(SD) baseline FEV_1 was 97.2% predicted (10.6%). Treatment difference for the mean change from baseline in FEV_1 at Day 29 was 7.0 percentage points ($P=0.0117$). Treatment difference for the mean change from baseline in sweat chloride at Day 29 was –45.9 mmol/L ($P<0.0001$). Treatment effect for the change from baseline in pooled CFQ-R Respiratory Domain score was 4.1 points versus placebo ($P=0.4020$). In the ivacaftor period, AEs and SAEs were reported in 13 and 2 subjects, respectively. In the placebo period, AEs and SAEs were reported in 15 and 1 subject, respectively.

Conclusions In CF patients with mild lung disease, ivacaftor improved ventilation inhomogeneity as measured by LCI and respiratory function as measured by percent predicted FEV_1. LCI is more sensitive to change in this mildly affected group of subjects and may be a useful outcome measure for future interventional trials in such patients.

S122 IS THERE A GENDER DIFFERENCE IN THE UK CF POPULATION?

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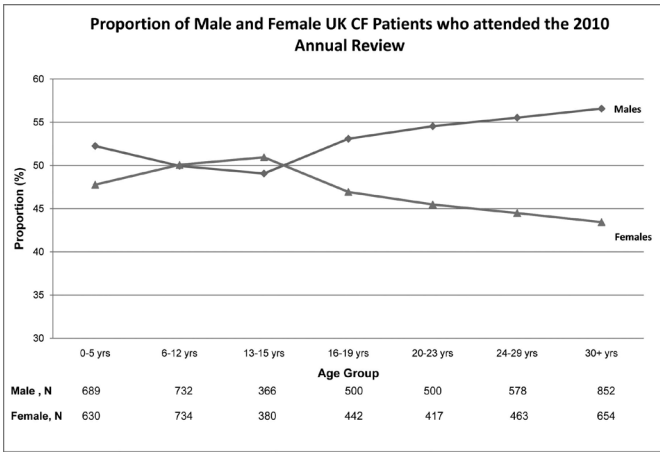
The literature is conflicting with regards to gender differences in the CF population with some studies reporting poorer prognosis in CF females. Variations in study design (cross-sectional v longitudinal and single v multi-centre), study populations (paediatric v adults) and small sample sizes may in part explain these differences. In an attempt to overcome some of these limitations we analysed the UK CF Trust Registry database, which collates data on the paediatric and adult UK CF population for evidence of gender differences.

Methods We used the 2010 annual review data and compared males and females in age-groups 0–5, 6–12, 13–15, 16–19, 20–23, 24–29 and 30+ years for lung function (FEV_1 and FVC (% predicted)), infection status, treatments (rhDNase, O_2 therapy, Non-Invasive Ventilation (NIV), IV antibiotics, supplementary feeding and pancreatic supplements), body mass index and associated complications (CF related diabetes, osteoporosis, liver disease).

Results Of the 7937 patients attending annual review in 2010 47% were female. The sex distribution differed by age whereby the proportion of females dropped from 16 years onwards (Figure 1). The mean FEV_1 was significantly($p<0.001$) lower in female teens aged 16–19 years compared to males (females: 71.0 (confidence interval 68.8–73.2), males 76.7 (74.6–78.8), but not in any other age group. Similar results were observed for mean FVC for which females aged 20–23 yrs also had significantly lower values than males. Teenage female salso had a significantly ($p<0.05$) higher use of rhDNase, gastrostomies, O_2, had more days/yr on IV antibiotics in hospital and had a significantly($p\leq0.001$) higher rate of CFRD. In addition, a significantly ($p<0.05$) higher percentage of females older than 13 had more home IV antibiotics. We did not detect any gender differences with respect to chronic bacterial colonisation(PA, SA, Bcepacia and MRSA).

Conclusions There is evidence of a more severe lung function deficit in teenage years. The reduced proportion of females in the adult cohorts is consistent with a survival difference. Further longitudinal analyses will help characterise the identified gender gap and effects on disease progression.

We thank the UK CF Trust for the registry data.



