Inflamm. res. 54 (2005) 51–56
1023-3830/05/020051-06
DOI 10.1007/s00011-004-1321-x

Inflammation Research

Sputum eosinophilia in idiopathic pulmonary fibrosis

S. S. Birring, D. Parker, S. McKenna, B. Hargadon, C. E. Brightling, I. D. Pavord, P. Bradding

Institute for Lung Health, Department of Respiratory Medicine, Glenfield Hospital, Leicester, LE3 9QP, United Kingdom, Fax: ++44 116 236 7768, e-mail: sb134@le.ac.uk.

Received 11 June 2004; returned for revision 20 August 2004; accepted by N. Boughton-Smith 24 September 2004,

Abstract. *Objectives and Design:* Cough is a common symptom in idiopathic pulmonary fibrosis that is difficult to treat and has a major impact on quality of life. We tested the hypothesis that the cough and increased cough reflex sensitivity seen in patients with idiopathic pulmonary fibrosis may be due to airway inflammation in a prospective, cross-sectional study.
Subjects and Methods: We measured the induced sputum inflammatory cell profile and cell-free supernatant inflammatory mediator concentrations in 15 patients with idiopathic pulmonary fibrosis, 17 healthy controls and 15 patients with chronic obstructive pulmonary disease.
Results: Both the geometric mean sputum differential eosinophil cell count and median eosinophilic-cationic-protein concentration were significantly higher in patients with idiopathic pulmonary fibrosis than controls (2.1% vs 0.3%; $p < 0.001$ and 1.1 mg/ml versus 0.2 mg/ml; $p = 0.03$ respectively). There were no significant differences in sputum eosinophil counts and eosinophilic-cationic-protein concentrations between patients with idiopathic pulmonary fibrosis and chronic obstructive pulmonary disease. Sputum leukotriene-B4 concentrations were significantly lower in patients with idiopathic pulmonary fibrosis ($p = 0.03$) and chronic obstructive pulmonary disease ($p = 0.008$) compared to controls.
Conclusions: Idiopathic pulmonary fibrosis is characterised by the presence of active eosinophilic airway inflammation raising the possibility that airway inflammation may contribute to symptoms such as cough.

Key words: Idiopathic pulmonary fibrosis – cryptogenic fibrosing alveolitis – chronic obstructive pulmonary disease – eosinophils – cough

Introduction

Idiopathic pulmonary fibrosis (IPF), also known as cryptogenic fibrosing alveolitis is one of the idiopathic interstitial pneumonias that typically presents insidiously, with the gradual onset of breathlessness [1]. Although breathlessness is the most recognised symptom, non-productive cough is reported in up to 86% of patients with IPF. This can be refractory to antitussive therapy and significantly impairs quality of life [2]. The mechanism of cough in IPF is unclear, but traction of airways secondary to reduced lung compliance and stimulation of pulmonary cough receptors by alveolar inflammation are thought to be possible mechanisms [3]. However, increased capsaicin cough reflex sensitivity in patients with IPF [4] suggests that cough may arise from airway abnormalities since cough receptors are most concentrated in the upper airways [5]. Airway cough receptors can be activated by both inflammatory and mechanical stimuli [5], and several inflammatory mediators have been implicated in the pathogenesis of cough which include histamine, prostaglandin E_2 (PGE_2), cysteinyl leukotrienes (cystLTs; LTC_4, LTD_4 and LTE_4), eosinophilic cationic protein (ECP) and interleukin 8 (IL-8) [6, 7]. However, most studies investigating inflammatory mechanisms in IPF have been limited to the lung parenchyma and distal airways.

In this study, we tested the hypothesis that there is large airway inflammation in patients with IPF by studying the inflammatory cell profile and inflammatory mediator con-

Correspondence to: S. Birring
Supported by: Supported by a grant from the British Lung Foundation and University Hospitals of Leicester NHS Trust. S.S.B. is a British Lung Foundation clinical research fellow.

