of which have also been isolated from CF patients from other UK hospitals. Isolates from the remaining 3 patients were unique. One additional isolate was identified as *M. abscessus* subsp. *bolletii*. No clear epidemiological connexions between patients within each cluster at our centre have been identified to date.

Conclusion These results provide further evidence that some strains of *M. abscessus* complex may be isolated from multiple CF patients. However, there were no clear epidemiological connexions between patients within clusters at our centre. The same strains have been isolated from patients at different UK CF centres. Further studies are required to determine the mode of acquisition of infection with these strains and whether there is a common environmental source of infection or cross infection between patients.

P200 PRELIMINARY EVALUATION OF THE FUNGAL AIRWAY MICROBIOME IN ADULT CYSTIC FIBROSIS BY NEXT-GENERATION SEQUENCING, CULTURE AND STAINING TECHNIQUES

¹IC Felton, ¹S Benson, ¹A Nicholson, ¹K Al-Shafi, ²P James, ²MJ Cox, ²AJ Walley, ²MF Moffatt, ¹D Bilton, ¹MR Loebinger, ¹NJ Simmonds, ²WO Cookson. ¹Royal Brompton and Harefield NHS Foundation Trust, London, UK; ²National Heart and Lung Institute, Imperial College London, London, UK

10.1136/thoraxjnl-2014-206260.329

Introduction The prevalence and diversity of fungal airway isolates is increasing in cystic fibrosis (CF). Amidst an extending spectrum of fungal complications, lack of standardised mycology methods and poor sensitivity of culture-dependent techniques renders interpretation of isolates challenging.

Aims To evaluate the diagnostic utility of fungal cytology and microbiology stains in addition to prolonged sputum culture from adult CF patients in comparison to standard mycology techniques.

Secondly, to develop a novel, next-generation sequencing assay targeting the ITS2 region of the fungal ribosomal-RNA gene to comprehensively profile the sputum fungal microbiota.

Methods Sputum samples were investigated by a panel of three mycology techniques: prolonged fungal culture (each examined at: Day7, D14, D21, D28); Calcofluor White (CFW) stain; Grocott's Methenamine Silver (GMS) stain. A cohort of samples was also subject to broad-spectrum fungal next-generation sequencing.

Results 25 adult patients provided 45 sputum samples. Four fungal species were cultivatable: *Candida* species (26.6%); *Aspergillus fumigatus* (4.4%); *Scedosporium apiospermum* (15.5%) and *Exophiala dermatitidis* (11.1%).

Prolonged culture significantly increased overall fungal prevalence by 22% compared to standard duration (D7) $p = 0.008$. A significant increase of 11.1% in *S. apiospermum* prevalence was observed $p = 0.02$, whilst all *E. dermatitidis* isolates required prolonged culture. The sensitivity of GMS and CFW stains (85% and 93%) compared favourably to standard duration culture (29%).

DNA extracted from a pilot group of these sputum samples ($n = 14/45$) was subject to PCR using barcode-indexed ITS2 primers designed for Illumina-MiSeq amplicon sequencing. Fungal taxa were detected in all samples, of which seven samples (50%) were negative after prolonged culture. Preliminary sequencing analysis of an extended sample cohort ($n = 30$) has detected 89 fungal taxa, from which only four species were cultured.

Conclusions Prolonged fungal culture is associated with a significant increase in fungal prevalence. The increased sensitivity is restricted to less common filamentous fungi associated with increasing pathogenicity: *S. apiospermum* and *E. dermatitidis*. The predictive value of stains in identifying samples positive at prolonged culture, but negative at standard duration illustrates their clinical utility.

Illumina-MiSeq ITS2-amplicon sequencing directly from sputum has identified a more diverse CF airways fungal microbiota. Preliminary analysis suggests that this is a highly sensitive tool for detecting fungi from sputum, including species which are refractory to standard and enhanced culture.

