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Colonization and Bronchiectasis in Patients with Advanced Chronic Obstructive Pulmonary Disease

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Abstract

Background: Pathogenic organisms in sputum of COPD patients are associated with increased risk of exacerbations and with a higher prevalence of bronchiectasis. This study evaluates the rate of colonization in patients with advanced COPD, along with severity of bronchiectasis.

Methods: A retrospective single-center evaluation of 378 patients with advanced COPD was performed in our pre-transplant outpatient clinic between October 2008 and June 2011. Lung function, exacerbation rate in the preceding 12 months and lung and sputum microbiology were analyzed. CT scans were assessed for bronchiectasis by blinded radiologists.

Results: Sputum expectoration was reported in 196 patients (52%). Potential pathogens were identified in 77 cases (20%), *Pseudomonas aeruginosa* (20%) and *Staphylococcus aureus* (21%) were the dominant bacterials. Patients with positive sputum cultures were more likely to be hospitalized due to exacerbation (p=0.01) and demonstrated more severe bronchiectasis in standardized qualitative CT examination (p=0.002). Ninety-four patients (25%) underwent lung-transplantation during the observation period. In the explanted lungs, evidence of potential pathogen was found in 21 patients (22%).

Conclusions: Within our cohort of advanced COPD patients, almost 1 of 5 patients exhibited a positive respiratory culture. Pathogenic bacterial colonisation correlated with more severe bronchiectasis and significantly increased hospitalization due to exacerbations.

Keywords: Colonization; COPD; Lung transplantation; Bronchiectasis

Abbreviations:

BAL: Broncho Alveolar Lavage; COPD: Chronic Obstructive Disease; CT: Computed Tomography (high resolution); ERS: European Respiratory Society; FEV1: Forced Expiratory Capacity in one second; GOLD: Global Initiative for Chronic Obstructive Lung Disease; IQR: Interquartile Ranges; PP: Potential Pathogen; QOL: Quality Of Life

Introduction

COPD is characterized by reduced FEV1 due to non-reversible airway obstruction and chronic cough. Subsequent clinical and radiological assessment has previously identified distinct emphysematous and obstructive subtypes [1].

Through increased use of high-resolution CT (HRCT) scanning, evidence of bronchiectasis has become increasingly apparent, with previous studies demonstrating a prevalence of between 4% [2] and 57% [3] of COPD patients.

Development of bronchiectasis appears dependent on declining pulmonary function [4] as well as airway colonization by potential pathogens (PP), particularly *Pseudomonas* species [3]. The relationship between bronchiectasis and bacterial colonization appears to reflect a self-perpetuating pro-inflammatory state.

The inherent destruction of normal airways inevitably predisposes to colonization [5]. Such colonization conceivably promotes a chronic airway inflammation resulting in further airway damage leading to progressive bronchiectasis [3].

Independent from its role in promoting bronchiectasis, bacterial airway colonization has been associated with increased exacerbation rates as well as increased hospitalization in COPD patients [3].

Existing data is however largely based on heterogeneous cohorts with regards to COPD stage and generally used non-standardized CT protocols for defining and sub-classifying bronchiectasis [2,4], which may well account for the variation in prevalence.

Previous studies, whilst demonstrating prognostic impact, have highlighted the need for comprehensive microbiological characterization in COPD patients with bronchiectasis, to facilitate the development of tailored therapies such as inhaled antibiotics or long-term macrolide prophylaxis [5].

Bacterial colonization may have additional implications for subsequent management in these patients. Lung transplantation has become an increasingly accepted treatment option in selected patients with end-stage COPD under 65 years [6].

Previous colonization represents a proven risk factor for increased infection rates as well as chronic allograft dysfunction (rejection) in lung transplant recipients [7].

In this study we assess bacterial colonization along with the presence and extent of bronchiectasis in patients with end-stage COPD. In the sub-group of patients who underwent lung transplantation information of bacterial status from explanted lung was reviewed.

Aim of the Study

Assessment of bacterial status of patients with advanced COPD and evaluation of CT morphologic changes in patients with versus patients without bacterial prove and assessment of outcome.

Methods

Study design and study population

In this retrospective single-center study, 378 patients with advanced COPD (GOLD III and IV) attending the lung transplantation evaluation clinic at Hannover Medical School between October 2008 and June 2011 were included. COPD was defined in accordance with GOLD criteria (www.goldcopd.org). All patients were ≥18 years old. The local Ethic Board proved the study (No 1120-2011).

