Airway Bacterial Load and FEV_1 Decline in Patients with Chronic Obstructive Pulmonary Disease

Tom M. A. Wilkinson, Irem S. Patel, Mark Wilks, Gavin C. Donaldson, and Jadwiga A. Wedzicha

Academic Unit of Respiratory Medicine, St. Bartholomew's and Royal London School of Medicine, St. Bartholomew's Hospital, London, United Kingdom

Chronic obstructive pulmonary disease (COPD) is characterized by an accelerated decline in lung function and progressive airway inflammation. Bacteria have been isolated from the lower airway of stable COPD patients, and airway inflammation has been related to bacterial load and type. The relationship between bacterial colonization, airway inflammation, and lung function decline remains uncertain. We studied 30 patients with COPD, mean (SD) FEV_1 0.947 (0.329), 34.8% (13.6%) predicted, for 12 months. Sputum collected at recruitment and the end of the study was analyzed for cytokines and for quantitative bacteriology. The decline in FEV_1 was 57.6 (137.6) ml $year^{-1}$. Bacterial growth was identified in all subjects, with an initial count of $10^{7.47(0.91)}$ cfu ml^{-1} rising to $10^{7.93(0.81)}$ cfu ml^{-1} at the end of the study ($p = 0.019$). FEV_1 decline was related to this increase in airway bacterial load ($r = 0.59$, $p = 0.001$). FEV_1 decline was greater in subjects who exhibited a change in the colonizing bacterial type compared with those with persistence of a single bacterial species over the study period ($p = 0.017$). Higher sputum interleukin (IL-8) was associated with greater declines in FEV_1 ($p = 0.03$). Rising airway bacterial load and species changes are associated with greater airway inflammation and accelerated decline in FEV_1. Bacterial colonization in COPD is an important factor in disease progression.

Keywords: chronic obstructive pulmonary disease; bacterial colonization; FEV_1 decline; airway inflammation

Chronic obstructive pulmonary disease (COPD) is characterized by an accelerated and progressive decline in lung function, which is not fully reversible (1–3). Smoking is the most important etiologic factor for COPD and is known to cause inflammation in the lung (4). However, smokers exhibit a variable rate of decline in lung function, suggesting that factors such as variability in smoking behavior, susceptibility to cigarette smoke, and other factors such as airway inflammation caused by bacterial colonization may contribute to the progression of COPD.

Patients with stable COPD exhibit increased airway inflammation (5, 6). The degree of airway inflammation is positively related to the severity of airway obstruction with more bronchial inflammation in patients with lower FEV_1 (4). Furthermore, higher levels of airway inflammation, as evidenced by high sputum neutrophil counts, were associated with a greater rate of decline in FEV_1 (7). The stimulus for increasing airway inflammation as lung function declines has not yet been determined.

(*Received in original form October 15, 2002; accepted in final form January 19, 2003*)

Supported by the Joint Research Board, St. Bartholomew's Hospital Special Trustees.

Correspondence and reprint requests should be addressed to Jadwiga A. Wedzicha, Academic Unit of Respiratory Medicine, Dominion House, St. Bartholomew's Hospital, London EC1A 7BE, UK. E-mail: j.a.wedzicha@qmul.ac.uk

This article has an online supplement, which is accessible from this issue's table of contents online at www.atsjournals.org

Am J Respir Crit Care Med Vol 167. pp 1090–1095, 2003
Originally Published in Press as DOI: 10.1164/rccm.200210-1179OC on January 24, 2003
Internet address: www.atsjournals.org

The lower airways of healthy individuals are sterile, but bacteria have been isolated in significant numbers in patients with clinically stable COPD, indicating the presence of lower airway bacterial colonization (LABC) (8–10). The presence of bacteria in the lower airway can result in a range of important effects on the lung, including activation of host defenses with release of inflammatory cytokines and subsequent neutrophil recruitment, mucus hypersecretion, impaired mucociliary clearance, and respiratory epithelial cell damage (11). Animal models of chronic bacterial infection in the lung have shown changes characteristic of those seen in COPD in terms of inflammatory cells, cytokine expression, and pathologic changes to both airways and alveoli (12). There is evidence that airway inflammation increases with higher airway bacterial loads determined from quantitative sputum cultures in patients with COPD (13). Thus, it has been suggested that chronic LABC contributes to progression of airways obstruction (14, 15).

Previous studies performed to evaluate the relationship between airway bacterial colonization, inflammation, and lung function have been cross-sectional in design and have not addressed the important relationship between these parameters and effects on disease progression. This study addresses the hypothesis that bacterial colonization leads to increased airway inflammation and thus contributes to the accelerated progression of airway obstruction. We have performed a prospective observational study in well-characterized patients with moderate to severe COPD to elicit the relationship between LABC using both quantitative and qualitative microbiologic techniques and the progression of airway obstruction.

