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BRONCHIECTASIS, EXACERBATION INDICES AND INFLAMMATION IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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ABSTRACT

Relationships between high-resolution computerised tomography (HRCT) findings in chronic obstructive pulmonary disease (COPD), and bacterial colonisation, airway inflammation or exacerbation indices are unknown. 54 COPD patients (mean (standard deviation) age 69 (7) years, forced expiratory volume in one second (FEV_1) 0.96 (0.33) litres, FEV_1% predicted 38.1 (13.9) %, FEV_1/forced vital capacity 40.9 (11.8) %, arterial partial pressure of oxygen 8.77 (1.11) kilopascals, 50.5 (33.5) smoking pack years) underwent high-resolution CT scans of the chest to quantify the presence and extent of bronchiectasis or emphysema. Exacerbation indices were determined from diary cards over 2 years. Quantitative sputum bacteriology and cytokine measurements were performed. 27/54 (50%) patients had bronchiectasis on HRCT, most frequently in the lower lobes (18/54, 33.3%). Patients with bronchiectasis had higher levels of airway inflammatory cytokines (p= 0.001). Lower lobe bronchiectasis was associated with lower airway bacterial colonisation (p = 0.004), higher sputum interleukin (IL) -8 levels (p = 0.001) and longer symptom recovery time at exacerbation (p = 0.001). No relationship was seen between exacerbation frequency and HRCT changes. Evidence of moderate lower lobe bronchiectasis on HRCT is common in COPD and is associated with more severe COPD exacerbations, lower airway bacterial colonisation and increased sputum inflammatory markers.

Word count: 200

Key Words: CT scanning, bronchiectasis, COPD, exacerbation, inflammation

INTRODUCTION

Patients with chronic obstructive pulmonary disease (COPD) are prone to exacerbations, which account for significant morbidity and mortality and are a key determinant of health-related quality of life [1]. There is considerable heterogeneity in the character, frequency and time course of COPD exacerbations, which cannot be accounted for solely on the basis of degrees of airways obstruction or disease severity. Mechanisms governing the natural history of COPD exacerbations remain poorly understood.

Lower airway bacterial colonisation (LABC) is a common clinical finding in COPD [2] and is increasingly recognised as an independent stimulus to airway inflammation [3]. We have recently shown that LABC can modulate the character and frequency of COPD exacerbations [4]. In addition, we have demonstrated that patients with frequent exacerbations have higher levels of induced sputum interleukin-6 (IL-6) and interleukin-8 (IL-8) in the stable state compared to those with infrequent exacerbations [5], suggesting the presence of heightened airway inflammation in this patient group. Symptoms of daily cough and sputum production have been shown to be factors predictive of frequent COPD exacerbations [1] and a recent study reported that 29% of patients with COPD who developed an exacerbation in primary care were found have some bronchiectatic changes when evaluated by CT scanning [6]. The implications of this finding are unknown, and the role of undiagnosed bronchiectasis in influencing relationships between exacerbation frequency, lower airway bacterial colonisation and airway inflammation in COPD has not been examined.

This study was designed to evaluate the prevalence and extent of bronchiectasis and emphysema on high resolution CT scanning (HRCT) in a well characterised group of stable patients with moderate to severe COPD, and to relate this to the presence of lower airway bacterial colonisation, levels of airway inflammatory markers, exacerbation frequency, severity and time course. Sputum samples were obtained from patients followed in the East London COPD Study. These patients completed daily diary cards for changes in symptoms and peak flow and reported exacerbations to the study team as previously described [7]. A validated exacerbation frequency was calculated for each patient and this was related to the detection of bronchiectasis and emphysema on HRCT in the stable state. Stable daily symptoms, symptoms at exacerbation and the recovery from exacerbations were also examined with reference to the distribution and severity of HRCT findings. Some of the results of this study have previously been reported in the form of abstracts [8,9,10,11].

METHODS

Word count: 1394

Study Subjects

54 stable patients with moderate to severe COPD were recruited as volunteers from those being followed up in the East London COPD (ELCOPD) Study, which is a prospective cohort based study of COPD exacerbations. Patients in the study were recruited on a sequential basis from the outpatients department of the London Chest Hospital, and were representative of patients with moderate to severe COPD in the United Kingdom who are referred from primary care to secondary care because of increasing disability due to COPD. Patients gave informed consent to undergo CT scanning and ethics approval was obtained from the East London and City Health Authority Research Ethics committee. Additional detail on these methods is available in an on-line data supplement.

COPD was defined as a forced expiratory volume in one second (FEV_1) less than 70% predicted for age and height, beta-2 agonist reversibility on predicted FEV_1 of less than 15 % and/or 200 ml with FEV_1/forced vital capacity (FVC) ratio of less than 70%. Patients with previously diagnosed or clinically evident bronchiectasis or other significant respiratory disease were excluded. Subjects were recruited when stable, without any evidence of an exacerbation for at least six weeks. Baseline measurements were made of height, weight, FEV_1, FVC and peak expiratory flow rate (PEFR) by rolling seal spirometer (Sensor Medic Corp, Yorba Linda, USA). Pulmonary diffusion was measured by single breath carbon monoxide/helium diffusion (Pulmolab 501, Morgan Medical, Ferraris, UK). Arterialised ear lobe blood gases were analysed for arterial oxygen and carbon dioxide partial pressures [12].

Sputum Sampling

Stable patients attended the research clinic in the morning. Patients and diary cards were examined to confirm the absence of an exacerbation over the preceding six weeks, as defined by our previously validated criteria [7]. If no spontaneous sputum sample was available, sputum induction was performed using the DeVilbiss UltraNeb2000 nebuliser (DeVilbiss Healthcare) with 3% saline as described previously [13]. Additional detail on this procedure is provided in an on-line data supplement. 15 of the sputum samples obtained have been used for an analysis of the relationship between bacterial load and FEV_1 decline in COPD [14].

At this clinic visit an appointment was also made to attend for a high resolution CT scan of the chest at a later date. Patients were asked to inform the study team if they developed symptoms of an exacerbation before or on the date of this scan, in which case it was rebooked for a period 6 weeks after this exacerbation.

Sputum Examination

Sputum samples were examined as soon as possible within two hours of collection. The sample was separated from contaminating saliva, one third was taken for quantitative bacterial culture and the remainder was processed using previously published methods [13]. Additional detail on these methods is available in an on-line data supplement. Sputum levels of interleukin-6 (IL-6) and interleukin-8 (IL-8) were measured using ELISA (R&D Systems Europe, Abingdon, Oxon, UK).

