prior to sampling. The bronchoscope was wedged in a subsegmental bronchus for
the BAL procedure. To minimize the risk of oropharyngeal contamination of the BAL sample this was performed in two steps. First, 20 ml of sterile pyrogen-free phosphate-buffered saline (PBS) with a pH of 7.3 and a temperature of 37°C was instilled and immediately aspirated into a siliconized glass container. This specimen, referred to as ‘pre-BAL’, was processed separately. Secondly, without moving the bronchoscope, a further 80 + 60 ml of PBS was instilled, immediately aspirated and pooled in another siliconized glass container. This sample is referred to as ‘BAL’. The pre-BAL and BAL samples were then immediately transported to the microbiological laboratory for processing.

BACTERIAL CULTURES

Quantitative methods for bacterial cultures were used. From the pre-BAL and BAL samples 0-01 ml were plated onto each of a series of plates with horse blood agar (two plates), modified Drigalski agar for Gram-negative bacteria, enriched haematin agar, Saboraud agar for yeast, horse blood chocolate agar supplemented with X and V factors, vancomycin and bacitracin for the selective growth of Haemophilus influenzae and a two-layer blood agar containing oxolinic acid and colistin for selective growth of streptococci. One horse blood agar plate was incubated anaerobically for 48 h, the plate for selective growth of streptococci anaerobically for 24 h, then aerobically for another 24 h. The remaining plates were incubated for 48 h aerobically with or without 5% CO2 as appropriate. All plates were incubated at 37°C. Routine bacteriological methods for identification of bacteria were used (16). The detection limit for the pre-BAL and BAL cultures were 100 colony forming units (cfu) x ml^-1.

Evaluation of pre-BAL and BAL sample representativity was done in a standardized manner as described and evaluated previously by Kahn et al. (17). Briefly, the pre-BAL and BAL fluid samples were centrifuged at 3000 rpm for 10 min. One droplet (~ 50 μl) of the cell-pellet was placed on a slide and Gram-stained. The preparations were examined by light microscopy with a power of magnification of 100 and the fraction of squamous epithelial cells in relation to total number of inflammatory cells was assessed. Samples containing <1% squamous epithelial cells were considered representative of the lower airways.

STATISTICAL CONSIDERATIONS

A StatView® 4-5 (Abacus Concepts, Berkeley, CA, U.S.A.) software package was used for the statistical analysis. Since most of the data did not show a normal distribution, data are presented as median and range unless otherwise stated. For comparisons between subject groups, a Wilcoxon rank sum test or a Fisher’s exact test was performed. For comparison between pre-BAL and BAL samples a test of confidence interval (CI) for two paired proportions using the continuity correction was applied (18). All tests were two-tailed and P-values < 0.05 were accepted as significant. A Spearman rank correlation coefficient was calculated to analyse possible correlations between culture results and clinical variables.

Results

A total of 68 recruited subjects (48 CB, 10 AS and 10 NS) fulfilled all inclusion criteria at the first visit. Of these, 59 underwent bronchoscopy and completed the study. Nine subjects, all CB patients with recurrent infectious exacerbations, did not complete the study. Two were excluded after the first visit due to opacities on chest radiograph. They were followed up in the tumour investigation programme of the clinic. After initial consent, seven subjects later declined to undergo fibre-optic bronchoscopy. There were no significant differences in demographic or clinical characteristics between the nine CB dropouts and the 39 CB patients who completed the study.

The demographic and clinical data of the study population are summarized in Table 1. The asymptomatic smokers differed significantly from the CB group with regard to smoking history with, on average, a lower number of pack-years. However, current smoking habits were not significantly different. Ventilatory lung function was significantly lower in the CB group. In the CB group, 14 out of 39 (36%) subjects had mild chronic airflow obstruction (median FEV1 % pred 70; range 61–79). This subgroup of subjects with chronic obstructive pulmonary disease (COPD) did not differ significantly in any other way from the 25 non-obstructive subjects within the CB group (data not shown).

