

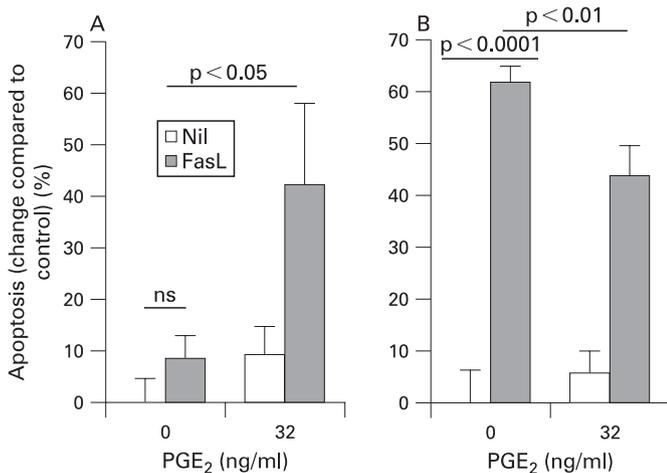
S132 THE ROLE OF COX-2 DERIVED PGE₂ IN MODULATING THE BALANCE OF APOPTOSIS IN THE EPITHELIAL-MESENCHYMAL TROPHIC UNIT: AN IMPORTANT MECHANISM IN THE PATHOGENESIS OF IDIOPATHIC PULMONARY FIBROSIS

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Background: In the lungs of patients with idiopathic pulmonary fibrosis (IPF), apoptosis is increased in alveolar epithelial cells (AEC) but reduced in fibroblasts. Patients with IPF have a decreased capacity to upregulate cyclooxygenase (COX)-2 and thus fail to induce synthesis of the anti-fibrotic prostanoid prostaglandin E₂ (PGE₂). We hypothesise that this reduction of PGE₂ in IPF modulates the paradox of increased AEC but reduced fibroblast apoptosis.

Methods: To assess apoptosis human fibrotic and control lung sections were TUNEL stained and immunostained for active caspase 3 and cleaved PARP. Primary type II AEC and fibroblasts were derived from IPF and control lung sections obtained at surgery. Apoptosis of AEC and fibroblasts was induced with Fas ligand (FasL 50 ng/ml). Cells were also treated with either the non-selective COX-1/2 inhibitor indomethacin (5 µg/ml), the COX-2 inhibitor NS398 (5 µg/ml) or PGE₂ (32 ng/ml). Competitive agonists/antagonists of the E-prostanoid receptors were used to determine cell signalling mechanisms. Apoptosis was detected by annexinV/propidium iodide and analysed by flow cytometry. Regulation of proteins in the apoptotic pathway, including Fas, Bcl-2, BAD and XIAP was assessed by quantitative reverse transcription PCR, Western blotting and immunohistochemistry. Wild type (WT) and heterozygous COX-2-deficient mice received oropharyngeal bleomycin (2 mg/kg) or saline and apoptosis at day 14 was assessed by TUNEL.

Results: Frequent AEC apoptosis was seen in IPF but not control lung tissue. Fibroblasts from IPF lung (nine lines) were resistant to FasL when compared with control lung fibroblasts (six lines). Control fibroblasts were protected from apoptosis by COX-2 inhibition. PGE₂ increased FasL induced apoptosis of fibrotic fibroblasts but reduced apoptosis in patient-derived fibrotic primary type II AEC. Consistent with this TUNEL staining showed increased AEC, but decreased fibroblast, apoptosis in COX-2-deficient mice compared with WT. Expression of EP2 and EP4 receptors is increased in IPF lung compared with control. PGE₂, via EP receptor signalling, decreases the expression of the key inhibitor of apoptosis XIAP.



Abstract S132 Representative results from one line of fibroblasts (A) and type II airway epithelial cells (B) derived from a patient with IPF. Fibroblasts are resistant to FasL induced apoptosis but are sensitized by the addition of PGE₂. AECs undergo apoptosis in response to FasL but are partially protected by exogenous PGE₂.

