Caffeine induced a concentration-dependent decrease in TGFβ activation in iHBECs but had no effect on TGFβ activation in lung fibroblasts. Furthermore, caffeine reduced expression of the TGFβ-inducible genes *PAI1* and *Col1A* and reduced *TGFB1* transcript in epithelial cells. Additionally, caffeine reduced TGFβ-induced proliferation of lung fibroblasts and reduced expression of pro-fibrotic genes including *COL1A* and *ACTA2*. Crucially, *ex vivo* treatment of fibrotic PCLS from bleomycin treated animals with caffeine caused a dose-dependent reduction in collagen deposition after five days. Caffeine had no effect on collagen deposition in PCLS isolated from saline treated animals nor did caffeine affect tissue viability in PCLS from either saline or bleomycin treated animals.

In conclusion, caffeine has anti-fibrotic effects in the lung via concomitant inhibition of epithelial TGF activation and fibroblast responses to TGFb.

S67 VITAMIN D DEFICIENCY DRIVES PULMONARY INFLAMMATION IN A HUMAN MODEL OF THE ACUTE RESPIRATORY DISTRESS SYNDROME INDUCED BY INHALED LIPOPOLYSACCHARIDE IN HEALTHY VOLUNTEERS

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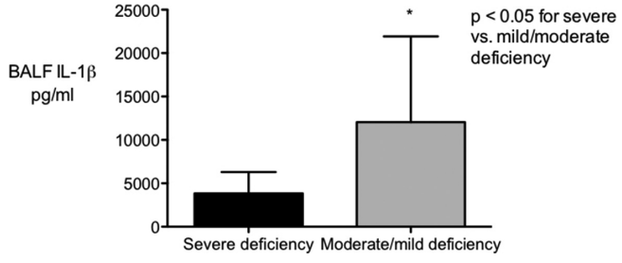
The acute respiratory distress syndrome (ARDS) is characterised by exaggerated alveolar inflammation. Vitamin D deficiency in an LPS induced murine model of ARDS results in exaggerated alveolar inflammation. However the role of vitamin D deficiency in pulmonary inflammation in humans is unclear. We hypothesised that in healthy volunteers with vitamin D deficiency, pulmonary inflammation would be increased following LPS inhalation.

Methods Healthy volunteers inhaled 50 micrograms of LPS and six hours later underwent bronchoalveolar lavage for measurement of cytokines. Plasma was collected at baseline and one day post LPS inhalation for measurement of vitamin D.

Results 28 participants were included. The mean age of volunteers was 26.2 +/- 5.5 years. All 28 patients were vitamin D deficient (plasma levels <50 nmol/l), with 89% (25/28) patients having severe vitamin D deficiency (<25 nmol/l). Vitamin D levels were significantly higher after LPS inhalation ($p < 0.002$). Levels of IL-1β in BALF were significantly higher in those with severe deficiency than those with mild/moderate deficiency (Figure 1; $p = 0.04$). Levels of IL-6, IL-8 or TNF-α did not differ between groups.



Abstract S67 Figure 1 Bronchoalveolar lavage fluid (BALF) levels of IL-1 beta were significantly elevated in volunteers with severe plasma vitamin D deficiency (<25 nmol/l) compared to those with mild or moderate deficiency (25–50 nmol/l)

Conclusions Vitamin D deficiency was highly prevalent in this population of healthy volunteers. The rise in vitamin D levels post LPS exposure may represent mobilisation of vitamin D from fat stores during inflammation though vitamin D metabolism and kinetics are complex and may differ in healthy volunteers and the critically ill. Severe deficiency correlated with increased alveolar inflammation.

Lung infection and primary ciliary dyskinesia

S68 A LONGITUDINAL STUDY CHARACTERISING A LARGE ADULT PRIMARY CILIARY DYSKINESIA COHORT

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Adult Primary Ciliary Dyskinesia (PCD) has not been well characterised. Patients have varied radiological severity of disease and lung function impairment and limited data is available regarding prognosis. In this retrospective study we describe and characterise a large adult PCD cohort, and identify determinates of disease progression using longitudinal lung function data.