Abstract S122 Figure 1

S123 THE ROLE OF NASAL POTENTIAL DIFFERENCE TESTING IN DIFFICULT CASES OF POSSIBLE CYSTIC FIBROSIS

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The European CF Diagnostic Working Group guidelines include nasal potential difference (nPD) testing when conventional tests (sweat chloride/*CFTR* mutation analysis) are inconclusive. This specialist measure of epithelial ion transport is available in relatively few centres throughout the UK and Europe and therefore may not be readily considered as a diagnostic option. We have been offering a clinical service for several years, for both our own patients and those referred in. Here, we report the results of a retrospective audit over a 2.5 year period with an aim to increase awareness and understanding of the test.

Between March 2009 and Sept 2011, 66 patients (58% female) underwent testing. Median (range) age was 18 (2–67) years. Children under the age of 8 were most commonly tested whilst under anaesthetic for a bronchoscopy. 59% of patients were referred from other centres. The majority of sweat chloride results (52%) were in the 30–60 mEq borderline range. 95% had undergone first-line CFTR mutation analysis: 62% had none, 37% had one and 1 subject had 2 mutations, one of which was considered a 'sequence variant'.

One child was unable to tolerate the procedure; 6% of traces were uninterpretable due to presumed nasal inflammation. Overall, the nPD results in the context of other features led to a diagnosis of variant/atypical CF in 18%; abnormality in chloride secretion was more sensitive than sodium absorption. We were able to rule out CF 'to the best of current diagnostic ability' in 60% including 6 of 8 patients diagnosed with CF in early childhood, but now considered so unlikely to have the disease that re-investigation was warranted. In a further 13% of cases, nPD was of good quality but was difficult to categorise; in addition to the full CFTR gene sequencing performed routinely, we will investigate these patients for mutations in genes such as ENaC.

Nasal PD was well tolerated and helpful in clarifying the diagnosis in the majority (78%) of patients referred. It should be considered in patients for whom, despite careful work up, a diagnosis of CF is difficult to confirm or rule out.

S124 THE ROLE OF PANCREATIC BETA-CELLS IN THE DIURNAL VARIATION OF GLUCOSE HANDLING IN CYSTIC FIBROSIS

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Introduction An impaired glucose tolerance later in the day may be an early sign of diabetes[1,2], suggesting that there is diurnal variation

in pancreatic endocrine function. This may be important in CF, where glucose handling is deficient even in those without established CF-related diabetes (CFRD). To look at this further, we assessed the response to a glucose challenge throughout the day in CF patients and compared it with healthy controls.

Method We compared 20 CF patients (17 pancreatic insufficient) without known CFRD with 6 healthy age and BMI matched controls. Following an overnight fast subjects consumed a standardised mixed meal (the gold standard measure of endogenous insulin secretion[3], providing an equivalent glucose load to a standard OGTT) at 0800, 1300 and 1800 hours on the same day. Blood glucose and insulin were measured over 120 minutes for each test meal and the area under the curve (AUC) calculated for the entire duration of each test. β-cell indices [β-cell function (%B), insulin sensitivity (%S), insulin resistance (IR)] were measured using the HOMA method[4].

Results See Table (mean±SEM). CF subjects had greater overall glucose levels throughout the day when compared to controls for all 3 tests ($p<0.005$). β-cell function was highest in the afternoon in the CF group in keeping with a lower $AUC_{glucose}$ at this time and there was a decrease in %S and an increase ($p=0.03$) in IR with progression of the day.

Conclusions This study demonstrates insulinopoenia and reduced insulin sensitivity in the CF population resulting in glucose intolerance. Although not the primary defect in CF, there is an increase in insulin resistance as the day progresses. The clinical implications of this study are important not only for the diagnosis of CFRD but also its management in terms of the timing and profiling of exogenous insulin administration.

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Abstract S124 Table 1 Blood Glucose and markers of pancreatic beta-cell function

	Controls			CF		
	Morning	Afternoon	Evening	Morning	Afternoon	Evening
$AUC_{Glucose}$ (mmol/L)	500±27	560±9	571±22	820±33	697±20	831±35
$AUC_{Insulin}$ (μU/ml)	4128±626	3662±548	3582±634	4005±573	3263±482	3449±555
% B	127±11	136±23	152±17	136±19	166±27	149±19
% S	109±22	112±29	71±9	91±8	78±9	77±10
IR	1.0±0.2	±0.2	1.6±0.3	1.4±0.2	1.7±0.3	2.1±0.5