Abbreviations:
IPF: Idiopathic pulmonary fibrosis
COPD: chronic obstructive pulmonary disease
FEV_1 = forced expiratory volume in 1 second
FVC: forced vital capacity
SE: standard error
SD: standard deviation
PG: prostaglandin
Cyst-LT: cysteinyl-leukotriene
ECP: eosinophilic cationic protein
IL: interleukin
HRCT: high resolution computerised tomography
UIP: usual interstitial pneumonia

centrations in induced sputum from patients with IPF compared to healthy control subjects. We also recruited patients with chronic obstructive pulmonary disease (COPD) as a disease control group, since it is a condition in which cough, heightened cough reflex sensitivity and large airway inflammation has been well described [8].

Methods

Subjects

Fifteen consecutive patients with IPF, 15 patients with COPD and 17 healthy controls were recruited from Glenfield Hospital outpatient clinics and from healthy volunteers between 2001 and 2002. The diagnosis of IPF was based on clinical and radiological criteria [9]. All patients had a suggestive clinical history, presence of fine inspiratory basal crackles and a high resolution CT scan (HRCT) confirming the presence of pulmonary fibrosis, with predominant reticular or honeycomb pattern distributed preferentially to the lung bases and subpleural regions. Patients with predominant ground glass patterns or those with features of diseases other than usual interstitial pneumonia (UIP) were excluded. Patients with a history of asbestos exposure, fibrogenic drug therapy or clinical evidence of an underlying connective tissue disease were also excluded. Normal subjects were asymptomatic, had no history of respiratory disease and had normal spirometric measurement [10]. Patients with COPD had symptoms of chronic airflow obstruction and fulfilled lung function criteria as set out by the NHLI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines [11]. Patients with COPD had post-bronchodilator forced expiratory volume in one second/forced vital capacity (FEV_1/FVC) < 70% and no substantial improvement in FEV_1 after taking 2.5 mg nebulized albuterol (< 15% or, if FEV_1 < 1.2 L, < 200 mL improvement) or following a corticosteroid trial (2-week course of 30 mg prednisolone daily, or 2 months high dose inhaled corticosteroids, with reassessment of symptoms and spirometry). No subjects had respiratory tract infection within the past 6 weeks, and no symptoms or past history of atopic disease. Patients with IPF and COPD were clinically stable for at least 2 months and had not received oral or inhaled corticosteroids or immunosuppressive therapy within two months prior to the study. Written consent was obtained from all patients and the protocol for this study was approved by the Leicestershire Research Ethics Committee.

Protocol and clinical measurement

Subjects attended on two occasions. At the first visit we assessed patient records, pulmonary function tests and chest radiography. Patients with IPF had lung function tests with a benchmark (P K Morgan, Chatham, UK) and lung volumes assessed by the helium dilution method. At the second visit subjects rated cough severity on a previously validated 100mm horizontal visual analogue scale [12], had spirometric measurement and sputum induction using methods that have been described previously [13, 14]. Sputum free of salivary contamination was selected and was mixed with four times its volume of 0.1% dithiothreitol. From the induced sputum sample, a differential cell count was obtained from a cytospin preparation stained with Romanowski's stain and a total cell count was determined using a haemocytometer. The cell-free supernatant was stored at –80 °C until analysis. Cell counting was performed by an experienced observer blind to the subject's clinical characteristics.

Mediator measurements

The concentration of mediators were determined in sputum supernatant by competitive enzyme immunoassays for Histamine (Immunotech, Marseille, France), PGE2 (R&D systems, Oxon, UK), cystLTs (Cayman Chemical, Ann Arbor, MI), Leukotriene B4 (R&D systems, Oxon, UK) and sandwich enzyme linked immunosorbent assay for IL-8 (Pharmingen, San Diego). The concentration of ECP was measured by fluoroimmunoassay (Unicap; Pharmacia, Milton Keynes, UK). The sensitivity of the assays were: Histamine: 50×10^{-3} ng/ml, PGE_2: 8.25×10^{-3} ng/ml, cystLTs: 13×10^{-3} ng/ml, LTB_4: 19.4×10^{-3} ng/ml, ECP: 18×10^{-3} ng/ml and IL-8: 0.8×10^{-3} ng/ml. The intra-assay coefficient of variability of the assays were 5 to 10% and the interassay coefficient of variability were 3–15% across the range of concentrations measured.