Conclusion: Failure to upregulate COX-2 and PGE₂ in IPF contributes to aberrant profibrotic changes in fibroblast and epithelial cell apoptosis. PGE₂ restores the sensitivity of fibrotic fibroblasts to FasL and does this, at least partly, by downregulating XIAP.

Epidemiology of obstructive lung disease

S133 BETA CRYPTOXANTHIN LEVELS CORRELATE WITH LUNG FUNCTION IN MIDDLE-AGED MEN

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Background: Beta cryptoxanthin is one of the pro-vitamin A carotenoids and is found in many yellow/orange fruits and vegetables. It is known to be an antioxidant and is reported to be a good biomarker of fruit and vegetable intake. We hypothesised that levels of serum beta cryptoxanthin would be related to FEV₁.

Methods: From 1991 to 1994, 2745 men aged 50–59 years were recruited into the Belfast arm of the Prospective Epidemiological Study of Myocardial Infarction (PRIME). 2010 of these men were rescreened at 10 years. In this study we describe the cross-sectional analysis of the 1208 men who had a valid spirometry trace (ERS/ATS criteria) and plasma sample at follow-up. Beta cryptoxanthin levels were measured using HPLC analysis. FEV₁ values at 10 years were modelled using simple linear regression, and adjusted for age, height, body mass index, smoking history, cholesterol level and social status.

Results: The men had a mean age of 64.4 years and 36.9% had never smoked. Serum beta cryptoxanthin levels were positively correlated with FEV₁ ($r = 0.23$, $n = 1208$, $p < 0.001$). In the crude analysis, for each nanomole per litre increment in serum beta cryptoxanthin levels, FEV₁ was 2.22 ml greater (95% CI 1.60 to 2.75). Following adjustment for covariates, for each nanomole per litre increment in serum beta cryptoxanthin levels, FEV₁ was 1.26 ml greater (95% CI 0.78 to 1.75, $p < 0.001$).

Conclusion: Serum beta cryptoxanthin levels are positively correlated with FEV₁ in this cohort of middle-aged men from Northern Ireland. This suggests that in this population a moderate increase in serum beta cryptoxanthin levels (achievable by a modest increase in dietary intake of fruit and vegetables) may have a protective effect on lung function.

S134 DIETARY PATTERNS AND ADULT ASTHMA: POPULATION-BASED CASE-CONTROL STUDY

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Introduction and Objectives: Epidemiological studies of diet and asthma have focused largely on relations with intakes of individual nutrients and foods or food groups and few studies have examined associations with dietary patterns. The main aim of this paper is to determine whether dietary patterns are related to asthma and related outcomes in adults.

Methods: We carried out a population based case-control study of adults aged 16–50 years in Greenwich, south London. Information about usual diet was obtained by food frequency questionnaire and we used principal components analysis to define five dietary patterns. We used logistic and linear regression, adjusting for confounding factors, to relate these patterns to asthma, asthma severity, rhinitis and chronic bronchitis in 601 cases and 856 controls.

Results: There were no statistically significant associations between dietary patterns and asthma or severity of asthma after controlling for confounders. Dietary patterns I (characterised by a

Abstract S134 Table Adjusted* odds ratio per quintile increase of pattern score

Pattern	Asthma	Chronic bronchitis
I	1.06 (0.93 to 1.20)	1.33 (1.09 to 1.62)
II	0.97 (0.87 to 1.08)	0.95 (0.82 to 1.10)
III	1.08 (0.93 to 1.23)	1.25 (1.01 to 1.55)
IV	1.04 (0.95 to 1.16)	1.01 (0.86 to 1.18)
V	0.93 (0.83 to 1.60)	0.92 (0.79 to 1.07)

*Adjusted for all other dietary patterns, age, sex, body mass index, social class, housing tenure, employment status, whether a single parent, smoking, passive smoking exposure at home, total energy intake, paracetamol and supplement use.

high intake of fish, salad vegetables, pasta, couscous and vegetable dishes) and III (associated with high intake of chips, processed meats, crisps, fried snacks, chocolate bars, sponge puddings, ketchup and cola) were positively associated with chronic bronchitis after adjusting for potential confounders. Dietary pattern II (which loaded highly on tomatoes and fruits) was positively associated with rhinitis (adjusted odds ratio per quintile increase of pattern score 1.75 (95% CI 1.08 to 3.27) (see table).