Novel mechanisms in lung fibrogenesis

S125 PRO-FIBROTIC EFFECTS OF MULTI-WALLED CARBON NANOTUBE EXPOSURE ON PRIMARY HUMAN ALVEOLAR TYPE II EPITHELIAL CELLS AND FIBROBLASTS

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Multi-walled carbon nanotubes (MWCNTs) are hollow fibre-like nanomaterials which are being investigated for use in drug delivery and as biosensors. However, due to their structural similarity to asbestos fibres, inhaled MWCNTs may elicit similar adverse health effects such as fibrosis and mesothelioma. Animal studies have suggested that this may be possible, however there is currently limited human data.

We hypothesised that the pro-fibrotic potential of MWCNTs would be determined by their physicochemical properties i.e length and concentration of impurities. We exposed primary human alveolar type II epithelial (ATII) cells and pulmonary fibroblasts to 30nm diameter CNTs of increasing length (0.2–2μm, 3–5μm and 10–30μm) and increasing purity (49%, 69% and >97%) for up to 96 hours. Oxidative stress, TGFβ release, soluble collagen release, and cell proliferation were measured in fibroblasts and release of VEGF, MCP-1, TGFβ and surfactant proteins (SP) A and D were measured in ATII cells.

MWCNTs induced oxidative stress in fibroblasts within 4h and a significant dose-dependent cell proliferation after 96h ($P<0.05$) that was not affected by MWCNT length or purity. Furthermore, there was a significant, dose-dependent, 4–7-fold increase in release of collagen, exceeding what could be accounted for by proliferation alone ($P<0.05$). There was a trend towards shorter and less pure CNTs inducing greater collagen release ($P<0.1$). Release of VEGF and MCP-1 from ATII cells was not induced by CNTs. However, TGFβ, SP-A and SP-D were released by ATII cells and were found bound to MWCNTs. More SP-A bound to the 0.6–2μm MWCNTs compared to the longer MWCNTs; the converse was true for SP-D. In addition the >97% pure MWCNTs bound more surfactant protein than the lower purity MWCNTs. Significantly more TGFβ was bound to the 10–30μm MWCNTs compared to shorter MWCNTs.

Our results demonstrate that MWCNTs can induce pro-fibrotic responses in primary human fibroblasts. Furthermore, our unique discovery of binding of TGFβ and surfactant proteins to MWCNTS suggests that this could exacerbate the fibrotic response if MWCNTs translocate across the epithelial barrier, due to the "Trojan horse" effect of MWCNTs delivering these mediators to the interstitium.

S126 INTERLEUKIN-1 ALPHA (IL-1α) RELEASED FROM INJURED LUNG EPITHELIUM IS A CRITICAL ALARMIN DRIVING ACTIVATION OF A POTENT INFLAMMATORY PHENOTYPE IN LUNG FIBROBLASTS

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Background Activation of the innate immune system plays a key role in exacerbations of chronic lung disease. Myeloid cells are classically considered to drive innate immune responses yet the potential of fibroblasts to act as immune cells has been postulated. We hypothesized that alarmins released from lung epithelium during environmental insults such as oxidant injury and viral infection might induce innate immune responses in lung fibroblasts.

Methods Human bronchial epithelial cells (BECs) and human lung fibroblasts (HLFs) were cultured from brushings taken from lung transplant recipients and resected lung tissue respectively. Cytokine concentrations were measured by ELISA or multiplex platform (MSD). Gene expression was assessed by qRT-PCR. Wild-type and *Il1a-/-* mice were infected with Influenza (PR8). Data were analysed using t-Student or Mann-Whitney U Test. Correlations were assessed using Spearman rank correlation coefficient.

Results Conditioned media from PBECs subjected to oxidant injury contained elevated levels of alarmins. Treatment of HLFs with conditioned media significantly upregulated proinflammatory cytokine expression. Anti-IL-1α or IL-1Ra significantly reduced induction of IL-8 (93% and 95%), IL-6 (90% and 91%), MCP-1 (92% and 93%) and GM-CSF (95% and 94%). Anti-IL-1β had no effect. *Co-stimulation with Poly I:C significantly accentuated the IL-1α induced inflammatory phenotype in HLFs.* Bronchoalveolar lavage (BAL) form