Pulmonary function tests

All patients underwent pulmonary function testing including spirometric or if possible bodypletysmography function testing and capillary blood gas analysis according to ERS guidelines [8]. A six-minute walk test was performed in all patients [9].

Assessment of clinical parameters and outcome parameters

At each attendance, patients underwent physical examination. Height, weight, heart and respiratory rate, oxygen requirements and current treatment were recorded. A questionnaire evaluating exacerbation rate (last 12 months), antibiotic treatment received and sputum colour used in our outpatient clinic. Patient quality of life was measured by visual analogue scale as previously described [10].

Microbiological status

To determine colonization status, all available microbiology reports for individual patients were reviewed. This consisted principally of sputum and bronchoalveolar lavage (BAL) results, with additional data from explanted lungs in patients who underwent transplantation.

The latter consisted of microbiological culturing a single bronchial ring from a single main bronchus. All isolated organisms were identified by standard laboratory methods. Patients were considered “colonized” if bacterial prove was obtained. In all cases of positive bacterial growth, antibiotic resistance testing was performed and the results collated.

Analysis of computed tomography scans

Multi-detector CT scans were performed in several institutions with varying scan protocols (slice thicknesses between 1.25 and 8 mm) and various quality. Digital computed tomography scans in sufficient quality were only available in 22 patients with bacterial colonization.

These and 22 CT scans of patients without colonization, that were randomly selected (by H.S.), were analysed by two blinded radiologists (S.D. and P.H.).

Morphologic description of bronchiectasis and emphysematous changes were reported in accordance to criteria described by Hansell et al. [11]. Additionally, a score to quantify degree of bronchiectasis was used as previously described by Smith et al. [12].

Relevant characteristics such as bronchiectasis, bronchial wall thickening, infiltrates and mucus plugging are listed in Table 1.

Table 1: Patients characteristics.

	all patients (n=378)	Colonized patients (n=77)	Non-colonized patients (n=301)	Significance
Age at first presentation, years (IQR)	54 (49; 58)	54 (47; 59)	54 (49; 58)	0.2
Lung transplantation, n (%)	94 (25)	34 (44)	60 (19)	0.0001
Age at transplantation, years (IQR)	55 (51; 59)	55 (50; 60)	55 (51; 59)	0.46
Gender: Male/Female	191/187	51/26	140/161	0.03
Body mass index (IQR)	23 (20; 26)	23 (22; 27)	23 (21; 26)	0.2

Packyears (IQR)	30 (20; 44)	30 (15; 40)	30 (20; 45)	0.3
Oxygen, n (%)	275 (73)	54 (70)	221 (73)	0.36
6 MWT, m (IQR)	264 (165; 374)	235 (110; 375)	271 (174; 374)	0.4
Respiratory infections per year, n (IQR)	2 (1; 3)	3 (1; 5)	2 (1; 3)	0.01
Respiratory infections with hospitalization per year, n (IQR)	1 (0; 1)	1 (0; 2)	1 (0; 1)	0.02
QoL – VAS (IQR)	4 (2.5; 5)	4 (3; 5)	4 (2; 5)	0.7
FEV1% predicted (IQR)	19 (15; 25)	20 (16; 25)	19 (15; 25)	0.4
FVC% predicted (IQR)	62 (51; 76)	61 (48; 74)	63 (53; 78)	0.4
pO2, mmHg (IQR)	69 (62; 77)	69 (59; 77)	69 (62; 77)	0.6
pCO2, mmHg (IQR)	43 (38; 49)	43 (38; 50)	43 (39; 49)	0.7

Statistical analysis

Numeric data are reported as median and inter-quartile ranges. Group comparisons were performed using the student's t test. All reported P values are two sided unless otherwise indicated.

For all analyses, P values less than 0.05 were considered statistically significant. Categorical variables were analyzed using chi-square tests. Correlation analysis was performed using univariate one way ANOVA analysis comparing bronchiectasis score against FEV1%, QOL, time from first presentation until transplantation, age, packyears, oxygen flow, infections per year.

Results

Patient characteristics are summarized in Table 2.

Table 2: CT findings.

