

Conclusions Molecular profiling identifies heterogeneity in the airway microbiome of COPD patients, with dominance of pathogens routinely identified at culture. However, a precise role for bacteria in COPD remains unclear.

S17 IMPACT OF COPD SEVERITY AND SPUTUM PRODUCTION ON ANTIBIOTIC RESISTANCE

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Introduction Bacterial infections are a well-known trigger for exacerbations of COPD. A variety of antibiotics are regularly prescribed for this group of patients but the risk and frequency of antibiotic resistance in the COPD population is less understood. Routine culture data can be evaluated to establish resistance prevalence and patterns.

Methods Culture data were collected from the sputum samples of 293 patients in the London COPD cohort over a period of 5 years (01/01/2006–31/12/2010) mean (\pm SD) months in study 28.4 (\pm 19.9); age 69.9 years (\pm 8.9); predicted FEV₁ 47.8% (\pm 16.5); male gender 58%; exacerbation samples 48.9%; sputum producers 77.5%. Identification of bacterial presence was established and where clinically indicated drug sensitivity tests (DSTs) were performed. A resistant sample was reported as any bacterial isolate resistant to at least one antimicrobial agent.

Results 92/293 (31.4%) patients had at least one bacteria positive sample over the study period. 87/92 (94.6%) patients had samples where DSTs were performed on bacteria positive samples. Resistance was observed in 69/87 (79.3%) patients. 30/293 (10.2%) patients were resistant to all samples where DSTs were performed. 227/293 (77.5%) of patients were sputum producers. There was no significant relationship between predicted FEV₁ and antibiotic resistance frequency in this cohort (X^2 -test; $p=0.577$). Patients who were classified as regular sputum producers were more likely to exhibit resistance in culture positive bacteria ($p=0.048$).

Conclusion Results from this analysis conclude that an estimated 23.5% of COPD patients will develop resistance to an antimicrobial agent within 28.4 months of follow-up with sputum producers being at a higher risk. This study highlights the importance of investigating sputum samples with determination of resistance patterns. Information on resistance patterns and transmission of resistance in COPD can allow more appropriate and targeted antibiotic therapy for COPD exacerbations with improved outcomes.

S18 A COMPARISON OF PREVALENCE AND LOAD OF AIRWAY BACTERIA IN COPD PATIENTS WITH PAIRED STABLE AND EXACERBATION STATE SAMPLES

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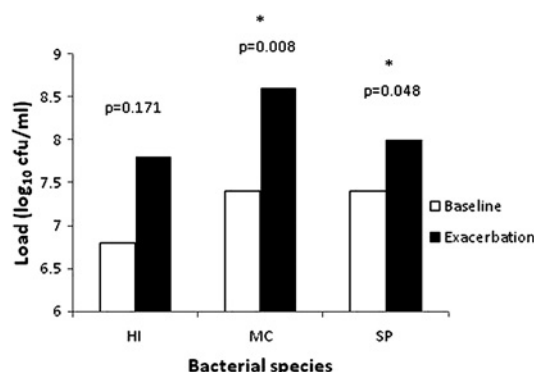
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Introduction Airway bacterial infections are associated with COPD exacerbations. The most frequently identified bacteria in COPD are *Haemophilus influenzae* (HI), *Moraxella catarrhalis* (MC) and *Streptococcus pneumoniae* (SP) (Wilkinson *et al*, 2006), though studies have used culture techniques, with little data available on PCR methodology in airway infection. Using the London COPD cohort, we aimed to assess and quantify bacterial prevalence and load via quantitative PCR, in paired baseline and exacerbation sputum samples.

Methods Quantitative PCR was utilised, measuring prevalence and load on paired baseline and exacerbation samples, with baseline samples obtained within 1 year prior to its paired exacerbation. SP, HI and MC gene targets were Spn9082; *Haemophilus influenzae* P4 lipoprotein gene; copB outer-membrane-protein gene, respectively. The baseline state was defined as being at least 6 weeks after, and 2 weeks before, an exacerbation. Exacerbation was defined as two consecutive days of at least two increased symptoms (Anthonisen criteria), at least one of which is a major symptom (dyspnoea; sputum purulence; sputum volume).

