

CD4+ and CD8+ cells producing IFN- $\gamma$ , TNF- $\alpha$  or dual responses was higher in all participants with TB compared with LTBI. CD4+IL-2+ cells were reduced by HIV co-infection, especially IFN- $\gamma$ +IL-2+ cells ( $p=0.008$ ) and this was apparent as a proportion of total cytokine response ( $p=0.016$ ).

**Conclusions** The proportion of CD8+ IFN- $\gamma$  or TNF- $\alpha$  responders was a more sensitive indicator of TB stage than CD4 responses. CD4 + IL-2 responses were vulnerable to HIV co-infection, possibly affecting CD8+ IFN- $\gamma$  and TNF- $\alpha$  responses at high viral loads, increasing susceptibility to active TB. These immune correlates of the TB spectrum and the MTB-specific T-cell deficiencies caused by HIV co-infection are important in rationalising treatment of co-infection as well as testing new vaccines and therapeutics.

## Cystic fibrosis: bench to bedside

### S44 LUNG CLEARANCE INDEX (LCI) AND FEV<sub>1</sub> CORRELATE EQUALLY WITH TREATMENT BURDEN AS MEASURED BY CYSTIC FIBROSIS QUESTIONNAIRE-REVISED (CFQ-R)

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**Introduction** LCI derived from multiple breath washout (MBW), measures the elimination of an inert marker gas during tidal breathing and is a sensitive measure of ventilation inhomogeneity in CF. LCI is more sensitive than FEV<sub>1</sub> and FEF<sub>25-75</sub> in detecting airways abnormalities and does not require a forced manoeuvre. The CFQ-R is a validated patient reported outcome used to assess health related quality of life (HRQoL) and patient perception of symptoms. There is a need to better understand the relationship between LCI, HRQoL and symptoms.

**Objective** To investigate the relationship between LCI, FEV<sub>1</sub> % pred, HRQoL and symptoms as measured by the CFQ-R.

**Methods** These data are part of a larger study investigating the role of LCI as a tool to monitor lung function longitudinally. Patients were recruited from the adult and paediatric CF centres in Belfast Health and Social Care Trust. Inclusion criteria: clinical diagnosis of CF; clinically stable (exacerbation free=4 weeks); informed consent. Age appropriate versions of the CFQ-R were used (patients >14 years, children aged 12 and 13, children aged 6–11). A parent questionnaire was completed in addition where appropriate (for children aged 6–13). The instrument yielded a score of 0–100 for each domain, with higher numbers indicating better function on various domains. Participants completed three MBW tests, using 0.2% sulphur hexafluoride and a modified Innocor device. LCI was reported as the mean of at least 2 acceptable tests. Spirometry was performed to ATS/ERS standards.

**Results** Data were collected for 21 patients (15M:6F), age range 6–51 yrs, mean (SD) 26.4 (13.7). Mean (SD) FEV<sub>1</sub> % pred was 77.1 (16.3). Mean (SD) LCI was 9.4 (2.5) (normal <7.5). LCI correlated negatively with FEV<sub>1</sub>% pred ( $r=-0.62$   $p=0.003$ ). The domain of treatment burden was significantly correlated with LCI ( $r=-0.67$   $p=0.001$ ) and FEV<sub>1</sub>% pred ( $r=0.69$   $p=0.001$ ). However no correlation was observed with respiratory symptoms or any other domain of the CFQ-R.

**Conclusion** Patients with a greater treatment burden are more likely to have more severe lung disease. The severity of CF lung disease as determined by FEV<sub>1</sub>% pred and LCI correlate equally with treatment burden. This further validates LCI as a useful measure of lung function.

### S45 CLINICAL EFFICACY OF SEASONAL INFLUENZA VACCINATION IN ADULTS WITH CYSTIC FIBROSIS

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**Introduction** Influenza vaccination produces an adequate serological response in adults with cystic fibrosis (CF)<sup>1</sup> and is a recommended part of routine CF care. There is little evidence to date, however, of a clinical benefit from influenza vaccination in this patient group. We compared prospectively the rate of influenza infection with vaccination status among 100 adults with CF over the 2010/2011 UK influenza season.

**Methods** 100 adults with CF were enrolled in a prospective observational study of respiratory viruses between December 2010 and March 2011. Sputum, nose- and throat-swabs for PCR-based virological analysis were sent every 2 months and additionally at onset of acute respiratory illness through to June 2011. Prior to enrolment, sputum was sent for virology at onset of pulmonary exacerbations as part of routine care. Details of influenza vaccination status were obtained from the CF centre's database and GP records. Previous infection with influenza A/H1N1 was determined from clinical records.