Analysis

Subject characteristics were described using descriptive statistics and expressed as means (standard deviation). The primary end points were inflammatory cell total and differential counts in sputum. Secondary endpoints were sputum mediator content. Comparisons across the three groups and between groups were undertaken using the Kruskal-Wallis test and the Mann-Whitney-U test and ANOVA and unpaired t-tests for non parametric data and parametric data respectively. Sputum differential eosinophil counts were log-transformed and described as geometric mean (log SEM). Total cell counts were expressed as cells per gram of sputum. A value of $p < 0.05$ was taken as being statistically significant.

Results

The subject characteristics and spirometry measurements are as shown (Table 1). All patients with IPF and 12 (80%) of patients with COPD reported symptoms of cough. Patients with IPF had a mean (SE) FEV_1 of 2.1(0.1)L, FVC 2.6(0.2)L and mean (SE) % predicted residual volume of 54(4)%, total lung capacity 71(5)%, diffusing capacity for carbon monoxide (DLco) 60(4)% and carbon monoxide diffusion coefficient (Kco) 89(8)%. Twelve (80%) patients with IPF had HRCT features of honeycombing, 6(40%) had traction bronchiectasis and all had subpleural reticular changes. No patients with IPF had HRCT features of emphysema. There were significant differences in the sputum differential and total eosinophil counts between the 3 groups ($p < 0.001$ and $p = 0.002$ respectively; ANOVA; Table 2). The geometric mean sputum differential eosinophil cell count was significantly higher in patients with IPF (2.1%) compared to controls (0.3%: mean difference 8.0 fold, 95% confidence interval of difference 3.1 to 20.5; $p < 0.001$) but not COPD (2.3%; Table 2). There was a similar increase in geometric mean absolute eosinophil cell counts (10^3) per gram of sputum in patients with IPF (22.9) compared to controls (1.1: mean difference 21 fold, 95% confidence interval 0.3 to 138; $p = 0.003$) but not COPD (19.7). Nine (60%) patients with IPF and 8 (53%) with COPD had a sputum differential eosinophil cell count greater than 1.9% (upper limit of normal range in our laboratory) [13]. Both differential and absolute eosinophil counts were significantly raised in COPD compared to normal subjects ($p < 0.001$ and $p = 0.003$ respectively), but there were no other differences in sputum differential or absolute cell counts between groups (Table 2).

The median concentration of ECP was significantly higher in patients with IPF (1.1 mg/ml) than controls (0.2 mg/ml; 95% confidence interval of difference 0.01 to 1.5; $p = 0.03$; Table 3, Fig. 1). The concentration of sputum ECP in patients with IPF did not correlate significantly with

Table 1. Subject characteristics.

	Normal	IPF	COPD
Number	17	15	15
Male (%)	13 (76)	10 (67)	11 (73)
Age (years)	67 (5)	68 (9)	71 (5)
Smoking status (%)			
Never	29	27	0
Former	71	73	80
Current	0	0	20
Symptom VAS score (mm)			
Breathlessness	0	59 (24)	50 (31)
Cough	0	40 (29)	40 (32)
FEV_1 % predicted	105 (13)	82 (15)*	43 (12)*
FVC % predicted	105 (12)	84 (15) *	62 (17) *
FEV_1/FVC (%)	78 (4)	80 (7)	51 (8)*

Mean (SD); IPF: idiopathic pulmonary fibrosis; COPD: chronic obstructive pulmonary disease (GOLD stages 2–4); Symptom VAS: visual analogue score (0–100 mm-worst symptom); FEV_1: Forced expiratory volume in 1 s; FVC: Forced vital capacity; *$p < 0.001$ vs normals by unpaired t-test.

Table 2. Sputum differential cells counts.

