or sensitised patients would benefit from antifungal treatment. To aid treatment decisions and to monitor response more accurate methods to detect *Aspergillus* in sputum are needed. This study aimed 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 sputasol 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 correlated to 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 2003 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.

S20 THE BACTERIAL CYTOSKELETON—A NEW ANTIMICROBIAL TARGET IN CYSTIC FIBROSIS PATHOGENS?

doi:10.1136/thx.2010.150912.20

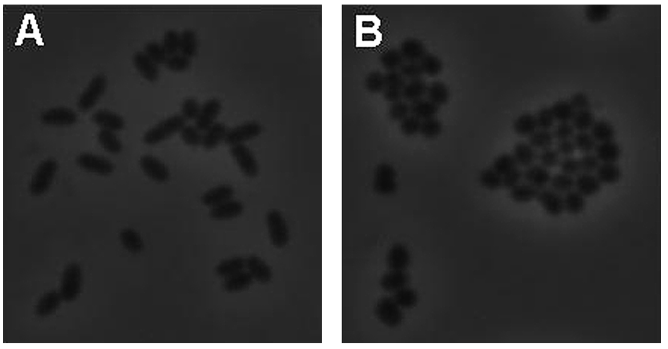
[1]S C Carnell, [2]J D Perry, [1]C M A Khan, [3]A De Soyza. *[1]Institute for Cell & Molecular Biosciences, University of Newcastle, Newcastle-upon-Tyne, UK; [2]Department of Microbiology, The Freeman Hospital, Newcastle-upon-Tyne, UK; [3]Institute of Cellular Medicine, University of Newcastle, Newcastle-upon-Tyne, UK*

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 find new bacterial targets for antimicrobials that provide functions essential for cell growth & replication. A major component of the bacterial cytoskeleton is the actin homologue MreB. MreB maintains bacterial cell shape by forming filaments under the bacterial inner membrane. A22 is a cell permeable compound that disrupts MreB, destabilising the bacterial cytoskeleton and altering the bacterial shape.

Aims To investigate the MreB bacterial cytoskeleton as a novel target for antimicrobials.

Methods We have tested a synthetic library of A22-related compounds and identified compound Q22 as a potential antimicrobial of interest against BCC and *Pseudomonas aeruginosa* strains. BCC bacteria have been grown in the presence of Q22 and a number of phenotypic changes observed.

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* (3 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, a virulence associated secretion system, 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.



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

Abstract S20 Figure 1

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).

S21 IDENTIFICATION OF *PSEUDOMONAS AERUGINOSA* INFECTION VIA VOLATILE ORGANIC COMPOUNDS IN SPUTUM HEADSPACE GASES

doi:10.1136/thx.2010.150912.21

[1]S Savelev, [2]J Perry, [3]S J Bourke, [1]R Taylor, [1]A J Fisher, [4]M Petrie, [1]P A Corris, [1]A De Soyza. *[1]Newcastle University, Institute Cellular Medicine, Newcastle upon Tyne, UK; [2]Dept Medical Microbiology Freeman Hospital, Newcastle upon Tyne, UK; [3]Cystic Fibrosis Unit, Royal Victoria Hospital, Newcastle upon Tyne, UK; [4]Newcastle University, Institute of Ageing & Health, Newcastle upon Tyne, UK*

Background *Pseudomonas aeruginosa* is a key respiratory pathogen with a distinctive odour in culture. An elevated level of hydrogen cyanide in the breath has been associated with the presence of *P. aeruginosa* in the airway, thus determining compounds specific to *P. aeruginosa* offers the possibility of a non-invasive diagnostic (breath) test.

Hypothesis Determining relevant to *P. aeruginosa* volatile compounds from sputum headspace gases offers target validation for the development of an electronic nose breath test for *P. aeruginosa*.

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.

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

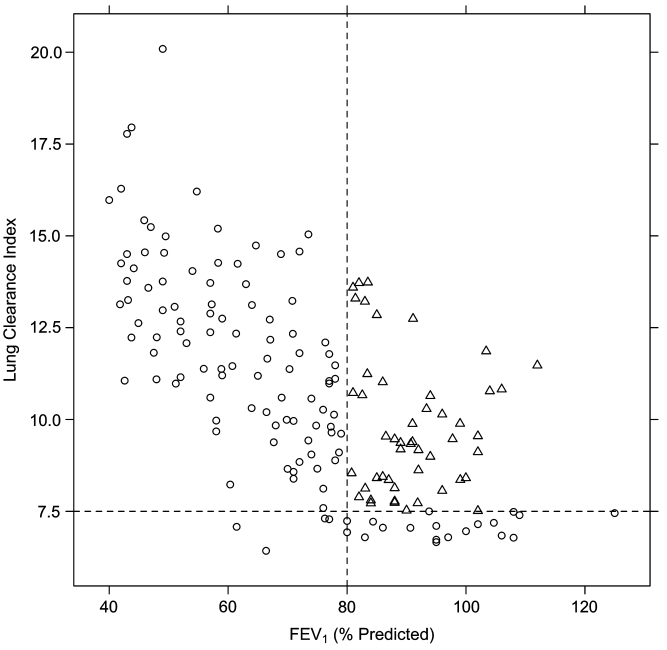
doi:10.1136/thx.2010.150912.22

[1]H S Sheridan, [2]N J Bell, [1]K A Macleod, [2]P A Reid, [3]A R Horsley, [4]G Davies, [4]C Saunders, [1]S Cunningham, [2]J A Innes, [4]J C Davies, [3]E W Alton. *[1]The Royal Hospital for Sick Children, Edinburgh, UK; [2]Western General Hospital, Edinburgh, UK; [3]UK CF Gene Therapy Consortium, UK; [4]Department of Gene Therapy, Imperial College, London, UK*

Introduction Lung Clearance Index (LCI) is a measure of lung gas mixing derived from the Multiple Breath Washout (MBW) test. We present LCI, FEV_1 and CT data from the Run-In Study, a longitudinal 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 (10–59.1) years. Validated LCI, FEV_1 and CT results were available for 167, 191 and 150 patients, respectively. Mean (SD) FEV_1 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 FEV_1 ($r=-0.68$, $p<0.001$), but was abnormally elevated in 72% of participants with normal FEV_1 (see Abstract S22 Figure 1; triangles indicate FEV_1 >80% and LCI >7.5). 95% CI for LCI in normal subjects 5.9 to 7.5. Both FEV_1 and LCI correlated with all CT measures ($p<0.001$), most strongly with extent of bronchiectasis. LCI correlated better than FEV_1 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 suggests that LCI is a more sensitive test of early CF lung disease, and correlates better with extent of bronchiectasis seen on CT, than FEV_1. Validation of data from subsequent study visits is in progress and will be reported at a future date.

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

doi:10.1136/thx.2010.150912.23

[1]S P Cummings, [1]A Nelson, [1]P J Purcell, [2]A De Soyza, [3]S J Bourke, [4]J D Perry. *[1]School of Applied Sciences, Ellison Building, University of Northumbria, Newcastle upon Tyne NE1 8ST, UK; [2]Lung Transplantation and Immunobiology Group, Newcastle University and the Freeman Hospital Newcastle upon Tyne, UK; [3]Adult Cystic Fibrosis Unit, Department of Respiratory Medicine, Royal Victoria Hospital, Newcastle upon Tyne, UK; [4]Department of Microbiology, Freeman Hospital, Newcastle upon Tyne, UK*

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 RNA*later*. 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.