Conclusions: We have not found any relation between dietary patterns and adult asthma. The findings for chronic bronchitis and rhinitis were unexpected and require replication.

S135 TRENDS IN FIRST HOSPITALISATION FOR CHRONIC OBSTRUCTIVE PULMONARY DISEASE IN MEN AND WOMEN IN SCOTLAND, 1991–2006

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Background: We describe the changing pattern of first hospitalisation for chronic obstructive pulmonary disease (COPD) in men and women in Scotland over a 15-year period.

Methods: The Scottish Morbidity Record was used to identify all first hospitalisations in adults aged 35 years and over in Scotland where COPD was coded in the principal diagnostic position at discharge from 1991 to 2006. Annual age and sex-specific rates of first hospitalisation were calculated using denominator data from the 1991 and 2001 censuses, with interpolation and extrapolation for the inter-census years. Direct standardisation was carried out using 2001 census data. Rates over time were modelled using Poisson regression.

Results: There were 80 242 first hospitalisations for COPD in Scotland between 1991 and 2006; 41 522 (52%) were in women. The mean age at admission in men was invariant over time at 70.5 years (SD 10.5). In women the mean age at first hospitalisation rose from 69.9 years (10.6) in 1991 to 70.5 years (11.5) in 2006 ($p=0.031$). Age standardised rates of first hospitalisation for COPD (per 100 000 population) increased in men from 175 (95% CI 167 to 182) in 1991 to 227 (218 to 235) in 1993, before falling to 176 (169 to 183) in 2006. In women rates increased from 124 (118 to 129) in 1991 to 217 (210 to 224) in 1999 before falling to 185 (178 to 192) in 2006. No significant change in the relative rate (RR) of first hospitalisation for COPD (2006 vs 1991) was found in men, RR 1.00 (0.81 to 1.24). However, a non-significant trend towards a reduction in RR in men aged 75 years and over was observed. In women the RR of first hospitalisation (2006 vs 1991) was 2.85 (2.68 to 3.02) overall, with increases observed across all age groups.

Conclusions: Despite an ageing population rates of first hospitalisation for COPD in men in Scotland appear to have stabilised. Indeed, a trend towards falling rates in elderly men is reported. Rates in women have more than doubled over the study period. However, there is some evidence that these may have peaked.

S136 WHAT SMOKING PREVALENCE CAN TELL US ABOUT HEALTH INEQUALITIES (OR VISA VERSA?)

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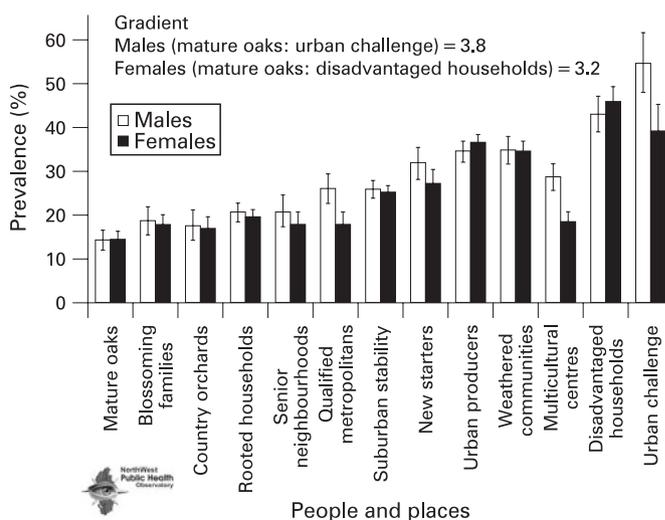
Introduction: Despite smokefree legislation and continuing declines in smoking prevalence, smoking remains the largest single killer in England: with approximately 85 000 deaths from smoking-related diseases each year. Effective targeting of local interventions to reduce smoking prevalence requires identification of communities who smoke the most but this intelligence is often lacking. It is time consuming and often prohibitively expensive to collect robust information on peoples' behaviours. Population segmentation enables "types" of people and places to be identified that can help the targeting of communities with particular health needs.

