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The intrabronchial microbial flora in chronic bronchitis patients: a target for N-acetylcysteine therapy?

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ABSTRACT: Chronic bronchitis is common among smokers, often together with recurrent infectious exacerbations. *Streptococcus pneumoniae* and *Haemophilus influenzae* are the pathogens traditionally considered most important. N-acetylcysteine (NAC) treatment has been shown to reduce the number of infectious exacerbations in patients with chronic bronchitis. The mechanism behind this is unknown. We attempted to characterize the intrabronchial bacterial flora in patients with chronic bronchitis in an infection-free interval, and to determine whether pharmacological and immunological factors effected the bacterial occurrence.

Twenty two smokers with non-obstructive chronic bronchitis, 19 smokers with chronic bronchitis and chronic obstructive pulmonary disease (COPD) and 14 healthy nonsmokers underwent bronchoscopy. To obtain uncontaminated intrabronchial samples, a protected specimen brush was used. Quantitative bacterial cultures and virus isolations were performed.

Significantly positive bacterial cultures (>1,000 colony-forming units (cfu)·ml^{-1}) were found only in the patients. *S. pneumoniae* and *H. influenzae* were found in five patients, and only in the patients without NAC treatment. The most common bacterium was α-haemolytic streptococcus. Negative cultures were more common in the healthy controls. Of the various factors examined, only NAC medication had an influence on bacterial numbers. Significantly fewer patients with NAC medication had positive cultures (3 out of 16) than in the group of patients without NAC therapy (15 out of 21).

Our results confirm that chronic bronchitis in smokers leads to increased intrabronchial bacterial colonization. We could also confirm that 1,000 cfu·ml^{-1} is an adequate cut-off level for significant bacterial growth when using the protected specimen brush. NAC medication was associated with low bacterial numbers.
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Chronic bronchitis is a common airway disease, characterized by persistent productive cough. The most important aetiological factor is tobacco smoke. Some smokers with chronic bronchitis also acquire chronic obstructive pulmonary disease (COPD), characterized by irreversible airway obstruction [1]. Smokers with chronic bronchitis have been shown to have damaged ciliated columnar epithelium, an increased number of goblet cells in their major and minor airways [2–5], and a change in the chemical composition of the mucus [6]. The functional result is impaired mucociliary clearance and productive cough.

About one third of the patients with chronic bronchitis are prone to recurrent infectious exacerbations, characterized by pronounced cough, increased sputum production and sputum purulence. Several studies have shown that oral N-acetylcysteine medication can reduce the rate of infectious exacerbations in patients with chronic bronchitis [7–11]. The mechanism for this is unknown.

Species of pathogenic bacteria commonly isolated from the sputum of patients with chronic bronchitis have been *Streptococcus pneumoniae*, non-encapsulated *Haemophilus influenzae* and, to some extent, *Moraxella catarrhalis*. These bacteria have been found both at exacerbations and during remissions [12–17]. The results of these studies must, however, be accepted with some reservations, due to methodological difficulties. Some studies are based on expectorated sputum cultures, and in others heterogeneous patient groups have been included. The intrabronchial microflora in chronic bronchitis and COPD has, therefore, not been well characterized. One study using transtracheal aspiration in patients with clinically stable chronic bronchitis and COPD, showed that 50% of the patients were colonized with bacteria belonging to the normal oropharyngeal flora. *S. pneumoniae* and *H. influenzae* were only found in low numbers and in a few patients, whilst the remaining 50% of the patients had sterile bacterial cultures [18]. Similar findings were

reported in a study using fibreoptic bronchoscopy together with a protected specimen brush (PSB) in patients with acute infectious exacerbations [19].

The present study was designed to identify the intrabronchial flora in two well-defined groups of smoking patients with chronic bronchitis during a stable phase in their disease, and to compare it to the flora of nonsmoking healthy controls. One group of patients had non-obstructive chronic bronchitis, and the other group had chronic bronchitis and COPD. We tried to determine whether airway medication and serum immunoglobulin levels could have an influence on bacterial occurrence. Comparisons were also made between patients with and without a history of recurrent infectious exacerbations. To obtain material from the large bronchi of each individual, a protected specimen brush (PSB) was used, and quantitative cultures for bacteria were performed. As viral infection has been shown to affect intrabronchial bacterial growth in chronic bronchitics [13, 20, 21], virus isolations were also performed.