METHODS

Patient Selection

Thirty patients with COPD were recruited from volunteers in the East London COPD cohort and gave informed consent. Ethics approval was obtained from the East London and City Health Authority Research Ethics committee. The inclusion criteria for this prospective cohort study have previously been published and include FEV_1 of less than 70% predicted and β_2 agonist reversibility of less than 15% of baseline and/or 200 ml. Patients were assessed clinically and with a chest radiograph at recruitment to ensure the absence of other significant respiratory disease (16). Patients completed daily diary cards, for symptoms and recorded peak expiratory flow. Exacerbations were diagnosed from the diary card data as previously described (16–18). We ensured that each patient had been clinically stable (exacerbation free) for at least 6 weeks before both recruitment and sampling at the end of the study by patient interview and review of diary cards.

Patients were followed prospectively for 1 year. Patients who suffered an exacerbation around the end of the study period were only sampled when they had been clear of exacerbation symptoms and had completed any exacerbation treatment for at least 6 weeks. The mean sampling interval (after allowance for ensuring patients were fully stable before the second sample point), therefore, was 1.11 years.

Measurement of Lung Function

Lung function was measured with a rolling seal spirometer (Sensor Medic Corp., Yorba Linda, California). Lung function measurements

TABLE 1. CHARACTERISTICS OF PATIENTS IN THE STUDY

Characteristic	Mean (SD)
Number, female/male	8/22
Age, years	66.43 (10.25)
FEV_1, L	0.95 (0.33)
FEV_1 percentage predicted	34.81 (13.61)
FVC, L	2.51 (0.70)
FEV_1/FVC percentage	38.40 (10.70)
PEF, L/min	218.07 (76.14)
Pa_{O_2}, kPa	8.55 (1.11)
Pa_{CO_2}, kPa	6.20 (1.00)
Pack-years of smoking	74.3 (66.5)
Inhaled steroid dosage	1.55 (0.92)
Beclomethasone equivalent, mg/day	

were taken between 9:30 A.M. and 11:30 A.M., 1 hour after the patient's usual bronchodilator medication inclusive of 200 μg of salbutamol via metered dose inhaler. At least three spirometry readings were taken at each visit, and the best performance was recorded.

Sputum Sampling

Sputum was sampled at the beginning and the end of the study. Immediately after lung function measurement, patients were asked to expectorate spontaneously sputum into a sterile pot. Patients unable to produce a sample of sputum spontaneously underwent sputum induction (17). Sputum samples containing less than 25 squamous epithelial cells per low-powered field and more than 25 leukocytes per high-powered field were accepted for processing. The sample was separated from saliva, and a portion was taken and analyzed for bacteriology (19); the remainder was processed using previously published methods (17, 20, 21) and analyzed for inflammatory cytokines (17, 21). Sputum interleukin (IL)-6 and IL-8 levels were measured using ELISA (R&D Systems, Abingdon, UK) (17). Twenty of the baseline samples have been used for an analysis of the relationship between LABC and exacerbation frequency (22).

Quantitative Bacterial Analysis

Samples were processed by using sputolysin. Serial dilutions were made and cultured on appropriate media. These were incubated for 18 hours at 37°C in an atmosphere of air +5% CO_2. After incubation, bacterial colonies were enumerated and subcultured for identification by standard methods (19, 22). The number of colony forming units per gram of sputum was calculated from the total number of colonies obtained and the dilution to give the total bacterial count for each sample expressed in cfu ml^{-1}.

Statistical Analysis

Normally distributed data are reported by means (SDs) and skewed data by medians (interquartile range [IQR]). Correlations were assessed using the Pearson or Spearman correlation coefficient (two tailed). Continuous variables with normal distributions were compared by *t*-test, whereas those with non-normal distributions were compared by the Mann-Whitney U or Wilcoxon signed ranks test.

During the analysis, patients were divided into groups dependent on exacerbation frequency during the study. Patients with an exacerbation frequency that was higher or lower than the median were termed "frequent" or "infrequent" exacerbators, respectively (16); p values of 0.05 or less were regarded as significant. The SPSS version 10.0 (SPSS Chicago, IL) statistical package was used for data analysis. An extended version of the methods is available in an online supplement.

RESULTS

Patient Characteristics

The baseline physiologic characteristics of the 30 patients who were recruited for the study are summarized in Table 1. The 30 patients were followed for a median (IQR) period of 1.05 (1–1.22) years to ensure all sampling data were collected in the stable state. The mean (SD) FEV_1 was 0.947 (0.329) L, and the predicted FEV_1 was 34.8% (13.6%), with a range from 13.80 to 69.95% predicted. Therefore, all patients can be classified as suffering from moderate to severe COPD according to Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria (3). Eleven of the patients were current smokers at the time of recruitment and did not alter their smoking habits during the study. Twenty eight of the patients were receiving inhaled steroids mean (SD) dosage 1.55 mg (0.92) beclomethasone equivalents; no changes to the dose of inhaled steroid occurred during the study. At the first sample point, 24 patients produced sputum spontaneously, the remainder being induced, compared with 22 patients expectorating spontaneously at the second sample point.