Quantitative Identification Of Bacteria

Sputum samples were processed by using sputolysin ("Sputasol", Unipath, Hampshire, UK) and cultured on appropriate media according to previously described protocols [4,15]. Additional detail on these methods is available in an on-line data supplement.

All estimations of inflammatory mediators and bacterial colonisation were performed by observers blind to the clinical characteristics of the patients in the study.

High Resolution CT Scans

Assessment of bronchiectasis

CT scans were performed from December 2000 to August 2001 using a HiSpeed XZ/i scanner (GE Medical Systems, Milwaukee, USA). High-resolution images were obtained in full inspiration at 1mm collimation at 1 cm intervals from the apices to the lung bases. The CT scans were interpreted for the presence of bronchiectasis by two radiologists experienced in the interpretation of HRCT (IV, RHR) and blinded to the patient's clinical grouping or microbiological status. The presence of bronchiectasis was based on the following criteria: non-tapering bronchus with internal diameter 110% or greater than the adjacent pulmonary artery or the presence of visible bronchi within 1 cm of the costal pleural surface or adjacent to the mediastinal pleural surface. Bronchiectasis was scored in each lobe by consensus using the grading system proposed by Smith et al [16] as follows: 0 if no bronchiectasis was present; 1 if less than 25% of bronchi were bronchiectatic; 2 if 25-49% of the bronchi were bronchiectatic; 3 if 50-74% of the bronchi were bronchiectatic and 4 if 75% or more of the bronchi were bronchiectatic. The lingula was graded as a separate lobe resulting in a maximum score of 24 per

patient. Previous studies have shown that over 50% of healthy volunteers may have at least one dilated bronchus on HRCT [17]. A similar finding was reported in 53% of normal subjects at altitude [18], a change likely to be driven by hypoxia, and elderly patients may also have a higher normal ratio of bronchus/artery [19]. Therefore only patients with a total bronchiectasis score of 2 or more were considered to have changes consistent with clinically significant disease for the purposes of this study. Patients with a score of 0 or 1 (less than 25% of bronchiectasis bronchi in one lobe) were considered "normal". Study subjects were categorised in this way for calculations of bronchiectasis prevalence and comparisons of other parameters.

Assessment of Emphysema

Emphysema was quantitatively assessed by determining the area of both lungs measuring less than -950 Houndsfield units (HU) [20] at 4 levels, including 5 cm above the carina, the carina, 5 cm below the carina, and 2 cm above the highest hemi-diaphragm. For each lung at each level the total area, emphysematous area less than -950 HU and mean lung density were assessed using standard CT software (GE Medical Systems). At each level the percentage of emphysematous lung was calculated. The overall degree of each patient's emphysema was expressed as a percentage of the total lung area assessed.

COPD Exacerbations

All patients maintained daily diary cards on which they recorded their indoor PEFR measured with a Mini-Wright peak flow meter (Clement Clarke International Ltd., Harlow, UK) after their morning medication. Patients also noted any appearance or increase in intensity of 'major' symptoms (dyspnoea, sputum purulence, sputum amount) or 'minor' symptoms (nasal

discharge/congestion, wheeze, sore throat, cough) over their chronic (stable) symptoms on their diary cards. Throughout the study patients were seen three monthly for diary card review and spirometry. A member of the study team saw patients within 48 hours of the detection of deterioration in symptoms and the diagnosis of an exacerbation was confirmed in each case. Exacerbations were identified according to defined criteria of any two major symptoms or one major and one minor symptom, as described above, on two consecutive days, the first of which was taken as the day of onset of exacerbation [1, 7]. Exacerbation numbers were calculated from diary cards for a moving 24-month period of follow up, encompassing one year before and one year after each study patient's CT scan, and covered the period from December 1999 to August 2002. The sum of the major and minor symptoms listed above gave a daily exacerbation symptom count. The difference in symptom count between exacerbation onset and a baseline, taken as the mean over days 14 to 8 preceding onset, was calculated. Exacerbation recovery was the time from onset for a 3-day moving average of the symptom count to equal or become less than the baseline. The time to recovery of symptoms was taken as an indicator of exacerbation severity.

Statistical Analysis

Normally distributed data were summarised by means (standard deviations, SD), and skewed data by medians (interquartile ranges, IQR). Continuous variables with normal distributions were compared by t-test (two-tailed, unpaired) whereas those with non-normal distributions were compared by the Mann-Whitney U or Wilcoxon signed ranks test as appropriate. Sputum cytokine levels and total bacterial counts were correlated using Spearman's rank correlation. The median exacerbation frequency for this group was 2.54 per patient per year, in agreement with previously published results [1], and this was taken as the cut-off point to divide patients into

either frequent or infrequent exacerbators. Median values of exacerbation severity (based on the time to recovery of symptoms), over the moving 24-month period referred to above, were computed for each patient. Generalized linear models, which allowed for their Poisson shaped distribution, were used to assess the relationship between the distribution and extent of bronchiectasis and exacerbation indices. Data analysis was performed in SPSS for Windows version 11 for STATA 5.0.

RESULTS

Patients

At the time of recruitment for the present study there were 79 patients in total being followed up in the East London COPD study, of which 54 were willing to undergo sputum sampling and a CT scan of the chest. Table 1 shows the baseline physiological characteristics of the 54 patients studied. 27 (50%) were daily sputum producers, 16 (29.6%) were current smokers and 51 (94%) were taking inhaled steroids at a median (interquartile range, IQR) beclomethasone equivalent dosage of 1.25 (0.8-2.0) mg per day. We have compared the baseline characteristics of these study subjects with those who did not undergo CT scans, and there was no statistically significant difference between the two groups with respect to FEV_1, FVC or any other physiological parameter. Subjects in the present study had been prospectively followed up for a median (IQR) 1197 (804-1941) days at the start of CT scanning in December 2000.

Table 2 compares the physiological characteristics of the frequent (n = 27) and infrequent (n = 27) exacerbators; the two groups were similar except, of course, in the number of exacerbations per year ($p < 0.001$).

44 patients underwent CT scanning within a median (interquartile range, IQR) 10.5 (4-32.5) days of stable sputum sampling. 10 patients developed exacerbations before or on the day of CT scanning resulting in deferral of the scan to a date at least 6 weeks later. For these 10 patients the median (IQR) number of days between sputum sampling and CT scanning was 66.5 (52.7-95.2) days.

The median (IQR) time from the last exacerbation to the time of CT scanning was 65 (45-92) days. The median (IQR) time to recovery of peak flow after the last exacerbation was 5 (1-15) days, and the median time to recovery of symptoms was 12 (6-16.5) days.

Exacerbations

A median of 672 (558-724) days of diary card data were analysed for patients included in the study. Two hundred and fifty exacerbations were documented over the study period of which 15, in 11 patients, required hospital admission. 84.7% of reported exacerbations were treated with oral antibiotics and 55.3% were treated with oral prednisolone.