Subjects and materials

Three groups of subjects were studied:

- Fourteen healthy nonsmoking volunteers with normal spirometry, had no history of pulmonary disease and no symptoms of upper or lower respiratory infection during the past four weeks.
- Twenty two current smokers with non-obstructive chronic bronchitis, characterized by productive daily cough for at least six consecutive months each year during the last 2 yrs, and with normal spirometry. Twelve had no history of recurrent exacerbations, and 10 had two or more infectious exacerbations per year during the past 2 yrs.
- Nineteen current smokers with chronic bronchitis and stable COPD (defined as a forced expiratory volume in one second (FEV_1) <80% predicted and a reversibility <15% on β_2-agonists). Eleven had no history of recurrent exacerbations, and eight had two or more infectious exacerbations per year during the past 2 yrs. An exacerbation was defined according to BOMAN *et al.* [8].

Of the fourteen healthy nonsmokers, none had any regular medication. Of the 22 non-obstructive chronic bronchitis patients, five used oral N-acetylcysteine (NAC) sporadically, eight used β_2-agonists for inhalation sporadically, two used ipratroprium bromide for inhalation sporadically, and none used theophylline preparations or steroids. Of the nineteen COPD patients, eight used oral NAC regularly (200 mg two or three times daily for more than one year), four used oral NAC at irregular intervals, eight used β_2-agonists for inhalation sporadically, and four used inhalation steroids regularly.

The duration of the bronchitis symptoms in the patient group varied from 2.5 yrs to 24 yrs, with a mean of 12.5 yrs. No patients had any antibiotic treatment or symptoms of respiratory infection during the past four weeks.

Criteria for exclusion were abnormal chest X-ray, bronchial hypersecretion caused by known factors other than cigarette smoking, a history of asthma or reversibility >10% on β_2-agonists, and treatment with antibiotics during the past four weeks. For safety reasons, patients with FEV_1 <50% predicted, or age >70 yrs, were also excluded. Ventilatory lung function (FEV_1) was measured with a Bernstein spirometer or Vitalograph (table 1).

Table 1. – Age, FEV_1 and cigarette consumption in controls and patients

	Healthy controls n=14	CB without COPD n=22	CB with COPD n=19
Age yrs	46 (26–66)	52 (36–68)	57 (38–70)
FEV_1 % pred	101±1	92±2	62±2
Smoking pack yrs	0	36±2	44±4

Data for age are presented as mean and range in parenthesis. Data for FEV_1 and cigarette consumption are presented as mean±SEM. CB: chronic bronchitis; COPD: chronic obstructive pulmonary disease; FEV_1: forced expiratory volume in one second.

The mean age and the mean maximum FEV_1 (in percentage of predicted normal) did not differ substantially between the healthy controls and the patients with non-obstructive chronic bronchitis. The patients with chronic bronchitis and COPD were significantly older than the healthy controls, and had a lower FEV_1 than the controls and the patients with non-obstructive chronic bronchitis. The smoking history of the two groups with chronic bronchitis was comparable (table 1).

The study design was approved by the Ethics Committee of Göteborg University, and the subjects gave their consent after written and oral information.

Methods

Bronchoscopy

Care was taken to follow the procedures described by WIMBERLEY and co-workers [22] to obtain uncontaminated bacterial cultures from the lower airways. Premedication was given with haloperidol, 5 mg orally, followed by 0.5–1 ml morphine-scopolamine *i.m.* Five ml of 1% tetracaine, without preservative, was nebulized with a pressurized nebulizer (Plug-in inhalator, Aiolos systems, Karlstad, Sweden) and inhaled in an upright position for local anaesthesia. All bronchoscopies were performed transorally by one of two experienced bronchoscopists, and with the patient in the supine position. Olympus flexible fibreoptic bronchoscopes of several models were used. One bronchial sample per subject was taken, and the patients were all in an infection-free interval in their disease.