	All patients (n=44)	Colonized patients (n=22)	Non-colonized patients (n=22)	Significance
Bronchiectasis, n	35	18	17	1
-Mild	23	7	16	0.01
-Severe	12	11	1	0.002
Score bronchiectasis, median (IQR)	6 (2; 12)	11 (2; 17)	5 (0.75; 6.25)	0.006
Bronchial wall thickening, n	37	19	18	0.5
Infiltrates, n	4	2	2	1
Pleural effusion, n	1	0	1	0.3
Mucus plugging, n	18	10	8	0.3
Tree in bud phenomenon, n	4	3	1	0.3
Homogenous emphysema, n	19	10	9	0.8

In total 378 patients were identified and included in the analysis. With regards to symptom profile, 51.7% had chronic cough with >5 ml/day sputum production. Patients without chronic cough and expectoration were regarded as not colonized.

Total seventy-seven patients demonstrated bacterial colonization (20%). In comparing the colonized and non-colonized cohorts, no differences were identified with regards to age, tobacco consumption, life-quality and functional status.

Men were more often colonized and colonized patients were more often lung-transplanted. The median exacerbation rate (see respiratory infections in Table 2) in the preceding 12 months for the entire cohort was 2 (IQR 1-3) with increased exacerbations in colonized patients (p=0.01).

Hospitalization rates were however significantly higher (p=0.02). Microbiological material was available in 151 patients, with the remaining 227 patients unable to provide specimens, the majority of who denied significant expectoration.

Sputum analysis was reviewed in 107 patients and tissue examination from explanted lungs was available in 94 patients (Figure 1).

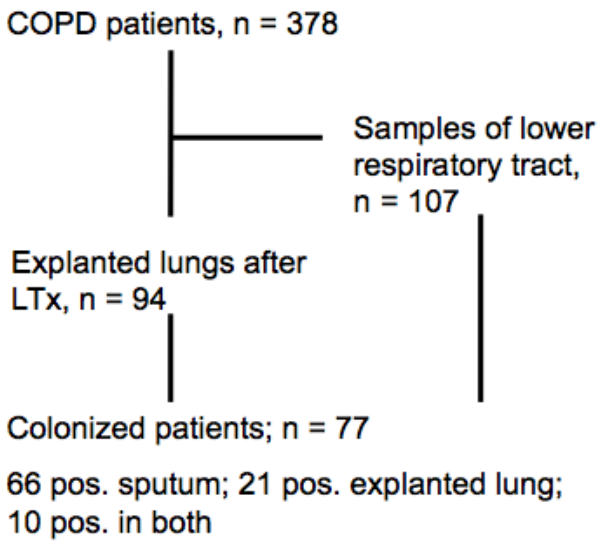

Figure 1: Flow chart of analysis.

In 22% of the histological lung examinations, pathogenic organisms were identified. Sputum analysis identified PP in 62% of patients. Considering the entire cohort collectively, sputum analysis revealed a colonization rate of 17%.

In 50 patients, both lower respiratory secretions and explanted lung tissue were available for comparison. Of the 94 transplant recipients, 63 previously denied significant expectoration and finally demonstrated no PP in the explanted lung.

A further 10/94 patients reporting sputum, tested negative for PP in sputum but demonstrated PP in the explanted lung. Only one expectorating patient had both sterile sputum and in the explantat.

The calculated sensitivity and specify of sputum in comparison to lung tissue was 0.77 and 0.64 respectively, returning a positive predictive value of 0.416. In patients with corroborating positive findings, 5 of 10 organisms were identical.

The commonest pathogenic organisms identified were *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Table 1). Eight patients cultured *Aspergillus*. Colonization with multiple organisms in lower respiratory samples was observed in 22 patients (Table 1).

Comparison between colonized patients exhibiting multiple vs. single PP revealed a tendency (p=0.058) towards lower QOL in patients with multiple PPs, with other baseline characteristics revealing no significant differences. We furthermore analyzed the resistograms of the isolated bacteria (Table 3).

Table 3: Microbiological findings and resistance analysis of sputum samples and findings from explanted lungs were shown. Multiple bacterial in a single patient were possible.

