P98 ACCURACY OF ROUTINE ANTIBIOTIC SUSCEPTIBILITY TESTING OF SPUTUM SAMPLES IN ADULT CYSTIC FIBROSIS (CF) PATIENTS COLONISED WITH PSEUDOMONAS AERUGINOSA (PSA)

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Background In CF patients with chronic Psa infection, the role of conventional antibiotic susceptibility testing (antibiotic disc diffusion on sputum subculture morphotypes according to BSAC protocols) is controversial, but many centres still rely on these methods when selecting treatment. However, morphotypes may exhibit up to six antibiograms, and this may contribute to the variable clinical response often noted. To look at this further, we performed additional Psa susceptibility testing on 26 sputum samples from 10 chronically infected CF patients provided over a 15-month period, and compared the results with those from the routine laboratory.
Methods For each sputum sample, 40 colonies proportionately representative of morphological subtypes (mean 2, range 1–4) on the Psa selective plates were cultured onto Columbia plates. From these, single colonies were mixed with sterile distilled water to attain a standard optical density (10 MacFarland units), and 10 µl spread onto iso-sense plates and incubated overnight with tobramycin, meropenem, colomycin, ceftazidime, ciprofloxacin, and piperacillin/tazobactam antibiotic discs. Antibiotic sensitivity (break point 50% of isolates) was determined by the zone of inhibition as per standardised BSAC protocols. In total, 6240 analyses were performed.
Results Although there was 100% concordance with the number of morphological subtypes of Psa with the routine laboratory, on multiple antibiotic susceptibility testing in 260 cases (25%) increased resistance was discovered. Overall, mean concordance between the routine diagnostic lab methodology and multiple antibiotic sensitivity testing was 70% (median 80%, IQR 60–100). However, in 15% of cases concordance was <50%, suggesting that more detailed testing may have altered the choice of antibiotics used.
Conclusion This study shows that routine microbiological methodology may under-represent antimicrobial resistance in Psa when patients are chronically infected. This may in part explain the clinical experience, and underlines the need for better microbiological techniques to aid the clinician in caring for these complex patients.

P99 SOCIAL DEPRIVATION AND CLINICAL OUTCOMES IN ADULT CF PATIENTS

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Introduction Economic and social deprivation is associated with increased morbidity and mortality, and this may be particularly important in chronic disease states such as cystic fibrosis, where there is a high burden of care. Despite this, there have been no studies assessing its impact on adult patients with CF. Our large adult CF clinic takes patients from a wide catchment area, including some of the most deprived areas in the country: we wished to study the impact of this deprivation on the health of our CF patients.
Methods We used the postcode-based Index of Multiple Deprivation (IMD) which takes into account seven domains (including income, education, housing and health-related outcomes), to assign Lower Super Output Areas (LSOAs) in a range of 0–32 486 (where a lower LSOA indicates increased deprivation) to adult patients attending our centre. LSOAs were then correlated with spirometry, BMI, clinic attendances, number and length of inpatient spells, treatment burden, diabetes, and ultimate outcome between 2004 and 2009. Data were analysed using the χ^2 test and bivariate correlations to calculate Pearson's coefficient, where appropriate.
Results Of 219 patients (mean age 27.8 years, range 17–65, 99 females), 113 (57%) lived in the lowest 20% of LSOAs (compared to only 20% nationally). We found no correlation between IMD score and FEV1, BMI, hospital admissions, diabetes and death, but there was an inverse relationship ($r^2=-0.153$, $p<0.05$) with the mean length of inpatient stay.
Conclusion Although many of our adult CF patients live in very deprived areas, this seems to have little impact on their health over a 5-year period, but this is at the expense of more inpatient care, a surrogate marker for more intensive treatment. It is therefore likely that these patients will consume more healthcare resource, and this may need to be factored into any proposed national policy allotting funding towards this complex group of patients.