Objective: To apply population segmentation methods to identify local communities at greatest risk of smoking-related diseases

Methods: Using national Health Survey for England (HSE) data, smoking measures were generated for two different small area geographical classifications—the index of multiple deprivation (IMD, 2004) and the geodemographic lifestyle classification, P2 people and places. These classifications are available at postcode or lower super output area (LSOA) and therefore every respondent to the HSE could be assigned to one group within each classification. A smoking prevalence "gradient" was generated for each classification, which was used to estimate the number of smokers in every LSOA and local authority in England. These measures were compared with other modelled estimates and data on smoking-related deaths.

Results: Smoking prevalence showed the expected strong relationship with deprivation (IMD, 2004): higher deprivation resulting in over double the proportion of people who smoked. This almost exactly matched the gradient of smoking prevalence identified using individual socioeconomic status. People and places showed an even stronger gradient with over three times more people smoking in the most deprived communities than in the most affluent (fig). Estimates of smoking prevalence generated by these methods showed very high correlations with the rate of deaths due to smoking ($R^2 = 0.72$). We will illustrate how these geodemographic classifications enable the identification of very localised communities with predicted higher levels of smoking.

Conclusions: Although accurate local measures of smoking status are needed to monitor the effectiveness of interventions, the targeting of campaigns and programmes to reduce smoking prevalence can usefully benefit from population segmentation techniques.



Abstract S136 Figure Smoking prevalence by geodemographic lifestyles group (Health Survey for England 2003–2005).

S137 THE EFFECT OF GENDER ON DECLINE OF LUNG FUNCTION OVER 9 YEARS: A POPULATION-BASED STUDY

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Objectives: To investigate the effect of gender on decline in lung function in a population-based cohort.

Methods: In 1991, 2633 individuals aged 18–70 years were randomly sampled from a local authority area in the UK. In 2000, 1334 of these individuals were re-studied. On both occasions, data were collected on forced expiratory volume in one second (FEV₁), forced vital capacity (FVC) and a variety of other exposures including a detailed smoking history. These data were analysed using linear regression to create age, sex and height-adjusted FEV₁ residuals for 1991 and 2000 and to estimate the effect of gender on decline in FEV₁ after adjustment for smoking status, smoking pack years and body mass index over this time.

Results: The study populations from 1991 and 2000 are described in the table. Over the 9-year period the unadjusted FEV₁ fell by 386 ml in men and 297 ml in women. After adjustment for potential confounding factors, this resulted in a difference of –74 ml (95% CI –37 to –111) for men compared with women. This effect was also observed after the analysis was restricted to those who had never reported smoking (excess decline for men –99 ml; 95% CI –169 to –30). However, after adjustment for baseline FEV₁, this difference ceased to be significant (excess decline for men –2 ml; 95% CI –46 to –43).

Conclusions: In this population-based cohort, men experienced a larger absolute decline in lung function than women. This effect is probably a consequence of the higher baseline lung function observed in men compared with women. This observation differs from previous reports of the effect of baseline lung function on subsequent decline in lung function.

Funding: British Lung Foundation and Asthma UK.

Abstract S137 Table Description of study population

	1991	2000
Total participants	2633	1346
Males, N (%)	1312 (50%)	667 (50)
Mean age (SD), years	44.4 (13.6)	46.3 (12.3)
Smoking status in 1991, N (%)		
Never	1306 (50)	714 (53)
Ex	730 (28)	391 (29)
Current	597 (23)	241 (18)
Mean FEV ₁ in 1991(SD), l	3.19 (0.92)	3.18 (0.84)
Mean FVC in 1991 (SD), l	3.94 (1.07)	3.94 (1.00)

S138 HETEROGENEOUS CLINICAL EXPRESSION OF EOSINOPHILIC AIRWAY DISEASES IN AN ADULT HOSPITAL CLINIC POPULATION

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Introduction: Eosinophilic airways inflammation is seen across a spectrum of different airway diseases with distinct pathophysiological and clinical features. Recognising these overlapping patterns is important for appropriate planning of clinical trials of specific anti-eosinophil therapy.

