

Clinical and laboratory features of chronic cough

Thesis submitted for the degree of
Doctor of Medicine
at the University of Leicester
by

Surinder S Birring BSc (Hons) MBChB (Hons) MRCP
2004

Clinical and laboratory features of chronic cough

Contents	i
Abstract	iv
Acknowledgements	v
Publications arising from this thesis	vi
List of tables and figures	x
1. INTRODUCTION	
1.1 Chronic cough in adults	
1.1.1 Introduction	1
1.1.2 Epidemiology	2
1.1.3 Cough reflex	2
1.1.4 Causes of chronic cough	3
1.1.5 Evaluation of a patient with chronic cough	15
1.1.6 Treatment of chronic cough	20
1.2 Idiopathic chronic cough	
1.2.1 Background	24
1.2.2 Airway inflammation	24
1.2.3 Association with autoimmune disease	26
1.2.4 Role of inflammatory mediators	27
1.2.5 Summary	27

1.3	Assessment of chronic cough	
1.3.1	Cough reflex sensitivity	29
1.3.2	Cough symptom scores	30
1.3.3	Cough monitors	31
1.3.4	Quality of life questionnaires	31
1.4	Hypothesis and aims	36
2.	METHODS	
2.1	General	
2.1.1	Peripheral blood tests	38
2.1.2	Skin tests	38
2.1.3	Symptom visual analogue scores	38
2.2	Respiratory physiology	
2.2.1	Spirometry and lung function tests	39
2.2.2	Airway responsiveness	39
2.2.3	Exhaled nitric oxide	39
2.2.4	Sputum induction	40
2.2.5	Capsaicin cough reflex sensitivity	44
3.	STUDIES	
3.1	Association of idiopathic chronic cough with organ specific autoimmune disease	
3.1.1	Idiopathic chronic cough and organ specific autoimmune diseases: a case-control study	45
3.1.2	Respiratory symptoms in patients with treated hypothyroidism and inflammatory bowel disease	53

3.2	Immunopathology of idiopathic chronic cough	
3.2.1	Idiopathic chronic cough: Association with organ specific autoimmune disease and bronchoalveolar lymphocytosis	62
3.2.2	Induced sputum inflammatory mediator concentrations in chronic cough	89
3.3	Unexplained airflow obstruction- a possible link with idiopathic chronic cough?	
3.3.1	Clinical, radiological and induced sputum features of chronic obstructive pulmonary disease in non-smokers. A descriptive study	103
3.4	Health status in patients with chronic cough	
3.4.1	Development of a symptom specific health status measure for patients with chronic cough: Leicester Cough Questionnaire (LCQ)	119
4.	CONCLUSIONS	
4.1	Summary of findings	132
4.2	Advances in the assessment of chronic cough	134
4.3	Problems encountered and criticisms	136
4.4	Future studies	137
Appendix I	Chronic cough questionnaire (case-control study)	139
Appendix II	Respiratory symptom questionnaire (hypothyroidism and IBD)	141
Appendix III	Leicester Cough Questionnaire (LCQ) version 1	145
Appendix IV	Final version of LCQ	158
Appendix V	Scoring for LCQ	161
REFERENCES		162

Clinical and laboratory features of chronic cough

Surinder Birring

Abstract

Chronic cough is a common presenting symptom to both general practice and respiratory clinics and in up to 20% of patients, the cough remains unexplained after extensive investigation and treatment trials. In this thesis, we have shown that patients with idiopathic chronic cough are predominantly female, have an onset of cough in middle age and have a high prevalence of organ specific autoimmune disease, particularly hypothyroidism. We found that idiopathic chronic cough is associated with a bronchoalveolar lavage lymphocytosis and have suggested that this is due to homing of activated lymphocytes from the primary site of autoimmune inflammation to embryologically related structures such as the airways, analogous to the mechanism thought to be responsible for airway complications of inflammatory bowel disease. The concept that chronic inflammation of foregut structures can be associated with airway symptoms, inflammation and damage is also supported by our finding of a striking excess of cases of treated hypothyroidism amongst a predominantly elderly non-smoking female population with fixed airflow obstruction and a 2-3 fold excess of respiratory symptoms in a cohort of patients with autoimmune hypothyroidism and another with inflammatory bowel disease. We have also shown raised histamine concentrations in airway secretions of patients with idiopathic chronic cough, which suggests a possible mechanism whereby airway inflammation results in cough and raises the possibility that antihistamines may have a therapeutic role in idiopathic chronic cough. Finally, recognising the major impact of chronic cough on health status and the paucity of objective measures available for the assessment of cough, we developed the Leicester Cough Questionnaire, which is a 19-item cough specific quality of life questionnaire.

ACKNOWLEDGEMENTS

I am indebted to my supervisor, Ian Pavord for his guidance and support over the past two years. His encouragement to pursue a wide range of studies has lead to a very rewarding and enjoyable period in research. I would also like to thank my co-supervisor, Andy Wardlaw for his expert supervision and encouragement, particularly with laboratory based aspects of the project. Chris Brightling has been a great help throughout my research and has played an important role in motivating research fellows in our department with his enthusiasm and expertise. Peter Bradding encouraged me to pursue new ideas and supported me in the laboratory. Ruth Green has provided advice and support as a colleague and friend. Ben Prudon and Ronnie Patel's help in cough reflex sensitivity measurement and data collection was invaluable. Debbie Parker, Sue McKenna, Beverly Hargadon, Will Monterio, and Natalie Neale were of considerable help in clinical characterisation of subjects. I am grateful for expert guidance from Fiona Symon and help from Simon Barlow with flow cytometry experiments and immunohistochemistry. Anna Murphy and Jane Scullion collected the data and helped in the venepuncture of patients for the case control study. Many departments of Glenfield Hospital have supported me during this thesis which include Institute for Lung Health laboratory, induced sputum team, respiratory medicine, respiratory physiology, pulmonary rehabilitation, outpatient clinic preparation team, medical secretaries, physiotherapy, integrated medicine, chemical pathology and radiology. This project would not have been possible without the generous financial support from grants awarded by the British Lung Foundation and University Hospitals of Leicester NHS Trust.

Finally a special thank you to my wife, Sharan for her patience, encouragement and support during those long days in the laboratory and writing up.

PUBLICATIONS ARISING FROM THIS THESIS

Papers

- 1) Birring SS, Prudon B, Carr AJ, Singh SJ, Morgan MDM, Pavord ID. Development of a symptom specific health status measure for patients with chronic cough: Leicester Cough Questionnaire (LCQ). *Thorax* 2003; 58:339-343
- 2) Birring SS, Morgan AJ, Prudon B, McKeever TM, Lewis SA, Falconer Smith JF, Robinson RJ, Britton JR, Pavord ID. Respiratory symptoms in patients with treated hypothyroidism and inflammatory bowel disease. *Thorax* 2003; 58:533-536
- 3) Birring, SS, Brightling CE, Bradding P, Entwisle JJ, Vara DD, Grigg J, Wardlaw, AJ and Pavord ID. Clinical, radiologic, and induced sputum features of chronic obstructive pulmonary disease in nonsmokers: a descriptive study. *Am J Respir Crit Care Med* 2002; 166:1078-1083
- 4) Brightling CE, Symon FA, Birring SS, Wardlaw AJ, Robinson R, Pavord ID. A case of cough, lymphocytic bronchoalveolitis and coeliac disease with improvement following a gluten free diet. *Thorax* 2002; 57: 91-92
- 5) Birring SS, Murphy AC, Scullion JE, Brightling CE, Browning M, Pavord ID. Idiopathic chronic cough and organ specific autoimmune diseases: a case-control study. *Respiratory Medicine* 2004; 98: 242-246
- 6) Birring SS, Brightling CE, Symon FA, Barlow SG, Wardlaw AJ, Pavord ID. Idiopathic chronic cough: Association with organ specific autoimmune disease and bronchoalveolar lymphocytosis. *Thorax* 2003; 58: 1066-1070
- 7) Birring SS, Parker D, Brightling CE, Bradding P, Wardlaw AJ, Pavord ID. Induced sputum inflammatory mediator concentrations in chronic cough. *Am J Respir Crit Care Med* 2004; 169: 15-19
- 8) Birring SS, Passant C, Patel RB, Prudon B, Murty GE, Pavord ID. Chronic tonsillar enlargement and cough: preliminary evidence of a novel and treatable cause of chronic cough. *Eur Respir J* 2004; 23: 199-201
- 9) Birring SS, Patel RB, Parker D, McKenna S, Hargadon B, Montero W, Pavord ID. Airway function and markers of airway inflammation in patients with treated hypothyroidism. *Submitted*.

- 10) Birring SS, Matos S, Patel RB, Prudon B, Evans DH, Pavord ID. The relationship between cough frequency, cough sensitivity and quality of life in patients with chronic cough. *Submitted.*
- 11) Birring SS, Parker D, McKenna S, Hargadon B, Brightling CE, Pavord ID, Bradding P. Sputum eosinophils in cryptogenic fibrosing alveolitis. *Submitted.*
- 12) Prudon B, Birring SS, Vara DD, Hall AP, Thompson JP, Pavord ID. Cough and glottic stop reflex sensitivity in health and disease. *In revision.*

Reviews

- 1) Birring SS, Berry M, Brightling CE, Pavord ID. Eosinophilic Bronchitis: Clinical Features, Management and Pathogenesis. *Am J Respir Med* 2003; 2: 169-173
- 2) Berry M, Birring SS, Brightling CE, Pavord ID. Cough and asthma. *Clin Exp Allergy* 2003;33:1481-3

Chapters

- 1) Birring SS and Pavord ID. An approach to respiratory symptoms: cough. In *Clinical Respiratory Medicine*. 2nd Ed 2004. Editors: Albert RK, Spiro SG, Jett RJ. Elsevier Science, PA, USA.
- 2) Birring SS and Pavord ID. Impact of cough on health status. Acute and chronic cough. Editors: Redington AE, Morice AH. Marcel Dekker Inc, NY, USA.

Letters

- 1) Birring SS and Pavord ID. COPD and Hepatitis C infection. *Chest* 2003;124:2035-6
- 2) Birring SS and Pavord. Gastro-oesophageal reflux and chronic cough. *Thorax*. *In press.*

Abstracts

- 1) Birring SS, Murphy AC, Scullion JE, Brightling CE, Browning M, Pavord ID. Idiopathic chronic cough and organ specific autoimmune diseases: a case-control study. *International Cough Symposium* 2001; P17
- 2) Birring SS, Brightling CE, Entwisle JJ, Vara DD, Bradding P, Wardlaw, Pavord ID. Chronic Obstructive Pulmonary Disease in non-smokers. *Am J Resp Crit Care Med* 2002; 165:A587
- 3) Birring SS, Prudon B, Carr AJ, Singh SJ, Morgan MDL, Pavord ID. Development of a disease specific health status measure for patients with chronic cough. *Eur Resp J* 2002; 20 (suppl 38): P2849
- 4) Birring SS, Morgan AJ, Prudon B, McKeever TM, Lewis S, Falconer Smith JF, Britton J, Pavord ID. Respiratory symptoms in patients with treated hypothyroidism. *Eur Resp J* 2002; 20 (suppl 38): P2832
- 5) Birring SS, Brightling CE, Symon FA, Barlow S, Wardlaw AJ, Pavord ID. Lymphocytic bronchoalveolitis in idiopathic chronic cough. *Thorax* 2002; 57(3):iii1 (Short listed for BTS young investigator prize)
- 6) Birring SS, Prudon B, Pavord ID. Relationship between capsaicin cough sensitivity and quality of life in patients with chronic cough. *Thorax* 2002; 57(3):iii23
- 7) Birring SS, Patel RB, Prudon B, Singh SJ, Morgan MDM, Pavord ID. Quality of life in chronic cough. *Am J Resp Crit Care Med* 2003;167:A315
- 8) Birring SS, Parker D, Brightling CE, Wardlaw AJ, Pavord ID. Induced sputum inflammatory mediator concentrations in idiopathic chronic cough. *Am J Respir Crit Care Med* 2003;167:A315
- 9) Birring SS, Passant C, Patel RB, Prudon B, Murty GE, Pavord ID. Cough and chronic tonsillar enlargement: The effect of tonsillectomy. *ERJ* 2003;22:172s
- 10) Birring SS, Parker D, McKenna S, Hargadon B, Brightling CE, Pavord ID, Bradding P. Sputum eosinophilia in cryptogenic fibrosing alveolitis. *ERJ* 2003;22:227s
- 11) Brightling CE, Symon FA, Birring SS, Pavord ID, Wardlaw AJ. Th2 cytokine expression in bronchoalveolar lavage T-lymphocytes in asthma

- and eosinophilic bronchitis without asthma. *J Allergy Clin Immunol* March 2002
- 12) Prudon B, Birring SS, Vara DD, Pavord ID. Repeatability of capsaicin cough reflex sensitivity measurement. *Thorax* 2002; 57(3):iii23
 - 13) Prudon B, Birring SS, Hall AP, Thompson JP, Pavord ID. Upper airway sensitivity in subjects with a chronic non-productive cough. *Thorax* 2002; 57(3):iii23
 - 14) Patel RB, Birring SS, Falconer Smith JF, Pavord ID. Airway function and markers of airway inflammation in patients with treated hypothyroidism. *Thorax* 2003;58:iii47
 - 15) Birring SS, Parker D, McKenna S, Hargadon B, Brightling CE, Pavord ID, Bradding P. Induced sputum mediator concentrations in COPD. *Thorax* 2003;58:iii87
 - 16) Matos S, Birring SS, Patel RB, Prudon B, Evans DH, Pavord ID. Day time cough counts using the Leicester Cough Monitor (LCM) in patients with chronic cough and healthy controls. *Thorax* 2003;58:iii48
 - 17) Birring SS, Patel RB, Singh SJ, Morgan MDL, Pavord ID. Assessment of quality of life in acute cough with the Leicester Cough Questionnaire. *Am J Respir Crit Care Med* 2004
 - 18) Birring SS, Matos S, Patel RB, Prudon B, Evans DH, Pavord ID. The relationship between cough counts and quality of life in patients with chronic cough. *ERJ* 2004

LIST OF TABLES

- 1.1 Common causes of chronic cough in specialist clinics
- 1.2 Symptoms, airway physiology and inflammation in untreated asthma and eosinophilic bronchitis
- 1.3 Clinical features, investigations and treatment of chronic persistent cough
- 1.4 Randomised controlled trials of anti-reflux therapy for gastro-oesophageal associated cough
- 1.5 Common pitfalls in the management of chronic cough
- 1.6 Suggested treatment trials for patients with idiopathic chronic cough
- 1.7 Adverse symptoms in patients with chronic cough
- 3.1 Distribution of age and sex among all cases and controls identified, all respondents to the questionnaire and those who had an autoantibody screen
- 3.2 Self-reported organ-specific autoimmune disease and presence of organ-specific autoantibodies in cases and controls
- 3.3 Subject characteristics (hypothyroidism and IBD)
- 3.4 Prevalence of respiratory symptoms (hypothyroidism and IBD)
- 3.5 Antibody concentration used for flow cytometry (surface markers)
- 3.6 Antibody concentrations used for immunohistochemistry
- 3.7 Subject characteristics (immunopathology study)
- 3.8 Median (range) differential cell counts in sputum, bronchial wash and BAL (%)
- 3.9 Median (range) bronchial biopsy subepithelial cell counts per mm²
- 3.10 Mean (SEM) of the proportion (%) of CD4+ (CD3+CD4+) and CD8+ (CD3+CD4-) T-cells that express chemokine receptors and activation markers in PBMC and BAL
- 3.11 Mean (SEM) of the proportion (%) of CD4+ (CD3+CD8-) and CD8+ (CD3+CD8+) PBMC and BAL T-lymphocytes cells expressing intracellular cytokine staining
- 3.12 Subject characteristics (inflammatory mediator study)
- 3.13 Sputum cells counts and exhaled nitric oxide levels
- 3.14 Sputum mediator concentrations
- 3.15 Patient characteristics I (unexplained COPD study)