HRCT Scans

Bronchiectasis Scores

27/54 (50%) of patients had significant detectable bronchiectasis (a total bronchiectasis score of >/= 2) on HRCT. The total bronchiectasis scores are shown in Figure 1. Additional detail on the individual results for each study subject is available in an on-line data supplement (Table 1). Of those in whom bronchiectasis was detected, the median score was 3 (range 2-14). In this group, 8/27 (29.6%) had a cumulative upper lobe score of >/=2 (range 2-5), 8/27 (29.6%) had a cumulative

middle lobe/lingular score of >/= 2 (range 2-5) and 18/27 (66.7%) had a cumulative lower lobe score of >/=2 (range 2-8) i.e. bronchiectasis was most frequently detected in the lower lobes.

The total bronchiectasis score was inversely related to the arterial partial pressure of oxygen (PaO_2), i.e. was higher with worsening hypoxemia (rho = -0.299, p = 0.03). No relationship was seen between the total bronchiectasis score and stable respiratory symptoms or spirometric measurements.

Emphysema Scores

The median (IQR) total emphysema score was 15.6% (13.6) with a range from 1.24 to 51.5%.

The total emphysema score was inversely related to the FEV_1 (rho = -0.3, p = 0.027), the FEV_1% predicted (rho = -0.34, p = 0.012) and the FEV_1/FVC ratio (rho = -0.426, p = 0.001). Higher emphysema score was also related to lower KCO (rho = - 0.365, p = 0.021). No relationship was seen between emphysema scores and chronic stable daily symptoms, pack years of smoking (p = 0.19), current smoking (p = 0.66) or other physiological parameters.

Bacterial Isolates

52 sputum samples were obtained from patients in the stable state, of which 43 (82.7%) were spontaneous and 9 (17.3%) were induced samples. 28/52 (53.8%) sputum samples yielded a positive culture of one or more potentially pathogenic microorganisms (PPMs) as previously defined [3]. Pathogens recovered included *Haemophilus influenzae* (10/28 or 35.7%),

Branhamella catarrhalis (7/28 or 25%), *Haemophilus parainfluenzae* (6/28 or 21.4%), *Pseudomonas aeruginosa* (5/28 or 17.9%), *Streptococcus pneumoniae* (4/28 or 14.3%), *Klebsiella* species (2/28 or 7.1%), *Staphylococcus aureus* (2/28 or 7.1%) and *Enterobacter species* (2/28 or 7.1%). These are shown in Figure 2. 8 of the 28 colonised patients (28.6%) were colonised by multiple organisms. "Non specific growth" (NSG) was defined as the isolation of non-potentially pathogenic microorganisms, which are not usually involved in respiratory infections in immunocompetent hosts (*Streptococcus viridans* group, *Neisseria* species, *Corynebacterium* species and coagulase negative *Staphylococci*) [21].

Sputum IL-8 levels were higher in samples colonised by a PPM than those with a finding of non-specific growth (median (IQR) IL-8 (pg/ml) = 4598 (2404-5581) and 3343 (1808-4538) respectively, p = 0.026) and were related to the total bacterial count (rho = 0.417, p = 0.03) in colonised samples. The total bacterial count was higher with worsening hypoxia (rho = -0.288, p = 0.04).

Bacterial Colonisation, Airway Inflammation And HRCT Findings

Prevalence of bronchiectasis and airway inflammation

Subjects with significant detectable bronchiectasis (total score $\geq$ 2) exhibited higher levels of airway inflammation than those with a score of 0 or 1 (Table 3). The median (IQR) sputum IL-8 level (pg/ml) in patients with significant bronchiectasis was 3939 (3173-5528) versus 3897 (1772-4733) in those without, p = 0.001. The median (IQR) IL-6 level (pg/ml) in patients with significant bronchiectasis was 113.2 (20.1-218.9) versus 50.2 (13.6-213) in those without, p = 0.001.

Distribution of bronchiectasis and lower airway bacterial colonisation

A specific relationship was seen between the extent of bronchiectasis in the lower lobes and the presence of lower airway bacterial colonisation. Subjects were categorised, as for the total bronchiectasis scores, into those patients with a total lower lobe score of 0 or 1 (n = 36/54 or 66.7%) and those with a total lower lobe score of 2 or more (18/54 or 33.3%). Lower lobe bronchiectasis was related to percentage colonisation by a PPM (chi square, p = 0.004, odds ratio 7, 95% confidence intervals 1.47-37.9, Figure 3) and to percentage colonisation by *Haemophilus influenzae* (chi square p = 0.041). Levels of airway inflammatory markers were also higher in patients with significant lower lobe bronchiectasis (Table 4). The median (IQR) sputum IL-8 level (pg/ml) in those patients with a lower lobe bronchiectasis score of 2 or more was 4681 (3258-5785) and in those with a score of 1 or less it was 3614 (1929-4663), p = 0.001. The median (IQR) sputum IL-6 level (pg/ml) in patients with a lower lobe bronchiectasis score of 2 or more was 96.2 (13.6-178) and in those with a score of 1 or less it was 62.6 (19.1-219), p = 0.03. These relationships were not seen when similar categories were applied to the degree of upper or middle lobe bronchiectasis.

No relationship was seen between the degree of emphysema on HRCT and bacterial colonisation or airway inflammation.

Relationships With Exacerbation Indices

Distribution/ extent of bronchiectasis and exacerbation severity

A specific relationship was seen between exacerbation severity, as assessed by the time to recovery of symptoms, and the extent of bronchiectasis in the lower lobes. Patients with a lower lobe bronchiectasis score of 2 or more took longer to recover their symptoms after an exacerbation than patients with a lower lobe score of 1 or less. The median time to recovery was 12 versus 10 days in each group respectively, $p = 0.001$, Table 4). These relationships were not seen when similar categories were applied to the degree of upper or middle lobe bronchiectasis.

No difference was seen in the proportions of exacerbations treated with oral prednisolone between patients with and without significant lower lobe bronchiectasis ($p = 0.193$, Poisson regression).

Bronchiectasis and exacerbation frequency

The total or lower lobe bronchiectasis scores were not related to exacerbation frequency or number. The number of exacerbations experienced by patients per year was related to the sputum IL-6 level ($p = 0.017$, Poisson regression), as previously reported [5].

Emphysema scores and exacerbation indices

Patients with a higher emphysema score had a reduced incidence of symptoms of increased sputum volume ($p = 0.001$), wheeze ($p = 0.015$) or sore throat ($p = 0.038$) at exacerbation. No relationships were seen between emphysema scores and other indices of exacerbation severity or exacerbation frequency.