Methods: We performed a retrospective observational study of 100 consecutive patients with airway diseases and a raised induced sputum eosinophil count (>3%) attending a specialist airway clinic. Patients returned a symptom questionnaire and visual analogue symptom score for their most prominent symptom on a 10 cm horizontal scale. They all underwent spirometry with reversibility if

pre-bronchodilator FEV₁/FVC was less than 0.7 or methacholine provocation (PC₂₀) if it was over 0.7. Sputum induction and processing was performed using standard methods. Asthma was identified in patients with a post-bronchodilator FEV₁ greater than 80% and FEV₁/FVC greater than 0.7 and either bronchodilator reversibility greater than 15% or a PC₂₀ less than 8 mg/ml. Patients with bronchodilator reversibility greater than 15% but FEV₁/FVC less than 0.7 were labelled as having mixed chronic obstructive pulmonary disease (COPD) and asthma and those with airflow obstruction without a significant bronchodilator were labelled COPD. Patients with predominant cough, without evidence of variable airflow obstruction or airway hyperresponsiveness were diagnosed as having eosinophilic bronchitis (EB).

Results: Asthma was diagnosed in 35 (35.3%), mixed COPD and asthma in nine (9.1%), COPD in 15 (15.2%) and EB in 40 (40.4%). The geometric mean sputum eosinophil count across all groups was 13.59% (SD 0.39). There was no significant difference comparing mean sputum eosinophil count across all four groups in this population (p = 0.849).

Conclusion: Eosinophilic airways disease presenting to secondary care is heterogeneous and a significant proportion does not fulfil standard criteria for asthma. The role of corticosteroids and novel anti-eosinophilic agent therapies under development requires consideration in a broader population of patients with airways disease and greater emphasis needs to be placed on identifying eosinophilic airway inflammation in patients presenting with airway symptoms.

Lung cancer: genetics and pathogenesis

S139 FOURIER TRANSFORM INFRARED SPECTROSCOPY AND METABOLIC PROFILING IN LUNG CANCER

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Introduction: We have shown that sputum is a feasible biofluid for analysis of biomarkers using Fourier transform infrared (FTIR) spectroscopic analysis and that FTIR can detect different patterns of metabolites between patients with lung cancer and healthy volunteers.¹

Aim: To analyse further the FTIR spectrometric patterns in more patients with lung cancer and also smokers deemed “high risk”. To identify what actual compounds may differentiate cancer from non-cancer sputum samples.

Methods: Loco-regional ethical permission was obtained.

Subjects: 26 patients undergoing bronchoscopy for suspected lung cancer provided spontaneous sputum samples. 16 eventually had biopsy confirmed non-small-cell lung cancer; 10 had no tumour at bronchoscopy and no evidence of cancer 6–12 months later (“high risk”). 25 control samples were also obtained from healthy volunteers and chronic obstructive pulmonary disease patients attending pulmonary rehabilitation.

Process: Bronchial epithelial cell presence was confirmed in the sputum by microscopy; cells were isolated by centrifugation and freeze dried before being processed in triplicate for FTIR (Vertex 70 FT-IR spectrometer).

Statistics: Multiplicative signal correction was performed to improve the signal-to-noise ratios on the baseline data followed by a hierarchical cluster analysis on the FTIR spectrometry generated wave numbers; further individual metabolite profiling was performed using electrospray injection (Micromass LCT) and comparing the wave numbers generated to the (ESI)-MS profiling chemical library.

Results: The cancer cases seemed to form a discrete cluster group away from healthy volunteers and the “high-risk” group. Metabolic fingerprinting detected differences in certain polyamines such as