Results Sixty-nine paired baseline and exacerbation sputum samples were obtained from 56 patients: mean (\pm SD) age 71.0 years (\pm 8.4); predicted FEV₁ 46.4% (\pm 17.0); male gender 60.4%; current smoker 30.2%. Bacteria were detected at significantly higher rate at exacerbation, being seen in 36/69 (52.2%) exacerbations, and 19/69 (27.5%) baseline samples (χ^2 -test; $p=0.003$). Mean bacterial load was significantly higher at exacerbation, with mean load of 8.3 (\pm 1.1) log₁₀ cfu/ml, compared with mean of 7.3 (\pm 1.8) log₁₀ cfu/ml at baseline (paired-samples t test; $p<0.001$), indicating a 10-fold overall-load increase at exacerbation. MC frequency increased significantly from 4.3% (3/69) at baseline to 17.4% (12/69) at exacerbation ($p=0.014$). Prevalence of HI (17.4% vs 26.1%) and SP (8.7% vs 20.3%) showed non-significant increases. Mean loads of SP and MC increased significantly from baseline to exacerbation ($p=0.048$; $p=0.008$, respectively).

Conclusion Prevalence and load of airway bacteria in COPD increases from baseline to exacerbation. This confirms that bacteria play an important role in exacerbation aetiology, implicating increasing bacterial load as a key underlying mechanism, and emphasises the importance of prompt antibiotic therapy at COPD exacerbation.



Abstract S18 Figure 1 Bacterial load at baseline and exacerbation of COPD as determined by quantitative PCR.

S19 MOLECULAR FINGERPRINTING AND METAGENOMIC ANALYSIS REVEALS A POLYMICROBIAL ELEMENT IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Introduction and objectives Chronic obstructive pulmonary disease (COPD) patients commonly exhibit a multi-factorial pathology with neutrophilic inflammation and chronic obstructive bronchiolitis. COPD patients suffer episodes of pulmonary exacerbations. The role of bacteria in exacerbations has been investigated in COPD using culture-dependent techniques. Unlike cystic fibrosis (CF), there are few molecular studies describing the possibility of a

microbial community in the COPD lung contributing to the pathogenesis of COPD.

Methods Nine clinically stable COPD patients attending the Freeman Hospital had a bronchoalveolar lavage fluid (BALF) taken. DNA extraction from these samples was performed using an Ultraclean® Microbial DNA Isolation Kit. DNA obtained from these samples was then used as template for conventional PCR. Both primer sets used targeted the universal bacterial and fungal V3 variable regions of the 16S rRNA gene and 28S rRNA gene respectively with attachment of a GC-clamp. Amplicons were then run out for analysis by denaturing gradient gel electrophoresis (DGGE) performed on a DCode System (BIO-RAD). Microbial DNA extracted from all nine BAL samples was then sent for 454 pyrosequencing to perform metagenomic analysis.

Results Molecular fingerprinting of BAL analysis by DGGE produced a distinct number of bands in each sample strongly indicating the presence of a diverse microbial community in the COPD infected lung. This was also seen in culture negative patients. Migration of bands present at the top of the denaturing gradient suggests that the lungs of COPD patients are heavily colonised with bacteria that have a low GC content such as *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Moraxella catarrhalis*. Metagenomic analysis of the nine BAL samples by 454 pyrosequencing supports this hypothesis by detecting numerous other bacterial taxa present.

Conclusions This preliminary study shows that the lungs of COPD sufferers are colonised with multiple species of bacteria and demonstrates that a complex microbial community is present. Metagenomic analysis performed demonstrates the key bacterial taxa which may be responsible for inducing the damaging inflammatory response and the differences in bacterial diversity shown in the nine patients studied. Thus a complex microbiota may elicit ongoing inflammation leading to lung function loss and destruction of the lung architecture.

Pulmonary thromboembolism: acute and chronic studies

S20 TIME-RESOLVED CT PULMONARY ANGIOGRAPHY CONTRAST TRANSIT TIME IN PATIENTS WITH PULMONARY EMBOLISM: A NOVEL FUNCTIONAL CT METRIC OF RIGHT HEART STRAIN?