DISCUSSION

This study was designed to evaluate structural changes of bronchiectasis seen on HRCT scanning in patients with moderate to severe COPD, and to relate these to a number of clinical parameters, including indices of exacerbation frequency and severity, airway inflammatory markers and the presence of lower airway bacterial colonisation. Fifty percent of these patients had evidence of significant radiological bronchiectasis, with HRCT changes being seen most frequently in the lower lobes. The presence of bronchiectasis was associated with increased airway inflammation, as measured by sputum levels of interleukin-6 and interleukin-8, but was not related to exacerbation frequency. The extent of bronchiectasis in the lower lobes was related to percentage colonisation by a potential pathogen in the lower airway, increased airway inflammatory markers and longer time to symptom recovery at exacerbation.

A number of previous studies have examined relationships between structural changes seen on HRCT scanning and functional or physiological parameters in COPD, notably in the context of alpha 1-antitrypsin disease [22, 23]. It is not known, however, if morphological changes in the airways or lung parenchyma in usual COPD in the stable state can be related to the number or severity of exacerbations experienced by patients, or to levels of airway inflammation. Recurrent COPD exacerbations are associated with a heightened airway inflammatory burden, and with the presence of lower airway bacterial colonisation [4,5], which in turn has been shown to be an independent stimulus to airway inflammation in COPD [24-28]. In addition, we have recently found that LABC in the stable state is associated with increased symptom counts and sputum purulence at exacerbation [4]. The possible role of unrecognized bronchiectasis in orchestrating such relationships in COPD has not been previously assessed.

The present study examined a well-characterised group of hospital outpatients with moderate to severe COPD. HRCT scans of the chest were performed on patients in the stable state and the extent of bronchiectasis and emphysema quantified. Patients filled in daily diary cards for peak flow and symptoms over two years and were seen three monthly for spirometry and diary card review. Exacerbations were validated clinically within 48 hours of onset and from diary cards according to our previously defined criteria [7]. By these means we were able to carefully examine relationships between HRCT findings, the frequency and severity of exacerbations, stable levels of airway inflammation and bacterial colonisation.

HRCT is now accepted as the imaging modality of choice for the evaluation of bronchiectasis, [29-38] and emphysema [39,40]. Thin-section CT has been shown to have discriminatory value in obstructive lung disease [41]. However there is no consensus to date on the role of HRCT in quantifying the structural changes of bronchiectasis in patients with COPD, and its use may have had a number of limitations in the present study. Previous studies of HRCT scanning in patients with clinical bronchiectasis [42,43] and one study of patients with cystic fibrosis [44], found significantly higher mean bronchiectasis scores than those seen in our cohort. The sensitivity and specificity of bronchiectasis detection by HRCT may therefore have been lower in our group of COPD patients, who had a relatively smaller burden of disease than subjects in other studies [45]. The extent of bronchiectasis has been shown to be negatively correlated with FEV_1 % predicted [16], suggesting that in patients with COPD bronchiectasis may develop in the presence of progressive airways obstruction. We found no relationship between the bronchiectasis score and spirometric measurements, however this may have been due to the

narrow spread of FEV_1, as all our patients had relatively severe COPD with a mean (SD) FEV_1 % predicted of 38.1 (13.9) %. This would also explain the relatively high prevalence of bronchiectasis found in our study. Comparison with a control group of subjects without COPD would have allowed a more detailed assessment of the accuracy of HRCT to score bronchiectasis or emphysema in our COPD patients. In view of the range of FEV_1 of our study subjects, the conclusions of this study are limited to patients with moderate to severe COPD.

An important finding in this study was the relationship between the detection of radiological bronchiectasis on HRCT and more severe COPD exacerbations, as assessed by time to symptom recovery. We have previously shown that exacerbation severity in COPD can be related to this parameter [7]. The extent of lower lobe bronchiectasis was also related in this study to the presence of lower airway bacterial colonisation. The presence of bacteria in the lower airway in COPD implies a breach of host defense mechanisms, which fuels a vicious cycle of structural damage, loss of epithelial cell integrity [46], impaired mucociliary clearance [47] and mucus hypersecretion [48]. This results in further mucosal injury and inflammation, which could thereby provide the mechanism for longer and more severe COPD exacerbations. The correlation between total bronchiectasis score and PaO_2 also suggests that alveolar ventilation to pulmonary perfusion imbalance could be a complementary mechanism to contribute to lower airway bacterial colonisation in COPD. The findings of this study therefore demonstrate that radiological evidence of structural damage in the COPD airway, which may be driven in part by lower airway bacterial colonisation, may have important clinical implications. These findings could also explain why antibiotics have been shown to be of limited efficacy in modifying outcome measures in studies of COPD exacerbations [49]. The presence of these CT changes

may provide a means of identifying those patients with COPD who are at risk of more severe COPD exacerbations.

In this study the emphysema score was associated with a lower incidence of increased sputum purulence, sore throat or wheeze at exacerbation. The extent of radiological emphysema has been shown to be related to physiological correlates such as airway obstruction, as found in this study, as well as measures of health status [22], and numbers of leukocytes in the small airways [50]. However little is known about relationships with exacerbation indices or symptoms. These findings suggest that the character of exacerbations may differ in COPD depending on the distribution of the pathophysiological abnormalities within the lung, with exacerbations associated with purulent sputum occurring more in the context of large airway damage and remodeling, as opposed to parenchymal disease. This requires confirmation in larger studies.

No relationship was seen in this study between radiological evidence of bronchiectasis or emphysema and exacerbation frequency. It is possible that the study was underpowered for detecting this relationship. However, we have previously shown that a significant proportion of exacerbations in our cohort of COPD patients have a viral aetiology, most commonly human rhinovirus infection [51]. Little information is available on how viruses and bacteria may interact in the COPD airway, and it is likely that mechanisms governing exacerbation frequency in COPD are multifactorial.

.There are a number of possible reasons why bronchiectasis was detected most frequently in the lower lobes in this study. In a previous study of patients with chronic purulent sputum

production [52] a predominantly lower lobe distribution of bronchiectasis was found in subjects with impaired mucociliary clearance, one of the impaired host defense mechanisms seen in COPD.In addition, in a study of patients with bronchiectasis of known aetiologies [53], a lower lobe distribution was most often seen in patients with a history of childhood viral infections, a suggested risk factor for COPD. It is possible that multiple physiological and pathological alterations, including damaged mucociliary transport, localized or diffuse peripheral obliteration of the bronchial tree or lung tissue scarring act in concert in COPD, in the context of an already disrupted lung parenchyma, to produce the structural changes of bronchiectasis seen on HRCT. Further longitudinal studies are now required to establish criteria for the detection of these structural changes and their significance in COPD, and how they may relate to the natural history of this condition.