FEV_1 Decline

The mean (SD) FEV_1 at recruitment was 0.947 (0.329) L and declined to 0.883 (0.367) L at the end of the 1.05 (1–1.22)-year sample interval. The mean annual rate of decline was 57.6 (137.6) ml per year; expressed as percentage of initial FEV_1, this equates to 6.08% of baseline FEV_1 decline per year.

The 30 patients had a total of 86 exacerbations during the study period, 40 (46.5%) of which were reported to the study team; the remainder of the exacerbations were diagnosed from diary card review, a proportion of which (17.4%) had been independently reported to a general practitioner. Fifty-two exacerbations received antibiotic treatment during the study. The median (IQR) exacerbation frequency in this study was 2.39 (1.95) exacerbations per year. Patients with an exacerbation frequency higher than this median (frequent exacerbators) had more severe airways obstruction with a mean FEV_1 of 0.86 L compared with infrequent exacerbators with a mean FEV_1 of 1.07 ($p = 0.05$).

Quantitative Bacteriology

All cultures of sputum samples grew significant numbers of bacteria ranging from $10^{5.4}$ to $10^{9.6}$ cfu ml^{-1}. The mean (SD) total bacterial count at sample 1 was $10^{7.47(0.91)}$ cfu ml^{-1} and rising to $10^{7.93(0.81)}$ in sample 2 ($p = 0.019$) or a rise from 29,512,092 cfu ml^{-1} to 85,113,804 cfu ml^{-1} when expressed without log transformation.

Bacterial Load and FEV_1 Decline

Patients with an increasing airway bacterial load demonstrated a more severe decline in FEV_1 over the study period compared with patients with stable or decreasing airway bacterial load who exhibited less marked declines or slight improvements in FEV_1. This relationship between FEV_1 decline and changes in bacterial load (Figure 1) was statistically significant in terms of absolute FEV_1 decline ($r = 0.593$, $p = 0.001$) and decline expressed as a percentage of baseline FEV_1 ($r = 0.633$, $p < 0.001$). The total bacterial count of the second sample was itself related to the absolute rate of decline over the study ($r = 0.560$, $p = 0.001$) (Figure 2). The total bacterial count of the first sample was not predictive of the subsequent decline in FEV_1 over the study ($r = -0.125$, $p = 0.369$).

A linear regression analysis of the relationship between bacterial load and FEV_1 decline revealed that a 10-fold increase (10^1 cfu ml^{-1}) in bacterial load is associated with an 82.4-ml decline in FEV_1 over the study period; the regression coefficient was 0.095 (95% confidence interval, 0.032–0.132) ($p = 0.002$). As the mean increase in bacterial load was from $10^{7.47}$ to $10^{7.93}$ cfu ml^{-1}, this represents a decline in FEV_1 attributable to the airway bacterial load of 33.3 ml/year for this patient group. A multivariate regression analysis of potential factors in the observed decline in lung function (L/year) was performed: regression coefficient (95% confidence interval), change in bacterial load (log cfu ml^{-1}) 0.0631 (0.034–0.133, $p = 0.003$), number of cigarettes

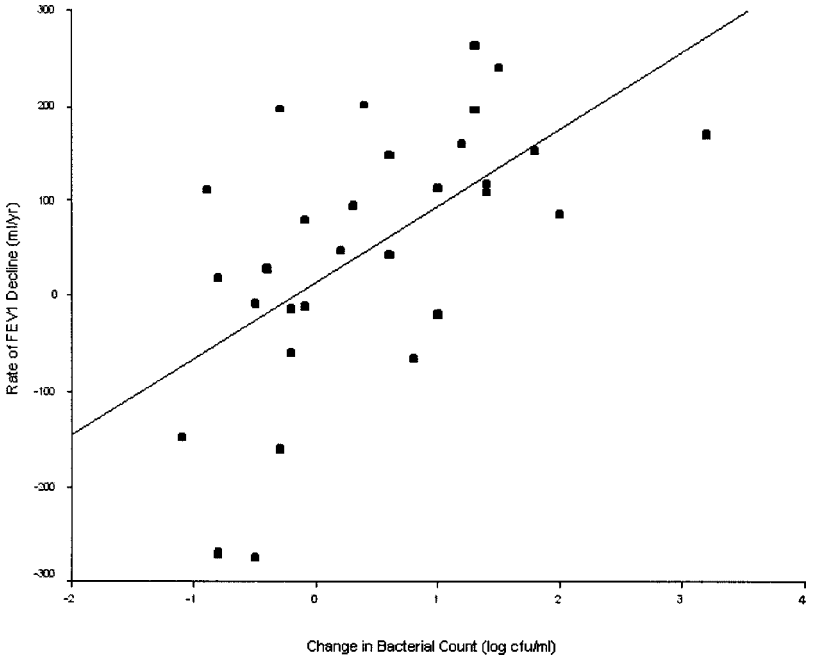

Figure 1. Correlation between change in FEV_1 and change in total bacterial count over study period. Figure shows decline in FEV_1 expressed as milliliters of loss (*negative values* indicate improving lung function) against log change in total bacterial count (*positive values* indicate increasing numbers of bacteria over study) ($r = 0.59$, $p = 0.001$).

smoked per day −0.011 (−0.11–0.89, p = 0.818), exacerbation frequency −0.002 (−0.27–0.022, p = 0.819), and baseline FEV_1 0.001 (−0.002–0.005, p = 0.368). The change in bacterial load and the decline in FEV_1 were the strongest and the only significant relationship in this analysis.