	Normal	IPF	COPD
TCC	1.3 (0.2)	2.4 (0.6)	2.0 (0.6)
Squamous cells (%)	10 (3)	7 (3)	6 (2)
Viability (%)	74 (5)	68 (7)	70 (6)
Differential cell counts (%)			
Neutrophils (%)	68.7 (4.2)	76.9 (2.4)	75.4 (6.1)
Eosinophils# (%)	0.3 (0.1)	2.1 (0.2)*	2.3 (0.1)*
Lymphocytes (%)	1.0 (0.2)	0.5 (0.2)	0.6 (0.1)
Macrophages (%)	27.8 (4.1)	15.9 (2.3)	17.7 (5.8)
Epithelial cells (%)	2.2 (0.5)	2.5 (0.8)	2.5 (1.1)
Absolute cell counts (10^3/g)			
Neutrophils	899 (196)	1996 (559)	1741 (571)
Eosinophils#	1.1 (0.3)	23 (0.3)	20 (0.3)
Lymphocytes	12 (3)	6 (2)	16 (6)
Macrophages	302 (64)	280 (63)	250 (101)
Epithelial cells	30 (9)	51 (23)	27 (9)

Data expressed as mean (SEM) except where indicated; # Geometric mean (log SEM); TCC: Total cell count × 10^6/ml; Absolute cell counts (10^3) per gram of sputum; *$p < 0.001$ vs normals by unpaired t-test.

Table 3. Sputum mediator concentrations.

	Normal	IPF	COPD
LTB_4 (ng/ml)	6.3 (9.5)	3.4 (3.3)*	1.5 (0.8)**
Histamine (ng/ml)	2.0 (14.2)	8.0 (9.3)	24.3 (94)**
IL-8 (ng/ml)	11.3 (15.4)	19.9 (46.7)	58.4 (103)**
PGE_2 (ng/ml)	1.5 (1.5)	1.2 (1.5)	1.7 (1.6)
Cyst-Leukotrienes (ng/ml)	1.5 (4.3)	1.9 (3.1)	1.7 (3.3)
ECP (mg/ml)	0.2 (0.3)	1.1 (1.7)*	0.4 (1.8)

Median (interquartile range); ECP: eosinophilic cationic protein; PG: Prostaglandin; IL: Interleukin; *$p < 0.05$; **$p < 0.01$ versus normal subjects by Mann-Whitney-U test.