P100 AN INVESTIGATION OF MUTATOR PSEUDOMONAS AERUGINOSA IN CHRONICALLY INFECTED CYSTIC FIBROSIS (CF) PATIENTS TREATED FOR PULMONARY EXACERBATION

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Background *Pseudomonas aeruginosa* with increased mutation rates due to defective DNA repair are reported in 24–73% of chronically infected patients with bronchiectasis, with or without cystic fibrosis (CF). Mutators may enhance *P aeruginosa* persistence in the lung by accumulating adaptive mutations including antibiotic resistance, and have been associated with worse lung function. In chronic infection the sputum can contain a heterogeneous population of *P aeruginosa* with different phenotypes, including variations in colony morphology (morphotype) and antibiotic susceptibility. Most studies on mutator prevalence however only tested one or two isolates per sputum. Investigation of the clinical relevance of mutator strains relies on accurately assessing the prevalence of hypermutators. We therefore performed a detailed investigation of mutators in sputum before and after antibiotic treatment for acute exacerbation.
Methods Six patients with CF, chronically infected with *P aeruginosa,* were tested before and after 2 weeks antibiotic treatment for exacerbation. Culture, antibiotic susceptibility testing and pulsed-field gel electrophoresis (PFGE) typing were performed using published methods.[1] Mutation rates were measured for up to four colonies of each morphotype. As the original phenotypic method for detecting mutators is very laborious,[2] we developed a method to screen pooled cultures using spiral plater quantitation.
Results 168 *P aeruginosa* isolates were tested (average 14 per sputum). Three patients had no mutators before or after antibiotics and three cultured mutators before and after antibiotics. One to four PFGE pulsotypes and two to six morphotypes were found in each sputum. Both mutators and non-mutators were present in a single sample with no association with morphotype or genotype. Mutators were more likely to be antibiotic resistant, but there was no obvious selection of mutators after 14 days antibiotics.
Conclusions With extensive testing, mutators were found in three of six patients with chronic *P aeruginosa* infection. The phenotypic and genotypic diversity observed means that multiple colonies of each morphotype should be tested to reliably detect mutators. Our method variation allows more extensive testing and may assist future clinical studies.

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P101 SURFACE PARASTERNAL INTERCOSTAL ELECTROMYOGRAM (SEMGPARA) AS A MONITORING TOOL IN CYSTIC FIBROSIS

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Lung function has been traditionally accepted as the primary monitoring tool in cystic fibrosis. The rate of change in lung function however, is slowing and is now as low as 1% per annum. Alternative monitoring tools to assess disease severity are therefore required. Measuring neural respiratory drive (NRD) using diaphragm electromyography (EMG) provides a sensitive measure of load on the respiratory system. The invasive nature of this technique limits is application, however measurement of NRD by sEMGpara is non-invasive and has potential clinical application in monitoring respiratory function in cystic fibrosis (CF).

Hypothesis That NRD measured by sEMGpara%max can be used to assess the change of ventilatory mechanics during an infective exacerbation in CF.

Methods Eight patients [median (range) 20 (20–25) years old, three females] with CF, admitted to hospital with an acute chest infection were studied. The studies were performed within 48 h of admission and on the day of discharge. At both time points spirometry and sEMGpara were measured. sEMGpara was recorded from bipolar surface electrodes placed 3 cm bilaterally from the midpoint of the sternum in the second intercostal spaces (positive electrode on the right side of the chest). The reference electrode was placed on the lateral aspect of the clavicle. For EMG analysis the root mean square (RMS) was calculated and peak RMS of the resting EMG was expressed as a percentage of peak RMS of the maximum (EMG % max) obtained during inspiratory capacity manoeuvres.

Results The median (range) length of stay was 10 (5–22) days. There was a significant reduction in median (range) sEMGpara% max between the first measurement and discharge [19.5 (8–28)% vs 13.5(6–18)% p=0.008]. The reduction in sEMGpara%max was coupled with an improvement in FEV_1% predicted [41 (20–62)% vs 46 (34–85)% p=0.02] and VC% predicted [70 (38–79)% vs 74 (45–90)% p=0.033] on discharge.