We retrospectively analysed 151 adult patients at a single tertiary centre. Overall mortality was 4.6% over a 7-year median follow-up period. Lung function decline was estimated at 0.49% FEV_1predicted/year. Older age at diagnosis showed moderate negative correlation with FEV_1%predicted at diagnosis ($r = -0.30$; $p = 0.01$) and increased *Pseudomonas aeruginosa* colonisation ($p < 0.01$) but not longitudinal FEV_1%predicted ($\beta = 0.001$; (95% CI:-0.35,0.35)). Within multivariate mixed models of FEV_1 adjusting for ciliary ultrastructure, HRCT scoring of severity of bronchial wall dilatation ($p < 0.01$) and extent of bronchiectasis ($p = 0.03$) showed evidence of modifying the decline in FEV_1 with age. Lung function decline additionally differed by ciliary ultrastructure ($p = 0.04$) with patients with microtubular defects having the greatest decline.

Our study reveals a large proportion of adult PCD patients are diagnosed late with lower FEV_1 and increased *P. aeruginosa* colonisation at diagnosis. Increased disease burden on HRCT and microtubular defects on ciliary ultrastructure predicts progressive lung function decline. This study highlights the need for early diagnosis alongside prospective multi-centre disease-specific trials to confirm triggers for lung function decline and identify potential novel therapeutic strategies.

S69 DEVELOPMENT OF AN *IN VITRO* ASSAY TO DETECT CHEMICALLY-INDUCED CHANGES IN CILIARY BEAT FREQUENCY

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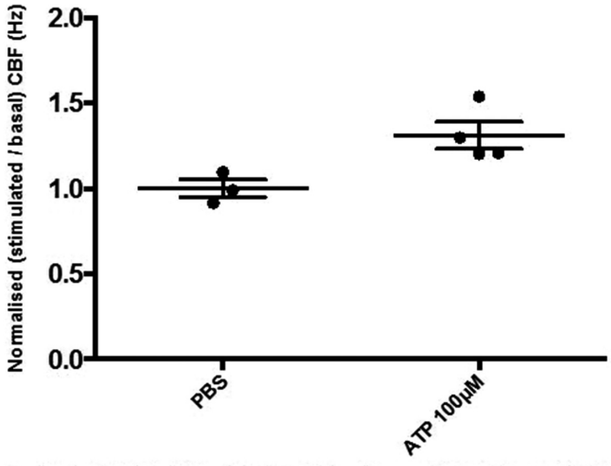
Techniques are well-established to quantify ciliary beat frequency (CBF), which is often reduced in patients with primary ciliary dyskinesia. This project aims to determine the impact of genetic

polymorphisms in the T2R38 (bitter taste) receptor in response to chemical ligands, which is predicted to lead to changes in CBF, which are transient and small in magnitude. These receptors are of interest as they have been shown to 'sense' quorum sensing molecules produced by *Pseudomonas aeruginosa* (Pa) and may thus be disease modifiers in patients with cystic fibrosis. This work describes the development of a rapid CBF assay with sufficient sensitivity to provide a read-out of airway epithelial cell responses to stimulation *in vitro*.

Air-liquid interface (ALI) cultures were obtained from surplus clinical diagnostic samples. Cells in ALI were exposed to phosphate buffered saline (PBS; negative control) and adenosine 5'-triphosphate (ATP; positive control). Experiments were conducted in temperature-controlled wells under a 40× light microscope. Cilia were imaged by high-speed video camera and CBF expressed as a ratio of stimulated/basal frequencies.

CBF increased in ALI cultures exposed to ATP (n = 4) but not PBS (n = 3), in experiments at 24–25°C; this effect was not seen at 37 °C. Mean ±SEM stimulated/basal CBF ratio was 1.00 ± 0.05 in the PBS-exposed cells, and 1.31 ± 0.08 in the ATP-exposed cells (p = 0.029). Inter-observer variability (n = 2) was lower than within-sample CBF variability (95% limits of agreement from -0.66 to 1.62 Hz). Intra-observer variability was good with 95% limits of agreement between -0.31 to 0.52 Hz.