Bacterial Isolates

The results of the qualitative bacteriology from sputum samples taken at the beginning (sample 1) and the end (sample 2) of the study are shown in Figure 3. The graph illustrates the relative frequency of each bacterial isolate expressed as a percentage of the 30 samples at each time point. Sixteen (53.0%) and 17 (56.6%) patients were colonized with a potentially pathogenic organism at recruitment and completion of the study, respectively. The remainder of patients' sputum produced nonspecific growth of bacteria, defined as growth of bacterial species not usually associated as respiratory pathogens in immunocompetent individuals such as *Streptococcus viridans* group, *Neisseria* spp, *Corynebacterium* spp, and coagulase negative staphylococci. The relative frequencies of individual bacterial species are as shown. Five and six subjects respectively displayed colonisation with more than one potentially pathogenic microorganisms at recruitment and completion. At each time point, *Haemophilus influenzae* was the most prevalent individual bacterial type present in 9 (30%) and 7 (23.3%) at samples 1 and 2, respectively.

The nature of bacterial colonization was dynamic with changes

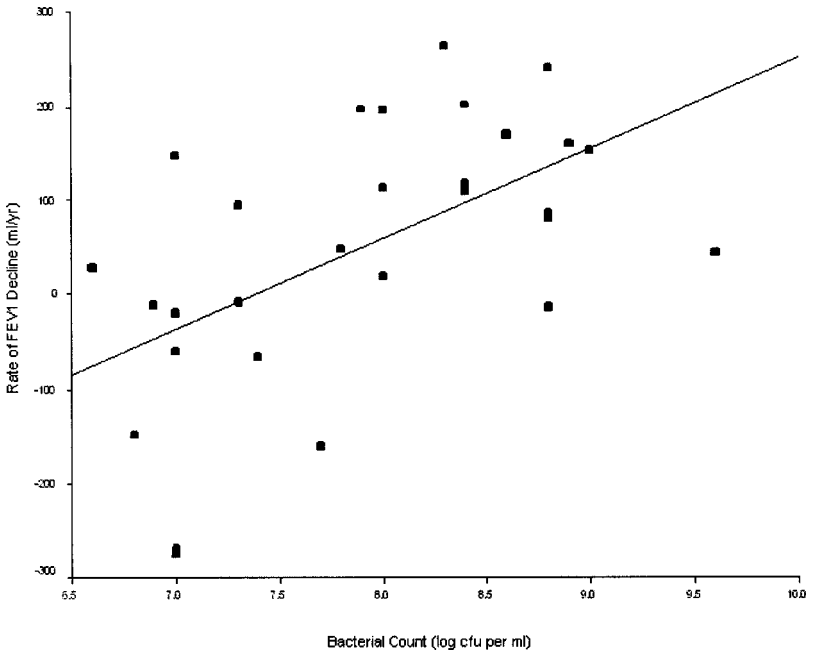

Figure 2. Correlation between rate of change in FEV_1 over study period (adjusted to ml/year) and total bacterial count at the end of the study. Figure shows decline in FEV_1 expressed as milliliters of loss (negative values indicate improving lung function) against log change in total bacterial count from sputum taken at the end of the study ($r = 0.56$, $p = 0.001$).