Clinical history and examination correlate poorly with HRCT features, with patients with bronchiectasis often being clinically indistinguishable from other study subjects [6,16]. A high prevalence of bronchiectasis has been demonstrated in an unselected group of patients with a primary care diagnosis of COPD [6], and studies of patients with alpha 1-antitrypsin disease have suggested that bronchiectasis may be present either concomitantly [54] or prior to the development of emphysema [55,56]. The alpha-1 status of our patients was not routinely ascertained, and none had clinical evidence of bronchiectasis on recruitment. However it is possible that some of these patients had bronchiectasis and then developed COPD with emphysema on top of this at a later date. Only 50% of our COPD patients reported daily cough and sputum production, and no relationship was seen in this study between these symptoms and bronchiectasis scores, suggesting that the HRCT findings in our patients were likely to represent

sub-clinical changes. Our findings therefore suggest that while HRCT continues to be an infrequently used tool in the assessment of COPD, sub-clinical bronchiectasis, which may nevertheless have important implications for some patients, is likely to remain undiagnosed.

In summary, this study has shown a high prevalence of radiological bronchiectasis in a group of moderate to severe COPD patients without clinical signs of this condition. Patients with moderate lower lobe bronchiectasis experienced more severe exacerbations, were more likely to exhibit lower airway bacterial colonisation and had heightened levels of airway inflammation. This suggests that high resolution CT scanning may be useful in identifying particular sub groups of patients with moderate to severe COPD, who are prone to more severe exacerbations and to the increased morbidity associated with these. Moreover, this study provides further evidence linking the presence of lower airway bacterial colonisation, and related structural airway changes, to important clinical parameters in COPD.

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LEGENDS FOR FIGURES

Figure 1

Total bronchiectasis scores: 27/54 (50%) of patients had significant detectable bronchiectasis (a total bronchiectasis score of 2 or more) on high resolution CT scanning

Figure 2

Potential pathogens isolated in 52 sputum samples

HI = *Haemophilus influenzae*; HP = *Haemophilus parainfluenzae*; SP = *Streptococcus pneumoniae*; BC = *Branhamella catarrhalis*; PA = *Pseudomonas aeruginosa;* K = *Klebsiella spp;* E= *Enterobacter spp;* SA=*Staphylococcus aureus*

Figure 3

Relationship between the extent of lower lobe bronchiectasis and colonisation by a potentially pathogenic micro-organism (PPM) with 95% confidence intervals, p = 0.004, odds ratio =7.0. Subjects were categorised, as for the total bronchiectasis scores, into those patients with a total lower lobe score of 0 or 1 (n = 36/54 or 66.7%) and those with a total lower lobe score of 2 or more (18/54 or 33.3%).

TABLE 1 **Baseline physiological characteristics of the patients studied; all data is quoted as mean (SD) except daily inhaled steroid dosage and annual exacerbation number for which median (IQR) values are shown**

Number	54 (36 M, 18 F)
Age in years (range)	69.04 (48 -83)
FEV_1 (litres)	0.96 (0.33)
FEV_1 % predicted	38.10 (13.9)
FEV_1 % reversibility	9.10 (12.0)
FVC (litres)	2.40 (0.73)
FEV_1 / FVC %	40.90 (11.8)
TLCO (% predicted)	55 (18.7)
VA (ml)	4772 (1237)
KCO (% predicted)	70 (26.5)
PaO_2 (kPa)	8.77 (1.11)
$PaCO_2$ (kPa)	5.94 (1.02)
Pack years of smoking	50.5 (33.5)
Daily inhaled steroids (mg)	1.25 (0.8-2.0)
Daily sputum producers (%)	27 (50%)
Exacerbations per year	2.54

FEV_1 = forced expiratory volume in one second; FVC = forced vital capacity; TLCO = transfer factor; VA = alveolar volume; KCO = transfer coefficient, PaO_2, $PaCO_2$ = arterial oxygen and carbon dioxide tensions in kilopascals. Daily inhaled steroid dosage was normalized per steroid potency and represents beclomethasone equivalent doses

TABLE 2 **Mean (SD) baseline physiological characteristics of the patients grouped by exacerbation frequency; no significant difference was found in any parameter other than of course the exacerbations/ year,** * = $p < 0.001$; § = median (IQR) values quoted

PARAMETER	EXACERBATION FREQUENCY	
MEAN (SD)	< 2.54 PER YEAR (n = 27)	> 2.54 PER YEAR (n = 27)
Age (years)	70.50 (7.04)	67.70 (6.93)
FEV_1 (litres)	1.03 (0.32)	0.89 (0.33)
FEV_1 % predicted	40.70 (13.4)	36.10 (14.2)
FEV_1/FVC %	40.52 (9.3)	41.10 (14.2)
TLCO (% predicted)	57 (16.7)	54 (20.4)
VA (ml)	4724 (1342)	4806 (1183)
KCO (% predicted)	77 (29.5)	65 (23.4)
PaO_2 (kPa)	8.80 (0.92)	8.75 (1.30)
Pack years of smoking	48.00 (29.2)	51.60 (37.6)
Daily inhaled steroids (mg)	1.0 (0.5-2.0)	1.6 (1.0-2.0) §
Daily sputum producers (%)	13	14
Daily dyspnoea (%)	23	22
Percentage with LABC (%)	25	29
Exacerbations/year	1.5 (1.0)	4.2 (2.6) *§
Total bronchiectasis score	2.0	1.0 §
Total emphysema score (%)	15.3	15.6 §

TABLE 3

Relationship between the presence of bronchiectasis on HRCT scanning and median (IQR) airway inflammatory cytokine levels. Study subjects were divided into those patients with a total bronchiectasis score of 0 or 1 and those with a total score of 2 or more (n = 27/54 or 50% in each group)

PARAMETER	TOTAL BRONCHIECTASIS SCORE		p (Poisson regression)
	0 or 1 (n = 27)	≥ 2 (n = 27)	
Stable sputum IL-8 level (pg/ml)	3897 (1772-4733)	3939 (3173-5528)	0.001
Stable sputum IL-6 level (pg/ml)	50.2 (13.6-213)	113.2 (20.1-218.9)	0.001

TABLE 4

Relationship between the extent of lower lobe bronchiectasis on HRCT scanning and median (IQR) levels of airway inflammatory markers, and exacerbation severity as assessed by median time to recovery of symptoms (days). Study subjects were divided into those patients with a total lower lobe score of 0 or 1 (n = 36/54 or 66.7%) and those with a total lower lobe score of 2 or more (18/54 or 33.3%).