Collection of samples

Without using the inner channel for suctioning of secretions, brush biopsies for bacterial and viral isolation were taken during bronchoscopy, using a telescoping double-catheter protected specimen brush (Microvasive catheter

no. 1650) [22]. Under direct inspection, the inner catheter of the PSB was advanced 3–4 cm into the lower trachea, the protective plug then dispelled, and the brush further advanced into one of the major bronchi to sample the specimen. The brush was transected with sterile scissors and placed in a screw-capped glass vial, containing 1 ml liquid transport medium [23]. The specimen was transported within 2 h to the microbiological laboratory and processed there.

Microbial analysis

The brush was vigorously shaken in the 1 ml vial with transport medium. This gives a dilution of approximately 1:100 (data not shown). From the medium, 0.1 ml was plated onto each of an aerobic and anaerobic blood agar plate, and selective media for *H. influenzae* and streptococci. The plates were incubated in 5% CO_2 at 35–37°C for 48 h. Routine bacteriological methods were used for identification of bacteria [24].

The possible interference of tetracain on the growth of *H. influenzae* and *S. pneumoniae* on solid media was tested in a control experiment. A paper disc saturated with 10 µl of tetracain (10 $g \cdot l^{-1}$) was placed on the agar surface of Petri dishes seeded with *H. influenzae* or *S. pneumoniae*. No inhibition of bacterial growth was found. This is in accordance with previous studies on lidocaine, where no significant negative effect on bacterial growth was found [25, 26].

In a control experiment, the capacity for the PSB to contain bacteria was tested using a *H. influenzae* ATCC 8468 strain and a *S. pneumoniae* CCUG 23261 strain. A brush was dipped into a suspension of approximately 10^6 colony-forming units (cfu)$\cdot ml^{-1}$ of each bacterial strain and then transferred to the transport medium and shaken. A serial dilution was made, and each dilution was plated on blood agar. After incubation overnight, the number of viable bacteria was about 10^3 cfu$\cdot ml^{-1}$ (data not shown). This gives a rough estimate of a dilution factor of about 1:1,000, which is in agreement with previous publications testing the bacterial accumulation capacity of the PSB [22].

All colony counts were expressed in terms of the 1.0 ml sample as received by the laboratory. A previously defined cut-off level delineating significant bacterial growth in the PSB sample of 1,000 cfu$\cdot ml^{-1}$ was used [27–30].

The rest of the transport medium was analysed at the virology department for virus cultivation and the presence of viral antigens from the following viruses: influenzae A and B, parainfluenzae 1–3, adenovirus and respiratory syncytial virus.

Viral analysis

Isolation and identification of virus was performed as described previously [31]. In short, samples were inoculated onto cultures of human embryonic lung fibroblasts, green monkey kidney (GMK)-AH1, A549 and Madin-Darby canine kidney (MDCK) cells. The cultures were incubated for two weeks, with weekly changes of maintenance medium.

For detection of respiratory syncytial virus (RSV) antigen, ABBOT's Testpack RSV (Abbot Diagnostics Division, north Chicago, Ill., USA) was used, according to the instructions from the manufacturer. Viral antigens from influenza A and B, parainfluenza 1–3 and adenovirus were detected in a solid phase enzyme-immunoassay as described by SARKKINEN and co-workers [32–34].

Immunoglobulin determinations

The serum levels of immunoglobulin G, M and A (IgG, IgM and IgA) as well as the IgG subclasses were assessed by radial immunodiffusion. Class specific polyclonal rabbit anti-human IgG, IgM and IgA (Dakopatts a/s, Produktionsvej 42, Glostrup, Denmark) and monoclonal antibodies specific for the four IgG subclasses (Oxoid Unipath Ltd, Wade road Basingstoke, Hampshire, UK) were used. The concentrations of immunoglobulin classes and IgG subclasses were expressed in $g \cdot l^{-1}$, by comparison with a standard.

Statistics

The number of bacteria recovered from the PSB is expressed as cfu$\cdot ml^{-1}$ of transport medium. Other results were estimated using the arithmetic mean and standard deviation (SD). For comparison of numerical data, Spearman's rank correlation test was used. Comparison of proportions of results between groups was made with Fisher's exact test. Stepwise logistic regression analysis was performed in the two patient groups for comparison of results of the quantitative bacterial cultures in relation to pharmacological and immunological factors.