**Results** Patients had a median age of 28 years (range 18–62). 88% had received the 2010/2011 seasonal influenza vaccine (A/California/7/2009/H1N1, A/Perth/16/2009/H3N2 & B/Brisbane/60/2008). 44% of the cohort had received the 2009 monovalent swine-origin influenza A/H1N1 vaccine and 8 patients had previously had PCR-confirmed swine-origin influenza. Over the study period there were 10 cases of influenza: 5 influenza A/H1N1, 4 influenza B and 1 dual influenza A/B infection. Among patients who received the 2010/2011 seasonal vaccine, 9/88 (10.2%) suffered influenza compared with 1/12 (8.3%) of those who had not been vaccinated (OR 1.25; 95% CI 0.14 to 10.9). All 9/9 patients who developed influenza despite being vaccinated were homozygous for the F508del mutation compared with 43/79 (55.7%) of vaccinated patients who did not develop influenza ( $p=0.009$ ). No significant difference was seen between these groups with regard to age, gender, BMI, lung function, diabetes mellitus or use of oral corticosteroids.

**Conclusions** Influenza vaccination may have limited clinical efficacy in adults with CF. The influence of CF genotype on susceptibility to influenza infection and response to vaccination requires further investigation.

## REFERENCE

1. Ong ELC, et al. Influenza vaccination in adults with cystic fibrosis. *BMJ* 1991;**303**:557.

### S46 A COMPARISON OF THREE DIFFERENT SPECIMEN TYPES FOR THE DIAGNOSIS OF VIRAL RESPIRATORY INFECTIONS IN ADULTS WITH CYSTIC FIBROSIS

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**Introduction** Respiratory viruses have been associated with increased symptoms and a decline in lung function in patients with cystic fibrosis (CF). The optimal means of diagnosis of respiratory viruses in CF is unclear. We compared the suitability of sputum,

nose- and throat-swabs for the diagnosis of respiratory viruses in adults with CF.

**Methods** 100 adults with CF provided sputum, nose- and throat-swabs every 2 months between December 2010 and June 2011 within a prospective observational study. Samples were repeated if new respiratory symptoms developed between regular visits. Specimens were analysed using PCR assays for adenovirus, influenza, metapneumovirus, parainfluenza, respiratory syncytial virus and rhinovirus. Positive viral identification rates for each specimen type were compared. FEV<sub>1</sub>, inflammatory markers and symptom scores for upper respiratory tract infection (URTI) and pulmonary exacerbation (PE<sub>x</sub>) were recorded at each visit.

**Results** 210 sets of samples were collected. A respiratory virus was identified at 52 (24.8%) visits of which rhinovirus accounted for 63%, influenza A 10.5%, metapneumovirus 9% and influenza B 7%. Among virus-positive cases, sputum was positive in 34 (65%), nose swab in 25 (48%) and throat swab in 21 (40%). A single specimen type was positive in 32 (62%) cases; two specimens in 12 (23%) and all three specimens in only 8 (15%). Sputum alone was positive in 17 (33%) cases, nose-swab alone in 8 (15%) and throat-swab alone in 7 (13%). An increasing number of positive specimens was associated with higher mean (SD) URTI scores (4.9 (5.0) vs 6.7 (6.6) vs 10.3 (5.5) for 0, 1 and ≥2 positive specimens respectively;  $p=0.046$  for ≥2 vs 1 specimens) and higher PE<sub>x</sub> scores (2.8 (2.8) vs 3.2 (2.2) vs 5.1 (1.5);  $p=0.002$ ). FEV<sub>1</sub>, CRP and WCC were similar between these groups. There were no significant differences in lung function, symptoms or inflammatory markers when viruses were detected in sputum compared with the upper airways.

**Conclusions** Sputum is superior to nose- and throat-swabs for the diagnosis of respiratory viruses in adults with CF but all three are required for optimal identification rates. Viral positivity in ≥2 specimens is associated with higher upper and lower respiratory symptom scores.