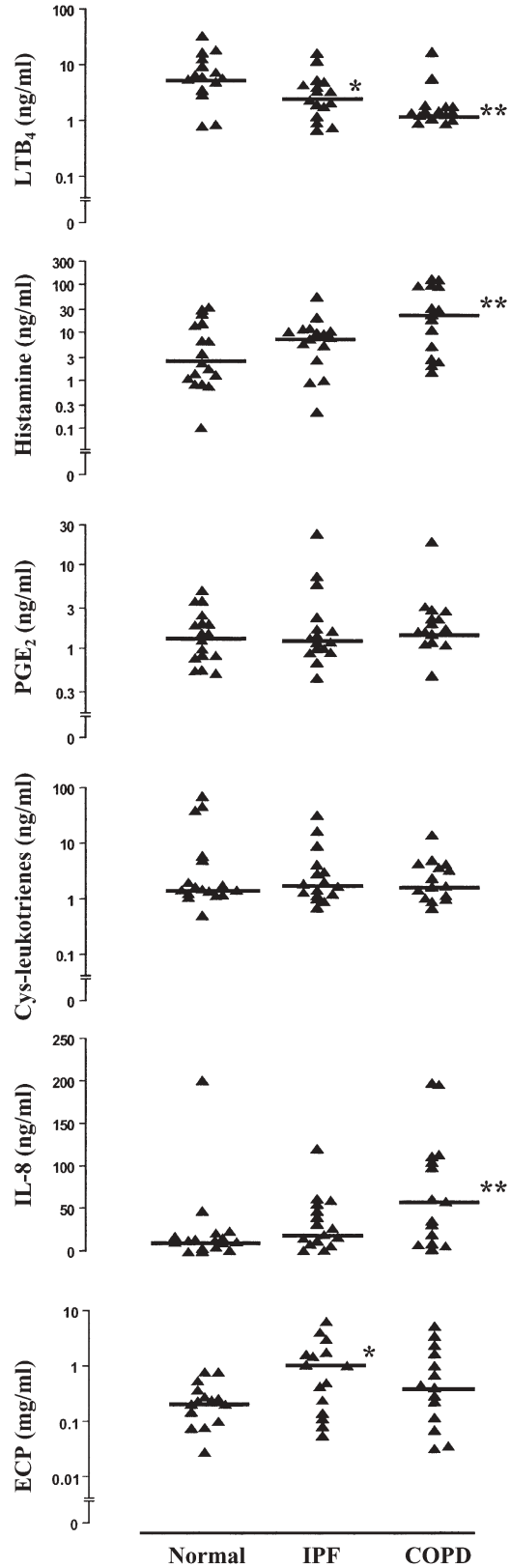

Fig. 1. Horizontal lines represent median concentrations (ng/ml) of Histamine, Leukotriene B_4 (LTB_4), prostaglandin E_2 (PGE_2), cysteinyl-Leukotrienes (cys-Leukotrienes), interleukin-8 (IL-8) and eosinophilic cationic protein (ECP; mg/ml). Subjects with idiopathic pulmonary fibrosis (IPF); chronic obstructive pulmonary disease (COPD). *$p < 0.05$; **$p < 0.01$ versus normals by Mann-Whitney-U test.

sputum eosinophil count (absolute or differential), cough visual analogue score or forced vital capacity. There were significant differences in LTB_4 concentrations between the 3 groups ($p = 0.008$, Table 3, Fig.1). The median LTB_4 concentration was significantly lower in patients with IPF (3.4 ng/ml) and COPD (1.5 ng/ml) than normals (6.3 ng/ml; IPF vs normals: 95% confidence interval of difference 0.5 to 6.4 ng/ml; $p = 0.03$; COPD vs normals: 95% confidence interval of difference 1.7 to 7.9 ng/ml; $p = 0.008$; Table 3, Fig. 1). There were significant differences in histamine and IL-8 concentrations between the 3 groups ($p = 0.03$ and $p = 0.02$ respectively). The median histamine and IL-8 concentrations were significantly higher in patients with COPD (24.3 and 58.4 ng/ml respectively) than normals (histamine: 2.0 ng/ml; 95% confidence interval of difference 1.4 to 78.1 ng/ml; $p = 0.006$; IL-8; 11.3 ng/ml; 95% confidence interval of difference 4.5 to 90.2 ng/ml; $p = 0.02$). There were no other differences in mediator concentrations between groups (Table 3, Fig. 1).

Discussion

This study has addressed in detail the inflammatory cell and mediator profile in induced sputum from patients with idiopathic pulmonary fibrosis. We have demonstrated for the first time that patients with IPF have a sputum eosinophilia and raised sputum eosinophilic cationic protein concentration compared to normal controls and similar to that seen in patients with chronic obstructive pulmonary disease. Our findings are consistent with the hypothesis that IPF is associated with active airway inflammation.

We chose to measure airway inflammation and estimate airway mediator production using induced sputum since this is a non-invasive, simple [15], safe [16], and responsive method [17], and previous studies have shown that sputum eosinophil counts and mediator concentrations can be measured repeatably [14, 15]. We have measured a wide spectrum of mediators with the potential to activate the cough reflex by a variety of mechanisms. Whilst it is unclear if sputum eosinophils originate from the bronchi or alveoli, induced sputum is thought to examine an airway compartment distinct from that sampled with nasal biopsies, bronchial wash, bronchoalveolar lavage and bronchial and transbronchial biopsies [18]. The inflammatory cell profile of our sputum samples was typical of that found in induced sputum, and distinct from that found in BAL [19] suggesting that the sputum eosinophils originated from the airways. Further studies should include bronchial and transbronchial biopsies to determine the site of eosinophilic inflammation in patients with IPF.