Results

Bacteriological results

The pattern of intrabronchial bacterial isolates was different between the patients and the controls (fig. 1). Isolates with significant growth (>1,000 cfu$\cdot ml^{-1}$) were found in 10 out of 37 patients, whereas none of the 13 controls had significant growth ($p<0.05$) (table 2). No differences were found in the lower range (<1,000 cfu$\cdot ml^{-1}$) between the two patient groups and the controls (table 2 and fig. 1).

Negative cultures (defined as <10 cfu$\cdot ml^{-1}$ of the transport buffer) were more common in the controls (7 out of 13) than in the patients with non-obstructive chronic bronchitis (7 out of 19), or chronic bronchitis and COPD (6 out of 18), the difference was, however, not statistically significant. Cultures with known respiratory pathogens, such as *S. pneumoniae* (found in three patients

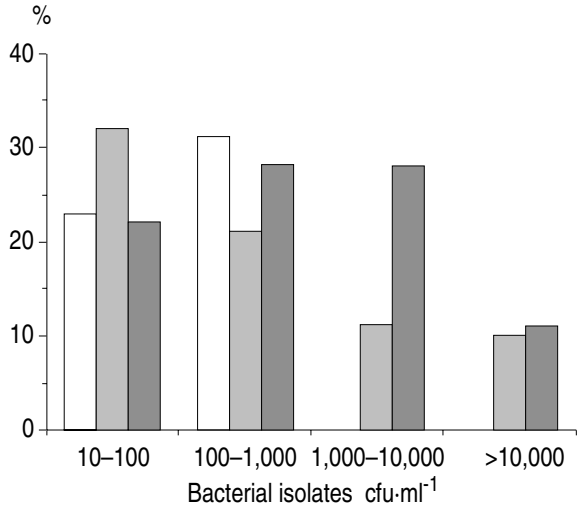

Fig. 1. – Percentage of individuals (healthy, chronic bronchitis and COPD) with bacterial isolates in intervals according to number of colony-forming units (cfu·ml^{-1}). None of the healthy subjects showed >1,000 cfu·ml^{-1}. ▭: healthy controls (n=13); ▨ : chronic bronchitis (n=19); ▬: chronic obstructive pulmonary disease (COPD) (n=18).

at 30, 200 and 50,000 cfu·ml^{-1}, respectively), and *H. influenzae* (in two patients at 250 and 30,000 cfu·ml^{-1}, respectively) were uncommon, and occurred only in the patients. *Moraxella catarrhalis* was not found in any culture. The most frequent bacterial group proved to be α-haemolytic streptococci. These were found in eight non-obstructive patients (where two had significant numbers), and in 11 obstructive patients (where five had significant numbers). Six controls had α-haemolytic streptococci, but only in low numbers.

Cultures of diphtheroid rods showed the same tendency towards higher numbers in the patient groups as did the results for α-haemolytic streptococci (table 2).

One culture from a control individual was excluded because of contamination during processing after bronchoscopy. Four patient cultures were excluded (additional topical anaesthesia had to be applied which increased the risk of contaminating the bronchial tree before cultures could be taken).

Virological results

Among the patients, all attempts at virus isolation and antigen detection were negative. One control individual was positive for respiratory syncytial virus by antigen detection only.

Quantitative serum immunoglobulin and subclass levels

Statistically significant lower mean IgG levels were found both in the patients with non-obstructive chronic bronchitis ($p<0.01$) and in the patients with chronic bronchitis and COPD ($p<0.05$) as compared to the healthy controls (table 3). Low mean levels of IgG subclasses were seen for IgG1 in the non-obstructive chronic bronchitis group ($p<0.05$), and for IgG2 in both the non-obstructive patients ($p<0.01$) and in the patients with COPD ($p<0.01$) (table 4).

Table 3. – Quantitative serum immunoglobulin levels in controls and patients

Subjects	IgG g·l^{-1}	IgA g·l^{-1}	IgM g·l^{-1}
Healthy nonsmokers	13.2 (2.1)	2.8 (1.0)	1.7 (0.5)
CB without COPD	10.5 (3.1)**	1.9 (0.9)**	1.6 (0.9)
CB with COPD	11.1 (3.2)*	2.7 (1.0)	2.1 (1.5)

Data are presented as mean and SD in parenthesis. *: $p<0.05$ between mean patient value and mean healthy value; **: $p<0.01$ between mean patient value and mean healthy value. IgG, IgA and IgM: immunoglobulin G, A and M. For further abbreviations see legend to table 1.