Germ	Isolates		Drug resistance
	Lower respiratory samples	Explanted lung	
Staph. aureus	19	5	16% MRSA
Pseudomonas	18	3	22% MDR
Stenotrophomonas	6	2	40% fluorochinolone-resistant
Haemophilus	8	-	
Klebsiella	7	-	0% ESBL
Serratia	6	-	
E. coli	4	-	
Other *Enterobacteriaciae*	4	-	
Enterococci	4	2	
Moraxella	3	-	
Streptococcae	4	1	
atyp. Mycobacteriae	4	1	
other	2	6	
Aspergillus	8	1	

CT-assessment for bronchiectasis was performed in 44 patients, with significant more severe bronchiectasis in patients with airway colonization (p=0.002), see Table 1.

Additional correlation analysis comparing bronchiectasis scoring and patient baseline characteristics did not demonstrate any significant findings, although a trend was found suggesting shorter time from first presentation until lung transplantation (p=0.09).

Treatment plans were available in 311 patients and corresponded with current GOLD treatment guidelines (www.goldcopd.org, December 2012), consisting of a combination of inhaled steroids, long acting β-sympathomimetics and anticholinergic drugs.

In the remaining patients, no medication plan was available. Oral steroids or steroid-equivalent drugs were recorded in 100 patients. Amongst patients receiving inhaled steroids, no significant increase in colonization or specifically *aspergillus* rates was observed.

Discussion

In evaluating the rates of bacterial colonization and bronchiectasis in patients with advanced COPD, the main findings of our study may be summarized as follows:

1. Only 77 (20%) from 378 patients awaiting lung transplantation for COPD exhibited airway colonization with potential pathogens. Even use of gold standard microbiological

assessment (tissue analysis from explanted lungs in 94 patients) identified PP in only 22% of patients.

2. Sensitivity and specificity of sputum culture was 77% and 64% respectively. A negative sputum result, therefore does not exclude chronic colonization.

3. Evidence of pulmonary bacterial colonization was associated with increased incidence of severe bronchiectasis (50% vs. 4.5%) and correlated with a significant increase in exacerbations requiring hospitalization (p=0.02) and lung transplantation (p=0.0001).

Whilst previous studies have alluded to different phenotypes within COPD patients [1], the current study focuses on the specific phenotypes of colonized and bronchiectatic patients. Colonized patients exhibited higher rates of sputum production and were more likely to be hospitalized due to infect exacerbations [1].

Whilst previous studies have examined the relationship between colonization, bronchiectasis and COPD [3,13], interpretation of findings has been limited due to small heterogenous study cohorts. Our data focused specifically on patients with end-stage disease. Over half of our cohort (52%) demonstrated significant expectoration. These findings concur with data from Patel et al, who observed daily cough and sputum production in 50% of patients with moderate or severe COPD [13]. Within our cohort, almost one third of expectorating patients demonstrated airway colonization. Colonization rates in our study were comparable with other recent observations [14,15]. Soler et al. reported BAL colonization rates of 33% in COPD patients [15]. Similar rates were observed by Monso et al. (25%), in 41 samples of patients with chronic bronchitis [14]. Previous studies have demonstrated both decreasing FEV1 and continued smoking as independent risk factors for lower respiratory bacterial colonization in advanced COPD [16].

In our study 94 patients (25%) underwent lung transplantation. Microbiological examination of the explanted lung was performed in all cases. With 22% of the specimens identifying pathogenic organisms. Obviously, 22% colonization rate revealed by intraoperative smear versus 62% by sputum (significant expectoration) can hardly be compared. Despite these findings, the calculated sensitivity of 77% and positive predictive value of 42%, sputum analysis remains a relevant screening tool in patients with chronic expectoration. Certainly, positive sputum results appeared relevant when compared with results obtained from explanted lungs.

Discordance in cultures between different sampling modalities for a variety of reasons: 1) Sputum contamination is known to occur in the upper respiratory tract [17,18], bronchoalveolar lavage is similarly predisposed to false positive results due to contamination [19]. 2) Comparing previous sputum results with explanted lung inevitably reflects different periods and is conceivably influenced by changes in treatment, particularly anti-microbial agents. 3) Although lung harvesting was performed under sterile conditions, contamination remains possible. 4) Colonization in COPD patients may vary at different times of year [15] and distinction between infection and colonization is not always possible.

Whilst lower respiratory samples offer better sensitivity than sputum, bronchoscopy cannot be considered a routine procedure in end-stage COPD, due to unacceptably high complication rates in patients with profoundly reduced FEV1 [20]. In contrast sputum sampling is relatively straightforward in such patients, especially given the reported corroboration in our colonization rates for sputum and explanted lungs [21].