- 3.16 Patient characteristics II (unexplained COPD study)
- 3.17 Lung function, induced sputum differential cell counts, and HRCT findings
- 3.18 LCQ1 item reduction reasons
- 3.19 Cronbach's alpha coefficients for each domain and total score for Leicester Cough Questionnaire
- 3.20 Spearman rank correlations between different health outcome measures and LCQ2
- 3.21 LCQ responsiveness: effect sizes of LCQ domains and cough VAS after treatment
- 4.1 Repeatability and responsiveness of outcome measures used to assess patients with chronic cough

LIST OF FIGURES

- 1.1 Diagnostic algorithm for patients with chronic cough
- 2.1 Sputum induction protocol
- 2.2 Sputum processing protocol
- 3.1 Odds ratio (95% confidence intervals) of reporting respiratory symptoms compared to controls in patients with treated hypothyroidism and inflammatory bowel disease
- 3.2 BAL lymphocyte differential cell count (%)
- 3.3 An example of a BAL lymphocytosis
- 3.4 An example of positive submucosal staining for lymphocytes (CD3)
- 3.5 Example FACS dot-plots from a normal control, a patient with idiopathic chronic cough and explained chronic cough for chemokine receptor CCR5 staining and isotypic control in BAL T-lymphocytes
- 3.6 Example FACS dot-plots from an individual normal control and a patient with idiopathic chronic cough for IL-4 intracellular cytokine staining and isotypic control in resting and stimulated PBMC T-lymphocytes
- 3.7 Median concentrations of Histamine, PGD₂, PGE₂, Cys-Leukotrienes and IL-8
- 3.8 Patients with airflow obstruction seen in a respiratory out-patient clinic over 2 years
- 3.9 Detailed lung function
- 3.10 Bland –Altman plot of LCQ total score repeated over two weeks in patients whose cough remained unchanged
- 4.1 Lymphocyte homing in idiopathic chronic cough

1 INTRODUCTION

1.1 CHRONIC COUGH IN ADULTS

1.1.1 Introduction

Cough is an important defence mechanism that clears the airways of secretions and prevents entry of foreign bodies and irritants to the lower respiratory tract. It is a universal experience in health but also a non-specific presenting feature of a number of respiratory conditions. Cough is one of the most common presenting symptoms to a general practitioner. Most cases result from viral and bacterial upper respiratory tract infection, are self-limiting and do not require further evaluation but a small proportion of patients have persistent cough that requires specialist opinion (Irwin & Madison 2000). Chronic cough is arbitrarily defined as a cough greater than three weeks in duration. It is present in 3% of the general population and is responsible for between 5 and 10% of respiratory outpatient referrals (Fuller & Jackson 1990; Janson et al. 2001a). Chronic cough is often perceived as a trivial problem but can be a disabling symptom associated with impairment of quality of life, and distressing associated symptoms such as musculoskeletal chest pains, syncope, incontinence, disturbed sleep, and social embarrassment.

The assessment of a patient with chronic cough is based on the so called 'anatomical diagnostic protocol.' This is a systematic evaluation based on the understanding that most cases are due to disease of the upper respiratory tract where cough receptors are most plentiful (Fuller & Jackson 1990). There is a general consensus that the cause of chronic cough in most patients with normal spirometry and chest radiography is asthma, eosinophilic bronchitis, gastro-oesophageal reflux, rhinitis or a combination of these (Brightling et al. 1999a; Irwin & Madison 2000). Many of these conditions can be recognised clinically and successful diagnosis and management is often possible without recourse to expensive or invasive investigations. This approach does seem to be successful and various series have reported a high rate of treatment success even in tertiary populations (Irwin, Corrao, & Pratter 1981).

1.1.2 Epidemiology

The exact prevalence of chronic cough in the population is difficult to estimate but varies between 3-40% (Cullinan 1992; Fuller & Jackson 1990; Loudon & Brown 1967). It is dependent on the method used to collect data and the characteristics of the population surveyed, in particular smoking prevalence and environmental and occupational pollution exposure. The control populations of angiotensin converting enzyme inhibitor trials suggest the prevalence of cough in the elderly population is around 3% (Inman 1986). In younger subjects, the prevalence of cough has been reported as 10% in 18,277 subjects aged 20 to 48 years from 16 countries interviewed for the European Community Respiratory Health survey (Janson et al. 2001a). In population surveys, men report cough more frequently (Cullinan 1992) but the majority of patients referred to specialist cough clinics are females (Morice & Kastelik 2003). This disparity is thought to arise in part from differences in smoking habits but also the fact that women have an intrinsically heightened cough reflex compared to men (Fujimura et al. 1996) and cough has a greater impact on their quality of life (Birring S.S. et al. 2003). In summary, cough is one of the most prevalent symptoms in any population and accounts for a substantial number of primary and secondary care consultations.

1.1.3 Cough reflex

An understanding of the cough reflex is important for the realisation of potential causes of chronic cough and because its assessment is a widely used outcome measure in clinical studies investigating chronic cough. Only a brief outline is given here since it is beyond the scope of this thesis and has been described in detail elsewhere (Morice, Kastelik, & Thompson 2001). Most cases of chronic cough are associated with heightened cough reflex sensitivity, particularly if the cough is dry (Choudry & Fuller 1992). This can be assessed formally using cough challenge tests incorporating tussive substances such as capsaicin or citric acid (Choudry & Fuller 1992 and section 2.2.5). A number of cough receptors have been described but bronchial C fibres, activated via vanilloid cough receptors, and rapidly adapting receptors are probably the most important (Hwang & Oh 2002; Widdicombe 1995). Sensitisation of cough receptors has been shown to occur in experimental animals and man after administration of various

inflammatory mediators such as prostaglandins and one plausible explanation for heightened cough reflex sensitivity and cough in patients with chronic cough is the presence of airway inflammation and increased concentrations of tussive mediators adjacent to cough receptors (Choudry, Fuller, & Pride 1989). Afferent nerve fibres pass to a central cough receptor in the medulla (Bolser & Davenport 2002) triggering a forced expiratory manoeuvre against a closed glottis followed by glottal opening and high velocity expiration. Factors influencing activity of the central cough receptor are poorly understood but opiates probably exert their anti-tussive effects here (Bolser & Davenport 2002). Successful treatment of the underlying cause of the cough has been shown to reduce cough sensitivity (O'Connell et al. 1994).

1.1.4 Causes of chronic cough

Most conditions implicated in causing chronic cough such as chronic obstructive pulmonary disease, lung cancer, foreign bodies, pulmonary tuberculosis, sarcoidosis, idiopathic pulmonary fibrosis and heart failure are obvious after clinical assessment, spirometry and a chest radiograph. Thus the majority of patients referred for investigation of chronic cough are non-smokers and have normal findings on physical examination, spirometry and chest radiography. Many specialist cough clinics have reported the causes of cough found in patients referred for further investigation; these are listed in Table 1.1. The cause in the majority of patients with cough referred to secondary care was asthma, gastro-oesophageal reflux or rhinitis. Between 10 to 30% have multiple causes for their cough (Irwin & Madison 2000).

Table 1.1 Common causes of chronic cough in specialist clinics.

AUTHOR	No patients (female)	Diagnosis (% of total)			
		Asthma/EB	GOR	Rhinitis	Idiopathic
(Irwin, Corrao, & Pratter 1981)	49 (27)	25	10	29	0
(Poe et al. 1989)	139 (84)	35	5	26	12
(Irwin, Curley, & French 1990)	102 (59)	24	21	41	1
(O'Connell et al. 1994)	87 (63)	6	10	13	22
(Smyrnios, Irwin, & Curley 1995)	71 (32)	24	15	40	3
(Mello, Irwin, & Curley 1996)	88 (64)	14	40	38	2
(McGarvey et al. 1998)	43 (29)	23	19	21	18
(Palombini et al. 1999)	78 (51)	59	41	58	0
(Brightling et al. 1999a)	91	31	8	24	7
(Simpson 1999)	86 (51)	6	22	28	2
(Birring S.S. et al. 2002)	150 (99)	32	15	13	17
(Birring et al. 2003a)	236 (142)	24	15	12	23

Cough variant asthma

Asthma is a condition characterised by airway hyperresponsiveness and inflammation that presents with variable symptoms of cough, dyspnoea and wheeze. A subgroup with an isolated chronic cough, known as cough variant asthma was originally described by Glauser in 1972 (Glauser 1972) and more clearly defined in 1979 by Corrao and colleagues (Corrao, Braman, & Irwin 1979). They described six patients with a dry cough without wheezing and airflow obstruction who had airway hyperresponsiveness and resolution of cough with oral theophylline or terbutaline (Corrao, Braman, & Irwin 1979). The cough

returned when the anti asthma medication was discontinued, suggesting this was a variant of asthma.

Cough variant asthma is a common cause of isolated cough, accounting for around 30% of cough referrals to specialist clinics (Table 1.1.). The differences in the prevalence of cough variant asthma seen in reported series may in part be due to the difficulty in diagnosing asthma. Serial peak flow recordings and spirometry with bronchodilator response are routine first line investigations but are often normal in cough variant asthma or even classical asthma (Cockcroft et al. 1997; Hunter et al. 2002). Demonstration of airway hyperresponsiveness by bronchoprovocation testing has been shown to be a more sensitive and specific index of variable airflow obstruction and can be the only abnormality seen (Hunter et al. 2002). Hunter et al compared methacholine challenge test with peak flow variability and bronchodilator reversibility in 69 patients with mild asthma and found that methacholine airway responsiveness had the best sensitivity and specificity (91% and 90% respectively) in the diagnosis of variable airflow obstruction.

Patients with cough variant asthma usually have a heightened cough reflex that is uncommon in patients with non-cough predominant asthma (Prudon et al. 2004). The airway inflammation in cough variant asthma is essentially similar to that seen in classical asthma (Niimi et al. 1998) and the reason for the different physiological association is unclear. Cough variant asthma is characterised by eosinophilic airway inflammation in sputum (Fujimura et al. 2003; Carney et al. 1997), bronchoalveolar lavage (Niimi et al. 1998; McGarvey et al. 1999), and bronchial biopsies (Niimi et al. 1998; Niimi et al. 2000). Furthermore, the degree of eosinophilic airway inflammation in bronchial biopsies correlates with the severity of cough (Niimi et al. 1998) implicating a role for eosinophilic airway inflammation in the pathogenesis of cough variant asthma. The mast cell is also thought to be important in the pathogenesis of cough variant asthma. Mast cell mediators in sputum such as histamine and prostaglandin D₂ are present in higher concentrations in patients with cough variant asthma (Birring et al. 2003b) and eosinophilic bronchitis (Brightling et al. 2000c; Gibson et al. 1998) than those with classical asthma (Brightling et al. 2000c), and antihistamines have been shown to be beneficial in cough associated with asthma (Rafferty 1990). Thus it is possible

that mast cells localising in the superficial structures, perhaps adjacent to sensory nerve endings, are particularly important in the genesis of heightened cough reflex sensitivity and cough in cough variant asthma.

Cough variant asthma can progress to classical asthma with more obvious wheezing and variable airflow obstruction, although it is unclear from longitudinal studies how frequent this transformation occurs. In some series up to one third of patients with cough variant asthma developed classical asthma symptoms (Johnson & Osborn 1991; Fujimura et al. 2003) although in another three year follow up of 63 patients with cough variant asthma, wheezing occurred in only 6% (Orejas Garcia & Pascual Pascual 1998). Although there are no longitudinal studies of decline in lung function specifically in cough variant asthma, increased subepithelial layer thickness in bronchial biopsies, thought to be a pathological feature of airway remodelling has been demonstrated in cough variant asthma, though not to the same degree as in classical asthma (Niimi et al. 2000). This implies that airway remodelling and possibly accelerated decline in lung function may occur in cough variant asthma in the same way as it occurs in classical asthma.

Eosinophilic bronchitis

Gibson et al. (Gibson et al. 1989), first described eosinophilic bronchitis without asthma as a cause of chronic cough in 1989. They presented a condition that manifests as a corticosteroid responsive chronic cough in non-smokers with none of the functional abnormalities seen in asthma. These patients typically had evidence of airway inflammation in the form of a sputum eosinophilia, hence the term eosinophilic bronchitis. The development of safe and non-invasive methods of assessing airway inflammation using induced sputum has allowed further characterisation of this condition. Studies where assessment of airway inflammation has been undertaken in chronic cough patients have shown that eosinophilic bronchitis without asthma may account for up to 10-15% of cases referred for specialist investigation although the incidence is likely to depend on the extent to which therapeutic trials of corticosteroids are undertaken in primary care (Brightling et al. 1999a; Carney et al. 1997).

Eosinophilic bronchitis is defined as a chronic cough in subjects with no symptoms or objective evidence of variable airflow obstruction, normal airway hyperresponsiveness (provocative concentration of methacholine producing a 20% decrease in FEV₁ [PC₂₀] > 16mg/ml) and a sputum eosinophilia (Brightling et al. 1999a). A sputum eosinophil count >3% is widely used as indicative of eosinophilic bronchitis as this is well outside our normal range (<1.9%) and is associated with a corticosteroid response in COPD and asthma (Pavord et al. 1999a;Pizzichini et al. 1998). The main features and differences between eosinophilic bronchitis, cough variant asthma and classical asthma are summarised in Table 1.2. Induced sputum is a safe, valid and repeatable measure of airway inflammation (Pavord et al. 1997) but does require same day processing for eosinophil quantification and cell viability, unlike routine cytology. Exhaled nitric oxide, another non-invasive marker of airway inflammation has been proposed as a simpler but more expensive alternative to induced sputum tests. Exhaled nitric oxide levels are usually higher in eosinophilic bronchitis (Berlyne et al. 2000) but its role in the diagnosis of eosinophilic bronchitis has not been formally evaluated.

One of the main interests in the pathogenesis of eosinophilic bronchitis is why an apparently similar pattern of airway inflammation is associated with different functional abnormalities in eosinophilic bronchitis and asthma. Both conditions are associated with a similar degree of sputum (Brightling et al. 2000c), bronchoalveolar lavage (Gibson et al. 1998) and biopsy (Brightling et al. 2002a) eosinophilia and similar degree of basement membrane thickening (Brightling et al. 2002a) in bronchial biopsy suggesting that the site within the bronchial tree is similar. Brightling et al have assessed the activation of airway inflammation in eosinophilic bronchitis by measuring sputum supernatant concentrations of various important effector mediators and found that eosinophilic bronchitis and asthma are both associated with increased levels of cysteinyl-leukotrienes and eosinophilic cationic protein (Brightling et al. 2000c). Interestingly, histamine and PGD₂ concentrations were only increased in eosinophilic bronchitis suggesting that activation of mast cells in superficial airway structures is a particular feature of this condition and raising the possibility that localisation of activated mast cells might differ in classical asthma and eosinophilic bronchitis. In support of this,

mast cell numbers in airway smooth muscle are increased in classical asthma, but not in eosinophilic bronchitis (Brightling et al. 2002a). Furthermore airway smooth muscle mast cell numbers inversely correlated with airway hyperresponsiveness. Thus a key factor determining the different functional association of airway inflammation in eosinophilic bronchitis and classical asthma might be the microlocalisation of mast cells with a predominant airway smooth muscle infiltration resulting in airway hyperresponsiveness and variable airflow obstruction, and an epithelial infiltration producing bronchitis and cough. The specific role of the mast cell in the bronchial epithelium of patients with eosinophilic bronchitis and its interactions with cough sensory afferents are not known and require further study.

Table 1.2 Symptoms, airway physiology and inflammation in untreated asthma and eosinophilic bronchitis.