Table 2. – Number of bacterial isolates in cultures from the bronchi of controls and patients

Bacterial species	CB without COPD n=19	CB with COPD n=18	Healthy controls n=13
S. pneumoniae	(1)	**1** (2)	(0)
H. influenzae	**1** (2)	(0)	(0)
M. catarrhalis	(0)	(0)	(0)
α-haemolytic streptococci	**2** (8)	**5** (11)	(6)
Coagulase negative staphylococci	**1** (5)	**1** (2)	(3)
S. aureus	(0)	(0)	(1)
Diphtheroid rods	**1** (2)	**2** (7)	(0)
Other*	**1** (3)	**3** (3)	(2)
Samples with no growth**	7	6	7

Numbers in bold denote significant bacterial isolates (>1,000 cfu·ml^{-1}). Numbers in brackets denote total number of bacterial cultures, both in the significant and the non-significant range. *: *Haemophilus parainfluenzae*, *β streptococcus type B*, two strains of *Neisseria* spp.; **: <10 cfu·ml^{-1} of transport buffer. For abbreviations see legend to table 1.

Table 4. – Quantitative serum immunoglobulin G subclass levels in controls and patients

Subjects	IgG1 g·l^{-1}	IgG2 g·l^{-1}	IgG3 g·l^{-1}	IgG4 g·l^{-1}
Healthy nonsmokers	7.8 (2.6)	5.5 (2.2)	0.8 (0.5)	0.5 (0.3)
CB without COPD	6.2 (2.2)*	3.5 (0.8)**	0.6 (0.3)	0.4 (0.3)
CB with COPD	6.5 (2.9)	3.5 (1.5)**	0.8 (0.5)	0.4 (0.2)

Data are presented as mean and SD in parenthesis. *: $p<0.05$ between mean patient value and mean healthy value; **: $p<0.01$ between mean patient value and mean healthy value. For abbreviations see legends to tables 1 and 3.

No deficiencies were found for either total serum levels of IgG, IgA or IgM. However, using the IgG subclass reference ranges from the normal population material published by OXELIUS [35], we found six subjects with IgG1 deficiencies (<4.2 g·l^{-1}), all in the two patient groups. No IgG2 deficiencies (<1.2 g·l^{-1}) were found. Eight IgG3 deficiencies (<0.4 g·l^{-1}), six in the patient groups and two among the healthy controls. The differences in number of subclass deficiencies between the subject groups were not statistically significant. There were no differences in immunoglobulin levels between the two patient groups with and without recurrent exacerbations.

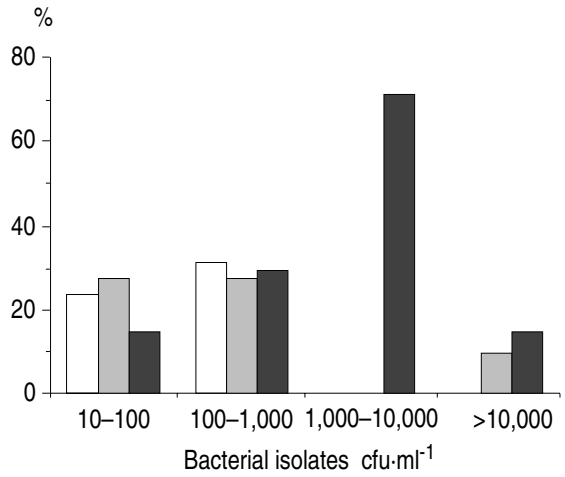

Fig. 2. – Percentage of individuals (healthy, chronic obstructive pulmonary disease (COPD) patients with and without N-acetylcysteine (NAC) treatment) with bacterial isolates in intervals according to number of colony-forming units (cfu·ml^{-1}). The patients with NAC medication had low bacterial counts. ☐: healthy controls (n=13); ▒: COPD and NAC (n=11); ▨: COPD and no NAC (n=7).