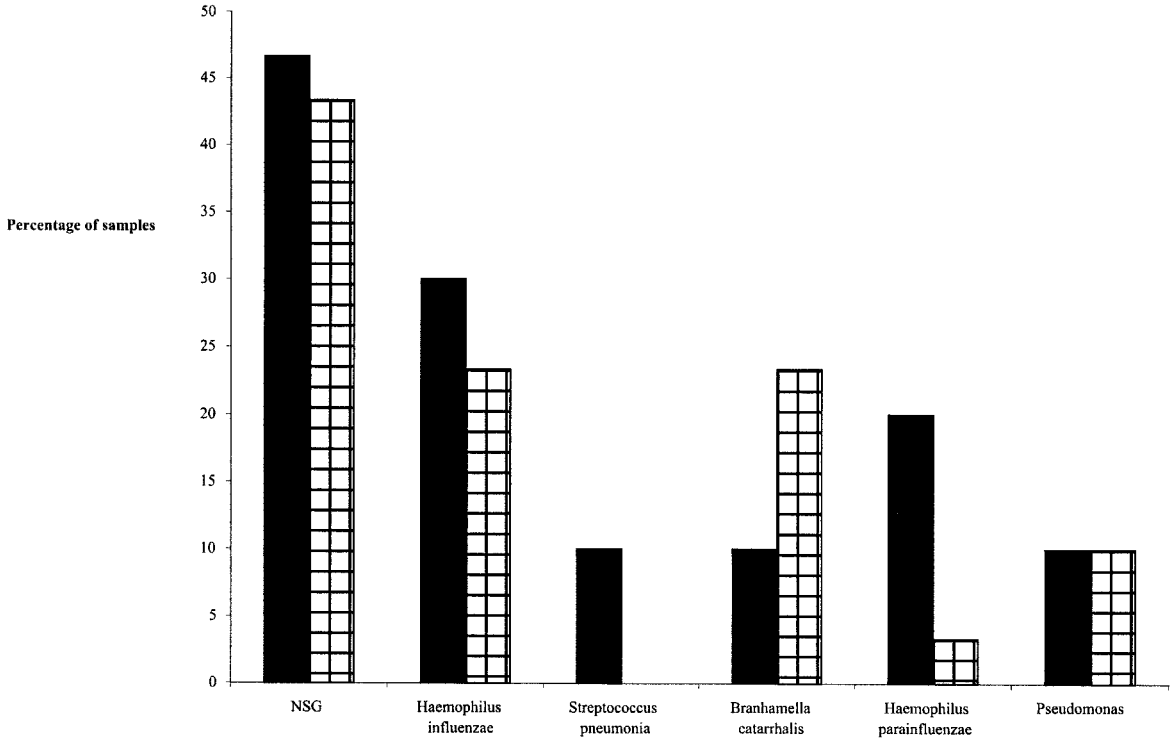

Figure 3. Relative frequency of bacterial isolates from first (*black bars;* n = 30) and second (*hatched bars;* n = 30) sputum sample expressed as a percentage of samples. NSG = nonspecific growth.

in the species of bacterial isolate over the sample interval. Fifty percent of the subjects grew entirely different bacterial species at each sample point, whereas the other 50% demonstrated persistence of a specific bacterial species or nonspecific growth across the sampling interval. Patients who demonstrated changes in colonizing bacterial species during the study exhibited higher mean bacterial loads ($10^{8.18}$ log cfu ml^{-1}) than those who maintained the same species in both samples ($10^{7.55}$ log cfu ml^{-1}, p = 0.03).

The decline in FEV_1 seen in the study group was significantly greater in those subjects with unstable bacterial type at a 102-ml (IQR 19–196, n = 18) decline in FEV_1 per year compared with a 3.6-ml (IQR −158–112, n = 12) decline per year in the group with persistence of one bacterial type at both time points (p = 0.017); this relationship with FEV_1 decline expressed as a percentage of baseline is illustrated in Figure 4. Changes in bacterial

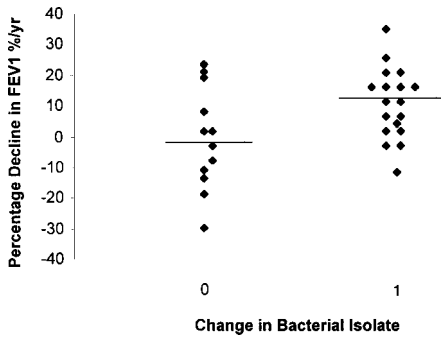

Figure 4. Differences in FEV_1 decline between subjects with persistent (0) and changing (1) bacterial isolate. 0 = Same bacterial species identified in sputum at beginning and end of study; 1 = Change in species of bacterial isolate; p = 0.017. Decline expressed as percentage of baseline FEV_1 (negative values indicate improving lung function).

species were not related to the number of cigarettes smoked per day (rho = 0.056, p = 0.767), the sputum collection method (induced or spontaneous) (rho = 0.012, p = 0.84), the recorded exacerbation frequency (rho= 0.149, p = 0.44), or the antibiotic usage during the study (rho = 0.048, p = 0.808). An analysis of each of the individual bacterial species and the associated lung function changes did not reveal any attributable significant differences in FEV_1 decline between subjects colonized with different bacterial species, although numbers in each subgroup were too small to draw any valid conclusions from this analysis.

Sputum Cytokines

Sputum IL-6 and IL-8 levels were measured on all samples. The median (IQR) IL-6 levels were similar 114 (283) pg ml^{-1} and 51 (297) pg ml^{-1} in samples 1 and 2, respectively, and IL-8 levels were 3,183 (1,688) pg ml^{-1} and 3,012 (1,684) pg ml^{-1}. Levels of sputum IL-6 and IL-8 were related to one another in each patient (rho = 0.378, p = 0.007). The absolute changes in IL-6 between samples 1 and 2 correlated with the changes in IL-8 seen between the two samples (rho = 0.542, p = 0.011). The sputum IL-8 levels were related to pack-years of smoking; those patients with IL-8 higher than the median having smoked for a mean of 100 pack-years and those with lower IL-8 for 42 pack years (p = 0.018). Patients exhibiting a decline in lung function exhibited higher overall sputum median (IQR) IL-8 levels 3,343 (1,592) pg ml^{-1} compared with those with stable or improving FEV_1 2,160 (2,050) pg ml^{-1} (p = 0.032). Similarly, patients with a higher bacterial load (greater than the overall mean, sample 1 and 2 combined) had higher overall IL-8 levels (2,938 pg ml^{-1}) compared with those with bacterial counts lower than the mean (2,329 pg ml^{-1}) (p = 0.05). There were no significant relationships between sputum IL-6 levels and bacterial counts or lung function decline.