	Symptom		Airway hyperresponsiveness	Airway Inflammation	
	Cough	Wheeze		Sputum Eosinophilia	Exhaled NO
Asthma	often	usual	usual	usual	usual
Cough Variant Asthma	Yes	No	usual	usual	usual
Eosinophilic Bronchitis	Yes	No	No	Yes	usual

NO: nitric oxide

The natural history of eosinophilic bronchitis is unclear. A ten year follow up evaluation of the twelve patients from the original reports of eosinophilic bronchitis suggests that this condition is generally benign and self-limiting (Hancox et al. 2001). However our experience is that most have persistent disease. Remission and progression to clinical asthma with wheezing and airway hyperresponsiveness is rare (3% and 9% respectively) although the development of fixed airflow obstruction occurred in 16% (Berry et al. 2003). Several studies have observed that 30-40% of patients with chronic obstructive pulmonary disease (COPD) without a history of asthma and with no bronchodilator reversibility have sputum evidence of an airway eosinophilia (Brightling et al. 2000a; Pizzichini et al. 1998). One possible explanation for the presence of eosinophilic airway inflammation in COPD without apparent pre-existing asthma is that eosinophilic bronchitis is a prelude to COPD in some patients. In support of this hypothesis there is one case report describing a patient with eosinophilic bronchitis and poorly controlled eosinophilic airway inflammation who developed fixed airflow obstruction over 2-3 years (Brightling et al. 1999b). The progressive irreversible airflow obstruction may have been due to remodelling of the airway secondary to the persistent eosinophilic airway inflammation in the presence of inadequate corticosteroid therapy. If this is true it has important implications in the early diagnosis and successful treatment of eosinophilic bronchitis.

One other corticosteroid responsive cough syndrome that has been described principally in Japan is atopic cough (Fujimura, Sakamoto, & Matsuda 1992). Atopic cough has been defined as an isolated chronic cough with no variable airflow obstruction or airway hyperresponsiveness and one or more objective indicators of atopy as defined by: blood or sputum eosinophilia, elevated total or specific IgE or positive skin prick tests (Fujimura et al. 2003). Most patients have heightened cough reflex sensitivity. The prevalence of atopic cough in Japanese patients referred to specialist cough clinics has been reported as high as 58% (Fujimura et al. 2003). Whether atopic cough should be considered a separate clinical entity is unclear since there is overlap with cough variant asthma in some patients reported to have atopic cough and others have suggested that it should be considered as part of the asthma syndromes (McGarvey & Morice 2003). Atopic cough differs from eosinophilic bronchitis in that there is absence of

bronchoalveolar lavage eosinophilia. Like eosinophilic bronchitis, most patients with atopic cough do not appear to develop typical asthma (Fujimura et al. 2003). Another corticosteroid responsive cough syndrome described in a Japanese patient with airflow obstruction and HRCT findings resembling diffuse panbronchiolitis is eosinophilic bronchiolitis (Takayanagi et al. 2001) but there are no reports of this condition in cough clinics outside Japan.

Gastro-oesophageal reflux related cough

Gastro-oesophageal reflux has been reported as the cause of chronic cough in up to 41% of patients referred to specialist cough clinics (Table 1.1). Symptoms suggesting gastro-oesophageal reflux and abnormalities of oesophageal function are common in patients with chronic cough of all age groups and the frequent clinical observation that effective treatment of gastro-oesophageal reflux is associated with improvement of cough supports a causal association (Irwin et al. 1989). Gastro-oesophageal reflux related cough is associated with the relaxation of the lower oesophageal sphincter and often occurs during eating, talking and on waking (Morice & Kastelik 2003). Although most patients recognise heartburn, dysphagia, sore throat, globus and dysphonia, up to a third of patients with gastro-oesophageal reflux related cough have no such symptoms (Irwin et al. 1989). It is not clear whether features in the history are helpful in the diagnosis of gastro-oesophageal reflux related cough. Mello et al (Mello, Irwin, & Curley 1996) found that the character, timing and the complications of cough were unhelpful in determining the cause of chronic cough whilst others suggest a detailed history is helpful (Morice & Kastelik 2003).

Gastro-oesophageal reflux can be diagnosed with 24-hour oesophageal pH and manometry studies with simultaneous patient diary cards to record cough events (Vaezi & Richter 1997). Oesophageal pH monitoring may show evidence of abnormal standard parameters of gastro-oesophageal reflux. A temporal relationship between cough and reflux is particularly suggestive of gastro-oesophageal reflux related cough (Irwin et al. 1993). However it has not always been possible to relate features on 24-hour oesophageal monitoring to cough that responds to treatment (Ours et al. 1999). It has been suggested that an alternative to oesophageal studies, particularly indicated in patients with obvious gastro-

oesophageal reflux symptoms, is a trial of high dose proton pump inhibitor for three months (Ours et al. 1999).

Several observations are widely quoted to support a causal relationship between gastro-oesophageal reflux disease and cough. Firstly, 24-hour oesophageal pH monitoring can demonstrate a temporal relationship between cough and reflux episodes, either simultaneously or shortly after a fall in pH (Irwin et al. 1993). Secondly, infusion of acid into the distal oesophagus causes cough in most patients with gastro-oesophageal reflux related cough (Ing, Ngu, & Breslin 1994). Thirdly, bronchoscopy and induced sputum findings in some patients with gastro-oesophageal reflux related cough is consistent with aspiration of gastric contents although how generally applicable these findings are is unclear (Parameswaran et al. 2000; Wolfe, Bone, & Ruth 1976). Finally, the improvement in cough with treatment of gastro-oesophageal reflux suggests a cause and effect relationship (Irwin & Madison 2002).

The pathophysiology of gastro-oesophageal reflux associated cough is poorly understood but microaspiration of oesophageal contents to the tracheobronchial tree and stimulation of a neural oesophageal-tracheobronchial reflexes are thought to be important (Ing, Ngu, & Breslin 1994; Jack et al. 1994). The fact that gastro-oesophagus reflux occurs mostly in distal than proximal oesophagus as assessed by 24-hour oesophageal pH studies suggests that aspiration of gastric contents is unlikely to be a key mechanism in most patients with gastro-oesophageal reflux related cough (Irwin et al. 1993). This suggests the presence of an oesophageal-tracheobronchial reflex activated by local irritation of cough receptors in the lower oesophagus. The lack of bronchoscopic features of aspiration in most patients with gastro-oesophageal reflux associated cough supports this reflex (Irwin et al. 1993). The oesophageal-tracheobronchial reflex can be activated by stimuli other than acid such as saline fluid and can be inhibited by local anaesthetic directly applied to the oesophagus (Ing, Ngu, & Breslin 1994). It is thought that gastro-oesophageal reflux exposes submucosal oesophageal cough receptors through epithelial injury. However, Ferrari et al have previously reported that heightened cough reflex sensitivity in patients with gastro-oesophageal reflux disease is related to the presence of reflux and not oesophagitis, suggesting that acid reflux rather than oesophageal damage per se is a key factor in the enhanced cough

response (Ferrari et al. 1995). Treatment of gastro-oesophageal reflux with proton pump inhibitors results in an improvement in cough reflex sensitivity (Benini et al. 2000). The interaction of this reflex interacts with other upper airway reflexes is unclear but it is conceivable that it is another expression of a global abnormality of upper airway reflexes (Prudon et al. 2004). Heightened cough reflex sensitivity is seen in the presence or absence of cough in patients with gastro-oesophageal reflux disease (Choudry & Fuller 1992; Ferrari et al. 1995) suggesting that other factors are involved in the pathogenesis of gastro-oesophageal reflux associated cough.

Several observations suggest that factors other than acid reflux into the oesophagus are important in the pathogenesis of cough in patients with gastro-oesophageal reflux. First, the infusion of saline into the oesophagus used as a control solution in experiments performed by Ing et al caused significant coughing in patients with gastro-oesophageal reflux related cough but not in healthy subjects (Ing, Ngu, & Breslin 1994). Moreover, the infusion of acid into the oesophagus did not result in cough in all patients (Ing, Ngu, & Breslin 1994; Irwin et al. 1993). Second, 24-hour oesophageal pH monitoring has been shown to be a poor predictor of therapeutic response to proton pump inhibitors in patients with gastro-oesophageal reflux related cough (Ours et al. 1999). Third, in randomised controlled trials of proton pump inhibitor therapy in patients with gastro-oesophageal reflux related cough, active treatment was not significantly better in suppressing cough than placebo (Kiljander et al. 2000; Ours et al. 1999)(Section 1.1.6). There are several potential explanations for these findings. Cough mediated by non-acid reflux, resistance to acid suppression, short treatment duration, and coexistence of other causes of chronic cough have been proposed (Irwin & Madison 2002; Morice & Kastelik 2003). Another potentially important factor is oesophageal dysmotility, which has recently been shown to be prevalent in patients with cough and gastro-oesophageal reflux symptoms and can be the only abnormality on 24-hour oesophageal pH and manometry studies in up to a third of such patients (Kastelik et al. 2003). Finally, a novel mechanism that may explain the discrepancies in 24-hour pH monitoring findings and varied treatment responses in patients with gastro-oesophageal reflux related cough is that gastro-oesophageal reflux and cough are merely a manifestation of a global

abnormality of upper airway reflexes, perhaps due to inflammation of embryologically related structures. The presence of airway inflammation in patients with gastro-oesophageal reflux associated cough is consistent with such a mechanism which may lead to heightened aero-digestive reflexes (McGarvey et al. 1999). Further studies are required to investigate the nature of inflammation in foregut derived organs and its relationship with the cough reflex.

Rhinitis associated cough

Rhinitis, often associated with sinusitis and post-nasal drip is one of the most common causes of chronic cough (Table 1.1). Allergy, vasomotor rhinitis, viral and bacterial infections and nasal polyps are potential causes of rhinitis with post-nasal drip. Patients usually report nasal congestion, nasal discharge, facial pain and may be aware of a post-nasal drip and the need to frequently clear their throat. Careful examination of upper airways may reveal nasal quality to the voice, nasal polyps, sinus tenderness, inflammation the posterior pharyngeal wall with evidence of draining secretions. Cough associated with silent post-nasal drip has been described in patients with an isolated cough and no other symptoms on the basis of an therapeutic response to antihistamines and decongestants (Pratter et al. 1993).

The pathophysiology of cough in rhinitis is poorly understood. The contribution of post-nasal drip to the chronic cough is controversial and may be difficult to establish. Mechanical stimulation of cough receptors from nasal and sinus secretions dripping into the hypopharynx and larynx has been suggested (Lalloo, Barnes, & Chung 1996). However the transport of mucus from the nose and sinuses to the pharynx is a physiological process present in all individuals. Many patients with rhinosinusitis sense post nasal drip and have a large amount of mucus and inflammation in the pharynx but do not have a chronic cough. Whether post-nasal drip causes cough or is merely a marker of upper airway pathology requires further study. Another mechanism for rhinitis associated cough that has been proposed is the extension of local inflammation to the pharynx and upper airways (Morice & Kastelik 2003). Rhinitis and asthma commonly occur together where the symptoms of rhinitis may be a marker of disease in the lower airway.

The diagnosis of cough associated with rhinitis is one of the most difficult to make of the cough syndromes because it is solely based on symptoms that occur frequently in the general population, lacks objective verification and commonly occurs with other causes of cough such as asthma. Investigations for rhinosinusitis include nasal endoscopy and x-ray or CT scan of the sinuses, which may reveal mucosal thickening and fluid levels. However these investigations have a low predictive value in the diagnosis of cough associated with rhinosinusitis.

Angiotensin converting enzyme inhibitor associated cough

It is 20 years since cough was recognised as a side effect of angiotensin converting enzyme inhibitor drugs (Sesoko & Kaneko 1985). Approximately 8% of patients taking angiotensin converting enzyme inhibitors develop a persistent cough (Berkin & Ball 1988). The risk is higher in females and is similar with all types of angiotensin converting enzyme inhibitors (Yeo, Foster, & Ramsay 1991). Cough is not seen with angiotensin converting receptor antagonists (Elliott 1999). Increased airway concentrations of airway tussive mediators such as bradykinins and prostaglandins are thought to be responsible for heightened cough reflex sensitivity and cough in patients with angiotensin converting enzyme inhibitor cough (McEwan, Choudry, & Fuller 1990; Morice et al. 1987). The cough usually resolves promptly after treatment withdrawal. Persistence may suggest asthma, the onset of which has been linked to the use of angiotensin converting enzyme inhibitors (Lunde et al. 1994).

Post- infectious cough

Community surveys suggest that most coughs related to upper respiratory tract infections resolve within three weeks (Curley et al. 1988). However, the cough can take several months to resolve in up to 25% of subjects. The infection in most cases remains unidentified but *Mycoplasma pneumoniae* and *Bordetella pertussis* have been implicated in adults (Davis et al. 1995). If infections are recurrent, bronchiectasis or host defence abnormalities should be considered.

1.1.5 Evaluation of a patient with chronic cough

An initial assessment of a patient with chronic cough is directed at finding a specific cause, assessing severity and initiating trials of treatment. A careful history and physical examination is paramount to the evaluation of a patient with chronic cough (Table 1.3, Brightling et al. 1999a). Details of the factors surrounding the onset of cough and associated symptoms, and a careful assessment of the upper airways and the respiratory system are particularly important. Basic initial investigations include up to date chest radiograph, spirometry and bronchodilator reversibility if appropriate. The history and physical examination is often unremarkable, in which case the evaluation of a patient focuses on the common conditions causing cough, namely rhinitis, asthma and gastro-oesophageal reflux. The investigation and treatment of common conditions causing chronic cough is summarised in Table 1.3. There is some evidence suggesting that the diagnostic and treatment success is higher when evaluating patients with chronic cough in specialist cough clinics than general respiratory clinics (McGarvey, Heaney, & MacMahon 1998).

In 1981, an anatomical diagnostic protocol was introduced to investigate patients with chronic cough in a systematic manner that was based upon evaluating the locations of receptors and afferent nerves of the cough reflex (Irwin, Corrao, & Pratter 1981). This approach has been widely adopted in specialist cough clinics and comprises of a combination of investigations and treatment trials of potential causes. One such adapted diagnostic algorithm is illustrated in Figure 1.1. Initial reports suggested that the success rate of the anatomically based approach to manage chronic cough may be as high as 99% in determining the cause of cough and its resolution (Irwin, Curley, & French 1990;Poe et al. 1982). Subsequently, several groups have failed to achieve such high success rates (McGarvey et al. 1998; O'Connell et al. 1994; Birring S.S. et al. 2002), possibly due to increasing diagnostic complexity, methodological differences and different referral patterns.

Another approach to investigate patients with chronic cough is to employ comprehensive investigations in the diagnostic work-up (McGarvey et al. 1998;Palombini et al. 1999). In the study by Palombini et al, patients underwent more than 12 different diagnostic procedures and were considered to be positive

for a diagnosis if a test result was abnormal. This led to diagnosis of multiple aetiologies in over 60% of the patients, compared to 26% reported by Irwin et al (Irwin, Curley, & French 1990). Other studies have shown that expensive and invasive investigations such as HRCT scanning and bronchoscopy in patients with an isolated chronic cough and normal chest X-ray have a low diagnostic yield (Poe et al. 1982) and hence should be reserved for patients with suggestive symptoms. In contrast to investigation based diagnostic algorithms, protocols based predominantly on treatment trials have been shown to be equally successful in managing patients with chronic cough (Simpson 1999). In a clinic using a protocol based principally on treatment trials Simpson et al (Simpson 1999) reported a success rate of 92% and multiple aetiologies in 15% of patients. A diagnostic approach based on symptom led treatment trials requires a thorough knowledge of the location of cough receptors and potential causes of chronic cough.

Diagnostic algorithms in many cough clinics have recently placed increasing emphasis on the measurement of airway inflammation in the detection and management of asthma and the recognition of eosinophilic bronchitis. Induced sputum is the investigation of choice to assess airway inflammation in clinical practice but this technique can be time consuming, labour intensive and is largely unavailable outside tertiary centres. Exhaled nitric oxide detection has recently been proposed as simple, quick but expensive technique to measure airway inflammation. Exhaled nitric oxide is produced by bronchial epithelial and inflammatory cells and is elevated in many respiratory conditions such as asthma (Berlyne et al. 2000), eosinophilic bronchitis (Brightling et al. 2003), bronchiectasis (Kharitonov et al. 1995) and viral infections (Kharitonov, Yates, & Barnes 1995). In asthma, the relationship between exhaled nitric oxide and eosinophilic airway inflammation is poor (Berlyne et al. 2000). This may be due to measurement of different types and site of airway inflammation by the two methods. In patients with chronic cough, exhaled nitric oxide has been shown to have positive and negative predictive values of 60 and 93% for detecting cough variant asthma (Chatkin et al. 1999). Hence in corticosteroids naïve patients with chronic cough, a low exhaled nitric oxide level suggests that cough variant asthma is unlikely to be present.