Analysis of possible factors modifying the bacterial flora in the patients

Spearman rank correlation test was used to analyse whether the age, duration of bronchitis symptoms, levels of immunoglobulins, and smoking habits of the patients had an influence on the intrabronchial bacterial flora. No significant covariation between the above mentioned factors and quantitative bacterial growth was found. Stepwise logistic regression analysis was performed to analyse results of significant growth with respect to age, duration of bronchitis symptoms, levels of immunoglobulins, smoking habits, patient group, NAC medication and other airway medication. Only NAC medication proved to be an independent explanatory factor influencing the bacteriological results. It was connected to low bacterial numbers, with the effect being most pronounced in the COPD patients ($p<0.01$). This difference between NAC treated and untreated COPD patients is illustrated in figure 2.

The patients with oral NAC medication did not differ significantly in age, smoking habits or FEV_1 from the patients without NAC (data not shown). Fisher's exact test was used to compare significant bacterial cultures in the two patient groups in relation to NAC medication. Taking the individual NAC medication into consideration when comparing the results of the total number of isolates with significant bacterial numbers in the two patient groups, 15 isolates were found in 21 patients with no NAC medication and only three in 16 patients with NAC medication ($p<0.01$) (table 5). This difference could also be shown when the numbers of individuals with one or more significant positive cultures in each group were compared (nine out of 21 compared to one out of 16, $p<0.05$).

In the patients with chronic bronchitis and COPD, one patient out of 11 using NAC had cultures with >1,000 cfu·ml^{-1}, whereas for the patients without NAC

Table 5. – Number of significant bacterial isolates (<1,000 cfu·ml^{-1}) in cultures from the bronchi of patients with chronic bronchitis

	CB without COPD		CB with COPD	
Bacterial species	No NAC n=14	With NAC n=5	No NAC n=7	With NAC n=11
S. pneumoniae	0	0	1	0
H. influenzae	1	0	0	0
α-haemolytic streptococci	2	0	4	1*
Coagulase negative staphylococci	1	0	1	0
Diphtheroid rods	1	0	1	1*
Other	1	0	2	1*

Results are presented with respect to N-acetylcysteine (NAC) medication. For abbreviations see legend to table 1. *: one patient with cultures of three bacterial species >1,000 cfu·ml^{-1}.

Table 6. – Number of significant bacterial isolates (>1,000 cfu·ml^{-1}) in cultures from the bronchi of patients with chronic bronchitis with and without COPD

	No NAC		With NAC	
Bacterial species	Exac n=9	No exac n=6	Exac n=12	No exac n=10
S. pneumoniae	1	0	0	0
H. influenzae	0	1	0	0
α-haemolytic streptococci	3	3	1*	0
Coagulase negative staphylococci	0	3	0	0
Diphtheroid rods	1	1	1*	0
Other	3	0	1*	0

Results are presented with regard to N-acetylcysteine (NAC) medication and the patient history of recurrent infectious exacerbations (Exac). *: one patient with cultures of three bacterial species >1,000 cfu·ml^{-1}.

medication five out of seven had bacterial cultures with >1,000 cfu·ml^{-1}. This difference was statistically significant ($p<0.01$).

A similar trend, although not statistically significant, was seen in the patients with non-obstructive chronic bronchitis and NAC medication, as compared to those without (table 5). Comparing the number of significant bacterial cultures from the two patient groups with and without a history of recurrent infectious exacerbations, no statistically significant differences were seen. This was also true when the effect of NAC had been taken into consideration (table 6).

Discussion

In the present study, we have adopted the model described by WIMBERLEY and co-workers [22, 27] to obtain representative, uncontaminated bacterial cultures from the lower respiratory tract. The degree of colonization was measured using quantitative bacterial cultures, and a previously defined cut-off level of 1,000 cfu·ml^{-1} indicating significant bacterial growth in the PSB sample [27–30]. In agreement with previous reports, the healthy controls were found only to have cultures <1,000 cfu·ml^{-1} (fig. 1). Cultures with >1,000 cfu·ml^{-1} were only found in the patients. Our results thus confirm that 1,000 cfu·ml^{-1} is an adequate cut-off level for significant bacterial growth when using the PSB.