Our findings contrast to previous studies using induced sputum to investigate airway changes in IPF [20–23]. Beeh et al found no significant difference in sputum eosinophil differential cell counts in patients with IPF compared to healthy controls [21]. However, most patients with IPF in that study were immunosuppressed with oral corticosteroids, azathioprine or cyclophosphamide. Hope-Gill et al found a non-significant increase in sputum eosinophil counts in patients with IPF compared to controls and a significant increase in sputum neutrophil counts [23]. However, they did not use age and smoking history matched controls [23]. This is important since we have previously shown that neutrophils numbers in sputum increase with age [24]. A BAL neutrophilia [25] and eosinophilia [26] has been reported in IPF, which is associated with a poor response to treatment and a greater decline of lung function [27] but these studies did not measure sputum cell counts.

IPF is a disease of unknown cause, which predominately affects the lung parenchyma resulting in interstitial fibrosis. A common and disabling symptom in IPF is cough. Cough receptors are most concentrated in the upper airways [5], which suggests that cough may originate in the bronchi. In support of this, it has been demonstrated that patients with IPF have heightened cough sensitivity to inhaled capsaicin, and that this is not simply a result of reduced lung volume [4]. A potential explanation for this observation is the presence of airway inflammation, but there has never been a detailed immunohistological study of the airways of patients with this disease. Since IPF may result from the lung response to inhaled agents [28] and is associated with smoking [29], it is plausible that it could also affect the airways. We found a significant sputum eosinophilia in patients with IPF compared to controls and 60% of patients had a sputum differential eosinophil count above the normal range [13]. Eosinophilic cationic protein was significantly raised in the sputum of patients with IPF compared to controls suggesting that this inflammation is active. The sputum eosinophila in patients with IPF was comparable to that found in patients with COPD, a condition in which the presence of airway inflammation is well established. No patient with IPF had HRCT or physiological features of emphysema or COPD so it is unlikely that the sputum eosinophilia seen in patients IPF was due to the presence of concomitant COPD. Our data therefore provides evidence of eosinophilic airway inflammation in patients with IPF and raises the possibility that cough may result from airway inflammation as seen in patients with a chronic cough due to an eosinophilic bronchitis [7].

In this study, we did not find a correlation between differential or absolute sputum eosinophil count and cough visual analogue score. This might simply be because sputum eosinophils and their products may be markers of airway inflammation but not directly involved in the cough response. However, there is also a poor relationship between other measures of cough such as cough reflex sensitivity and cough symptoms [30], suggesting that this may result in part from the subjective nature of self-reported symptoms. The assessment of cough is hampered by a striking paucity of well-validated outcome measures, in particular ambulatory cough recorders [31]. Future studies need to incorporate objectives measures of cough such as cough recorders when they are available.

COPD and IPF have some common risk factors (occupational dust exposure and smoking) [29, 32]. Our findings suggest that these conditions are associated with similar airway inflammation. We did not confirm earlier reports that COPD is associated with sputum neutrophilia [33, 34], although previous studies in COPD have not used an age-matched control group. This together with the fact that our patients were stable and free from recent exacerbations likely explains why we could not detect a difference in sputum

neutrophilia between COPD and normal subjects in this study. In this study we also measured histamine concentrations and report for the first time that they are clearly elevated in patients with COPD compared to normal subjects, suggesting on-going mast cell or possibly basophil activation in this disease [35]. Previous studies have found elevated sputum LTB_4 concentrations during COPD exacerbations [36] but we found lower concentrations of sputum LTB_4 in stable COPD. The explanation for this is not clear. Lastly, although neutrophil cell counts were not elevated in patients with IPF or COPD in this study, we did not measure neutrophil activation markers in sputum supernatants which needs to be considered in future studies.

In summary we have found that patients with IPF have a sputum eosinophilia. Our findings raise the possibility that eosinophils may contribute to the sensitisation of the cough sensory pathway in IPF airways through the release of toxic mediators including ECP. Since cough responds to treatment with steroids in some patients with IPF, it will be of great interest to assess whether response to steroids correlates with sputum eosinophil counts as has been shown in COPD.