PARAMETER	LOWER LOBE BRONCHIECTASIS SCORE		
	0 or 1 (n = 36)	$\geq$ 2 (n = 18)	p (Poisson regression)
Stable sputum IL-8 level (pg/ml)	3614 (1929-4663)	4681 (3258-5785)	0.001
Stable sputum IL-6 level (pg/ml)	62.6 (19.1-219)	96.2 (13.6-178)	0.03
Time to recovery of symptoms following exacerbation (days)	10	12	0.001

FIGURE 1

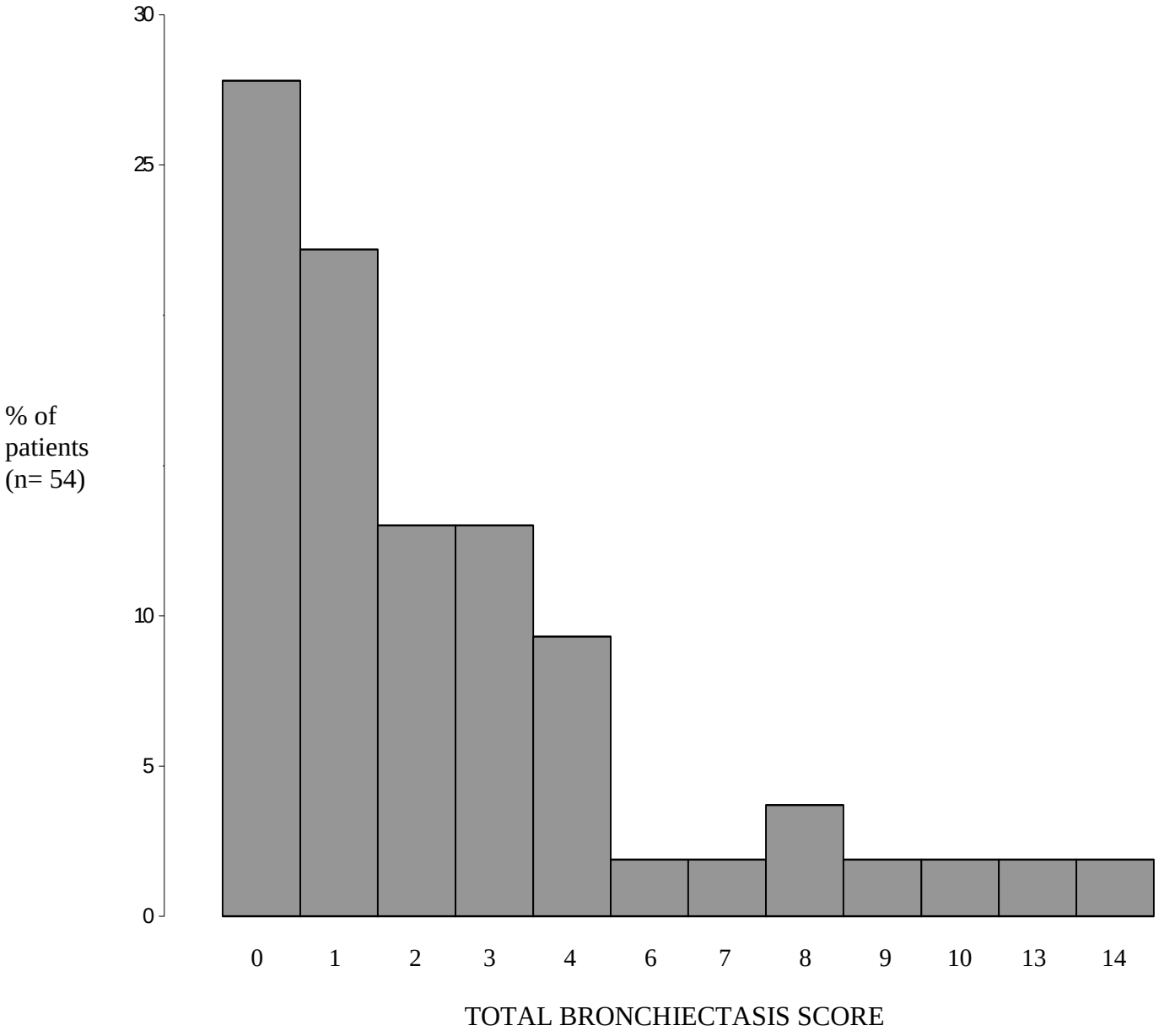

30
25
10
5
0
% of patients (n= 54)
0
1
2
3
4
6
7
8
9
10
13
14
TOTAL BRONCHIECTASIS SCORE