We could also confirm earlier reports that chronic bronchitis in smokers leads to increased bacterial colonization. Significantly more patients than controls had bacterial findings above the cut-off level. The bacteria cultured in the present study included the known respiratory pathogens *H. influenzae* and *S. pneumoniae*, but also other members of the normal oropharyngeal flora, such as α-haemolytic streptococci and diphtheroid rods. In fact, these last bacterial species were more common. It is unlikely that the finding of oropharyngeal bacteria in significant numbers (>1,000 cfu·ml^{-1}) in the bronchi of the patients could be due to bronchoscopic contamination. Both the careful sampling technique, and the marked differences in bacterial numbers between the patients and the controls, in spite of the uniform specimen taking procedure, speaks against contamination. Furthermore, our results are similar to other reports, where large numbers of oropharyngeal bacteria have been isolated in patients with chronic bronchitis, both in infection-free intervals [14, 18], and during acute exacerbations [19, 36]. It cannot be excluded that the bronchial colonization of large numbers of oropharyngeal bacteria, including species traditionally regarded as harmless, could be of pathogenic importance in chronic bronchitis and COPD. We have found no earlier studies of viral influence in chronic bronchitis during an exacerbation-free period. We found no viruses in the present study. Viral infections have, however, been associated with acute exacerbations of chronic bronchitis [13, 20, 21].

It is not known why some patients with chronic bronchitis develop recurrent infectious exacerbations. In this study, two possibilities were studied. Firstly, that patients with a history of exacerbations in an infection-free interval have a different intrabronchial bacterial colonization pattern than do patients without exacerbations. There were no signs of this in our study. Secondly, immunoglobulin deficiency was considered as an explanation. Deficiencies in IgG and IgA, as well as some of the IgG subclasses, have been associated with chronic airway infections [37, 38]. Although we did find a few IgG1 and IgG3 deficient subjects in the study, the intrabronchial bacterial flora of these individuals did not differ from that of the rest of the subjects. We found statistically significant lower mean values of IgG, IgG1 and IgG2 in the patients with chronic bronchitis and COPD as compared to the controls. The clinical significance of this is, however, uncertain.

An interesting finding was that the ratio of significant intrabronchial bacterial growth was lower in the patients with chronic bronchitis treated with oral NAC medication than in the patients without this therapy. The difference was most pronounced for the patients with COPD and statistically significant even with our relatively small number of patients. For the 11 COPD patients with NAC medication, this was so evident that the results of their bacterial cultures was almost a reflection of those of the 13 healthy controls (fig. 2). The seven COPD patients without NAC had an intrabronchial flora similar to the non-obstructive chronic bronchitis group, where only a

minority (five out of 19) of the patients used NAC, and at irregular intervals. Positive cultures with *H. influenzae* and *S. pneumoniae* occurred only in the patients without NAC. Analysis of patient factors showed that the patients with oral NAC medication did not differ significantly in age, smoking habits or FEV_1 from the patients without NAC.

Several studies have shown that oral NAC medication can reduce the rate of infectious exacerbations and days lost from work in patients with chronic bronchitis [7–11]. The mechanism behind this is unclear. Our results suggest an effect of NAC on intrabronchial bacterial growth. We have previous results showing that NAC has a negative effect on bacterial adhesive capacity, indicating this as a possible mechanism behind its exacerbation reducing properties [39]. This has also been shown by NIEDERMAN *et al*. [40]. The anti-adhesive action of NAC could possibly reduce the number of bacteria in the oropharynx, a potential reservoir for infections in the bronchi. A direct effect on the bacteria in the bronchial mucosa is more unlikely, since NAC has not been detected in bronchoalveolar lavage fluid or plasma after oral intake [41, 42]. These results, implying NAC interference with bacterial growth, also make it more difficult to interpret data from earlier studies, where possible concurrent treatment with NAC has not been described. It seems that the natural bacterial flora in patients with chronic bronchitis should only be studied in subjects not taking NAC.

To summarize, this study shows that patients with chronic bronchitis are more commonly colonized intrabronchially than healthy controls. Our data indicate that the exacerbation-reducing effect of NAC medication could be connected to an effect on the bacterial flora. This finding needs to be further evaluated in a controlled study.

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