

ABSTRACTS OF SOCIETIES

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Oral Abstracts

Lung Clearance Index, Measured with a Novel Photoacoustic Gas Analyser, is a More Sensitive Measure of Airways Dysfunction in Adults with CF than Spirometry.

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Background: The advent of novel therapies for mild cystic fibrosis (CF) lung disease necessitates the development of improved measures of early CF airway damage. Spirometry is insensitive to changes in small airway function, and correlates poorly with anatomical changes demonstrated on CT. Ventilation heterogeneity is known to be sensitive to changes in small airway function, but is difficult to assess routinely. We have adapted a novel, compact photoacoustic gas analyser to measure inhomogeneity of ventilation by inert gas multiple breath washout using 0.2% sulphur hexafluoride (SF₆) as an inert non-absorbed tracer gas. **Methods:** Multiple breath washouts were performed in 46 healthy controls and 25 adult patients with CF. Lung clearance index (LCI) is defined as the number of times lung volume has to be exchanged during quiet breathing to reduce tracer gas concentration to 1/40th of the starting value and was calculated using custom built software.

Results: The group mean (SD) for LCI in controls was 6.72 (0.41), with 95% limits of normality calculated as 5.92-7.51. The group mean (SD) for CF patients was 12.79 (3.62), $p < 0.0001$ compared to controls. LCI was independent of FEV₁ in controls but increased with reducing FEV₁ % in CF patients ($r^2 = 0.65$, $p < 0.0001$). There were 8 CF patients with FEV₁ within the normal range, but all of these had elevated LCI, 5 of these substantially so (figure 1 see www.smj.org.uk). Mean coefficient of variation for intra-visit repeat measures of LCI was 3.5% for controls and 4.6% for CF patients. **Conclusions:** We have demonstrated that LCI can be measured reliably and reproducibly using this novel gas analyser. Results show significant ventilation heterogeneity in those with normal spirometry making it superior for the detection of early disease and of treatment effects in mild patients. We now plan to use this technique to assess airway function longitudinally, though the potential for this device extends far beyond CF.

This abstract won the Methven Prize for best abstract.

Monocyte-based Gene Therapy with the Cationic Host Defence Peptide Elafin increases Clearance of *Pseudomonas aeruginosa* (PA01) from Murine Lung by increasing Macrophage Bacteriocidal Activity

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Background: Neutrophil-mediated lung injury is central to the pathogenesis of severe pneumonia.¹ Elafin is a potent endogenous inhibitor of HNE², is anti-microbial³ and immunomodulatory.⁴ Importantly we have shown that adenoviral delivery of elafin to healthy murine lungs increases clearance of *Pseudomonas aeruginosa* (PA01).⁵ However 'naked' adenovirus (Ad) induces an inflammatory response and delivery of these vectors to the inflamed lung in patients is notoriously difficult. Therefore our research has focused on elucidating the molecular mechanisms by which elafin produces these effects and the potential of monocyte-macrophages as vectors for *in vivo* elafin gene therapy. **Method:** Bone marrow derived macrophages (BMDM) matured for 6 days were transduced with Ad lac Z (control) or Ad elafin for 24 hours at an MOI of 100. For *in vivo* macrophage based gene therapy, mice (n=3) were given 1.5×10^7 cfu/ml PA01 i.t and then 6 hours later were given 2.5×10^5 transduced BMDM i.t. Eighteen hours later mice were killed and lungs lavaged. For *in vitro* work BMDM were exposed for up to 2 hours with combinations of PA01 alone and elafin treated PA01. Supernatants were isolated and TNF_α assayed by ELISA, and the amount of viable PA01 detected by plating serial dilutions on LB agar. **Results:** Mice that received elafin transduced macrophages had detectable elafin in their lavage fluid (up to 6000 pg/ml) and had 1000 fold lower counts of viable PA01. *In vitro* pre-incubation of PA01 with elafin (10-100 nM) before exposing them to BMDM increased bacterial killing by 30% compared to BMDM alone. Levels of TNF_α in the supernatants were decreased in those exposed to elafin. **Conclusion:** Our results provide proof of concept data for the use of monocytes to deliver therapeutic transgenes to the lung. Further our data suggest that elafin increases PA01 clearance by modulating macrophage bacteriocidal activity.