An assay has been developed to detect rapid changes in CBF in ALI cultures using ATP as a positive control. Further work is being undertaken to a) optimise this assay in epithelial cells in suspension, thus increasing throughput, and b) assess more relevant chemicals and culture media. Once optimised, this assay will be used to study the effects of Pa quorum sensing molecules on ciliated epithelial cells *in vitro*, from patients of varying TAS2R38 genotypes.



Ratio of stimulated/basal CBF in ALI cultures exposed first to PBS, followed by PBS (n=3 cultures) or ATP 100µM (n=4 cultures).

Abstract S69 Figure 1

S70 EXPERIMENTAL HUMAN PNEUMOCOCCAL COLONISATION IS AN ASYMPTOMATIC EVENT IN HEALTHY ADULTS

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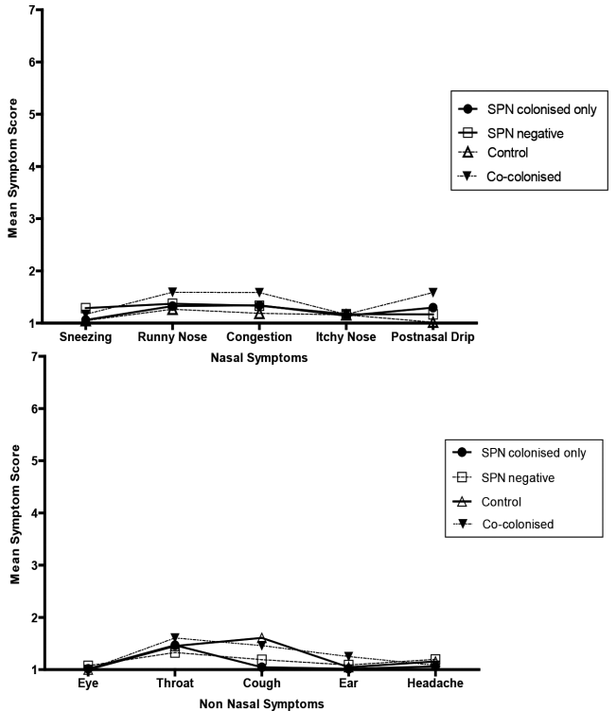
Background Pneumococcal colonisation is a necessary precursor for pneumococcal diseases. Previous studies have suggested that pneumococcal colonisation in children is symptomatic and that there is a relationship between symptom severity/frequency and colonisation density. The literature refers to colonisation in adults as an asymptomatic event but no studies have used robust methodology. Using the Experimental Human Pneumococcal Colonisation (EHPC) model, we investigated whether pneumococcal colonisation (or co-colonisation with a respiratory virus) is symptomatic in healthy adults.

Methods Non-smoking healthy adults aged 18–60 years old were recruited and inoculated intranasally with 0.1 ml per naris of pneumococcus (serotype 6B, 23F) or saline (control). Nasal and non-nasal symptoms were monitored pre-inoculation and for 7 days post-inoculation using a modified Likert score. Symptom severity scoring ranged from 1 (none) to 7 (cannot function). Area under the curve (AUC) was calculated for each participant. Mean values were compared using ANOVA between the groups.

Results Data from 53 participants were analysed. 45 were inoculated with pneumococcus and 8 with saline. In total, 14 became experimentally colonised. Colonised and non-colonised participants reported similar symptom severity scores (Figure 1). Mean severity scores for both nasal and non-nasal symptoms did not significantly differ according to colonisation status ($p > 0.05$), nor was there any significant association with any particular symptom. In the 14 experimentally colonised participants, density of colonisation did not correlate with symptom severity scores ($R^2 = 0.03$, $p = 0.89$). 6 participants were co-colonised (with pneumococcus and a respiratory virus), although they showed a trend towards experiencing more non-nasal symptoms than other groups, their mean symptom severity was still occasional only ($p > 0.05$).



Abstract S70 Figure 1 Mean Symptom Scores for Nasal and Non-nasal Symptoms. SPN = *Streptococcus pneumoniae*. Control = saline. Co-colonised = colonised with *Streptococcus pneumoniae* and virus