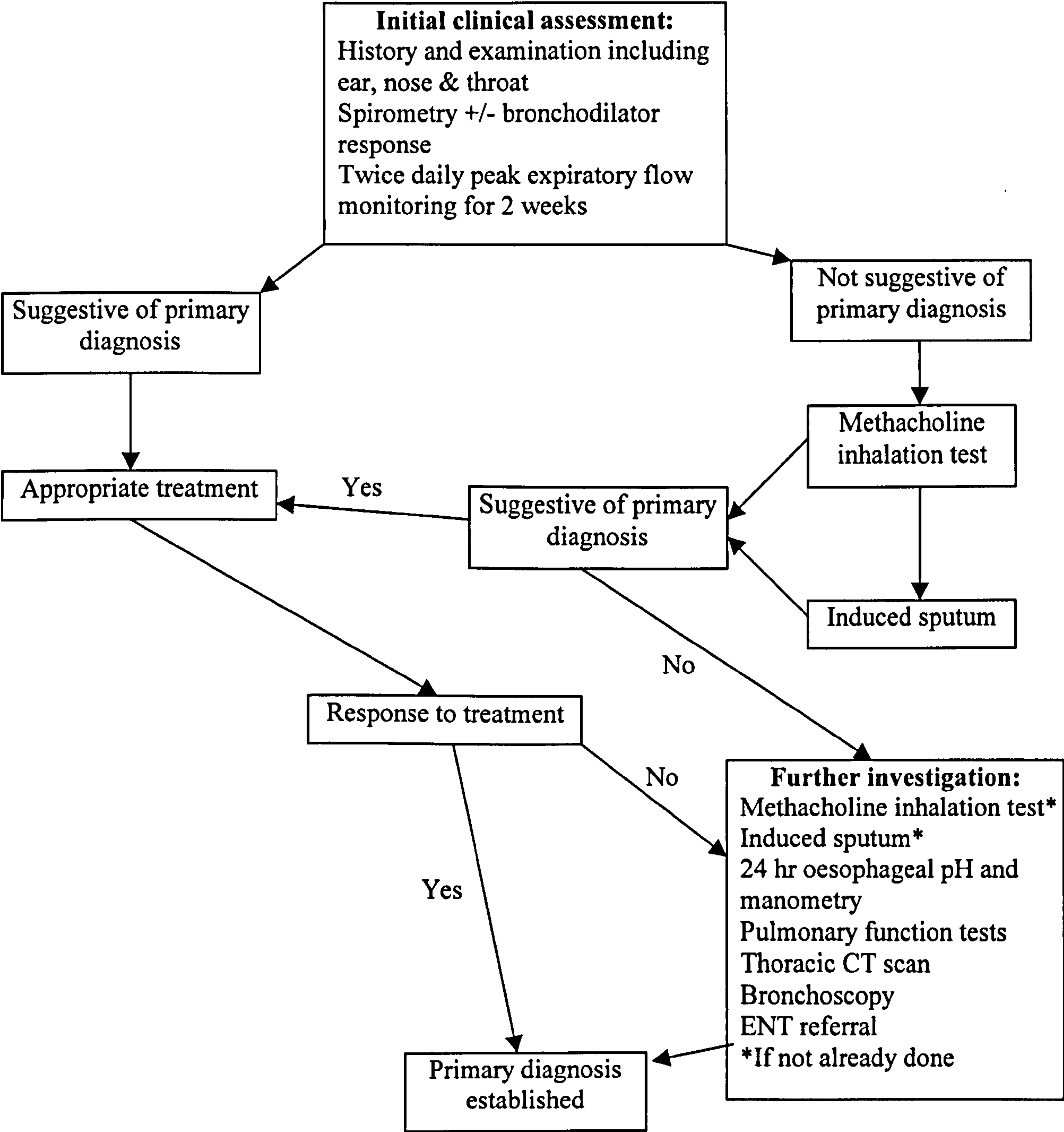
An important limitation of all diagnostic strategies for chronic cough is the lack of objective tools to validate the presence of cough and assess its severity. Treatment trials form an integral part of investigating a patient with chronic cough and are more easily interpreted when combined with attempts to validate the effects of treatment of the underlying cause and objective assessment of cough severity before and after treatment. Examples of the latter include cough visual analogue scores, cough reflex sensitivity, cough counts and quality of life assessment. These objective tests of cough severity are discussed in section 1.3. Another limitation of older diagnostic strategies is that they did not include investigations for previously unrecognised causes of chronic cough such as eosinophilic bronchitis (Brightling et al. 1999a) and oesophageal dysmotility (Kastelik et al. 2003). This may explain in part the large differences seen in the prevalence of common conditions causing chronic cough reported in the literature.

Table 1.3 Clinical features, investigations and treatment of chronic persistent cough

Diagnosis	History	Examination	Investigations	Treatment
Rhinitis	Rhinorrhea, nasal obstruction, sinus pain, sneezing, nasal itch, postnasal drip	Nasal secretions, nasal or pharyngeal mucosal inflammation	Sinus X-ray/CT showing mucosal thickening and/or fluid level	Topical budesonide/BDP 100 µg twice daily. In selected cases: topical ipratropium bromide 40 µg twice daily, topical xylometazoline HCL 0.1%, oral antibiotics, oral antihistamine
Eosinophilic bronchitis	No wheezing, dyspnea	No signs of airflow obstruction	FEV ₁ > 80% pred. FEV ₁ /FVC > 75%. Maximum within-day PEF variability over 2 wk < 20%. PC ₂₀ > 8 mg/ml. Sputum eosinophil count > 3%	Inhaled budesonide/BDP 400 µg twice daily with prednisolone 30 mg daily for 14 d in selected cases
ACE inhibitor-induced cough	Cough onset temporarily related to starting ACE inhibitor			Drug withdrawal. Substitution of alternative if appropriate.
Gastroesophageal reflux	Heartburn, flatulence, waterbrash		Barium swallow, endoscopy, and 24 h esophageal manometry and pH in selected cases	Weight reduction, elevation of head of bed, avoidance of eating within 2 h of bedtime, acid suppression. Prokinetic agent in selected cases
Asthma	Episodic wheezing, dyspnea, and/or chest tightness	Polyphonic expiratory wheeze	One or more of the following: > 15% increase in FEV ₁ after inhaled salbutamol 200 µg, maximum within-day PEF variability over 2 wk > 20%, PC ₂₀ < 8 mg/ml	Inhaled budesonide/BDP 400 µg twice daily with prednisolone 30 mg daily for 14 d in selected cases. Inhaled β ₂ -agonist as required
Postviral	Onset following viral upper respiratory tract infection			Observation
Chronic bronchitis	Productive morning cough > 3 mo/yr for more than 1 yr. Smoking history	Coarse crackles		Cessation of smoking

Definition of abbreviations: ACE = angiotensin converting enzyme; BDP = beclomethasone dipropionate; CT = computed tomography; PC₂₀ = provocative dose of methacholine causing a 20% decrease in FEV₁.

Figure 1.1. Diagnostic algorithm for patients with chronic cough.



1.1.6 Treatment of chronic cough

Treatment directed at the specific cause of chronic cough is summarised in Table 1.3. Using the anatomical diagnostic protocol, success rates of up to 98% in the management of chronic cough have been reported (Irwin, Curley, & French 1990). The success rate goes down to approximately 80% in specialist cough clinics, possibly due to the complexity of cases referred (McGarvey et al. 1998). Reassessment of the patient after treatment and excluding additional aggravating factors or causes form an integral part of managing a patient with chronic cough. A common dilemma faced by physicians managing patients with chronic cough is that the diagnosis of cough often depends on successful trials of treatment, which if unsuccessful leads to the difficult question as to whether the underlying condition has not responded or is not responsible for the cough. However, the use of objective tests to make a diagnosis and careful validation of the effect of therapy for the underlying condition minimises this problem. Therapy for common causes of chronic cough are discussed below.

Cough variant asthma

Cough due to asthma responds well to bronchodilators (Irwin et al. 1997) and inhaled corticosteroids (Cherian, Greenberger, & Patterson 1994; Doan, Patterson, & Greenberger 1992). A response typically occurs within 1 to 2 weeks of starting therapy and reaches a maximum after 8 to 10 weeks. Leukotriene antagonists have recently been shown in a randomised controlled study to reduce symptoms and cough reflex sensitivity in patients with cough variant asthma (Dicpinigaitis, Dobkin, & Reichel 2002). High dose antihistamines have been demonstrated to dramatically reduce cough in seasonal asthma (Rafferty et al. 1990) but have not been specifically investigated in cough variant asthma. The duration of asthma therapy remains unclear but return of the cough on gradual withdrawal of therapy suggests long-term therapy may be necessary. Patients with cough variant asthma often have coexisting rhinitis or post-nasal drip, and a complete response may not be seen until all potential aggravating factors are treated.

Eosinophilic Bronchitis

Inhaled corticosteroids are the mainstay treatment of cough due to eosinophilic bronchitis. Like cough variant asthma, evidence for the efficacy of corticosteroids in eosinophilic bronchitis comes from uncontrolled trials (Brightling et al. 2000b; Gibson et al. 1995). Rarely oral corticosteroids are required to suppress eosinophilic airway inflammation and cough (Brightling et al. 1999b).

Gastro-oesophageal reflux related cough

Self-help measures for gastro-oesophageal reflux related cough include weight reduction, high protein low fat diet, avoidance of tight clothing, elevation of headrest during sleep and reduced alcohol, tobacco, caffeine and chocolate intake although the basis for this advice is unclear (Irwin et al. 1998). Drug therapy for acid suppression has been advocated as the best treatment for gastro-oesophageal reflux related cough (Morice & Kastelik 2003). Many studies have shown some improvement in chronic cough with anti-reflux therapy but most are not placebo controlled and are largely from one group in the USA (Irwin et al. 1989; Irwin et al. 1993; Irwin, Corrao, & Pratter 1981; Irwin, Curley, & French 1990; Mello, Irwin, & Curley 1996; Poe et al. 1989; Pratter et al. 1993; Rolla et al. 1998; Smyrniotis, Irwin, & Curley 1995; Waring et al. 1995). However, two double-blind, placebo-controlled trials of proton pump inhibitor therapy have demonstrated that the impact on cough is far from clear (Kiljander et al. 2000; Ours et al. 1999). Table 1.4 summarises the findings of these studies. Both studies demonstrated that anti-reflux therapy was not significantly better than placebo. Ing et al (Ing, Ngu, & Breslin 1992) found similar findings with the histamine-2 receptor antagonist ranitidine in a randomised placebo controlled trial that has been published in abstract form only. The authors of these studies have suggested that these surprising findings may result from a carry over effect of anti-reflux therapy into the placebo phase (Ing, Ngu, & Breslin 1992; Kiljander et al. 2000). Further studies using objective markers of cough severity are required to investigate the role of proton pump inhibitors in chronic cough.

Table 1.4 Summary of randomised placebo controlled trials of proton pump inhibitors in patients with chronic cough and pathological gastro-oesophageal reflux on 24-hour oesophageal pH studies

Study	No	Drug	Duration (months)	Improvement in cough	
				Placebo	Omeprazole
Ours et al (1999)	17	Omeprazole 40mg BD	3	0/9 (0%)	1/8 (13%)
Kiljander et al (2000)	21	Omeprazole 40mg OD	2	13/21 (62%)	16/21 (76%)

Outcome measure: cough scores

Other forms of treatment for gastro-oesophageal related cough include prokinetic agents such as metaclopramide and anti-reflux surgery. Both are included in current treatment guidelines on the basis of weak data (Irwin et al. 1998). Anti-reflux surgery has been demonstrated to be effective in patients with an objective diagnosis of gastro-oesophageal reflux related cough that is unresponsive or only partially responsive to medical therapy, in uncontrolled studies (Irwin et al. 2002;Novitsky et al. 2002). Surgery for gastro-oesophageal reflux related cough is uncommon in the Europe and further studies are required to evaluate the role of anti-reflux surgery.

Rhinitis

There are no randomised, placebo-controlled studies of therapy for chronic cough secondary to rhinitis. Treatment strategies for cough secondary to rhinitis are based on studies investigating acute cough, rhinitis without cough and the evaluation of patients seen in specialist cough clinics. Topical corticosteroids are the main stay treatment for cough due to rhinitis. Where nasal obstruction is prominent, initial treatment with topical decongestant sprays may be necessary and antibiotics should be administered if infection is suspected. Topical

ipratropium bromide is often helpful if rhinorhea is prominent (Bronsky et al. 1995) and antihistamines are useful when sneeze and nasal itch is prominent and when there is coexisting atopy (Irwin, Corrao, & Pratter 1981;Irwin, Curley, & French 1990;Pratter et al. 1993;Smyrnios, Irwin, & Curley 1995). Surgical treatment may be necessary when there are obvious anatomical abnormalities

In conclusion, management of chronic cough with a careful history and physical examination and use of an anatomical diagnostic protocol can be rewarding in most cases. When evaluating a patient with unexplained cough, it is important to use objective tests and recognise common pitfalls in managing chronic cough (Table 1.5). Successful treatment of the underlying disorder can achieve significant improvements in all aspects of quality of life.

Table 1.5 Common pitfalls in the management of chronic cough.

- Incorrect diagnosis
- Not recognising multiple causes of cough
- Lack of objective evidence for the diagnosis of asthma
- Prolonged and aggressive treatment may be required before cough improves
- Poor treatment compliance
- Post viral and gastro-oesophageal associated cough may take many months to resolve
- Inappropriate labelling of psychogenic cough
- Failure to assess the impact of cough on quality of life

1.2 IDIOPATHIC CHRONIC COUGH

1.2.1 Background

A significant proportion of patients with chronic cough remain unexplained after extensive investigations and treatment trials (Table 1.6), variously estimated at between 5 to 20% of referrals (Table 1.1). A diagnosis of idiopathic chronic cough is made after all common causes of chronic cough have been excluded by investigation and treatment trials. Particular attention is paid to exclude asthma, gastro-oesophageal reflux and rhinitis especially when there are no suggestive symptoms. Patients with idiopathic chronic cough are predominantly middle-aged females, usually present in their fifth to seventh decade and have objective evidence of abnormality in the airways such as heightened cough reflex sensitivity (Prudon et al. 2004) and inflammation (McGarvey et al. 1999). They suffer considerable physical and psychological morbidity. Many patients with idiopathic chronic cough are labelled with a diagnosis of psychogenic cough although there is little evidence to support this view and it is perhaps more likely that any abnormal illness behaviour is secondary to the adverse impact of cough on psychosocial aspects of quality of life. Therapy for idiopathic chronic cough is disappointing and is largely limited to non-specific antitussive therapy such as dextromethorphan, codeine and drugs with weak evidence of benefit such as baclofen and nebulised local anaesthetics (lidocaine, mepivacaine) (Almansa-Pastor 1996). Referral to a physiotherapist for cough management advice can be very helpful.

1.2.2 Airway inflammation

Several studies employing different techniques to assess airway inflammation suggest that airway inflammation is present in patients with idiopathic chronic cough. These abnormalities include a sputum neutrophilia and increased concentration of interleukin-8 in induced sputum (Jatakanon et al. 1999), increased bronchoalveolar lavage mast cell numbers (McGarvey et al. 1999) and a bronchoalveolar lavage (McGarvey et al. 1999) and bronchial biopsy lymphocytosis in patients with idiopathic chronic cough (Boulet et al. 1994; Lee et al. 2001). The cause of airway inflammation in patients with idiopathic chronic cough is unclear but one possibility in women is that it may result from hormonal

changes at the time of the menopause that alter lung immunity (Mund et al. 2001). Mund et al have shown that there is an increase in bronchoalveolar lavage CD4 T lymphocytes and CD4/CD8 T lymphocyte ratio in healthy women aged greater than 43 years old compared to younger women which was not seen in blood lymphocytes and have suggested that the changes in lung immunity around the menopause may explain the female predominance in pulmonary disorders such as chronic dry cough.