For references see www.smj.org.uk

The Use of D-Dimers in the Exclusion of Venous Thromboembolism in a District General Hospital

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Introduction: The use of D-dimers and clinical probability scores in the exclusion of venous thrombo-embolic disease is

well established. The aim of this audit was to determine how D-dimers were being used in our district general hospital and ensure that appropriate protocols were in place to allow the correct usage of the test and clinical probability scores.

Methods: All D-dimer results from a three-month period were obtained and cross-referenced with the results of radiological tests from the same admission. D-dimers were analysed using a quantitative latex agglutination process (IL test™) and the cut-off for a positive test was 285.0 ng/ml. Case notes were retrospectively reviewed for clinical probability scores and appropriateness of D-dimer request. Follow-up hospital admission data for 3 months for development of thromboembolic events was analysed. **Results:** 342 valid D-dimer tests were analysed: 179 (52%) were positive and 163 negative. Of the positive D-dimers, 101 patients had subsequent investigation with either ultrasound Doppler (US) scan or CT pulmonary angiogram (CTPA). 35 events of venous thromboembolism were identified: 23 had deep venous thromboses (DVT) and 12 had pulmonary thromboemboli (PTE). 78 individuals with positive D-dimers had no subsequent investigation. Of the 163 patients with negative D-dimers, 150 were discharged and 13 had a subsequent US examination or CTPA. No evidence of PTE was seen in CTPA and only one US showed evidence of DVT. Of those 150 patients with negative D-dimer test who were discharged, there were 16 emergency medical readmissions within 3 months. No evidence of DVT or PTE was diagnosed. This gives a negative predictive value of D-dimer testing of 99.4%. In the subset of patients who had a positive D-dimer and no further investigation or a negative D-dimer and subsequent investigation, none had a documented clinical probability score. **Conclusions:** Our study shows that D-dimers have a high negative predictive value when used in a 'real-life' clinical setting in a district general hospital. However, the use D-dimer testing appears inappropriate with lack of clinical probability scoring. Clinical probability scores will be made mandatory for requests in future for D-dimer tests, US and CTPA to encourage more appropriate use of these investigations.

Sputum Trace Metals Are Biomarkers of Disease Activity in Inflammatory and Suppurative Lung Diseases

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Rationale and Objectives: Induced sputum provides information about inflammatory status in respiratory diseases. Sputum cytology facilitates the assessment of asthma and

COPD, and sputum fluid contains important biomarkers of inflammation such as cytokines. The presence of increased sputum iron in inflammatory lung disease is well-documented. We hypothesised that other sputum metals may be affected by airway inflammation and investigated their value as biomarkers.

Methods: Induced sputum was obtained from 20 healthy control subjects and patients with inflammatory pulmonary diseases: 23 with cystic fibrosis (CF) bronchiectasis, 16 with bronchiectasis, 17 with asthma and 23 with COPD, and subjected to inductively coupled plasma optical emission spectrometry to quantify iron, zinc, manganese and copper. Sputum from 14 CF patients was also analysed through an exacerbation cycle. **Measurements and Main Results:** Sputum zinc differentiated CF and bronchiectasis from controls with $p < 0.001$ at the following levels in $\mu\text{g/l}$ (SEM): Control 17.6 (3.0), bronchiectasis 112.1 (20.6), CF 150.0 (23.4), COPD 34.6 (7.1), Asthma 36.2 (13.6). Sputum iron also differentiated CF and bronchiectasis from controls at $p < 0.001$. Levels of manganese and copper were numerically lower, but were elevated for CF ($p < 0.05$), bronchiectasis and asthma ($p < 0.01$) versus controls for manganese, and were elevated for all diseases ($p < 0.05$) compared with controls for copper. Sputum zinc level decreased significantly following antimicrobial therapy for an exacerbation in CF subjects from 236 $\mu\text{g/l}$ (47.1) to 140 (30.1) ($p < 0.0086$). **Interpretation:** Sputum zinc and iron represent markers of airway inflammation in CF and bronchiectasis, but zinc has better potential to monitor disease activity. While there is a wealth of information about the significance of iron in lung inflammation, the role of fluctuating zinc levels revealed by this study merit further investigation. **Conclusions:** Sputum zinc and iron represent markers of airway inflammation in CF and bronchiectasis, but zinc has better potential to monitor disease activity. The roles of manganese and copper are less clear and merit further investigation.