P201 PNEUMOCYSTIS JIROVECCII PREVALENCE IN A LARGE UK ADULT CYSTIC FIBROSIS CENTRE

¹HD Green, ¹R Bright-Thomas, ¹PJ Barry, ¹A Horsley, ²K Mutton, ¹AM Jones. ¹Manchester Adult Cystic Fibrosis Centre, University Hospital of South Manchester, Manchester, UK; ²Clinical Virology Department, Central Manchester University Hospitals NHS Trust, Manchester, UK

10.1136/thoraxjnl-2014-206260.330

Introduction and objectives *Pneumocystis jirovecii* (PJ) is an atypical fungus that causes pneumonia in immunocompromised patients. Its role in patients with cystic fibrosis (CF) is unclear. Its reported prevalence in CF ranges from 1–22% but has never been determined in the UK. Here we present preliminary cross-sectional data from an ongoing study at a UK adult CF centre

Abstract P201 Table 1 Comparison of characteristics for patients with positive and negative samples

<i>Pneumocystis jirovecii</i> result			
	Negative	Positive	Sig.
Number	93	7	NS
Demographics			
Male gender, n (%)	52 (55.9)	4 (57.1)	NS
Mean age (yr), SD	33.8 ± 11.1	32.5 ± 10.1	NS
Spirometry and laboratory results			
Mean FEV ₁ (% predicted), SD	56 ± 20.1	67 ± 29.5	NS
Mean BMI (kg/m ²)	22.1 ± 3.2	22.2 ± 2.1	NS
WCC (x10 ⁹ /L) at recruitment	9.3 ± 3.1	9.3 ± 2.8	NS
CRP (mg/L) at recruitment	13.0 ± 16.8	7.9 ± 7.5	NS
Chronic colonisation, n (%)			
<i>Pseudomonas aeruginosa</i>	59 (63.4)	5 (71.4)	NS
<i>Staphylococcus aureus</i>	24 (25.8)	1 (14.3)	NS
<i>B. cepacia</i> complex	14 (10.3)	2 (28.6)	NS
<i>Achromobacter xylosoxidans</i>	6 (6.4)	0	NS
<i>Ralstonia</i> species	5 (5.4)	0	NS
NTM	5 (5.4)	1 (14.3)	NS
<i>Aspergillus fumigatus</i>	4 (4.3)	1 (14.3)	NS
<i>Pandora</i> species	1 (1.1)	1 (14.3)	NS
MRSA	1 (1.1)	1 (14.3)	NS
Treatment, n (%)			
Inhaled antibiotics	72 (77.4)	6 (85.7)	NS
Inhaled steroids	59 (63.4)	6 (85.7)	NS
Oral steroids (continuous)	12 (12.9)	1 (14.3)	NS
Prophylactic macrolide	77 (83.0)	5 (71.4)	NS
Azole therapy	8 (8.6)	0	NS
Co-trimoxazole past 3 months	32 (34.4)	0	0.06

Key: SD = standard deviation, WCC = serum white cell count, *B. cepacia* complex = *Burkholderia cepacia* complex, NTM = Non-tuberculous mycobacteria, MRSA = Methicillin resistant *Staphylococcus aureus*, NS = not significant, p values <0.1 are shown.

serving >400 patients, to establish prevalence and potential risk factors for infection.

Methods Sputum samples were obtained from 100 randomly selected CF outpatients and sent for routine microbiology and *PJ* DNA PCR assay at enrolment, subsequent visits, and pulmonary exacerbations requiring intravenous antibiotics within 4 months. Data were recorded for demographics, co-morbidities symptom score, spirometry, inflammatory markers, and prophylactic or recent therapeutic antibiotic therapy. Univariate comparisons were made between sputum *PJ* positive and negative patients. Chi square tests were used for categorical comparisons and independent sample t-tests for continuous independent variables.

Results Of the 100 patients, 4 of 100 had a positive sputum *PJ* PCR at baseline. 50 patients had a routine follow up sample between 1 and 4 months: 2 were positive for *PJ*. 22 patients had a sputum sample analysed at the onset of a pulmonary exacerbation of which 1 was positive for *PJ*. Hence, a total of 7 of 100 patients had a single positive sample by PCR for *PJ*. No patient has had >1 positive sample. None of the baseline parameters were significantly different between *PJ* positive and *PJ* negative patients at the level *p*

Conclusion These results suggest that *PJ* is not an important infecting pathogen in this UK cohort of CF patients. This may be due to frequent use co-trimoxazole for pulmonary exacerbations and high prevalence of prophylactic macrolide antibiotic therapy at our centre compared to other published studies. These preliminary data were underpowered to accurately compare baseline characteristics between *PJ* positive and *PJ* negative patients.