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Introduction and Objectives Acute right ventricular (RV) failure is known to cause death in patients with pulmonary embolus due to circulatory collapse. CT pulmonary angiogram (CTPA) is now

considered the gold standard test for the detection of pulmonary emboli, the technique provides excellent structural detail, however provides limited functional information. This aim of this was to assess time-resolved CTPA contrast transit times (TT) as a potential functional CT marker for the detection of right heart strain in patients with PE.

Methods We retrospectively reviewed consecutive patients who underwent CTPA at our institution over a 2-month period. Scans were performed on a Phillips Brilliance 16-slice scanner with a 4 ml/s OptirayTM 300 pressure injection. TT was defined as the time from the start of the injection to the scan trigger at the threshold of 150 hounsfield units measured from ROI analysis at the main pulmonary artery. Established CT structural imaging metrics were scored for comparison.

Results 56 consecutive patients were identified with evidence of pulmonary embolic disease or normal thoracic CT appearances from CTPA scans. One patient with PE was excluded as the CTPA scan was non-diagnostic. TT, RV septum to free wall distance, RV/LV ratio and PA diameter were all significantly elevated in patients with pulmonary embolus compared to patients with a normal CTPA. On analysis of bivariate correlation, TT had a statistically significant positive correlation with hepatic reflux, PA diameter and RV/LV ratio. Notably, the TT and RV/LV ratio demonstrated a significant direct linear correlation ($p=0.001$).

Conclusions This study supports previous evidence of existing markers, such as RV/LV ratio, being useful imaging marker in pulmonary embolic disease. It also suggests that TT, could be a new useful functional marker of right heart strain. The importance of further research into this field is highlighted, and particularly into TT as a haemodynamic prognostic indicator in acute pulmonary embolism.

S21 OBJECTIVE AND PATIENT REPORTED OUTCOMES OF LONG TERM MANAGEMENT OF PATIENTS WITH CHRONIC THROMBOEMBOLIC PULMONARY HYPERTENSION (CTEPH): A SINGLE CENTRE EXPERIENCE

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Objective To determine long-term objective and Patient Reported Outcomes at a single centre in patients with CTEPH (distal or residual post Pulmonary endarterectomy).

Method A retrospective study of all incident patients with CTEPH (2000–2011). Data obtained from the service database—6-minute walk test (6MWT), CAMbridge Pulmonary Hypertension Outcome Review (CAMPBOR) symptom, activity and Quality of Life (QoL) scores, and NTproBNP. Results obtained at baseline (at the time of diagnosis, pre-commencement of first targeted therapy), 3 months,

Abstract S21 Table 1 Summary of change scores over time for CTEPH cohort

	6MWT	CAMPBOR symptoms	CAMPBOR activity	CAMPBOR QoL	NTproBNP
CTEPH	n=98	n=75	n=77	n=76	n=64
3-month change score mean (SD)	30.03 (77.2)†	1.1 (3.8)*	0.7 (3.4)*	1.1 (4.1)†	714.9 (2342.2)†
CTEPH	n=97	n=80	n=79	n=79	n=60
1-yr change score mean (SD)	37.03 (72.69)†	1.8 (4.6)†	0.7(4.3)	1.6 (4.7)†	891.6 (1815.5)†
CTEPH	n=80	n=62	n=61	n=61	n=50
2-yrs change score mean (SD)	35.1 (76.1)†	0.9 (4.4)	0.1 (4.6)	1.1 (5.1)	904.7 (1832.80)†
CTEPH	n=61	n=56	n=56	n=54	n=39
3-yrs change score mean (SD)	40.4 (74.6)†	1.3 (4.6)*	0.1 (5.2)	0.3 (4.3)	1009.4 (2083.9)†
CTEPH	n=44	n=34	n=34	n=34	n=29
4-yrs change score mean (SD)	18.8 (97.2)	0.9 (3.6)	0.3 (4.8)	0.4 (4.9)	780.1 (1695.1)*

Wilcoxon signed rank test * Significant ($p<0.05$), † Highly significant ($p<0.01$).