The normal adult human lung contains large numbers of lymphocytes, largely memory T cells in the bronchial lamina propria and the peripheral alveolar and interstitial regions of the lung. T cells recirculate from the blood to tissue and back to the blood. Naive T cells exclusively traffic through secondary lymphoid tissue such as lymph nodes and bronchus-associated lymphoid tissue (BALT) via specialised postcapillary venules called high endothelial venules. In contrast, memory T cells, in addition to migrating into lymph nodes, can also transmigrate through the nonspecialised postcapillary venules of the systemic circulation where they preferentially return, or "home," to regions or microenvironments of the body similar to those where antigen was initially encountered. Lymphocyte homing is controlled by adhesion molecules and chemoattractant signals expressed by high endothelial venules in an organ-specific manner (Baggiolini 1998; Rossi & Zlotnik 2000). The chemoattractant signals for lymphocytes are principally chemokines which bind to chemokine receptors expressed on the lymphocyte surface. The chemokine receptors involved in T lymphocyte homing to the lungs in health and disease is unclear.

T cells are commonly divided into T-helper (CD4) and T-cytotoxic (CD8) cells. T-helper cells are further subdivided into T-helper 1 and T-helper 2 cells on the basis of the cytokine profile released by them (Th1: interferon-gamma, interleukin 2; Th2: interleukin 4 and 5). Excessive Th1 responses are thought to result in the development of autoimmune disease whereas the Th2 pathway is implicated in the pathogenesis of allergic disorders such as asthma (von Andrian & Mackay 2000). Th1 cells are thought to have a different chemokine receptor profile to Th2 cells (Th1: CXCR3; Th2: CCR3), hence selected lymphocyte homing responses may be involved in the pathogenesis of T cell mediated disorders. Although increased numbers of lymphocytes have been reported in bronchoalveolar lavage and

bronchial biopsies in patients with idiopathic chronic cough (Lee et al. 2001;McGarvey et al. 1999) the lymphocyte phenotype, activation status, cytokine profile and chemokine receptor expression has not been studied. Studying the airway lymphocyte in more detail may lead to better understanding of the mechanism of lymphocyte trafficking to the lung in patients with idiopathic chronic cough.

1.2.3 Association with autoimmune disease

The onset of cough in the fifth to seventh decade, female predominance and the nature of the airway inflammation in patients with idiopathic chronic cough suggest to us the possibility of an autoimmune process. Autoimmune disorders are thought to arise from a polarisation of CD4 cells towards Th1 responses via cytokines and activation of CD8 cytotoxic cells (Mackay 2000). The reduction of female sex hormones such as oestrogen and progesterone at the time of the menopause is thought to be responsible for the change in immunity seen in women but not men at this time and the emergence of autoimmune disease predominantly in women (Rose & McKay 1998).

A recent case-report has raised the possibility that idiopathic chronic cough may have an autoimmune basis and is associated with organ-specific autoimmune disease (Brightling et al. 2002c). A patient with idiopathic chronic cough and heightened cough reflex sensitivity had bronchoscopic evidence of lymphocytic airway inflammation in association with coeliac disease. The cough, heightened cough reflex sensitivity and lymphocytic airway inflammation improved markedly on a gluten free diet suggesting a causal relationship between coeliac disease and cough. Coeliac disease is associated with intense inflammation of the small bowel and a plausible explanation for the lymphocytic airway inflammation was homing of activated T cells into the pulmonary compartment from the primary site of autoimmune inflammation (Pozzilli, Carotenuto, & Delitala 1994). Analogous mechanisms are thought to be responsible for the respiratory complications seen in other immune mediated disorders such as inflammatory bowel disease and rheumatoid arthritis (Bonniere et al. 1986;Gabbay et al. 1997). Interestingly, cough has also been associated in two patients with inflammation of the thyroid gland (Irwin, Pratter, & Hamolsky 1982), a common site for autoimmune disease.

It is not known if idiopathic chronic cough is associated with other autoimmune disorders and if patients with autoimmune disease have an increased prevalence of cough.

1.2.4 Role of inflammatory mediators

The mechanism of cough associated with the different patterns of airway inflammation seen in idiopathic chronic cough is unclear, but one possibility is release of pro-tussive mediators and activation of sensory nerve endings in the airways (Choudry, Fuller, & Pride 1989). Mast cell derived mediators may be particularly important since there is evidence of increased mast cell numbers in bronchoalveolar lavage fluid from patients with idiopathic chronic cough (McGarvey et al. 1999), which release significantly higher concentrations of histamine when stimulated in-vitro compared to mast cells from normal subjects (Forsythe et al. 2000). An important role for histamine and other mast cell derived mediators in the pathogenesis of idiopathic chronic cough is also supported by studies showing that high dose antihistamines improve distilled water induced cough in patients with this condition (Tanaka et al. 1996). Other inflammatory mediators implicated in the pathogenesis of idiopathic chronic cough include interleukin-8, a cytokine associated with neutrophilic inflammation (Jatakanon et al. 1999), prostaglandin E₂, a prostanoid with tussive properties when inhaled (Choudry, Fuller, & Pride 1989) and the sensory neuropeptide calcitonin gene related product (CGRP) stored in human airway nerves (O'Connell et al. 1995).

1.2.5 Summary

Up to 20% of referrals to a cough clinic with chronic cough remain unexplained. The prevalence of idiopathic chronic cough varies between clinics possibly due to differing complexity of referrals made. Patients with idiopathic chronic cough are predominantly female, have an onset of cough around the middle age, have heightened cough reflex sensitivity and airway inflammation that could be consistent with an autoimmune process. Whether patients with idiopathic chronic cough have associated organ specific autoimmune disorders or if patients with autoimmune disease have a higher prevalence of chronic cough is not known. The nature of the airway inflammation and its source and the inflammatory mediator

profile in airway secretions of patients with idiopathic chronic cough is not known. The long term consequences of idiopathic chronic cough have not been studied. An intriguing possibility is that idiopathic chronic cough leads to fixed airflow obstruction as a result of longstanding airway inflammation similar to that described in a patient with chronic cough due to eosinophilic bronchitis (Brightling et al. 2000b). In our experience, patients with unexplained fixed airflow obstruction are predominantly elderly females, often with a preceding chronic cough, suggesting that idiopathic chronic cough and unexplained airflow obstruction may be related.

Table 1.6 Suggested treatment trials for patients with idiopathic chronic cough

Condition	Therapy	Duration
Gastro-oesophageal reflux	Lansoprazole 30mg OD	3 months
Rhinitis	Mometasone nasal spray 200mcg OD each nostril	2 months
Asthma	Pulmicort turbohaler 400mcg bd Salbutamol inhaler 2 puffs as required	2 months

ASSESSMENT OF CHRONIC COUGH

The management of patients with chronic cough is currently hampered by a striking paucity of objective tools to validate the presence of cough and assess its severity. Subjective measures such as questionnaires and cough scores are of value in understanding the impact of cough on an individual and providing more details on the nature of the cough but have limited use in clinical practice because they lack standardisation and cannot be used to measure inter-individual differences. Potential tools to assess cough in clinical practice include cough reflex sensitivity, cough scores, cough monitors and quality of life questionnaires, which are discussed below.

1.3.1 Cough reflex sensitivity

Cough reflex sensitivity measurement was initially used as a model for antitussive drug development and has been the most extensively studied tool to assess cough (Prime 1961). It is a relatively simple, safe and practical technique that is often likened to measuring bronchial reactivity in asthma. Citric acid and low chloride solutions can be used to measure cough reflex sensitivity but capsaicin is the agent of choice because it is more tolerable and there is less tachyphylaxis (Morice, Kastelik, & Thompson 2001). The actions of capsaicin on C fibre sensory nerves are mediated through the stimulation of a membrane-bound receptor known to be a ligand gated non-selective cation channel termed transient receptor potential vanilloid 1 (TRPV1). When stimulated, the TRPV1 receptor results in increased permeability to sodium and calcium ions with neuronal depolarisation and propagation of action potentials. The TRPV1 receptor is also sensitive to protons, heat and anandamide and capsazepine is an antagonist. The measurement of capsaicin cough reflex sensitivity is described in section 2.2.5.

The cough reflex has been studied in a number of respiratory diseases with and without cough. Increased cough reflex sensitivity is found in a wide variety of conditions causing chronic cough (Choudry & Fuller 1992; Prudon et al. 2004). Although patients with stable asthma do not usually cough and have a normal cough reflex, increased cough reflex sensitivity is seen in a small subgroup with cough variant or cough predominant asthma (Choudry & Fuller 1992). Female gender has been associated with increased cough reflex sensitivity but age and

atopy have no effect on the reflex (Fujimura et al. 1996;Prudon et al. 2004). Patients with a productive cough are more likely to have a normal reflex (Choudry & Fuller 1992) but others have shown this is not always the case (Doherty et al. 2000b;Prudon et al. 2004). Recent studies have reported a wide variation of cough reflex sensitivity in healthy subjects without a cough, and the test is repeatable (Prudon et al. 2004).

The large overlap between health and disease suggest that cough reflex sensitivity measurements do not have a role in the diagnosis of chronic cough. A potential use for cough reflex sensitivity testing is to monitor the response to treatment and disease progression. Successful treatment of the cough usually results in a return towards normal cough reflex sensitivity (Brightling et al. 2000b;O'Connell et al. 1994). In a patient with chronic cough, a 1.5 doubling dose change in C2 (concentration of capsaicin that causes 2 coughs) could be regarded as significant since it is greater than two standard deviations outside the range of within subject differences in repeatability testing (Prudon et al. 2004). Further studies need to examine the relationship between cough sensitivity and other markers of cough such as cough counts. Currently, cough reflex sensitivity measurement is largely limited to the research setting, and in the development of antitussive drugs.

1.3.2 Cough symptom scores

The simplest method to assess cough severity is to ask the patient how the cough affects daily living, frequency and intensity of cough episodes and their appreciation of cough severity. This subjective assessment can be formalised with patient diaries, cough symptom scores (0-no cough; 5-distressing cough most of day; (Chung 2002)) and linear cough symptom scales. The most widely used cough symptom scale is the visual analogue score (0-100mm) which is easy to administer and has been shown to be repeatable (Brightling et al. 2001). The 95% confidence interval of repeatability is a 15mm change in visual analogue score (Brightling et al. 2001). The limitation with all these measurements is that they are subjective in nature and not validated for use in chronic cough.

1.3.3 Cough monitors

The recording of cough events is potentially the best method to validate and assess cough in patients. Preliminary studies report large differences between health and disease suggesting this technique will be useful for validating the presence of cough (Matos et al. 2003). Several attempts to develop practical, automated cough recording system have been largely unsuccessful. A variety of methods have been used to count cough events which include manual counts by an observer in a nonambulatory subject (Eccles, Morris, & Jawad 1992), manual counting from tape recordings (Sevelius & Colmore 1966), and automated counting from combined sound and abdominal electromyographic signals (Hsu et al. 1994). Current cough monitors lack good specificity and sensitivity due to difficulty in differentiation from other cough like sounds such as throat clearing and are not practical for 24-hour ambulatory recording due size and method of recording. These difficulties need to be overcome before cough recorders are widely used.

1.3.4 Quality of life questionnaires

Chronic cough is often perceived as a trivial problem but can be a disabling symptom associated with significantly impaired quality of life (French et al. 1998). Currently, there are no tools to measure cough specific quality of life. There is no consensus on the definition of quality of life as it is affected by health but the definition of health by the World Health Organisation in 1947 as “a state of complete physical, mental and social well-being, and not merely the absence of disease” is widely quoted (World Health Organisation 1947). Health status or health related quality of life measurement is a means of quantifying the impact of disease on patients’ daily life and general well-being in a standardised and objective manner.

The assessment of health status has become increasingly important in respiratory disease and the use of various disease specific quality of life questionnaires has grown to become standard endpoints in many randomised controlled trials and clinical studies. Health status has been studied most extensively in asthma and chronic obstructive pulmonary disease by development

of disease specific questionnaires (Jones et al. 1992;Juniper et al. 1993). Less is known about the effects of chronic cough on health status.

Cough has wide-ranging effects on health status. The reasons why patients with chronic cough seek medical advice are poorly understood but may relate to worry about the cough, embarrassment, self-consciousness, and the presence of associated symptoms such as nausea and exhaustion (French et al. 1998). In acute cough, the adverse effects in health status result from physical symptoms and are transient. In contrast, the impact of chronic cough on health status is varied, being minimal in some patients who may not even seek medical attention and a disabling symptom in others, associated with impairment of quality of life comparable to other chronic respiratory disorders such as chronic obstructive pulmonary disease. The most commonly affected domains of health are physical, psychological and social (Table 1.7). Patients with chronic cough frequently report musculoskeletal chest pains, sleep disturbance and hoarse voice but more marked symptoms such as blackouts, stress incontinence and vomiting can occur. Psychological problems include worrying about serious underlying diseases such as cancer and tuberculosis. The impact of cough on social well-being depends on individual circumstances. The cough can cause difficulties in relationships, avoidance of public places, disruption at work and in severe cases time off work. The wide-ranging and potentially profound effects of cough on health status highlight the importance of a detailed history of associated symptoms and concerns when assessing patients with chronic cough.

There are several reasons why quality of life measurement should be included in clinical assessment of patients and clinical trials. Quality of life measures can be used to facilitate communication with patients and establish information on the range of problems that affect them. The impact of the illness on health and treatment preferences often differs between patient and physician and therefore quality of life considerations should take the patient's perspective into account. Quality of life measurement is particularly helpful when assessing treatments that are invasive or have significant side effects. Health status measures in clinical trials can be used to study aspects of health affected by therapy and compare treatments.

Table 1.7 Adverse symptoms in patients with chronic cough.

SYMPTOM
PHYSICAL
Musculoskeletal pains
Hoarse voice
Nausea and vomiting
Dizziness and syncope
Headaches
Sleep disturbance
Lack of energy
Stress incontinence
PSYCHOLOGICAL
Embarrassment
Anxiety
Depressed
Fear of serious illness
SOCIAL
Avoidance of public places
Interrupted conversation
Relationship difficulties
Disturbance of partner's sleep
Interference with occupation or daily tasks
Interference with recreational activities

The simplest method to assess quality of life is to ask the patient (Fayers & Machin D 2000). Drawbacks to this are that some observers are poor judges of patients' opinions. Assessment of patients with quality of life instruments is essentially similar to a structured clinical history although the outcome parameter is an objective, validated, and quantifiable measurement. Quality of life domains are usually measured separately to assess emotional and psychological well-being as well as physical and practical aspects of daily life. Questionnaires are divided into generic or disease specific. Generic instruments are intended for general use, irrespective of the illness and have the advantage that scores from patients can be compared to other conditions and even healthy subjects (Juniper 1997). However, these instruments do not focus on issues related to patients with the condition and lack responsiveness to specific interventions. This has led to the development of disease specific quality of life questionnaires in many areas of medicine.

The properties of a quality of life questionnaire should satisfy the basic principles of any measure that is to be clinically useful. These are primarily validity, repeatability, sensitivity, responsiveness and interpretability (Higginson & Carr 2001). Validation is an assessment of the extent to which the instrument measures quality of life. This usually involves comparison of the questionnaire to other objective parameters that may reflect disease severity and other quality of life instruments in the intended population. Repeatability assesses the random variability of the measure. Ideally the questionnaire should be repeatable over time in patients whose quality of life is unchanged. Sensitivity is the ability of an instrument to detect differences between patients and responsiveness is the ability to detect clinically meaningful changes within a patient, such as those that result from therapeutic intervention. The questionnaire scores must be clinically relevant if it is to be used in clinical practice. Finally, the measure must be simple, brief and easy to score if it is intended for clinical practice as well as research.

In summary, chronic cough has profound effects on quality of life and there is a pressing need for cough specific quality of life measures for the objective assessment of health status in the clinical setting and in the evaluation of new antitussive therapies in clinical trials. The use of well validated cough specific quality of life measures should complement other objective measures of chronic cough such as cough monitors and cough sensitivity.

To summarise, chronic cough is a common condition that causes significant physical and psychological morbidity in patients. It is a condition that causes much difficulty for the specialist, particularly its diagnosis and assessment. There are several aspects of chronic cough that are poorly understood. Firstly, the assessment of chronic cough is limited by the lack of objective measures for diagnosing and monitoring cough. Secondly, the treatment of cough is largely based on expert opinion or uncontrolled trials, and there is a paucity of randomised controlled trials with well-validated outcome measures to guide the clinician. Finally, a particularly difficult problem for clinicians is the significant proportion of patients with chronic cough that remain unexplained after extensive investigations and treatment trials. This thesis will address and discuss some of these important issues.

