Methods Adult patients were recruited from specialist bronchiectasis and Cystic Fibrosis clinics. The gold standard for diagnosing *P. aeruginosa* infection was positive sputum cultures. 72 sputum samples were analysed. A sputum sample was kept in a glass vial with a cap containing septum. The septum was pierced with a solid phase microextraction (SPME) fibre allowing sampling of the headspace for 50 min at 37°C before transferring the fibre into gas chromatography mass spectrometry. AnalyserPro software (automated peak capture software) and manual identification were used to identify relevant to *P. aeruginosa* specific compounds in the headspace of sputum.

Results 32 samples grew *P. aeruginosa* either on its own or mixed with other species. 2-nonanone was a marker of *P. aeruginosa* in sputum headspace gas with sensitivity of 72% and specificity of 88%. Cyanide was not detected. However, a combination of manually identified 2-nonanone with 17 other volatile compounds as identified by AnalyserPro, increased sensitivity in detection of *P. aeruginosa* to 91% with specificity of 88%.

Conclusion Optimal sampling and capture protocols still need refinement: we were unable to detect the prior noted biomarker Cyanide. These data however demonstrate the potential for rapid and accurate diagnosis of *P. aeruginosa* infection from sputum samples. In contrast to the 48+ hour turnaround for standard microbiological culture, these results were available within 1–2 h. It also provides a library of compounds as targets to validate in a future study of breath testing.

Abstract S22 Figure 1

Conclusions Results from this large cohort suggests that LCI is a more sensitive test of early CF lung disease, and correlates better with extent of bronchiectasis seen on CT, than FEV1. Validation of data from subsequent study visits is in progress and will be reported at a future date.

Abstract S23 A COMPARATIVE STUDY OF POLYMICROBIAL DIVERSITY IN CF AND NON-CF BRONCHIECTASIS

Introduction Bronchiectasis is a dilation of the peripheral airways with subsequent mucus hypersecretion. Bronchiectasis can be either genetic, that is cystic fibrosis (CF) or described as non-CF bronchiectasis (eg, idiopathic or post infectious bronchiectasis). Recently, many studies have demonstrated polymicrobial bacterial communities are present in the lower respiratory tract (LRT) of cystic fibrosis (CF) sufferers. These studies have identified complex microbial communities that are affected by many factors including age; CFTR genotype and antibiotic therapy. One prior abstract noted greater diversity in non-CF bronchiectasis as compared to CF (Bilton et al, 2009) though the sample size was small. Our aim is to extend prior work by comparing the metabolically active bacterial and fungal communities present in sputum samples from CF patients with those from non-CF bronchiectasis.

Methods Adult CF and non-CF bronchiectasis patients provided spontaneously expectorated sputum samples which were treated with RNAsafe. RNA was extracted from sputum samples and reverse transcribed to cDNA; this was the template for bacterial and fungal community PCR amplification using universal 16S or 28S primer sets. Amplicons were analysed by denaturing gradient gel electrophoresis (DGGE) which separates double stranded DNA based upon bacterial and fungal genomic GC content sequence. Common pathogens were identified such as *Pseudomonas aeruginosa* and *Haemophilus* spp. by comparison to a 16S or 28S standard ladder from pure cultures.
Results We have compared CF and non-CF bronchiectasis (n=36 combined). Polymicrobial communities were observed in all CF and non-CF bronchiectasis patients. However, CF patients demonstrated a greater bacterial diversity with a mean of 14.77 species per sample (range 6–21) than non-CF bronchiectasis patients who had a mean of 9.67 species per sample (range 4–14). However, fungal communities were similar between CF and non-CF bronchiectasis patients with 73.3% and 75% of patients harbouring fungi in their LRT respectively. Similarly, CF patients had a mean of 1.58 fungal species per sample (range 0–4) whilst non-CF bronchiectasis patients had a mean of 1.16 fungal species per sample (range 0–5).

Conclusions We note a complex microbiota in the lungs of both CF and non-CF bronchiectasis patients. In contrast to other studies using DNA based molecular analysis we note an increased microbial and non-CF bronchiectasis patients. In contrast to other studies

Paediatric infectious diseases

**S24**

**DIFFERENTIATED PRIMARY BRONCHIAL EPITHELIAL CELL (pBEC), MONOCYTE DERIVED MACROPHAGES (MdM) AND MONOCYTE DERIVED DENDRITIC CELLS (MoDCs): TRANSWELL CO-CULTURE: RESPIRATORY SYNCYTIAL VIRUS (RSV) INFECTION OF THE APICAL AND BASOLATERAL SURFACES**

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**Introduction and Objectives** RSV causes winter epidemics of respiratory disease. Active infection is virtually absent in summer months. Infected ciliated airway epithelial cells, local macrophages and dendritic cells secrete cytokines including interleukins (IL) 6 and 8, promoting a strong neutrophilic response that is important in disease clearance and airway inflammation. In *vitro*, RSV is capable of infecting MoDCs. RSV inhibits their maturation and can remain dormant in these cells. Dormant RSV can be stimulated to replicate with exogenous nitric oxide. These MoDCs are then able to re-infect HeLa cells (a lab strain of immortalised cervical cells). The following hypothesis was explored: *Infected dendritic cells act as a reservoir for RSV over summer months.*

**Aim** The aim of this study was to investigate the effect of RSV on pBEC and MoDC cell lines across a semi permeable membrane in the presence of MdMs.

**Methods** Primary bronchial epithelial cells were seeded in the apical part of the transwell model at 1×10^5^ cells per ml and differentiated over 21 days on an air liquid interface. MoDCs were seeded on the basolateral part of the transwells at 1×10^6^ cells/ml. MdM were seeded on top of the epithelial layer in selected experiments (see Abstract S24 Figure 1). Red fluorescent RSV (rr-RSV) was added to the apical side at a concentration of 1×10^6^ plaque forming units (pfu)/ml with un-infected MoDCs on the basolateral side, and uninfected pBECs in the apical side were cocultured with MoDCs previously infected with rr-RSV at 5×10^5^ pfu/ml. Controls were uninfected pBECs with uninfected MoDCs or with just media on basolateral side. Red fluorescence (marker for active infection) was measured at 24, 48 and 168 h by flow cytometry.

**Results** Directly exposed pBECs and MoDCs were infected at 24 h. Indirectly exposed pBECs were infected at 48 h. Indirectly exposed MoDCs were infected only when MdMs were present on the overlying epithelial cell layer.

**Conclusions** RSV is able to infect MoDCs and pBEC across a semi-permeable membrane in our *in vitro* model of airway epithelium, supporting MoDCs as a potential reservoir of RSV.
S23 A comparative study of polymicrobial diversity in CF and non-CF bronchiectasis

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