FIGURE 2

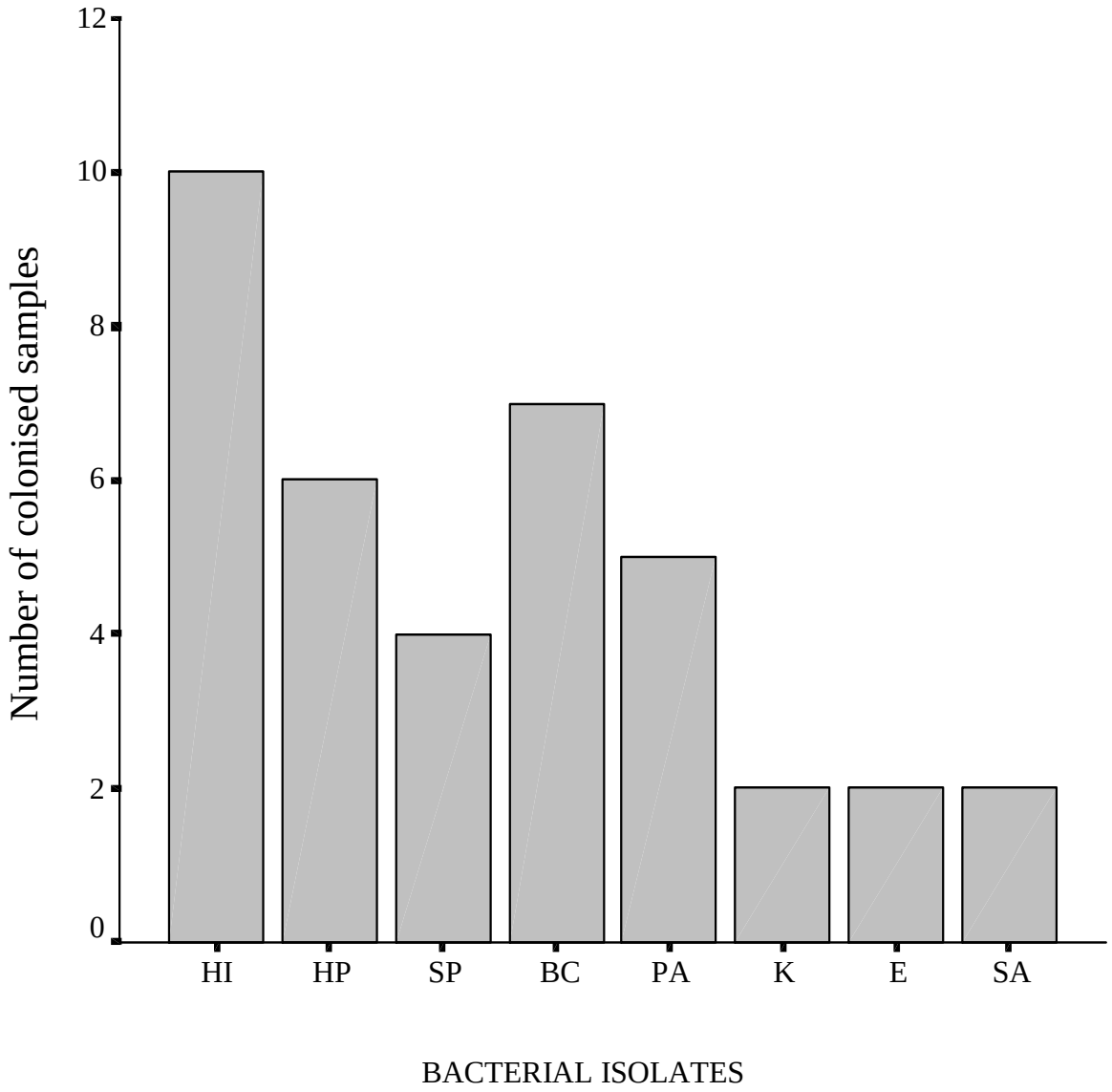
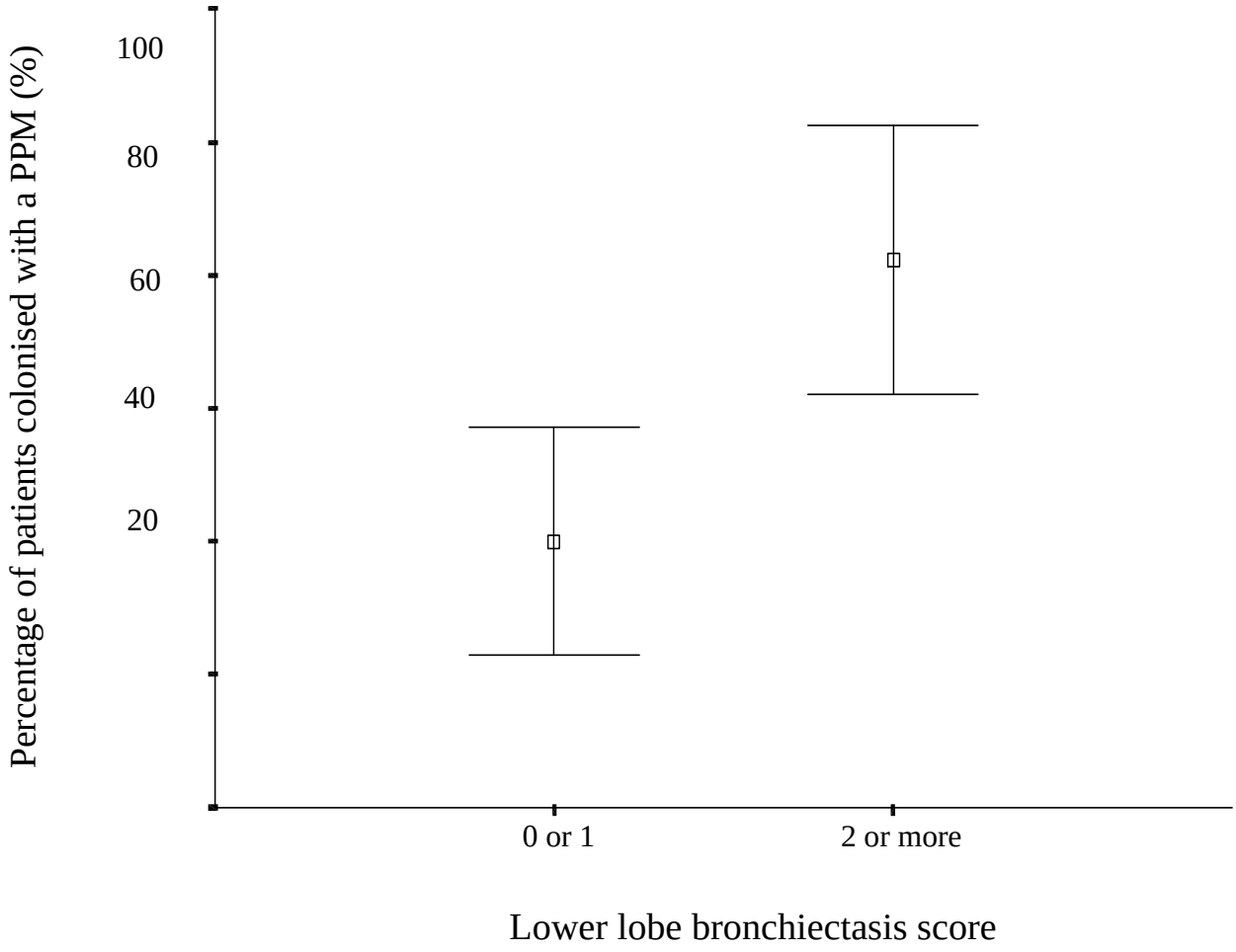

12
10
8
6
4
2
0
Number of colonised samples
HI
HP
SP
BC
PA
K
E
SA
BACTERIAL ISOLATES

FIGURE 3


Percentage of patients colonised with a PPM (%)
100
80
60
40
20
0 or 1
2 or more
Lower lobe bronchiectasis score

BRONCHIECTASIS, EXACERBATION INDICES AND INFLAMMATION IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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On-line data supplement

METHODS

Study Subjects

54 stable patients with moderate to severe COPD were recruited as volunteers from the larger group of patients being followed up in the East London COPD study. This is a prospective cohort based study of COPD exacerbations, which has been running at the London Chest Hospital since 1995. Patients in the East London COPD study are recruited on a sequential basis from the outpatients department of the London Chest Hospital, and are representative of patients with moderate to severe COPD in the United Kingdom who are referred from primary care to secondary care because of increasing disability due to COPD. Any patient with COPD (defined according to the British Thoracic Society guidelines) who is being followed up in the outpatients department of the London Chest Hospital, and who is willing to fill in diary cards and to take part in a long term study, is recruited into the East London COPD study. Patients are recruited sequentially to maintain a rolling cohort size of approximately 100 study subjects, and to replace any dropouts. As part of the East London COPD study all patients maintain daily diary cards on which they document daily symptoms and peak flow, and report exacerbations to the study team.