1.3 HYPOTHESIS

I hypothesise that idiopathic chronic cough has an autoimmune basis.

I hypothesise that patients with idiopathic chronic cough have an increased prevalence of organ specific autoimmune disorders and that patients with organ specific autoimmune disease have an increased prevalence of respiratory symptoms.

I hypothesise that patients with idiopathic chronic cough have lymphocytic airway inflammation due to homing of lymphocytes from primary sites of autoimmune inflammation and have increased concentrations of inflammatory mediators in the airways, particularly mast cell products.

AIMS

(1) Association of idiopathic chronic cough with organ specific autoimmune disease

- To assess the prevalence of organ specific autoimmune disease in patients with idiopathic chronic cough.
- To assess the prevalence of respiratory symptoms in patients with hypothyroidism.

(2) Immunopathology of idiopathic chronic cough

- Determine the type, intensity and site of inflammation in the airways of patients with idiopathic chronic cough.
- To characterise the T cell phenotype, activation state, cytokine profile and chemokine receptor expression in idiopathic chronic cough.
- To assess the degree of activation of the inflammatory response in idiopathic chronic cough by measuring potential pro-tussive mediators in airway secretions.

(3) Unexplained airflow obstruction- a possible link with idiopathic chronic cough?

- Describe the clinical, radiological and induced sputum features of non-smoking patients with fixed airflow obstruction.

(4) Health status of patients with chronic cough

- Develop a cough specific quality of life questionnaire for patients with chronic cough.

2 METHODS

Only methods common to multiple studies are described in this section. Detailed methodology is described in section 3 for each study.

2.1 GENERAL

2.1.1 Peripheral blood tests

Venous blood samples were taken to measure peripheral blood lymphocyte count, angiotensin converting enzyme (ACE) level, α_1 -antitrypsin level, Immunoglobulin levels (IgG,A,M), total IgE and radioallergosorbent tests to *Dermatophagoides pteronyssinus*, cat epithelium, dog dander and timothy grass. Blood samples were also taken for an autoantibody screen; antinuclear (in-house indirect immunofluorescence), rheumatoid factor (nephelometry, Dade Behring BNII protein analyser, UK), islet cell (in-house indirect immunofluorescence), adrenal (indirect immunofluorescence, Biodiagnostics Ltd, Worcestershire, UK), parietal (in-house indirect immunofluorescence), endomysial (indirect immunofluorescence, Binding Site Ltd, Birmingham, UK) and thyroid peroxidase autoantibodies (fluorescent ELISA system, Pharmacia Diagnostics, Milton Keynes, UK).

2.1.2 Skin tests

Allergen skin prick tests were carried out to *Dermatophagoides pteronyssinus*, cat fur and grass pollen solutions with normal saline and histamine controls (Bencard, UK). A positive response to an allergen on the skin prick tests was recorded in the presence of a weal >2mm more than the negative control.

2.1.3 Symptom visual analogue scores

Symptom scores were recorded using a 100mm visual analogue (0mm: no symptom; 100mm: worst symptom ever) and the symptoms recorded included dyspnoea, cough, sputum production and wheeze. (Appendix III)

2.2 RESPIRATORY PHYSIOLOGY

2.2.1 Spirometry and lung function tests

Spirometry was performed with a Vitalograph spirometer (Vitalograph, Buckinghamshire, UK). Bronchodilator reversibility was assessed 15 minutes after administration of 2.5mg salbutamol nebulised via a Flaem Nuova Type II nebuliser (Deva Medical, Runcorn, Cheshire) with a median particle size of 2 μ m and the patient breathing tidally or 200 μ g salbutamol inhaled via a volumatic. FEV₁ was recorded as the better of three successive readings within 100 mL. Lung-function tests were done with a benchmark (P K Morgan, Chatham, UK) and lung volumes assessed by the helium dilution method.

2.2.2 Airway responsiveness

Airway hyperresponsiveness was assessed using the standard tidal breathing method to determine the provocative concentration of methacholine causing a 20% fall in FEV₁, recorded as the PC₂₀FEV₁ (Juniper, Cockcroft, & Hargreave 1994). Following the measurement of the baseline FEV₁ subjects inhaled saline followed by doubling concentrations of methacholine (0.03-16 mg/ml) via a Wright's nebuliser (flow 0.13ml/min driven by dry compressed air; gift from Fisons, Leicestershire, UK). Additional concentrations of methacholine were used in some studies of 32, 64, 128 mg/ml. The subject was instructed to breathe tidally for 2 minutes with a noseclip. The FEV₁ was measured 30 and 90 s after the nebulisation was completed. If the FEV₁ fell less than 20%, the procedure was repeated with the next higher concentration. If the FEV₁ fell more than 20% from baseline (or the highest concentration has been given), no further methacholine was given. Methacholine PC₂₀FEV₁ concentration was calculated by linear interpolation of log dose response curve.

2.2.3 Exhaled nitric oxide

End exhaled nitric oxide (eNO) was measured as the mean of three blows by a chemiluminescent technique (Logan, UK). Subjects exhaled at a flow rate of 250ml/s with a sampling rate of 250ml/min.

2.2.4 Sputum induction

Instructions for Patients

Prior to commencing sputum induction, the procedure was fully explained to the patient including specific instructions for:

- Spitting out saliva generated during inhalation of saline into a discard container.
- Blowing their nose and rinsing their mouth and swallowing the water prior to trying to expectorate sputum. (It is important that the subject moves quickly through this procedure to prevent loss of sputum due to swallowing).
- Effective expectoration: it is necessary to explain and demonstrate the technique for coughing up sputum and moving sputum from the back of the throat, forward to the specimen container.
- Reminder not to swallow the sputum as it comes up the bronchial tree.
- Guidance on posture: sitting straight upright during nebulisation, and leaning forward during expectoration.

Sputum induction protocol

Subjects were pre-treated with either inhaled salbutamol 200 µg or nebulised salbutamol 2.5mg 10-30 minutes before sputum induction to minimise bronchoconstriction. Sputum was induced using 3, 4 and 5% saline inhaled in sequence for five minutes via an ultrasonic nebuliser (Medix, Harlow, UK; output 0.9 ml/min; mass median diameter 5.5 µm). Subjects were asked to breath tidally, taking a slightly deeper breath every minute. After each inhalation subjects blew their noses and rinsed their mouths to minimise nasal contamination and expectorated sputum into a sterile pot. FEV₁ was measured after each inhalation. If the FEV₁ fell by more than 10% but less than 20%, the same concentration of saline is administered. If the FEV₁ fell by more than 20% of the best post-bronchodilator value, or if significant symptoms occurred, the nebulisation was

stopped and the patients were treated with repeat short-acting β -agonist. (Figure 2.1)

Safety Procedures During the Induction

Inhaled hypertonic saline is a bronchoconstrictor stimulus so sputum induction using ultrasonically nebulised hypertonic saline should be carried out with care. The usual laboratory resuscitation apparatus plus nebulised salbutamol was readily available. A doctor either performed the procedure or was nearby during each procedure.

Protocol sputum processing

Sputum free from salivary contamination was selected and weighed. To the selected sputum was added 4X volume/weight of 0.1% dithiothrietol (DTT) (Sigma, Poole Dorset). The sputum was dispersed by gentle aspiration into a Pasteur pipette, vortex for 15 s and 15 minutes rocking on a bench spiromix. After the addition of an equal volume of Dulbecco's phosphate buffered saline (D-PBS) (Sigma, Poole, Dorset) the sputum suspension was filtered through 48 μ m nylon gauze and centrifuged 2000rpm (790g) for 10 minutes. The sputum supernatant was removed and stored at -80°C for future mediator assay. The cell pellet was resuspended in a small volume of PBS. An aliquot was removed and a total cell count, squamous cell contamination and viability were assessed using a Neubauer haemocytometer by the trypan blue exclusion method. The cell suspension was adjusted with PBS to 0.5-0.75 $\times 10^6$ cells/ml and cytopins were prepared from 75 μ l aliquots at 450rpm (18.1g) for 6 minutes using a Shandon III cytocentrifuge) (Shandon, UK). The cytopins were stained in neat Romanowski stain for 5 minutes and fixed in dilute stain for 25 minutes. A differential cell count was obtained by counting >400 non-squamous cells on a Romanowski stained cytopin (Figure 2.2).

Figure 2.1 Sputum induction protocol

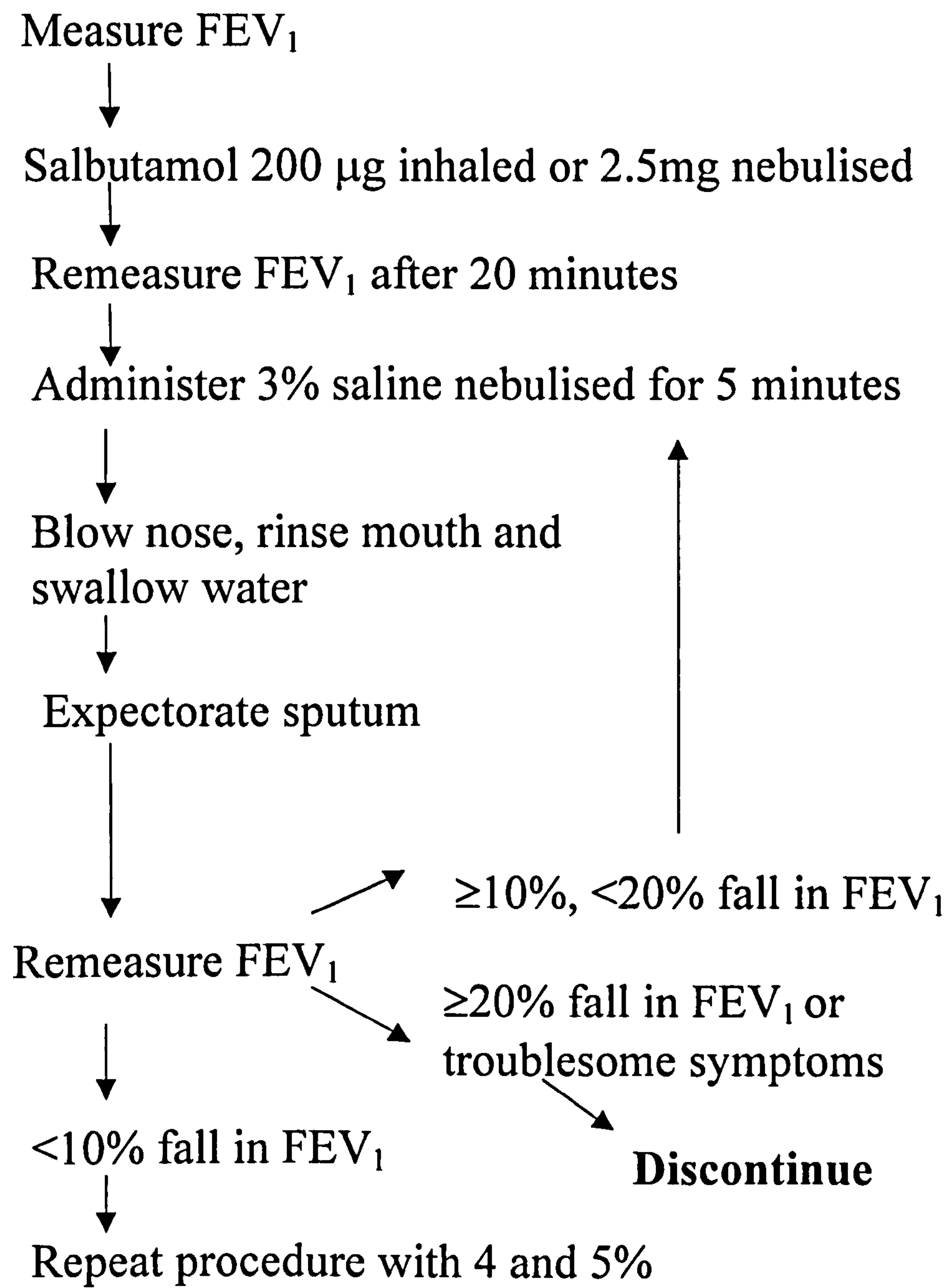
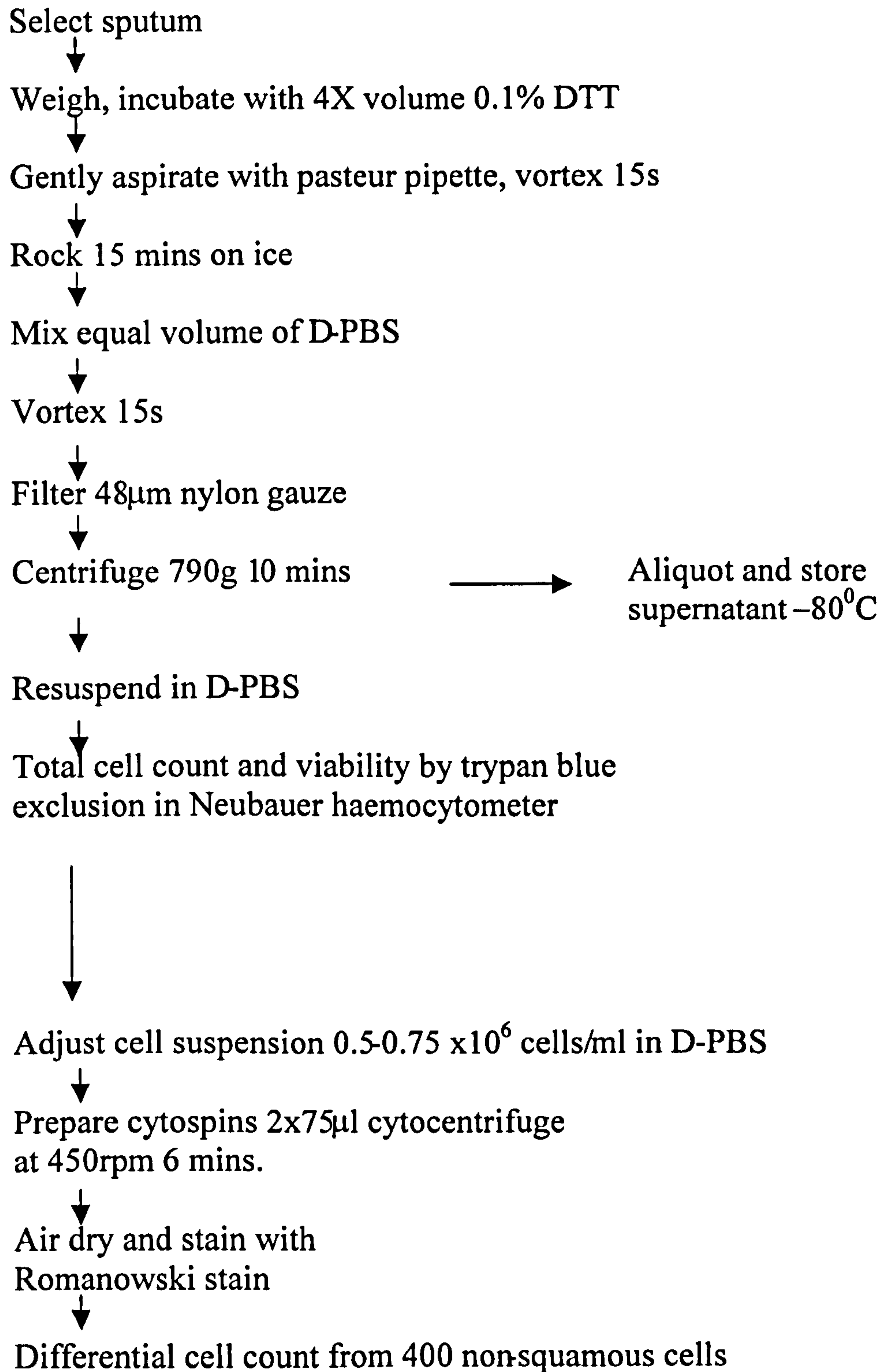


Figure 2.2 Sputum processing protocol



Romanowski stain preparation:

Dissolve 1.5g Azure-B-thiocyanate in DMSO at 37°C and 0.5g Eosin in 300ml methanol at room temperature. Slowly add the Azure blue solution to the Eosin and store away from light.