Our findings revealed that airway colonization was associated with higher rates of lung transplantation, and hospitalisation rates. These results broadly concur with Patel et al. who reported a positive correlation between lower airway bacterial colonization and exacerbation frequency [22]. In contrast to previous reports that associated tobacco exposition with rates of bacterial colonization [14,22], we observed no difference. Given the requirement for tobacco abstinence prior to lung transplantation evaluation, rates of active smoking in our cohort were extremely low, with elevated Hb-CO being identified in only 4% of patients.

With respect to sputum analysis, the most common pathogenic organisms were *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Table 3), contradicting previous studies, which reported *Haemophilus influenzae* as the most frequent pathogen [22]. The discordance most likely reflects the end-stage nature of the COPD patients in our study, exemplified by the fact that 22 patients exhibited multiple bacterial colonization and 7 patients demonstrating multi-resistant organisms (Table 3). Similarly the detection of *Aspergillus* in eight patients (4%) is in line with advanced disease. Until now, little was known about *Aspergillus* colonization rates in advanced COPD, with previous studies making no reference to fungal colonization [13,21,22]. Aquino et al. examined the association of COPD with Invasive Aspergillosis (IA) when new infiltrates occurred. *Aspergillus fumigatus* was recovered by culture in 4,2% in the context of acute infections [23]. An association between *Aspergillus* colonization and the use of inhaled steroids could not be established in the current cohort.

In assessing the relationship between lower airway bacterial colonization and the development of bronchiectasis on CT in COPD patients, limited previous data exists. Previous studies have examined the association between changes in CT and functional parameters in COPD, specifically in relation to α1-antitrypsin disease [24] but did not quantify severity of bronchiectasis [13]. Regarding bronchiectasis in moderate to severe COPD, Martinez-Garcia et al. reported a prevalence of 57,6%, with a frequency distribution similar to that of Patel et al. (50%) despite using heterogenous and non-standardized radiological assessment [3,13]. In contrast, we employed a standardized bronchiectasis CT protocol and focused on end-stage patients. The prevalence of bronchiectasis in our study was 79%. This higher rate may merely reflect the advanced COPD stage in our cohort, as suggested by Martinez-Garcia et al., who observed greater prevalence of bronchiectasis (70%) in patients with severe functional impairment (FEV1 <50%) [3]. Interestingly, differentiation between mild and severe

bronchiectasis revealed important differences between non- and colonized patients, with significantly higher bronchiectasis scores being recorded in colonized patients (11 vs. 5; p=0.006). These findings suggest that colonization is a risk factor for severe bronchiectasis in patients with advanced COPD.

This retrospective study has several strengths and limitations. Currently, it represents the first study comparing lung tissue and lower respiratory samples in a large number of patients with advanced COPD. Nevertheless, CT scans were analyzed in 44 patients but under blinded conditions. The small number of CT examinations results from either insufficient quality or date of CT scan (long period between CT scan and last presentation to the ambulance). A limitation is that in lower respiratory samples, a quantification of colonization forming units was not possible and no repetitive cultures were obtained by the patients.

In conclusion, our data provides another perspective on the truly heterogeneous nature of COPD and the growing acceptance for the need of individual tailored therapy. We could demonstrate that expectorating patients are at higher risk of developing bacterial colonization that may cause structural pulmonary changes. Whilst sputum sensitivity is limited, it remains the simplest and safest means of assessment in expectorating patients. In the absence of sputum availability or in cases of persisting clinical suspicion, we recommend performing a CT to assess for the presence and severity of bronchiectasis. Patients with severe bronchiectasis present a special phenotype of COPD and should treat like patients with non-cystic fibrosis-bronchiectasis [25,26].

Statement of Interest

The authors declare that they have no competing interests.

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Authors' Contributions

HS and JR conceived and designed the study, performed the statistical analysis, interpreted the data and drafted the manuscript. GA participated in the study design and revised the manuscript critically for important intellectual content. SD interpreted the data and drafted the manuscript and revised the manuscript critically for important intellectual content. MG revised the manuscript critically for important intellectual content. JG interpreted the data and contributed to the drafting of the manuscript. FCB revised the manuscript critically for important intellectual content. TW contributed to the study design, supervised the study and revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

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