Background  
Burkholderia cepacia complex (BCC) bacteria are opportunistic pathogens which cause severe lung infections in cystic fibrosis (CF) patients. Treatment of BCC infections is difficult due to the inherent multidrug resistance of BCC. There is a pressing need to identify CF patients with Aspergillus colonisation, using real time PCR, and examine the relationship of colonisation to markers of sensitisation.

Methods  
108 adult CF patients provided a sputum sample and a blood sample. Serological tests included total IgE, specific A. fumigatus IgE and specific A. fumigatus IgG performed by Phadia ImmunoCAP® assay, and A. fumigatus precipitins by counter immunoelectrophoresis. Sputum was homogenised with sputatal and sonication. 10 µl was cultured on sabouraud agar (Oxoid, UK) for 72 h. The remaining sample was used in a commercial real time PCR assay, MycAssay Aspergillus. Patients on antifungal treatment were excluded from serological data analysis.

Results  
30% of the 108 sputum samples were positive for Aspergillus species by standard culture whereas 80% were positive for Aspergillus species by PCR. 15 patients were on antifungal therapy of whom 7 were PCR positive. Of the serological tests, only specific IgG related to a positive PCR. Using a ROC curve, a specific IgG level above 65 mg/l gave 85% sensitivity and 100% specificity for positive PCR. 12 patients met the 2005 consensus minimum criteria for ABPA. All were PCR positive supporting the use of antifungals for ABPA. 38 patients were sensitised to aspergillus (specific IgE >0.4 kUa/l), 28 of these were PCR positive. A group of 32 patients was identified that had a rise in specific IgG and positive PCR but no IgE rise. They may represent ‘aspergillus bronchitis’. All patients with negative serology were PCR negative.

Conclusion  
Real time PCR can accurately identify CF patients with Aspergillus in their sputum, including those in whom antifungal therapy is inadequate. However, PCR alone cannot distinguish between ABPA, sensitisation and colonisation. Positive PCR correlates to a specific IgG >65 mg/l. A randomised trial of antifungal therapy is required to determine if there is clinical benefit in treating PCR positive patients.

Results  
Q22 inhibited growth of all 9 BCC species tested, including B. cenocepacia. A reduction in growth rate and cell morphology changes were also observed (Abstract S20 Figure 1). Higher concentrations of Q22 were required to exert B. cenocepacia growth effects (30 µg/ml Q22) when compared to P. aeruginosa (5 µg/ml Q22), probably due to the presence of two mreB genes in the B. cenocepacia genome. BCC bacteria lipopolysaccharide (LPS) is known to play an important role during infection. We analysed the LPS profile of BCC bacteria grown in the presence of Q22 and selected strains show profile differences when compared to untreated bacteria. The influence of Q22 treatment on bacterial motility and Type 3 secretion was assessed. However, growth inhibition masked motility analysis and differences observed in secreted protein profiles could not be attributed to Type 3 secretion. The growth conditions required for induction of Type 3 secretion in vitro remain undefined.

Abstract S20 Figure 1

A, B. cenocepacia J2315 grown in LB with no additives;  
B, B. cenocepacia J2315 grown in LB containing 30ug/ml Q22

Conclusion  
In vitro MreB is an attractive new target for novel antimicrobials. Further analysis of current observations and additional phenotypic analysis will be required to dissect the nature of Q22-induced changes. Work supported by Newcastle-Upon-Tyne Hospitals Special Trustees and Italian CF Research Foundation (FFC).
**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. AnalyzerPro 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 AnalyzerPro, 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.

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**Introduction** Lung Clearance Index (LCI) is a measure of lung gas mixing derived from the Multiple Breath Washout (MBW) test. We present LCI, FEV1 and CT data from the Run-In Study in preparation for a multi-dose trial of nebulised gene therapy for CF.

**Methods** MBW, spirometry and low-dose HRCT chest were performed as part of the first Run-In Study visit. LCI was reported as the mean result from at least two technically acceptable sulphur hexafluoride MBW tests performed using a modified Innocor gas analyser. Spirometry was performed to ERS standards. CT scans were assessed by two independent radiologists for extent and severity of bronchiectasis, wall thickening, presence of small and large airway plugs, and gas trapping.

**Results** 191 patients attended visit 1, mean (range) age 22.6 (10–59) years. Validated LCI, FEV1 and CT results were available for 167, 191 and 150 patients, respectively. Mean (SD) FEV1 was 72 (19)% predicted. Mean (SD) LCI was 10.7 (2.7). With mean intravisit coefficient of variation of 4.9%. LCI correlated negatively with FEV1 (r = −0.68, p < 0.001), but was abnormally elevated in 72% of participants with normal FEV1 (see Abstract S22 Figure 1; triangles indicate FEV1 >80% and LCI >7.5). 95% CI for LCI in normal subjects 5.9 to 7.5. Both FEV1 and LCI correlated with all CT measures (p < 0.001), most strongly with extent of bronchiectasis. LCI correlated better than FEV1 with extent of bronchiectasis, r = 0.72 (p < 0.001) vs r = −0.61 (p < 0.001), respectively.

**Abstract S22 Figure 1**

**Conclusions** Results from this large cohort suggest 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.

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**Introduction and Objectives** 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 RNAse. 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.

**S22 LUNG CLEARANCE INDEX, FEV1 AND CT FINDINGS IN CYSTIC FIBROSIS: DATA FROM THE UK CF GENETIC THERAPY CONSORTIUM RUN-IN STUDY**

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

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**Introduction** 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 RNAse. 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.

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S21 Identification of *Pseudomonas aeruginosa* infection via volatile organic compounds in sputum headspace gases

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