Dilute Romanowski stain:

62 ml 10mM HEPES buffer pH 7.2

3.5 ml DMSO

4.6 ml Romanowski stain

2.2.5 Capsaicin cough reflex sensitivity

Cough sensitivity was assessed using the capsaicin cough challenge (Choudry & Fuller 1992). Serial dilutions of capsaicin (Sigma, UK) were used to produce doubling concentrations from 0.49 to 500µM/L. Subjects inhaled at just below their functional residual capacity doubling concentrations of capsaicin in a sequential order at 1-minute intervals via a nebuliser attached to a breath-activated dosimeter delivering an output of 8µL. The inspiratory rate was standardised by maintaining an inspiratory pressure of 1cm of H₂O above baseline, measured by a manometer attached to the inspiratory limb. Cough counting, facilitated by tape recording, was done for 30 seconds after exposure to each dose, and the investigation ended when the subject coughed 5 or more times in response to one dose, or received a dose of the highest concentration. The concentration of capsaicin causing two coughs (C₂) and five coughs (C₅) were calculated by linear interpolation of log dose response curve.

3 STUDIES

3.1 Association of idiopathic chronic cough with organ specific autoimmune disease

3.1.1 Idiopathic chronic cough and organ specific autoimmune diseases: a case-control study

ABSTRACT

The marked female predominance in cases of idiopathic chronic cough and its association with mild chronic lymphocytic airway inflammation suggests an underlying autoimmune process. We set out to test the hypothesis that idiopathic chronic cough is associated with other organ-specific autoimmune diseases in a case-control study. 22 patients with idiopathic chronic cough and 65 community matched controls for age and sex who responded to a self-administered questionnaire were asked about the presence of autoimmune disease, other medical problems and drug history. All subjects were invited to have a blood test for an autoimmune screen. 13 out of 22 (59%) patients with idiopathic chronic cough and 8 out of 65 (12%) age and sex matched controls reported organ specific autoimmune disease (odds ratio 8.8; 95% confidence interval 2.4 to 31.8, $p < 0.001$). Organ specific autoantibodies were present in a significantly higher proportion of cases than controls (40% vs 13%; $p = 0.047$). These findings suggest a relationship between idiopathic chronic cough and organ specific autoimmunity.

INTRODUCTION

Cough is one of the commonest causes of presentation to general practice. At any one time 20% of the UK population have a troublesome cough and sufferers consume 75 million doses of over the counter anti-tussive medication annually (Fuller & Jackson 1990). Most cases are mild and self-limiting but a small proportion have persistent troublesome cough and require specialist review. Several series have shown that a cause of persistent cough can be identified relatively simply in 80-99% of cases with most causes due to one or more of asthma, gastro-oesophageal reflux and rhinitis with post-nasal drip (Brightling et

al. 1999a;Irwin, Curley, & French 1990;McGarvey et al. 1998). However in up to 20% of patients, the cough remains unexplained even after extensive investigation and treatment trials (McGarvey et al. 1998).

Bronchoscopic studies have shown some evidence of chronic airway inflammation with a mononuclear cell infiltrate in the airway mucosa of patients with idiopathic chronic cough (Boulet et al. 1994). The nature of the airway inflammation and the female predominance of patients with chronic cough suggested to us the possibility of an autoimmune process. We have tested the hypothesis that patients with unexplained chronic cough have a higher incidence of organ-specific autoimmune disease in a case-control study.

METHODS

Cough diagnostic algorithm

Patients were recruited from consecutive patients attending a specialised cough clinic run by Dr Pavord between 1998 and 1999 which receives referrals from primary and secondary care largely confined to a population of 970,000 within Leicestershire. Investigations were carried out according to a standardised algorithm (Brightling et al. 1999a) and figure 1.1. The protocol for investigation and treatment, and criteria for accepting diagnosis were as previously described (Brightling et al. 1999a;Irwin et al. 1998) and figure 1.1. We defined idiopathic chronic cough as a cough lasting >3 weeks in association with normal clinical examination (including Ear Nose Throat), normal chest x-ray and CT scan, normal lung function tests, negative methacholine inhalation test (provocative concentration: $PC_{20} FEV_1 > 16$ mg/ml), normal peak expiratory flow variability, no sputum eosinophilia and no pathological gastro-oesophageal reflux or evidence of temporal association between cough and gastro-oesophageal reflux on 24 hour oesophageal pH monitoring. Patients had normal macroscopic findings on fiberoptic bronchoscopy and all had extensive negative treatment trials including trials of inhaled and systemic corticosteroids (Brightling et al. 1999a).

Subjects

150 patients were seen in our cough clinic (mean age 51 years [range 22-90], females 66%) of which 25(17%) fulfilled our criteria for idiopathic chronic cough. The other primary causes of cough were cough variant asthma (15%), gastro-oesophageal reflux (15%), rhinitis with post-nasal drip (13%), eosinophilic bronchitis (17%), post-viral cough (13%), chronic bronchitis and/or bronchiectasis (8%) and angiotensin converting enzyme inhibitor cough (2%). Identification of controls was carried out using the method described by (Hubbard et al. 1996). Briefly, four controls matched for age and sex were obtained for each case that responded. These controls were drawn from the list of the case patient's general practitioner and were selected randomly if more than four suitable subjects were available; if four controls of the same age were not listed with the general practitioner the age matching was widened to within 5 years.

Questionnaire and blood tests

On obtaining permission to enter each case and their matched controls from their general practitioner, a questionnaire was posted to each subject's home. The questionnaire asked specifically about the presence of the following conditions: hyperthyroidism, hypothyroidism, diabetes, pernicious anaemia, Addison's disease, alopecia, vitiligo, coeliac disease, Crohn's disease, ulcerative colitis, premature menopause, autoimmune hepatitis, other medical problems and drug therapy. The presence of self-reported organ specific autoimmune diseases was validated (in cases) by review of medical notes. The subjects were also invited to have a blood test for an autoantibody screen (section 2.1.1).

Analyses

Matched case-control analysis of the incidence of organ specific autoimmune disease was done by conditional logistic regression using the EGRET computer statistical package (Seattle, USA). The effect of variables significantly associated with cough were analysed in a multivariate model. The incidence of organ specific autoantibodies in each group was compared by chi-square test. The study had ethics approval from the Leicestershire ethics committee.

RESULTS

We obtained the general practitioner's permission to contact 25 idiopathic chronic cough patients and 88 controls. 22 (88%) of the cases and 65 (74%) of the controls responded to the questionnaire. The distribution of age and sex among all cases and controls, those that responded to the questionnaire and those that had an autoantibody screen were similar and not statistically significant (table 3.1). The median duration of cough in cases was 12 months (range 7 - 360 months). The response to all questions asking about organ specific autoimmune disease is outlined in table 3.2. Diseases suggesting organ specific autoimmunity were reported by 13 cases (59.1%), and 8 controls (12.3%, table 3.2). Matched case-control analysis showed the difference to be highly significant (odds ratio 8.8; 95% confidence interval 2.4 to 31.8, $p < 0.001$). A significantly higher proportion of the cases had organ-specific autoantibodies present than controls (40% vs 13%; $p = 0.047$; table 3.2). In the cases, positive thyroid autoantibody titres ranged from 1:100 to 1:6400 (strong positive titre $> 1:100$). In general, the presence of organ specific autoantibodies was consistent with self-reported organ specific autoimmune disease. Four patients with explained chronic cough ($n = 125$) had organ specific autoimmune disease or positive autoantibodies (3.2%). Unexpectedly, non-steroidal anti-inflammatory drug (NSAID) use for more than two months was reported more commonly by cases than controls (45.5% vs 12.3%; odds ratio 5.6; 95% confidence interval 1.7 to 18.2, $p < 0.01$). Both associations (autoimmune disease and NSAID use) remained significant and were essentially unchanged when analysing using a multivariate model.

Table 3.1 Distribution of age and sex among all cases and controls identified, all respondents to the questionnaire and those who had an autoantibody screen.

	No	MEN		WOMEN		Overall mean(SD) age(years)
		No(%)	Mean(SD) Age(years)	No(%)	Mean(SD) Age(years)	
ORIGINAL SAMPLE						
Cases	25	7 (28)	54.2 (5.4)	18 (72)	55.4 (3.0)	55.1 (2.6)
Controls	88	21(24)	60.9 (1.6)	67 (76)	58.7 (1.7)	59.2 (1.3)
RESPONDENTS						
Cases	22	5 (23)	58.8 (3.3)	17 (77)	57.0 (3.1)	57.5 (2.5)
Controls	65	12(18)	63.3 (2.1)	53 (82)	55.7 (1.9)	57.1 (1.6)
AUTOANTIBODY SCREEN						
Cases	15	3 (20)	63.0 (4.6)	12 (80)	59.1 (3.6)	59.9 (3.0)
Controls	24	5 (21)	51.6 (9.6)	19 (79)	52.8 (2.9)	52.6 (2.9)

Table 3.2 Self-reported organ-specific autoimmune disease and presence of organ-specific autoantibodies in cases and controls.

Autoimmune disorder	Controls (no) n=65	Cases (no) n=22
Hypothyroidism	2	5
Hyperthyroidism	3	0
Diabetes mellitus type1	2	3
Pernicious anaemia	0	2
Celiac disease	1	1
Vitiligo & premature menopause	0	1
Vitiligo & chronic autoimmune hepatitis	0	1
Addison’s Disease	0	0
Inflammatory Bowel Disease	0	0
Alopecia	0	0
Total	8	13
Autoantibodies	Controls (no) n=24	Cases (no) n=15
Thyroid peroxidase	0	4
Parietal cell	1	1
Endomysial	1	1
Islet cell	1	0
Adrenal	0	0
Total	3	6

DISCUSSION

We found a significantly higher prevalence of organ-specific autoimmune disorders in patients with idiopathic chronic cough. There was a similar difference in the prevalence of organ specific autoantibodies between the two groups. These findings support our hypothesis that idiopathic chronic cough is associated with the presence of organ-specific autoimmune disease.

The controls represented a randomly selected group and were not excluded if they had a cough, smoked or had relevant occupational exposure. This was to ensure that we were not selecting a healthy group with an artificially lower prevalence of potential causative factors such as organ-specific autoimmune disease. As with all case control studies, our findings might be influenced by recall bias in controls. However, the drug histories from controls did not suggest underreporting of autoimmune disorders. Furthermore, the prevalence of organ-specific autoimmune disease seen in the control population was greater than expected from general population surveys (3-8%) (Mackay 2000; Rose & McKay 1998) and from the explained cough group (3.2%), suggesting that if anything, there was over-reporting of autoimmune disease in controls. Another possibility is that the cases may have different health behaviour, increasing the likelihood of seeking medical help and receiving a diagnosis of organ-specific autoimmune disease. However the significantly higher prevalence of objective markers of organ-specific autoimmunity in cases suggests this is not the sole explanation for the association although we cannot discount the possibility that the increased prevalence of NSAID use is due to this mechanism.

This is a small study, so we were not able to compare the strength of association between different organ-specific autoimmune disorders and idiopathic chronic cough. Larger studies are required to investigate the possibility that particular types of autoimmune diseases are associated with cough. Autoimmune thyroid disease was the most prevalent organ-specific autoimmune disorder in cases with idiopathic chronic cough. On the basis of our findings, we cannot exclude the possibility that the association is exclusively due to a link between autoimmune thyroid disease and idiopathic chronic cough. Chronic cough has been reported as

a presenting feature of thyroiditis in two patients (Irwin, Pratter, & Hamolsky 1982). In both the cough improved following treatment of the inflammatory thyroid disorder and was thought to be due to a local irritative effect of the inflamed thyroid. It is possible that local effects of a goitre are responsible for cough in some patients.

The lungs and many of the organs involved in organ-specific autoimmune disorders share common embryological origins as foregut derivatives and it is possible that homing of activated T cells into the pulmonary compartment as well as the primary site of autoimmune inflammation may cause airway wall inflammation and cough. Similar mechanisms are thought to be responsible for the lymphocytic airway inflammation seen in inflammatory bowel disease (Bonniere et al. 1986). Our recent description of a case of chronic cough associated with bronchoscopic evidence of lymphocytic broncho-alveolitis which resolved following treatment of coeliac disease (Brightling et al. 2002c) provides some support for this mechanism. An alternative and intriguing mechanism is that the cough might be due to a hitherto unrecognised autoimmune bronchitis and that the association with other diseases simply reflects the well-recognised association between different organ specific autoimmune diseases. The failure of the cough to respond to corticosteroids in the treatment trials our patients were subjected to is not surprising since autoimmune hypothyroidism, pernicious anaemia, insulin dependent diabetes mellitus and other organ specific autoimmune disorders differ from systemic autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis in that they do not typically respond to corticosteroids (Rose & McKay 1998).

Unexpectedly we identified long-term NSAID use in more cases than controls, independent of the presence of autoimmune disease. This difference was identified as a result of a post-hoc analysis and was not a primary outcome so we are cautious about interpretation of this data. The association is at first slightly surprising since prostaglandins are thought to be endogenous tussive mediators (Choudry, Fuller, & Pride 1989) and NSAIDs have been shown to inhibit Angiotensin Converting Enzyme Inhibitor chronic cough (McEwan et al. 1989).

However, the net effect of NSAIDs on airway prostaglandin production is far from clear and it is possible that in certain circumstances these drugs alter airway prostaglandin production so as to have a protussive effect. An alternative possibility is that NSAIDs cause airway inflammation, perhaps involving a similar mechanism to NSAID induced colitis (Koch 2001). Finally we cannot exclude the possibility that patients used these drugs to ameliorate the musculoskeletal complications of coughing.

In conclusion, we have identified a relationship between idiopathic chronic cough and organ-specific autoimmune disease. Further studies are required to investigate this relationship in more detail, determine whether it is associated with airway inflammation, investigate the mechanisms involved and test this hypothesis that other unexplained airway conditions are caused by similar mechanisms.

3.1.2 Respiratory symptoms in patients with treated hypothyroidism and inflammatory bowel disease

ABSTRACT

We have previously shown that patients with idiopathic chronic cough and unexplained airflow obstruction in non-smokers have an increased prevalence of hypothyroidism and other organ-specific autoimmune disorders. Whether patients with hypothyroidism have an increased prevalence of respiratory symptoms is unknown. We assessed the prevalence of respiratory symptoms in 124 patients with treated hypothyroidism recruited from primary and secondary care, 64 outpatients with inflammatory bowel disease and 1346 control adults recruited randomly from the electoral register in a case-control study. Respiratory symptoms and smoking history were assessed by a respiratory symptom questionnaire. After adjustment for age, sex and smoking, symptoms of breathlessness and sputum production were more prevalent in both patient populations than controls (odds ratios [95% confidence intervals] for hypothyroidism and inflammatory bowel disease; breathlessness: 3.1 [2.1-4.6] and 3.4 [2.0-6.0]; sputum: 2.7 [1.6-4.5] and 2.5 [1.2-5.0]). Cough during the day and night was significantly more prevalent in hypothyroidism (1.8 [1.2-2.9]) and approached significance for inflammatory bowel disease (1.8 [1.0-3.4] respectively). Wheeze and nocturnal cough were no more prevalent in disease population compared to controls. In conclusion, we found a significantly increased prevalence of respiratory symptoms in both patients with hypothyroidism and inflammatory bowel disease. These findings support the hypothesis that there is a link between autoimmune hypothyroidism and respiratory disease.

INTRODUCTION

In a recent case-control study, we noted hypothyroidism and other organ specific autoimmune disorders were eight times more likely to be present in patients with idiopathic chronic cough than age and sex matched controls (section 3.1.1). The strongest association was with autoimmune hypothyroidism and the subjects were treated with thyroxine implying that the association is independent

of thyroid hormone status. A link between thyroid disease and respiratory disease independent of thyroid hormone status is supported by a prospective community study showing that the odds ratio of death from respiratory disease was 2.8 in subjects with normal thyroxine level, but suppressed thyrotropin levels (Parle et al. 2001).