Experience Identifying Patients for AntilgE Therapy at a Problem Asthma Clinic

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We have screened 117 patients over a 9-month period in order to identify suitable patients for a pragmatic unblinded antilgE study in which patients are randomised to therapy with Omalizumab or continued therapy without this in a ratio of 2:1, after an 8-week run-in period of treatment optimisation.

We have identified 15 (13%) possible candidates:

| | |
|-------------------------------------|----|
| Entered into study | 6 |
| Eligible, considering participating | 8 |
| Declined | 1 |
| Uncategorised | 5 |
| Ineligible | 97 |

The reasons for ineligibility are as follows:

| | |
|--|----|
| ● Low exacerbation rate (<2 documented within 12 months) | 37 |
| ● Total IgE level <30 , or all RAST/SPTs negative | 34 |
| ● Total IgE >700 (range 752 – 3095) | 12 |
| ● FEV1 criteria not fulfilled ($<80\%$ for study) | 4 |
| ● History of anaphylaxis | 2 |
| ● Significant smoking history, otherwise eligible | 5 |
| ● Other ($<$ BTS step 3 therapy & otherwise eligible, not asthma) | 3 |

These findings, in a clinical setting in which the majority of eligible patients might have been expected, highlight the small numbers of patients likely to require this therapy as part of routine clinical practice and emphasises that this therapy is targeted at the small number of patients with ongoing allergic asthma, despite BTS step 3 treatment whose total IgE levels fit within the relatively narrow range in which the published studies have shown benefit.

MRSA - an Issue for Non-Invasive Ventilation

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Infection control is of major importance in current medical practice, particularly with regard to Methicillin Resistant Staphylococcus Aureus (MRSA). With increasing use of, and indications for, non-invasive ventilation (NIV) this intervention offers further potential for cross contamination.

Following identification of MRSA on a routine screen of a non-invasive ventilator air filter in a Medical High Dependency unit a prospective infection control audit was conducted. The issue was highlighted to staff, followed by screening of all machines after each use, together with patient filter changes every 24 hours, and air filter changes between patients.

Over a 6-month period 4 NIV machines were screened following an agreed infection control protocol, resulting in 179 samples, out of which 20 swabs produced growth on bacteriological culture. There were six cultures of staphylococcus aureus but identification of all positive samples clarified these as probable skin or environmental contamination with no evidence of MRSA within filters or airflow tubing on the machines.

Reassuringly, although our prospective screen revealed 11 cultures of staphylococci most were of only a light growth, none was an MRSA isolate, and none of the total of 20 positive results was felt by the infection control team to be of clinical significance or offered potential risk to patients being treated with NIV. The isolates probably represent skin or environmental contamination, as has been shown in a previous studies of cross-contamination, but this again emphasises the importance of adequate education, and implementation of effective and

comprehensive infection control measures to reduce the risk of cross-contamination and potential for hospital-acquired infection.

We conclude that although non-invasive ventilation offers potential for cross infection this does not appear to be a clinical problem provided adequate implementation of a comprehensive infection control policy. Appropriate machine decontamination and hand cleansing is essential in combating the issues surrounding cross contamination, and there remain scope for improvement and development within this area of practice.