Conclusion These findings support the hypothesis that NRD measured by sEMGpara%max has potential as a clinical tool to assess changes in ventilatory function in patients with CF following an acute exacerbation.

P102 QUANTITATIVE BIOLOGICAL IMAGING OF PLASMID DNA IN LIVE HUMAN AIRWAY EPITHELIAL CELLS FOLLOWING NON-VIRAL GENE TRANSFER

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We are interested in non-viral gene therapy for cystic fibrosis (CF). It is widely accepted that in addition to extracellular barriers responsible for inefficient uptake, there are key intracellular obstacles to the nuclear delivery of the therapeutic plasmid DNA (pDNA). Thus, we are investigating the intracellular fate of pDNA following transfection, using the clinically relevant cationic Genzyme Lipid (GL) 67 formulation, using three-dimensional Spinning-Disk real-time confocal, combined with transmission electron microscopy (TEM) to track, quantitate and provide high resolution 'snapshots' of pDNA at the single molecule level in transfected primaryhuman airway epithelial cells (AECs) grown at the air–liquid interface (hALI). The pDNA was tagged with fluorescent, photostable semiconductor quantum dots (Qdot-pDNA) or 1.4 nm gold nanoparticles (Au-pDNA) for use in fluorescence or TEM studies, respectively. Both confocal microscopy and TEM experiments demonstrate that Lipid GL67 was able to transfect AECs with Qdot- and Au-pDNA. The number of gold spots in the nuclei of Au-pDNA-transfected AECs compared with those in unconjugated-pDNA-transfected control cells was significantly higher ($p<0.05$, n=5 independent experiments). Approximately 50% of the total internalised pDNA localised to nuclei within 1 h post-transfection in both confocal (123 AECs, eight independent experiments) and TEM (40 AECs, five independent experiments) studies. Thus, within 1 h pDNA is equally distributed between the cytoplasm and the nucleus in well differentiated human ALIs following non-viral-based gene transfer. Experiments are now underway to track the intracellular trafficking of the pDNA at earlier time points.

P103 ORAL CONTRACEPTIVE USE DOES NOT AFFECT CF DISEASE SEVERITY

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Several studies using a variety of in vitro models indicate that sex hormones such as oestrogen can alter ion transport across epithelial cells by either directly affecting CFTR or altering the activity of alternative chloride channels; such effects may in part explain the gender-difference in disease severity observed in some studies. However, published data are inconsistent with several studies postulating beneficial and others detrimental effects of oestrogen on CF ion transport abnormalities. A large proportion of women with CF regularly use oral contraceptives (OC), but the effect of OC use on disease severity has not been systematically studied. Here, we assessed the effects of OC use in a retrospective study. The data included annual follow-up information from 681 women born between 1937 and 1992 of whom 42% have taken OC for varying periods of time. Data regarding OC use is currently available from 1981 to 2010. We performed an *inter-patient* analysis comparing average yearly changes in FEV_1% and body mass index (BMI) and total days of intravenous (IV) antibiotic use over a 5-year period between matched cohorts of OC users (n=57), (median age at start of study period: 23 (16–45), median FEV_1% at start of study period: 56.2 (20.4–111.1)), and OC non-users (n=57) (median age at start of study period: 22 (17–44), median FEV_1% at start of study period: 48.4 (12.8–119.6)). We found no differences between the groups (median change in FEV_1%: users: −1.87 (−11.5 to 10.4), non-users: −1.03 (−11.8 to 17.9); median change in BMI: users: 0.051 (−1.1 to 1.6), non-users: −0.065 (−1.5 to 3.3); median total days on antibiotics: users: 49 (0–308), non-users: 42 (0–378)). We next performed an *intra-patient* analysis of the same outcomes over a 3-year period on and a 3-year period off OC in the same patient (n=23–27), but again did not detect any differences in any of the clinical outcomes studied. In conclusion, OC use in CF females did not affect FEV_1%, BMI or intravenous antibiotic usage in this study; our findings suggest that there is no evidence of a clinically significant effect on CF outcomes.