Sputum Sampling

Stable patients attended the research clinic in the morning for baseline sputum sampling. They were questioned and diary cards were examined to confirm the absence of an exacerbation over the preceding six weeks, as defined by our previously validated criteria [E1]. Measurement of oxygen saturation (Minolta Pulsox 7, DeVilbiss Healthcare, Heston, Middlesex, UK) and spirometric tests were performed on arrival and repeated 10 minutes after premedication with 200 mcg inhaled salbutamol via a metered dose inhaler. Patients were instructed to blow their noses and rinse their

mouths out with water before expectorating sputum into a sterile pot. If no spontaneous sputum sample was available, sputum induction was performed. This was done using the DeVilbiss UltraNeb2000 nebuliser (DeVilbiss Healthcare) with 3% saline as described previously [E2]. This nebuliser produced an aerosol output of approximately 2 ml/min with a mean particle size 0.5 – 5 mcm in diameter. After seven minutes of nebulisation measurements of oxygen saturation and spirometric tests were performed and, if no sputum was produced, nebulisation was continued for a further seven minutes if the FEV_1 had not fallen by more than 20%.

Sputum Examination

Sputum samples were examined as soon as possible within two hours of collection. The sample was separated from contaminating saliva, one third was taken for quantitative bacterial culture and the remainder was processed using previously published methods [E2,3,4]. Half of the selected portion of the sputum was mixed with four times its weight of 0.1% dithiothretol (DTT) solution, vortexed for 15 seconds and then rocked for 15 minutes. A weight of Hank's balanced salt solution (HBSS) equal to that of the sputum plus DTT was then added and the whole mixture was rocked for a further 5 minutes. The suspension was then filtered through 50 micrometre (mcm) nylon gauze to remove mucus and debris and centrifuged at 790*g* (2000revolutions per minute) at 4 ^{o} Centigrade (^{o} C) for 10 minutes. This resulted in the formation of a cell pellet and supernatant solution. The other half of the selected portion of the sputum was mixed with nine times its weight of phosphate buffered saline (PBS) and agitated with silicanised 1-2 mm in diameter glass beads (BDH Chemicals Ltd, Poole, Dorset, UK). This was then filtered and centrifuged as before to provide a cell pellet and supernatant. The supernatants from both processes were decanted and stored at –70 ^{o} C. Levels of interleukin-6 (IL-6) and interleukin-8 (IL-8) were measured in the PBS processed

supernatants using quantitative sandwich immunoassay techniques, i.e. ELISA (R&D Systems Europe, Abingdon, Oxon, UK) and expressed as pg/ml.

Quantitative Identification Of Bacteria

Samples were processed by adding an equal weight of sputolysin ("Sputasol", Unipath, Hampshire, UK) and several glass beads (1-1.5 mm in diameter) and were incubated for 30 minutes at 37 ^{o} C, during which samples were vortexed for 5-10 seconds intermittently. Ten fold serial dilutions of the homogenised sample were made in Brain Heart infusion broth and 100 mcl aliquots plated out onto the surface of a range of different media including blood agar, chocolate agar, MacConkey agar and cysteine lactose electrolyte deficient agar. These were incubated for 18 hours at 37 ^{o} C in an atmosphere of air + 5% carbon dioxide. After incubation, bacterial colonies were enumerated and sub cultured for identification by standard methods [E5]. The number of colony forming units/g of sputum was calculated from the number of colonies obtained and the dilution of the sputum, as previously reported [E6].

All estimations of inflammatory mediators and bacterial colonisation were performed by observers blind to the clinical characteristics of the patients in the study.

RESULTS

Table E1

Individual bronchiectasis scores for each study subject. Bronchiectasis was scored in each lobe by consensus as follows: 0 if no bronchiectasis was present; 1 if les than 25% of bronchi were bronchiectatic; 2 if 25-49% of the bronchi were bronchiectatic; 3 if 50-74% of the bronchi were bronchiectatic and 4 if 75% or more of the bronchi were bronchiectatic. The lingula was graded as a separate lobe resulting in a maximum score of 24 per patient.

STUDY NUMBER	BRONCHIECTASIS SCORES						
	RUL	RML	RLL	LUL	LINGULA	LLL	TOTAL
1	0	0	1	0	0	0	1
2	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
4	0	3	4	0	2	4	13
5	3	4	4	0	1	2	14
6	2	1	0	3	1	2	9
7	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0
9	0	0	1	0	0	0	1
10	0	0	0	0	0	0	0
11	0	1	0	0	0	0	1
12	1	0	1	0	0	0	2
13	0	0	0	1	0	0	1
14	0	0	1	0	0	1	2
15	0	0	2	0	0	4	6
16	0	0	0	0	0	0	0
17	1	0	0	0	0	0	1
18	1	0	1	0	0	2	4
19	0	0	1	0	0	1	2
20	1	0	0	1	0	1	3
21	.	4	4	0	0	0	8
22	2	1	3	1	0	3	10

STUDY NUMBER	BRONCHIECTASIS SCORES						
	RUL	RML	RLL	LUL	LINGULA	LLL	TOTAL
23	0	1	2	0	0	0	3
24	0	0	1	0	0	1	2
25	1	1	0	0	0	0	2
26	0	0	0	0	0	0	0
27	0	1	0	0	0	0	1
28	0	1	1	0	1	0	3
29	0	1	0	0	0	0	1
30	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0
32	2	0	0	2	2	1	7
33	0	0	1	1	0	1	3
34	0	0	1	0	0	0	1
35	0	1	2	0	0	0	3
36	0	0	0	0	0	0	0
37	0	0	0	1	0	0	1
38	0	0	0	0	0	0	0
39	0	0	1	0	0	0	1
40	0	0	0	0	0	0	0
41	0	0	1	0	0	1	2
42	0	0	0	0	0	0	0
43	0	0	1	0	0	0	1
44	1	0	1	1	0	1	4
45	2	0	1	1	0	0	4
46	0	0	0	0	0	0	0
47	0	1	1	0	0	1	3
48	0	1	0	0	0	0	1
49	1	2	0	1	0	0	4
50	0	0	2	0	0	2	4
51	0	0	0	0	0	0	0
52	1	0	4	0	1	2	8
53	1	0	0	0	0	1	2
54	0	2	1	0	0	0	3

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E2. 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

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