HAEMATOLOGY AND CLINICAL CHEMISTRY

There were no significant differences in haemoglobin level, eosinocyte and platelet counts between the groups. Total leucocyte (WBC) and neutrophil (PMN) counts were significantly higher in all smokers compared with never-smokers (WBC: 7.0 ± 1.6 vs. 5.1 ± 0.9 x 10^9/l, P < 0.001; PMN: 3.9 ± 1.4 vs. 2.7 ± 0.4 x 10^9/l, P < 0.01, mean ± SD) and in CB patients compared with never-smokers (WBC: 7.0 ± 1.5 vs. 5.1 ± 0.9 x 10^9/l, P < 0.001; PMN: 4.0 ± 1.3 vs. 2.7 ± 0.4 x 10^9/l, P < 0.01, mean ± SD). However, no abnormal values of clinical significance for any of the haematological parameters were observed. All but one measurement of CRP were below the detection limit, i.e. < 10 mg l^-1. The exception was one otherwise asymptomatic subject in the CB group with a CRP value of 39 mg l^-1.

BACTERIOLOGICAL ANALYSES

Methodological evaluation

Pre-BAL and BAL samples were obtained in 58 and 57 out of 59 subjects, respectively. Twelve out of 58 (21%) pre-BAL samples had >1% squamous epithelial cells on examination of Gram-stained slides. The corresponding figure for BAL samples was three out of 57 (5%). The proportion of samples with this sign of oropharyngeal
contamination was significantly lower in BAL than in pre-BAL ($P = 0.039$, 95% CI: 0.03; 0.28). The percentages of samples in pre-BAL and BAL with growth of bacteria in all subjects and divided by smoking status are shown in Fig. 1. The proportion of cultures with growth of bacteria was significantly lower in BAL than in pre-BAL samples (51% vs. 74%, $P = 0.002$). There was a greater difference in never-smokers than in smokers (40% vs. 20%) between the proportions of cultures with growth of bacteria in pre-BAL and BAL samples but this difference was not significant.

**Bacteriological results**

There were no significant differences between the study groups in the fraction of cultures with bacterial growth (positive), or in the numbers of bacteria in positive cultures either in pre-BAL or in representative BAL samples. The

---

**TABLE 1. Demographic and clinical data of the study population**

<table>
<thead>
<tr>
<th></th>
<th>Never-smokers</th>
<th>Asymptomatic smokers</th>
<th>CB with exacerbations</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>10</td>
<td>10</td>
<td>39</td>
</tr>
<tr>
<td>Age, years</td>
<td>55 (37–64)</td>
<td>44 (34–60)</td>
<td>51 (37–68)</td>
</tr>
<tr>
<td>FEV$_1$ % pred</td>
<td>103$^a$ (91–120)</td>
<td>113$^b$ (95–129)</td>
<td>85 (61–111)</td>
</tr>
<tr>
<td>Pack-years</td>
<td>0</td>
<td>25$^c$ (11–62)</td>
<td>35 (13–88)</td>
</tr>
<tr>
<td>Current smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cigarettes x day$^{-1}$</td>
<td>0</td>
<td>15 (10–30)</td>
<td>20 (10–40)</td>
</tr>
<tr>
<td>Male/female</td>
<td>5/5</td>
<td>3/7</td>
<td>9/30</td>
</tr>
<tr>
<td>Duration of CB (years)</td>
<td>0</td>
<td>0</td>
<td>10 (4–30)</td>
</tr>
<tr>
<td>No. of exacerbations in 2 years</td>
<td>0</td>
<td>0</td>
<td>6 (4–20)</td>
</tr>
</tbody>
</table>

Data are presented as median values with range in parenthesis. CB: chronic bronchitis; FEV$_1$: forced expiratory volume in 1 sec; $a$: never-smokers vs. CB group, $P < 0.001$; $b$: asymptomatic smokers vs. CB group, $P < 0.001$; $c$: asymptomatic smokers vs. CB group, $P < 0.01$.

---

**FIG. 1. Percentage of cultures with growth of bacteria in pre-BAL and BAL.** $\square$: All samples; $\blacksquare$: smokers; $\triangledown$: never-smokers; BAL: bronchoalveolar lavage.