Acknowledgements. We would like to thank the subjects who participated in the study, Professor John Cookson for help with patient recruitment and Natalie Neale, William Monterio, Dhiraj D Vara and the Department of Respiratory Physiology for assistance in the clinical characterisation of some of the patients. Funding: British Lung Foundation and University Hospitals of Leicester NHS Trust. Surinder Birring is a British Lung Foundation Clinical Research Fellow.

Conflicts of interest. None declared.

References

[1] du Bois RM, Wells AU. Cryptogenic fibrosing alveolitis/idiopathic pulmonary fibrosis. Eur Respir J Suppl 2001; 32: 43s–55s.
[2] Crystal RG, Fulmer JD, Roberts WC, Moss ML, Line BR, Reynolds HY. Idiopathic pulmonary fibrosis. Clinical, histologic, radiographic, physiologic, scintigraphic, cytologic, and biochemical aspects. Ann Intern Med 1976; 85: 769–88.
[3] Widdicombe JG. Neurophysiology of the cough reflex. Eur Respir J 1995; 8: 1193–202.
[4] Doherty MJ, Mister R, Pearson MG, Calverley PM. Capsaicin induced cough in cryptogenic fibrosing alveolitis. Thorax 2000; 55: 1028–32.
[5] Fuller RW, Jackson DM. Physiology and treatment of cough. Thorax 1990; 45: 425–30.
[6] Choudry NB, Fuller RW, Pride NB. Sensitivity of the human cough reflex: effect of inflammatory mediators prostaglandin E2, bradykinin, and histamine. Am Rev Respir Dis 1989; 140: 137–41.
[7] Brightling CE, Ward R, Woltmann G, Bradding P, Sheller JR, Dworski R et al. Induced sputum inflammatory mediator concentrations in eosinophilic bronchitis and asthma. Am J Respir Crit Care Med 2000; 162: 878–82.
[8] Doherty MJ, Mister R, Pearson MG, Calverley PM. Capsaicin responsiveness and cough in asthma and chronic obstructive pulmonary disease. Thorax 2000; 55: 643–9.
[9] The diagnosis, assessment and treatment of diffuse parenchymal lung disease in adults. Introduction. Thorax 1999; 54 Suppl 1: S1–14.
[10] Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl 1993; 16: 5–40.
[11] Pauwels RA, Buist AS, Calverley PM, Jenkins CR, Hurd SS. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop summary. Am J Respir Crit Care Med 2001; 163: 1256–76.
[12] Brightling CE, Monterio W, Green RH, Parker D, Morgan MD, Wardlaw AJ et al. Induced sputum and other outcome measures in chronic obstructive pulmonary disease: safety and repeatability. Respir Med 2001; 95: 999–1002.
[13] Brightling CE, Ward R, Goh KL, Wardlaw AJ, Pavord ID. Eosinophilic bronchitis is an important cause of chronic cough. Am J Respir Crit Care Med 1999; 160: 406–10.
[14] Pizzichini E, Pizzichini MM, Efthimiadis A, Evans S, Morris MM, Squillace D et al. Indices of airway inflammation in induced sputum: reproducibility and validity of cell and fluid-phase measurements. Am J Respir Crit Care Med 1996; 154: 308–17.
[15] Pavord ID, Ward R, Woltmann G, Wardlaw AJ, Sheller JR, Dworski R. Induced sputum eicosanoid concentrations in asthma. Am J Respir Crit Care Med 1999; 160: 1905–9.
[16] Hunter CJ, Ward R, Woltmann G, Wardlaw AJ, Pavord ID. The safety and success rate of sputum induction using a low output ultrasonic nebuliser. Respir Med 1999; 93: 345–8.
[17] Pizzichini MM, Pizzichini E, Clelland L, Efthimiadis A, Mahony J, Dolovich J et al. Sputum in severe exacerbations of asthma: kinetics of inflammatory indices after prednisone treatment. Am J Respir Crit Care Med 1997; 155: 1501–8.