DISCUSSION

This is the first longitudinal prospective study to assess directly the relationship between lower airway bacterial load and decline in lung function in patients with moderate to severe COPD. We have demonstrated a significant relationship between the sputum bacterial load and disease progression in COPD, showing that the rate of decline of FEV_1 was proportional to the rise in colonizing bacterial load over the 1-year study. Individuals who exhibited changes in the nature of bacterial colonization suffered from faster declines in lung function than those with persistence of one or more bacterial species. Quantitative estimations showed that subjects with higher or rising bacterial loads similarly demonstrated greater declines in FEV_1 compared with those with lower or decreasing airway bacterial loads. These falls in FEV_1 were also associated with elevated levels of the potent neutrophil chemo attractant IL-8.

The lower airways of healthy nonsmoking individuals are sterile, although a number of studies have identified bacteria in the lower airways of patients with stable COPD (8–10). The prevalence of LABC is increased by active smoking and with progressive airways obstruction (9, 10), and as our patient group had more severe COPD, this explains the high prevalence of LABC found. Bacterial colonisation is proinflammatory and can result in a range of pathologic effects that are deleterious to lung function, including mucus hypersecretion and embarrassment of mucociliary clearance (23, 24). Bacteria can affect the airway epithelium directly and via the recruitment of neutrophils (25, 26) with release of excessive amounts of neutrophil derived proteases resulting in damage to airway epithelial cells (14). Airway inflammation has been shown to increase as airway obstruction worsens, but bacterial colonization was not originally considered as an explanation for this finding (4). Bacterial colonization has been shown to be detrimental to lung function in a number of pathological conditions, including cystic fibrosis and bronchiectasis (27, 28). Evidence that LABC contributes to worsening lung function comes from a study that found that *H. influenzae* colonization was associated with increased airway inflammation in patients with chronic bronchitis and airflow obstruction compared with patients with chronic bronchitis but without airflow obstruction, where airway inflammation was reduced (29). We have again confirmed that higher airway bacterial load is associated with greater airway inflammation in terms of sputum IL-8 levels. Furthermore, patients exhibiting a decline in FEV_1 during the study had higher levels of IL-8 than those with a stable or improving FEV_1. This finding suggests a mechanistic link between airway bacterial load, airway inflammation, and the associated deleterious effects on FEV_1.

We have previously reported that some patients with COPD develop frequent exacerbations, and this patient group has increased stable airway inflammatory cytokines (IL-6 and IL-8) compared with those with a history of infrequent exacerbations (30). Patients with frequent exacerbations also demonstrate a faster decline in FEV_1 than infrequent exacerbators, with exacerbations contributing to approximately 25% of the observed lung function decline in COPD (31). Analysis of data from the Lung Health Study (32) revealed that lower respiratory illnesses in smokers are deleterious to FEV_1 and lends further support to the hypothesis that lower airway infection and associated inflammation contribute to lung function decline.

Previous longitudinal studies of the mechanisms of lung function decline have used possible surrogate markers of airway infection such as mucus hypersecretion. The Copenhagen City Heart Study found that chronic mucus hypersecretion was associated with an excess FEV_1 decline of 22.8 ml/year in men and 12.6 ml/year in women together with increased risk of hospitalization (33). As bacterial colonization is associated with mucous secretion (34), and patients with mucous secretion have more airway inflammation (7); this again provides support for the role of LABC in the accelerated decline of FEV_1. We have found that the presence of bacterial colonization is directly related to exacerbation frequency, and patients with colonization have longer and thus more severe exacerbations (22). As patients with a past history of frequent exacerbations have increased airway inflammation (30), the nature of stable bacterial colonization may be an important factor in disease progression due to the effect of exacerbations.

In this study, the relationships between FEV_1 decline and features of LABC were strongest in the analyses, which included measures of "instability" of LABC such as changes in bacterial load and type. It is possible that such changes generate a renewed stimulus to inflammation in the airway, in turn causing a more rapid decline in lung function. Indeed, there is increasing evidence that bacterial colonization is a highly dynamic process and that changes in bacterial type are associated with the etiology of exacerbations (35). Bacterial colonization itself is likely to be affected by exacerbations and their treatment, but to what degree this is remains uncertain. The interrelationships between host defenses and bacterial infection in the stable state and at exacerbation are highly complex. We postulate that bacterial colonization may accelerate FEV_1 decline by both increasing airway inflammation in the clinically stable state and by affecting the FEV_1 decline due to more frequent and severe exacerbations. This study suggests a significant effect of bacterial colonization on disease progression in COPD.