**FIG. 2. Percentage of individuals with bacterial isolates in intervals according to number of colony-forming units (cfu $\times$ ml$^{-1}$) in, a) pre-BAL and b) BAL. $\square$: never-smokers; $\blacksquare$: asymptomatic smokers; $\blacksquare$: chronic bronchitis with recurrent exacerbations. Dotted line marks cut-off for colonization. BAL: bronchoalveolar lavage.
percentages of cultures in each study group with bacterial isolates in different intervals according to number of cfu $\times$ ml$^{-1}$ are shown in Fig. 2(a and b). In pre-BAL, potential respiratory pathogens were isolated in two subjects: $H.\ influenzae$ 10$^4$ cfu $\times$ ml$^{-1}$ in one CB subject, and $Staphylococcus aureus$ 500 cfu $\times$ ml$^{-1}$ in one AS subject. These pathogens were not isolated in the corresponding BAL samples. In all other cases bacteria belonging to the normal oropharyngeal flora ($\alpha$-haemolytic streptococci, $Neisseria$ spp. coagulase negative staphylococci, Corynebacterium spp. etc.) were isolated, with $\alpha$-streptococci dominating quantitatively. No strictly anaerobic bacteria were isolated.

Three BAL samples had more than 1% squamous epithelial cells, two from the CB group and one from the NS group. They were accordingly excluded from further analyses because of suspected contamination. No subject in the NS group had a BAL culture with $> 10^4$ cfu $\times$ ml$^{-1}$.

Significant growth, i.e. bacterial colonization, was defined using a cut-off determined by the highest number of bacteria isolated in a culture from a representative BAL sample in the NS group ($10^4$ cfu $\times$ ml$^{-1}$). Accordingly, bacterial colonization was found in six out of 10 (60%, 95% CI: 30; 90) subjects in the AS group and in seven out of 35 (20%, 95% CI: 7; 33) in the CB group. In the CB group, three out of 14 (21%) obstructive and four out of 21 (19%) non-obstructive subjects were colonized. There was no significant difference between the AS and CB groups in the numbers of bacteria in colonized subjects. However, the fraction of colonized subjects was significantly greater in the AS group ($P < 0.05$, 95% CI: 7; 73). Taking all smokers together, intrabronchial colonization was found in 13 out of 45 (29%, 95% CI: 16; 42) subjects. Colonized subjects did not differ significantly from non-colonized subjects with respect to amount of current smoking or number of pack-years (data not shown).

**ANALYSIS OF POSSIBLE FACTORS MODIFYING THE LOWER AIRWAY BACTERIAL FLORA**

There was no significant correlation between bacterial numbers in BAL samples and duration of chronic bronchitis, number of exacerbations the past 2 years, FEV$_1$, smoking history or current smoking habits. In the smoking groups colonized subjects were compared with non-colonized with respect to the above mentioned clinical variables. No significant differences between the groups were found.

**Discussion**

The main finding in this study is that not only patients with chronic bronchitis and a history of infectious exacerbations, but also asymptomatic smokers with normal ventilatory lung function can be colonized with bacteria in the lower airways. To our knowledge, no previously published studies of airway colonization have included a group of asymptomatic smokers (7,9–12,19–21), and since the AS group in our study is small, caution is needed when interpreting our results. In spite of the small group size, we feel that our results put earlier studies in perspective and indicate that future studies on airway colonization in CB and COPD should include both an AS and a NS group. Interestingly, bacterial colonization was not more frequent in heavy smokers, a connection found in a previous study (20), nor was there any correlation between colonization and number of exacerbations, or degree of airflow obstruction in the CB group.

The fraction of colonized smokers in the present study (29%), is comparable to the fraction of colonized patients with CB in earlier studies by Riise et al. (24%) (9) and two studies by Monsó et al. (25% and 22%) (7,12). However, the results differ when the colonizing bacterial species are considered. We found no respiratory pathogens, whereas in earlier studies respiratory pathogens were isolated in a few (9), or in as much as half or more of the colonized subjects (7,12). One possible explanation is that the earlier studies sampled the large proximal airways using PSB and therefore their results may not be naturally comparable with ours. Another might be the fact that we included an AS group with normal lung function, and only 14 out of 35 CB-patients with mild chronic airflow obstruction, whereas in the studies by Monsó et al. all had moderate or severe airflow obstruction.

An essential methodological problem in all studies investigating the microbiology of the lower airways is the risk to contaminate the samples with oropharyngeal flora. Due to this, cultures of expectorated sputum has been shown to be inadequate both in diagnosing lower airway infections (22,23) and in studies of lower airway colonization in patients with obstructive CB (20). Transtracheal needle-aspiration through the cricoid membrane was proven a more reliable method by Irwin et al. (20). However, this method obtains specimens from the trachea and does not reach the peripheral airways, and oropharyngeal secretions are sometimes inhaled during the procedure (19).