[18] Pavord ID, Pizzichini MM, Pizzichini E, Hargreave FE. The use of induced sputum to investigate airway inflammation. Thorax 1997; 52: 498–501.
[19] Brightling CE, Symon FA, Birring SS, Bradding P, Wardlaw AJ, Pavord ID. Comparison of airway immunopathology of eosinophilic bronchitis and asthma. Thorax 2003; 58: 528–32.
[20] Beeh KM, Beier J, Kornmann O, Buhl R. Neutrophilic inflammation in induced sputum of patients with idiopathic pulmonary fibrosis. Sarcoidosis Vasc Diffuse Lung Dis 2003; 20: 138–43.
[21] Beeh KM, Beier J, Kornmann O, Buhl R. Sputum matrix metalloproteinase-9, tissue inhibitor of metalloprotinease-1, and their molar ratio in patients with chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis and healthy subjects. Respir Med 2003; 97: 634–9.
[22] Beeh KM, Beier J, Haas IC, Kornmann O, Micke P, Buhl R. Glutathione deficiency of the lower respiratory tract in patients with idiopathic pulmonary fibrosis. Eur Respir J 2002; 19: 1119–23.
[23] Hope-Gill BD, Hilldrup S, Davies C, Newton RP, Harrison NK. A study of the cough reflex in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2003; 168: 995–1002.
[24] Thomas RA, Green RH, Brightling CE, Birring S.S., Pavord ID. The influence of age on induced sputum differential cell counts in normal subjects. Chest. In press.
[25] Hiwatari N, Shimura S, Sasaki T, Aikawa T, Ando Y, Ishihara H et al. Prognosis of idiopathic pulmonary fibrosis in patients with mucous hypersecretion. Am Rev Respir Dis 1991; 143: 182–5.
[26] Fujimoto K, Kubo K, Yamaguchi S, Honda T, Matsuzawa Y. Eosinophil activation in patients with pulmonary fibrosis. Chest 1995; 108: 48–54.
[27] Rudd RM, Haslam PL, Turner-Warwick M. Cryptogenic fibrosing alveolitis. Relationships of pulmonary physiology and bronchoalveolar lavage to response to treatment and prognosis. Am Rev Respir Dis 1981; 124: 1–8.
[28] Hubbard R, Lewis S, Richards K, Johnston I, Britton J. Occupational exposure to metal or wood dust and aetiology of cryptogenic fibrosing alveolitis. Lancet 1996; 347: 284–9.
[29] Gross TJ, Hunninghake GW. Idiopathic pulmonary fibrosis. N Engl J Med 2001; 345: 517–25.
[30] Birring SS, Prudon B, Carr AJ, Singh SJ, Morgan MD, Pavord ID. Development of a symptom specific health status measure for patients with chronic cough: Leicester Cough Questionnaire (LCQ). Thorax 2003; 58: 339–43.
[31] Chung KF. Assessment and measurement of cough: the value of new tools. Pulm Pharmacol Ther 2002; 15: 267–72.

[32] Mullen J, Hodgson MJ, DeGraff CA, Godar T. Case-control study of idiopathic pulmonary fibrosis and environmental exposures. J Occup Environ Med 1998; 40: 363–7.
[33] Louis RE, Cataldo D, Buckley MG, Sele J, Henket M, Lau LC et al. Evidence of mast-cell activation in a subset of patients with eosinophilic chronic obstructive pulmonary disease. Eur Respir J 2002; 20: 325–31.
[34] Maestrelli P, Richeldi L, Moretti M, Fabbri LM. Analysis of sputum in COPD. Thorax 2001; 56: 420–2.
[35] Cosio Piqueras MG, Cosio MG. Disease of the airways in chronic obstructive pulmonary disease. Eur Respir J Suppl 2001; 34: 41s–9s.
[36] Biernacki WA, Kharitonov SA, Barnes PJ. Increased leukotriene B4 and 8-isoprostane in exhaled breath condensate of patients with exacerbations of COPD. Thorax 2003; 58: 294–8.