The multivariate regression analysis of the data from this study did not find a significant influence of active cigarette smoking on FEV_1 decline over 1 year. Indeed, although it is established that cigarette smoking is a risk factor for bacterial colonization and itself leads to increased airway inflammation, the effects of smoking cessation on airway inflammation in severe COPD may not be as clearly defined as in a milder patient group. In this colonized COPD population with severe airways disease, lower airway inflammation may persist despite smoking cessation (36). Thus, the most significant influence on airway inflammation in this study group and consequent FEV_1 decline may be the airway bacterial load.

Many studies of lung function decline have largely been performed using patients diagnosed with bronchitis or airflow obstruction (1, 33). Therefore, understanding of the natural history of lung function decline in more severe COPD is based largely on an extrapolation of observations from patients with milder COPD. Quantitative assessment of airway bacterial load and the related sputum markers of inflammation suggests a threshold level of colonisation in the order of 10^5–10^6 cfu ml^{-1} above which LABC is a persisting drive to airway inflammation (13). However, the degree of airways obstruction at which the clinically significant effects of LABC occur and how bacterial colonization affects the natural history of COPD in the longer term remains uncertain and requires further study.

It is possible that the findings of changes in total bacterial count and FEV_1 may represent changes associated with unrecorded exacerbations. However, diary cards were used to record all changes in symptoms on a daily basis and could therefore be used to detect all exacerbations both reported and unreported as previously described (16). Furthermore, direct questioning of patients at each study visit was used to clarify symptomology and use of rescue medication during the previous 3 months. Therefore, exacerbations both reported to the study team or primary care requiring extra medication and those unreported but recorded on diary cards were included in assessment of baseline status. There was no identifiable relationship between

the changes in FEV_1 observed and the frequency or timing of exacerbations in this study. However, this study was not powered to investigate the relationship between exacerbation numbers and lung function decline demonstrated previously by our group (16). Assessment of diary cards for the 6-week symptom-free period effectively assured that patients had returned to baseline before sampling.

This study has shown that LABC is an important determinant of decline of lung function in this group of COPD patients with moderate to severe disease. These findings suggest that appropriate antimicrobial therapy in colonized patients may have an important therapeutic effect, offering an opportunity to alter the natural history of this highly prevalent disease. Studies performed over a longer period are required to investigate further the interactions between LABC, smoking, and exacerbations and their effect on the accelerated decline in lung function, which is characteristic of COPD.

Acknowledgment: The authors acknowledge the contribution of Angela Whiley for her expert assistance in the bacteriologic analysis.