This data has been accepted for presentation at the European Society Scientific Meeting in Munich 2006

Fluvastatin Selectively Inhibits Hypoxic Proliferation & Activation of p38 MAP Kinase in Pulmonary Artery Fibroblasts: Implications for Pulmonary Hypertension Treatment

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Background: Excessive pulmonary vascular cell proliferation is a key aspect in the development of severe pulmonary hypertension. Exploring the differential effects of any proposed antiproliferative treatment on the cell types resident to the pulmonary artery is important if we are to learn how best to exploit these drugs. Statin drugs have antiproliferative effects and reverse pulmonary hypertension in animal models. In particular, we have reported fluvastatin inhibition of hypoxia-induced pulmonary adventitial fibroblast (PAF) proliferation (Carlin et al, STS, 2005). It is unknown whether statins would be effective in the treatment of pulmonary hypertension in humans at standard doses or which statin would be best suited to this indication. Also unknown is whether established or novel therapies would complement or simply duplicate the effects of statins and whether we should expect all forms of pulmonary hypertension to respond similarly. To address some of these questions we studied proliferative responses of PAFs, pulmonary artery smooth muscle cells (PASMCs) and systemic adventitial fibroblasts (SAFs) to incremental doses of serum, platelet-derived growth factor and acute hypoxia (5%). We compared the effects of different statins across a range of doses. The cellular mechanisms in the PAF-hypoxia model were assessed by studying effects of statins, prenyl intermediates and related inhibitors on proliferation and MAP kinase activation.

Methods: Proliferation of vascular cells was assessed by (3 H) thymidine uptake and cell counting. MAP kinase activation was assessed by Western blot analysis. **Results:** Fluvastatin at pharmacological doses inhibited hypoxic proliferation and p38 MAP kinase phosphorylation in PAFs. This effect was reversed by the prenyl compound geranylgeranyl pyrophosphate and mimicked by a geranylgeranyl transferase inhibitor, suggesting

that hypoxia-induced p38 phosphorylation is mediated via a GTPase protein such as RhoA or Rac1. The Rho kinase inhibitor hydroxyfasudil had no effect. PSMCs and SAFs showed no increased proliferation in acute hypoxia. Serum and PDGF-induced proliferation of PAFs, PSMCs and SAFs was only influenced by fluvastatin at doses 10-100 fold higher than achieved *in vivo*, with no evidence of a circulation specific effect. Simvastatin and atorvastatin had similar effects to fluvastatin, but in contrast to fluvastatin the doses required are much greater than those achieved *in vivo*, in humans. **Conclusion:** An important hypoxic signaling pathway in RPAFs has been further characterised and it is selectively inhibited by fluvastatin at pharmacological dosage. Fluvastatin would seem to have specific potential for hypoxia-associated pulmonary hypertension.

The Impact of a Legislative Ban on Smoking in Public Places on the Quality of Health, Pulmonary Function and Inflammation of Bar-Workers in Scotland

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Context: Scotland has recently introduced a legislative ban on smoking in confined public places. **Objective:** To investigate the impact of this ban on the health of bar-workers. **Design:** A prospective observational study. **Participants:** Non-smoking bar workers in Tayside. **Measurements:** Exposure to environmental smoke, symptoms, pulmonary function, and airway and systemic inflammation one month before then one and two months after the introduction of the ban. **Results:** The percentage of bar-workers with respiratory or sensory symptoms fell by 26% (95% CI -13.8 – -38.1) and 32.5% (-19.8 – -45.2) at one and two months respectively ($p < 0.001$). FEV₁ increased by 8.2% (3.9 – 8.0) and 5.1% (2.1 – 8.0) of predicted ($p < 0.005$) at one and two months, with significant changes in both asthmatic and non-asthmatic workers. Serum cotinine levels fell by 1.93ng/ml (-2.83 – -1.03) and 2.23ng/ml (-3.10 – -1.34) at one and two months ($p < 0.001$). The total white cell and neutrophil count was reduced by 630 cells/ μ l (-1010 – -260, $p = 0.002$) and 410 cells/ μ l (-740 – -90, $p = 0.028$) respectively at two months. Compared with baseline, asthmatic and rhinitic bar-workers also had less airway inflammation at one month with a 0.8 fold reduction (0.67 – 0.96, $p = 0.036$) in exhaled nitric oxide, and better Juniper quality of life scores by 7.3 points (0.1 – 14.6, $p = 0.049$). **Conclusions:** Banning smoking in public places resulted in significant early improvements in symptoms, pulmonary function and circulating neutrophils in nonsmoking bar-workers. Asthmatics also had reduced airway inflammation and improved quality of life.