P202 STUDYING THE RELATIONSHIP BETWEEN MATRIX METALLOPROTEINASES AND LUNG TISSUE DAMAGE DURING A CLINICAL EXACERBATION OF CYSTIC FIBROSIS

¹HAP Passman, ²TD Daniels, ²PE Elkington. ¹University of Southampton Faculty of Medicine, Southampton, UK; ²University Hospital Southampton NHS Foundation Trust, Southampton, UK

10.1136/thoraxjnl-2014-206260.331

Background Cystic Fibrosis (CF) is the commonest fatal autosomal recessive condition of Caucasians. The lung in CF is characterised by chronic bacterial colonisation, inflammation, and lung tissue destruction. Matrix Metalloproteinases (MMPs) are proteases that are expressed in inflammatory states that degrade lung matrix. Whilst MMPs are known to be raised in many inflammatory lung conditions, their role in CF is poorly understood.

Aims The study aimed to compare lung function and MMP levels throughout an exacerbation.

Methods Sputum samples and clinical information were obtained with written consent from 23 adult subjects with CF. Samples were collected at day 0, day 14 and week 6 of an exacerbation of CF. Samples were frozen, before Luminex bioassay and IL-8 ELISA was performed. The sputum concentration of MMP 1, 2, 3, 7, 8, 9,10,12,13 and IL-8 were measured.

Results A statistically significant increase in IL-8 concentration (pg/ml) occurred between day 14 and week 6 ($p = 0.0387$). The concentration of MMP-9 decreased from day 0 to day 14 ($p = 0.006$), and then rose significantly between day 14 and week 6 ($p = 0.0412$). However, the concentration of MMP-9 at week 6 was significantly less than at day 0 (0.0453). A significant decrease in the concentration of MMP-8 occurred between day

Abstract P202 Figure 1 The sputum concentration of MMP-9 (pg/ml) at sample time. Values at 3000 represent values above the level of detection. Significant values are represented with a line and an asterisk (*). A significant decrease in MMP-9 concentration was found between Day 0 and Day 14 ($p = 0.006$). An increase in MMP-9 concentration was seen between day 14 and week 6 ($p = 0.0412$). There was a statistically significant decrease in MMP-9 concentration between day 0 and week 6 ($p = 0.0453$)

0 and day 14 ($p = 0.0124$); and between day 0 to week 6 ($p = 0.0426$).

Conclusions MMPs and other inflammatory markers are raised during exacerbations, and fall with treatment. High proteolytic activity may lead to a worsening lung function and contribute significantly to structural changes within the CF lung. Furthering our understanding of this diverse group of proteases could lead to potential novel therapeutic targets which could help prevent irreversible lung damage.

P203 DEVELOPMENT OF AN OPTIMAL F/HN PSEUDOTYPED SIV VECTOR FOR CF GENE THERAPY

¹SC Hyde, ¹Ewfw Alton, ¹AC Boyd, ¹MM Connolly, ¹M Chan, ¹JC Davies, ¹LA Davies, ¹S Gea-Sorli, ¹U Griesenbach, ²M Hasegawa, ¹JA Innes, ²M Inoue, ¹G McLachlan, ¹C Meng, ¹IA Pringle, ¹SG Sumner-Jones, ²SG Tsugumine, ¹DR Gill. ¹UK Cystic Fibrosis Gene Therapy Consortium, Oxford, London and Edinburgh, UK; ²DNAVEC Corporation, Tsukuba, Japan

10.1136/thoraxjnl-2014-206260.332

We are developing lung gene transfer vectors to treat acquired and inherited lung disorders such as cystic fibrosis, and have identified two platforms for efficient respiratory gene delivery: one non-viral system based on CpG-free plasmid DNA combined with cationic lipids (pDNA/GL67A), which has recently completed evaluation in a Phase IIb clinical study; and one novel viral system based on a recombinant simian immunodeficiency virus pseudotyped with the F/HN proteins of Sendai virus (rSIV. F/HN) to promote airway cell uptake. Here we report on the development of a "third generation" rSIV. F/HN vector suitable for use in the clinic. The vector is manufactured by transient transfection of cultured human cells using five producer plasmids, all of which have been engineered to be pharmacopoeia compliant. A variety of vector configurations, including a range of enhancers/promoters and transgenes, were evaluated in a panel of airway models. rSIV. F/HN vectors directed high-level, robust gene expression in fully differentiated human airway cells,