The broncopscopically guided PSB method for obtaining uncontaminated samples of the lower airways first discarded by Wimberly et al. (24) has been extensively used in the diagnosis of lung infections. In this method, topical anaesthesia of the larynx and proximal parts of the bronchial tree is achieved through inhalation of sterile local anaesthetic preceding the bronchoscopy, and no further local anaesthetic is then given through the bronchoscope until after completion of the bacteriological sampling. Although bronchi at the subsegmentary level can be reached with this method, it is a method mainly sampling the large conducting airways.

Fiberoptic bronchoscopy and BAL performed with sufficiently large volumes (minimum 100 ml) samples the peripheral airways and a large area of the airway mucosa (25), and is therefore an attractive method for the study of bacterial colonization in the peripheral airways. However, it has previously been shown that when the inner channel of the bronroscope is used to administer topical anaesthesia, BAL samples from healthy non-smoking subjects can be
contaminated with bacteria of oropharyngeal origin, albeit in low numbers (26). This is also the case when the Wimberly method of anaesthesia is used before PSB sampling (22,24). Occasional recovery of bacteria from the lower airways in healthy never-smoking subjects can, apart from contamination during the sampling procedure, be due to intermittent aspiration of small numbers of bacteria from the oral cavity. In the diagnosis of acute pulmonary infections this problem can be overcome by bacteria from the oral cavity. In the diagnosis of acute pulmonary infections this problem can be overcome by establishing a cut-off for significant growth, usually \( \geq 10^3 \text{cfu ml}^{-1} \) for PSB and \( \geq 10^5 \text{cfu ml}^{-1} \) for BAL (17,27), validated by comparison with other diagnostic criteria. Defining significant growth in studies of bacterial colonization is a more complex task since no clinically defined condition to compare a proposed bacteriological cut-off with exists. The PSB cut-off for significant growth in pulmonary infections \((10^3 \text{cfu} \times \text{ml}^{-1})\) was, however, shown to perform well also in one study of airway colonization (9), but no corresponding cut-off for BAL has so far been established. In other previous studies the cut-off level for colonization was arbitrarily chosen regardless of whether PSB or BAL was used (7,10–12,21). In contrast to this, we used culture results from the control group of healthy never-smokers to establish a cut-off for significant growth, i.e. colonization.

In the present study, conventional methods for local anaesthesia of the larynx and the bronchial tree were used and alternative methods to control and minimize oropharyngeal contamination and evaluate sample representativity were adopted. Discarding the first aliquot of BAL fluid to minimize the risk of contamination has been practised by other researchers (10,28), and the present study indicates the rationality of this procedure. The differences in culture results between pre-BAL and BAL samples point in this direction, especially when the NS and the smoking groups are compared (Fig. 1). Furthermore, presence of \( >1\% \) squamous epithelial cells in BAL samples accurately identified heavy oropharyngeal contamination in a methodological study by Kahn et al. (17), and this method has since been used by several researchers (28–30). In the present study, the rate of contamination according to this criterion was much higher in the pre-BAL than in the BAL samples (21% vs. 5%), which indicates that discarding the first aliquot may decrease the risk of contamination in the BAL cultures. All BAL samples with this sign of contamination were accordingly excluded from further analysis. Furthermore, we decided to let the highest number of bacteria present in a NS sample define the cut-off for colonization. By applying these strict criteria for colonization we feel convinced that the number of colonized subjects is not overestimated. It does however increase the risk that individuals colonized with low numbers of bacteria are overlooked. We are aware that the described method needs further evaluation, but nevertheless propose its use as an alternative method in studies of bacterial colonization of peripheral airways.

To conclude, our data suggest that smoking is associated with colonization of the lower airways with bacteria belonging to the normal oropharyngeal flora, even in the absence of symptomatic smoking-induced inflammatory airways disease, i.e. chronic bronchitis. In addition, our data suggest that colonization with respiratory pathogens is uncommon in patients with CB without severe airflow obstruction.

Acknowledgements

The authors would like to thank study nurse Ann-Marie Hilmersson and biotechnician Brit–Marie Essman for their excellent work in connection with this study.

The work was performed at the Dept. of Respiratory Medicine and Allergology, Sahlgrenska University Hospital, Göteborg, Sweden.

The study has received financial support from the Swedish Heart-Lung Foundation and the Göteborg Medical Society.

References