References

1. Fletcher C, Peto R. The natural history of chronic airflow obstruction. *BMJ* 1977;1:1645–1648.
2. Statement ATS. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1995; 152:S78–S119.
3. Pauwels RA, Buist AS, Calverley PM, Jenkins CR, Hurd SS. GOLD: the global strategy for the diagnosis, management and prevention of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2001; 163:1256–1276.
4. Di Stefano A, Capelli A, Lusuardi M, Balbo P, Vecchio C, Maestrelli P, Mapp CE, Fabbri LM, Donner CF, Saetta M. Severity of airflow limitation is associated with severity of airway inflammation in smokers. *Am J Respir Crit Care Med* 1998;158:1277–1285.
5. Riise GC, Ahlstedt S, Larsson S, Enander I, Jones I, Larsson P, Andersson B. Bronchial inflammation in chronic bronchitis assessed by measurement of cell products in bronchial lavage fluid. *Thorax* 1995;50: 360–365.
6. Keatings VM, Collins PD, Scott DM, Barnes PJ. Differences in interleukin-8 and tumor necrosis factor-alpha in induced sputum from patients with chronic obstructive pulmonary disease or asthma. *Am J Respir Crit Care Med* 1996;153:530–534.
7. Stanescu D, Sanna A, Veriter C, Kostianev S, Calcagni PG, Fabbri LM, Maestrelli P. Airways obstruction, chronic expectoration and rapid decline of FEV_1 in smokers are associated with increased levels of sputum neutrophils. *Thorax* 1996;51:267–271.
8. Monso E, Ruiz J, Rosell A, Manterola J, Fiz J, Morera J, Ausina V. Bacterial infection in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1995;152:1316–1320.
9. Zalacain R, Sobradillo V, Amilibia J, Barron J, Achotegui V, Pijoan JI, Llorente JL. Predisposing factors to bacterial colonization in chronic obstructive pulmonary disease. *Eur Respir J* 1999;13:343–348.
10. Monso E, Rosell A, Bonet G, Manterola J, Cardona PJ, Ruiz J, Morera J. Risk factors for lower airway bacterial colonization in chronic bronchitis. *Eur Respir J* 1999;13:338–342.
11. Murphy TF, Sethi S. Bacterial Infection in chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1992;146:1067–1083.
12. Vernooy J, Dentener MA, van Suylen RJ, Buurman WA, Wouters EM. Long-term intratracheal lipopolysaccharide exposure in mice results in chronic lung inflammation and persistent pathology. *Am J Respir Cell Mol Biol* 2002;26:152–159.
13. Hill A, Campbell EJ, Hill SL, Bayley LDL, Stockley RA. Association between bacterial load and markers of airway inflammation in patients with stable chronic bronchitis. *Am J Med* 2000;109:288–295.
14. Wilson R. The pathogenesis and management of bronchial infections: the vicious circle of respiratory decline. *Rev Contemp Pharmacother* 1992;3:103–112.
15. Wedzicha J. Airway infection accelerates decline of lung function in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2001;164:1757–1758.
16. Seemungal TAR, Donaldson GC, Paul EA, Bestall JC, Jeffries DJ, Wedzicha JA. Effect of exacerbation on quality of life in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1998;157:1418–1422.
17. Bhowmik A, Seemungal TAR, Sapsford RJ, Devalia JL, Wedzicha JA. Comparison of spontaneous and induced sputum for investigation of airway inflammation in chronic obstructive pulmonary disease. *Thorax* 1998;53:953–956.
18. Anthonisen NR, Manfreda J, Warren CPW, Hershfield ES, Harding GKM, Nelson NA. Antibiotic therapy in exacerbations of chronic obstructive pulmonary disease. *Ann Intern Med* 1994;106:196–204.
19. Barrow GI. Feltham RK. Cowan and Steel's manual for the identification of medical bacteria, 3rd ed. Cambridge, UK: Cambridge University Press; 1993.
20. Popov T, Gottschalk R, Kolendowics R, Dolovich J, Powers P, Hargreave F. The evaluation of a cell dispersion method of sputum examination. *Clin Exp Allergy* 1994;24:778–783.
21. Pizzichini E, Pizzichini MM, Efthimiadis A, Hargreave FE, Dolovich J. Measurement of inflammatory indices in induced sputum: effects of selection of sputum to minimize salivary contamination. *Eur Respir J* 1996;9:1174–1180.
22. Patel IS, Seemungal TAR, Wilks M, Lloyd-Owen SJ, Donaldson GC, Wedzicha JA. Relationship between bacterial colonisation and the frequency character and severity of COPD exacerbations. *Thorax* 2002; 57:759–764.
23. Sethi S, Murphy TF. Bacterial infection in chronic obstructive pulmonary disease in 2000: a state of the art review. *Clin Microbiol Rev* 2001; 14:336–363.
24. Adler KB, Hendley DD, Davis GS. Bacteria associated with obstructive pulmonary disease elaborate extracellular products that stimulate mucin secretion by explants of guinea pig airways. *Am J Pathol* 1986; 125:501–514.
25. Ras G, Wilson R, Todd H, Taylor G, Cole P. Effect of bacterial products on neutrophil migration in vitro. *Thorax* 1990;45:276–280.
26. Noguera A, Batles S, Miralles C, Iglesias J, Busquets X, MacNee W, Agusti AG. Enhanced neutrophil response in chronic obstructive pulmonary disease. *Thorax* 2001;56:432–437.
27. Packe GE, Hodson ME. Changes in spirometry during consecutive admissions for infective pulmonary exacerbations in adolescent and adult cystic fibrosis. *Respir Med* 1992;86:45–48.
28. Angrill J, Agusti C, de Celis R, Rano A, Gonzalez J, Sole T, Xaubet A, Rodriguez-Roisin R, Torres A. Bacterial colonisation in patients with bronchiectasis: microbiological pattern and risk factors. *Thorax* 2002; 57:15–19.
29. Bresser P, Out TA, van Alphen L, Jansen HM, Lutter R. Airway inflammation in non obstructive and obstructive chronic bronchitis with chronic Haemophilus influenzae airway infection. *Am J Respir Crit Care Med* 2000;162:947–952.
30. Bhowmik A, Seemungal TAR, Sapsford RJ, Wedzicha JA. Relation of sputum inflammatory markers to symptoms and physiological changes at COPD exacerbations. *Thorax* 2000;55:114–120.
31. Donaldson GC, Seemungal TA, Bhowmik A. Wedzicha JA. The relationship between exacerbation frequency and lung function decline in chronic obstructive pulmonary disease. *Thorax* 2002;57:847–852.
32. Kanner RE, Anthonisen NR, Connett JE. Lower respiratory illnesses promote FEV_1 decline in current smokers but not ex-smokers with mild chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2001;164:358–364.
33. Vestbo J, Prescott E, Lange P. Association between chronic mucus hypersecretion with FEV_1 decline and chronic obstructive pulmonary disease morbidity. *Am J Respir Crit Care Med* 1996;153:1530–1535.
34. Stockley RA, O'Brien C, Pye A, Hill SL. Relationship of sputum colour to nature and outpatient management of acute exacerbations of COPD. *Chest* 2000;117:1638–1645.
35. Sethi S, Evans N, Brydon JB, Murphy TF. New strains of bacteria and exacerbations of chronic obstructive pulmonary disease. *N Engl J Med* 2002;347:465–471.
36. Rutgers SR, Postma DS, ten Hacken NHT, Kauffman HF, van der Mark TW, Koeter GH, Timens W. Ongoing airway inflammation in patients with COPD who do not currently smoke. *Thorax* 2000;55:12–18.