#### S47 PSEUDOMONAS AERUGINOSA INDUCES APOPTOSIS IN HUMAN DENDRITIC CELLS: A POTENTIAL MECHANISM TO EVADE PULMONARY IMMUNE RESPONSES

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**Introduction** Airway colonisation with *Pseudomonas aeruginosa* (PA) represents a hallmark of cystic fibrosis (CF). Mechanisms by which PA establishes pulmonary infection are undoubtedly complex and, in part, will reflect the capacity to interfere with host defense mechanisms. Dendritic cells (DCs) represent the most potent antigen-presenting cells in the lungs, with the unique ability to prime naïve T cells. PA-induced apoptosis has been demonstrated in epithelial cells and macrophages. We examined the capacity of PA to induce cell death in human dendritic cells, the cytotoxicity of clinical PA isolates, and the impact on antigen-presenting capacity.

**Methods** CD14<sup>+</sup> monocytes were isolated from peripheral blood of healthy controls ( $n=11$ ) and individuals with CF ( $n=5$ ). Monocyte-derived DCs were generated by culture in the presence of IL-4 and GM-CSF. DCs were infected with live PA including isogenic laboratory strains of PA103 and PA isolates derived from the sputum of patients with CF. Heat-inactivated PA were utilised to evaluate the role of bacterial membrane components. Presence of early apoptosis and established cell death was analysed via annexin-V and 7-AAD incorporation, respectively. Cytotoxicity was further demonstrated via LDH release into the supernatant. Co-stimulatory molecules, CD40 and CD86, were measured via flow cytometry.

**Results** PA readily induced apoptosis and cell death in human DCs, with cytotoxicity seen within 3 h of infection. Induction of apoptosis by PA was an active process requiring live organisms, but was not dependent on a functional type III secretion system. A significant decrease in viable DCs was seen in response to infection with clinical PA strains at 3 h and 20 h compared with laboratory PA103 strains ( $p<0.05$  and  $p<0.001$ , respectively). Due to increased cytotoxicity of clinical PA isolates, post-infection DCs demonstrated no increase in co-stimulatory molecule expression compared with uninfected DCs ( $p>0.05$ ).

**Conclusions** These data demonstrate that human dendritic cells are susceptible to apoptosis induced by *P. aeruginosa*, with clinical isolates of PA demonstrating high levels of cytotoxicity, and a subsequent reduction in DC antigen-presenting capacity. Elimination of these important antigen-presenting cells could lead to impairment of immune responses and thus a factor in the establishment of chronic PA colonisation in the CF lung.

#### S48 MUCOIDY AND THE MICROBIOME: COMMUNITY COMPOSITION IN RELATION TO THE PRESENCE OF CULTURABLE, MUCOID PSEUDOMONAS AERUGINOSA

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**Introduction and Objectives** Cystic fibrosis (CF) and non-cystic fibrosis bronchiectasis (BX) are chronic airway diseases with significant microbial involvement. The presence of mucoid *Pseudomonas aeruginosa* (mPA) is associated with worse outcomes in these patients. We hypothesised that the presence of this organism would also influence the microbial community structure. We used high throughput sequencing to identify microorganisms present in sputum from these patients, and to associate culture data of mPA with this analysis.

**Methods** Expecterated sputum was collected from 16 patients (9 CF, 7 BX) and DNA extracted using standard protocols. PCR of the bacterial 16S rRNA gene and subsequent sequencing using a Roche 454 GS Junior sequencer was compared with standard clinical culture. Non-parametric t test was used to identify significant differential abundance of taxa in the molecular data (Bonferroni-corrected).

**Results** In the pilot study, 35 000 high quality 16S rRNA sequences were generated and could be assigned to 240 different bacterial taxa. Both CF and BX samples were dominated by the  $\gamma$ -proteobacteria. The presence of culturable *Paeruginosa* in a sample had no significant effect on either community composition or structure. However, Principal Coordinate Analysis indicated that the presence of mPA in clinical culture was associated with a different community structure. Though there were no significant differences between the diversity of mPA positive samples ( $p>0.15$ ), there were significant differences in the abundance of particular species; *Achromobacter* spp. and *Pseudomonas* spp. increased in relative abundance ( $p<0.001$  in each case) and *Haemophilus* spp., *Stenotrophomonas* spp. and *Staphylococcus* spp. decreased in mPA positive vs mPA negative samples ( $p<0.001$  in each case).

**Conclusions** Deep sequencing of sputum samples from CF and BX patients revealed a relationship between culture positivity for mPA and the presence of other known pathogens such as *Achromobacter* spp., suggesting an alternative mechanism for worse outcomes in these patients. This pilot has been extended to a larger cohort of 120 patients in order to confirm the result and data will be presented at the meeting.