The mechanism for the possible association between autoimmune hypothyroidism and respiratory disease is unclear, but one possibility is homing of activated inflammatory cells from the primary site of autoimmune inflammation to the lung. An analogous mechanism is thought to be responsible for respiratory complications of inflammatory bowel disease (Bonniere et al. 1986). However, the link between inflammatory bowel disease and respiratory morbidity is based on case reports and case series and there is no data from controlled studies showing an association (Camus et al. 1993). In this study we set out to investigate the prevalence of respiratory symptoms in patients with treated hypothyroidism and compare the prevalence and nature of respiratory morbidity to that seen in a cohort of patients with inflammatory bowel disease and a control population in a case-control study.

METHODS

Subjects

175 patients with treated hypothyroidism were randomly chosen from the Leicestershire Thyroid Register. This is a register of patients diagnosed with primary hypothyroidism from primary (48%) and secondary care (52%). All patients at the time of diagnosis had elevated thyroid stimulating hormone levels with suppressed thyroxine levels not due to treatment or surgery. At the time of completion of the respiratory questionnaire, all patients were on appropriate thyroid replacement therapy and were biochemically euthyroid. Patients with inflammatory bowel disease were recruited prospectively from consecutive patients meeting criteria, attending a gastroenterology outpatient clinic between September to December 2001. All patients had ulcerative colitis or Crohn's disease confirmed histologically and were at various stages of control. Disease activity was assessed and scored by a consultant gastroenterologist using the

Simple Index of Crohn's Disease Activity (Harvey & Bradshaw 1980) and a Simple Clinical Colitis Activity Index (Walmsley et al. 1998). Controls comprised 1346 individuals aged 27-80 years from a local authority area of Nottingham who had participated in a previously described study investigating the effect of diet on decline of lung function (Britton et al. 1994;McKeever et al. 2002). They were originally identified by systematic sampling from a random starting point in the electoral register. Their area of residence was similar in socio-economic and geographic characteristics to the area from which the hypothyroid and inflammatory bowel patients were recruited. Patients were asked for their consent to participate and the protocol was approved by the Leicestershire Ethics Committee.

Questionnaire

Patients with hypothyroidism completed a questionnaire on smoking, respiratory symptoms (breathlessness: [Do you ever have trouble breathing?]; cough: [Do you usually cough during the day, or at night, in the winter?]; sputum: [Do you usually bring up any phlegm from your chest during the day, or at night, in the winter?]; nocturnal cough: [Have you ever been woken by an attack of coughing at any time in the last 12 months?] and wheeze: [Have you had wheezing or whistling in your chest in the last 12 months]) and recent upper respiratory tract infections sent by mail, outside the flu and grass pollen allergy season. The questionnaire was adapted from the IUATLD questionnaire (Burney et al. 1989; appendix II). Control subjects and patients with inflammatory bowel disease completed the same questionnaire during a visit to hospital or local clinic.

Analysis

Multiple logistic regression was performed in STATA version 7 (Stata Corporation, Texas, USA) to estimate the chance of having respiratory symptoms in the presence of hypothyroidism and inflammatory bowel disease independent of age, sex and smoking (status and pack years). The hypothyroidism and inflammatory bowel disease groups were restricted to those within the age range of the control population for analysis.

RESULTS

124 (71%) patients with hypothyroidism, 1346 (51%) controls and 64 inflammatory bowel disease patients (22 with Crohn's disease) completed the questionnaire. 106 (86%) patients with hypothyroidism were female and 16 (13%) of patients self reported the presence of a goitre. In other respects, the characteristics of subjects between groups were similar (table 3.3). After adjustment for age, sex and smoking, symptoms of breathlessness and sputum production were more prevalent in both patient populations than controls (odds ratios [95% confidence intervals] for hypothyroidism and inflammatory bowel disease: breathlessness 3.1 [2.1-4.6], $p < 0.001$ and 3.4 [2.0-6.0], $p < 0.001$; sputum 2.7 [1.6-4.5], $p < 0.001$ and 2.5 [1.2-5.0], $p = 0.01$; figure 3.1). Cough was significantly more prevalent in hypothyroidism (1.8 [1.2-2.9], $p < 0.01$) and approached significance for inflammatory bowel disease (1.8 [1.0-3.4], $p = 0.057$). Wheeze and nocturnal cough were no more prevalent in disease population compared to controls (table 3.4, figure 3.1). These odds ratios remained significant when hypothyroid patients with a goitre and patients with ulcerative colitis were excluded. Patients with Crohn's Disease were 1.4 – 3.0 times more likely to report respiratory symptoms compared to ulcerative colitis and there was a trend for those with active inflammatory bowel disease to report more symptoms than those whose inflammatory bowel disease was inactive although the difference did not reach statistical significance. There were no significant differences between groups in the presence of a head cold, sore throat, flu or chest infection in the previous week.

Table 3.3 Subject characteristics.

	Controls	Hypothyroidism	Inflammatory Bowel disease
	n=1346	n=124	n=64
Male (%)	668 (50)	18 (15)	29 (45)
Age (years)	56 (12)	56 (14)	45 (14)
Smoking-			
Never (%)	679 (50)	62 (50)	30 (47)
Current (%)	155 (12)	11 (9)	11 (17)
Ex-smokers (%)	512 (38)	51 (41)	23 (36)
Pack-years smoking	19 (21)	18 (18)	17 (13)

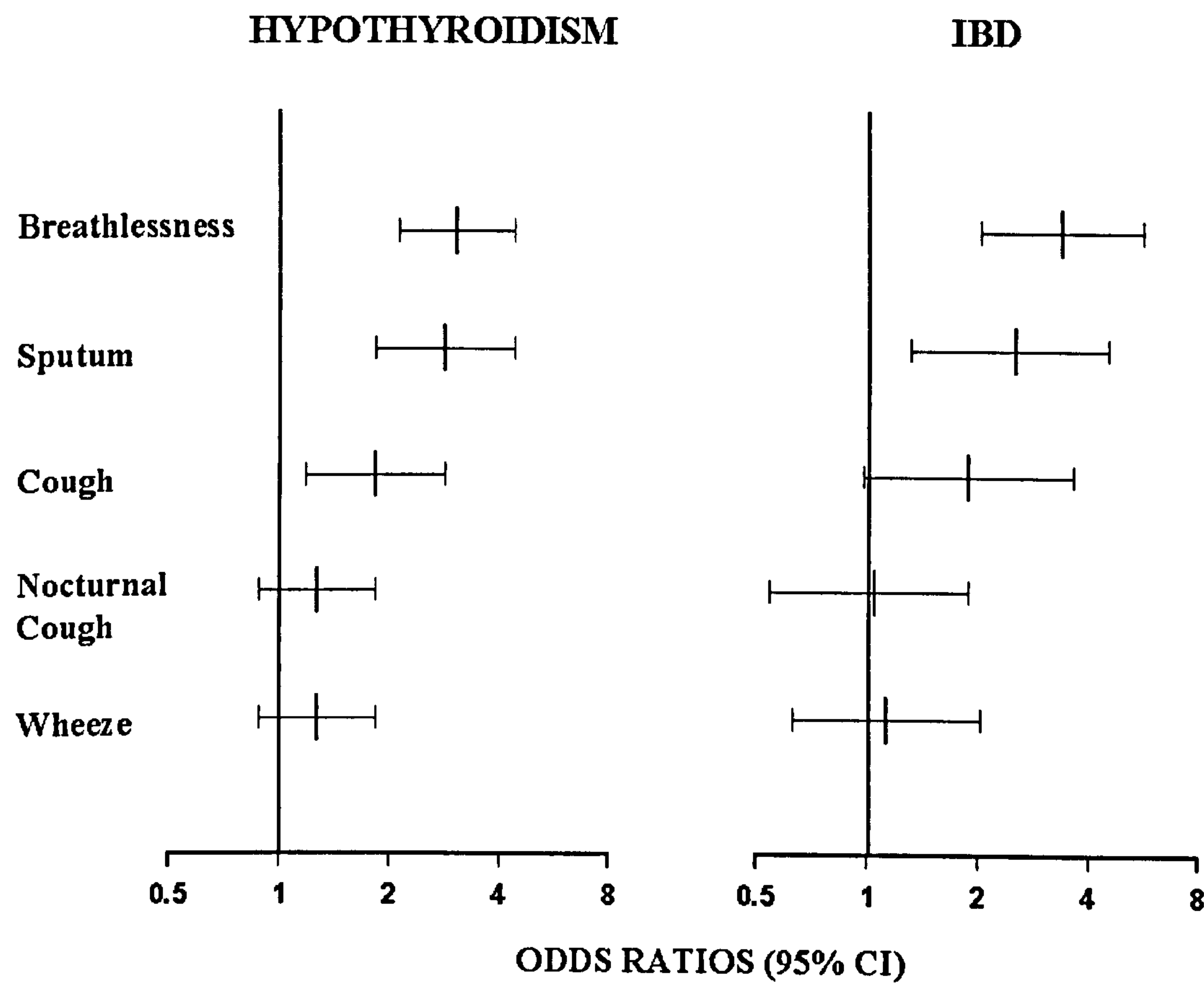
Age and pack-year smoking: mean (SD). Mean for pack-years does not include non-smokers.

Table 3.4. Prevalence of respiratory symptoms.

	Controls	Hypothyroidism	IBD
	(n=1346)	(n=124)	(n=64)
Breathlessness	215 (16)	49 (40)	23 (36)
Sputum	131 (10)	23 (19)	11 (18)
Cough	212 (16)	31 (25)	15 (24)
Nocturnal cough	334 (25)	39 (32)	15 (23)
Wheeze	343 (26)	35 (29)	17 (27)

Number (%). See text for definition of symptoms.

Figure 3.1 Odds ratio (95% confidence intervals) of reporting respiratory symptoms compared to controls in patients with treated hypothyroidism and inflammatory bowel disease (IBD) after correction for age, sex and pack-year smoking.



DISCUSSION

We have shown a significantly increased prevalence of respiratory symptoms in subjects with hypothyroidism and inflammatory bowel disease. The increase seen in hypothyroidism was to a similar degree to that seen in inflammatory bowel disease for breathlessness, cough and sputum production. This is the first demonstration of increased respiratory morbidity in subjects with treated hypothyroidism.

Our data supports the widely held view that inflammatory bowel disease is associated with respiratory morbidity. There was a suggestion that patients with Crohn's disease and active inflammatory bowel disease had more respiratory symptoms than patients with ulcerative colitis and non-active disease, which is contrary to previous reports (Camus & Colby 2000). However, the main purpose of the inflammatory bowel disease group was to serve as a disease control group and this study was not sufficiently powered to investigate differences within the inflammatory bowel disease group. Larger studies are required to investigate the differences in respiratory complication between Crohn's disease and ulcerative colitis and their relationship with bowel disease activity.

There was a striking similarity in the profile of reported symptoms in hypothyroidism and inflammatory bowel disease suggesting a common underlying pathophysiology of these symptoms. The absence of wheeze and nocturnal symptoms argue against asthma: the symptom profile would be more consistent with chronic obstructive pulmonary disease and chronic bronchitis. In support of this, the airway conditions associated with inflammatory bowel disease include chronic bronchitis, chronic obstructive pulmonary disease and bronchiectasis (Camus et al. 1993) and hypothyroidism has been linked to cough (section 3.1.1). We have considered the possibility that the respiratory symptoms in patients with treated hypothyroidism may be due to the presence of a goitre though this seems unlikely because the association between hypothyroidism and respiratory symptoms remained significant even after excluding those with a goitre. The presence of airway symptoms such as sputum production and cough

increases our confidence that we are detecting respiratory disease and not cardiac complications of hypothyroidism.

A causal association between organ specific autoimmune disease such as hypothyroidism and respiratory disorders is plausible. Autoimmunity is the commonest cause of hypothyroidism, accounting for over 90% of noniatrogenic cases (Rose & McKay 1998). The lungs and many organs involved in autoimmune disorders share common embryological origins as foregut derivatives and it is possible that homing of activated inflammatory cells into the pulmonary compartment as well as the primary site of autoimmune inflammation may cause airway wall inflammation and symptoms. An alternative and intriguing mechanism is that the respiratory symptoms might be consequence of a hitherto unrecognised autoimmune bronchitis and that the association with other diseases simply reflects the well-recognised association between different organ specific autoimmune diseases. The concept that some airway disorders are due to immune dysregulation is supported by the presence of cough, airflow obstruction and lymphocytic airway inflammation in Crohn's disease (Bonniere et al. 1986; Camus et al. 1993), coeliac disease (Brightling et al. 2002c), rheumatoid arthritis (Geddes et al. 1977), Sjogren's disease (Wallaert et al. 1987) and post-transplant chronic lung rejection (Yousem 1993). Furthermore, as well as being associated with organ-specific autoimmune disease, idiopathic chronic cough is associated with an airway lymphocytosis (Boulet et al. 1994; Lee et al. 2001).

One limitation of the study is a possible reporting bias with control subjects reporting fewer symptoms than patients since they had attended twice over ten years and were perhaps more likely to be healthy. However, we feel this is unlikely to have had an important effect as the prevalence of respiratory symptoms in control subjects was similar to that seen in a recent large European population survey which included subjects from the United Kingdom (Janson et al. 2001a). Moreover we noted a selective reporting of symptoms in both disease groups, which would not be consistent with systematic recall bias. A further concern is that patients recruited from hospital clinic were more likely to have multiple pathology than those recruited from the general population (Berkson's

fallacy) (Berkson J. 1946). This might explain the increased respiratory morbidity in inflammatory bowel disease but is less likely to explain the findings in hypothyroidism since half the subjects were recruited from primary care. Nevertheless, future studies should investigate whether there is a difference in respiratory symptoms in our patient categories compared to a hospital based control group. Finally, bias due to mismatch of social class, concurrent upper respiratory tract infection and seasonal allergy is unlikely since the study was carried out at a similar time of year for all subject groups, in neighbouring regions with similar social class distribution, and there was no excess of colds or flu in either patient group.

In summary, we have shown that patients with autoimmune hypothyroidism and inflammatory bowel disease have a higher prevalence of respiratory symptoms than controls. Furthermore, the pattern of respiratory symptoms in both immune mediated disorders was strikingly similar suggesting that they may share similar pathogenic mechanisms. Further studies are required to investigate the pathophysiology of the excess respiratory morbidity involved and its relationship to the underlying inflammatory disorder.

3.2 Immunopathology of idiopathic chronic cough

3.2.1 Idiopathic chronic cough: Association with organ specific autoimmune disease and bronchoalveolar lymphocytosis

ABSTRACT

We have recently reported a strong association between organ specific autoimmune disease and idiopathic chronic cough and have suggested that cough may be due to airway inflammation secondary to aberrant homing of activated lymphocytes to the lung. We set out to test the hypothesis that idiopathic chronic cough is associated with lymphocytic airway inflammation in an immunopathological study. We performed bronchoscopy, bronchial biopsies, bronchoalveolar lavage (BAL) and peripheral blood and BAL flow cytometry in 19 patients with idiopathic chronic cough, 14 with explained chronic cough and 11 normals. The mean duration of cough in patients with idiopathic chronic cough was 7 (0.5-50) years. Organ specific autoimmune disease or positive autoantibodies were present in 8/19 patients with idiopathic cough, 1/14 patients with explained cough and 1/11 normal subjects. Median BAL differential lymphocyte counts were significantly higher in patients with idiopathic cough (10.0%) than normals (6.3%, 95% confidence interval of difference 1.5 to 11.9%, $p=0.01$) and patients with explained cough (5.0%, 95% CI of difference 2.0 to 10.4%, $p=0.001$). There were no differences in bronchial biopsy T lymphocyte counts between groups. The mean(SEM) proportion of CD3+ peripheral blood mononuclear cells (PBMC) expressing CD4 was significantly higher in normals than patients with idiopathic cough (69(3)% vs 58(3)%, mean difference 11%, 95% CI of difference 2, 20%, $p<0.02$) but not explained chronic cough (63(2)%). There were no differences in BAL T lymphocyte phenotype between groups. We have found a BAL lymphocytosis in some patients with idiopathic chronic cough. The association of idiopathic chronic cough with organ specific autoimmune disease raises the possibility that this might be due to lymphocyte homing from the primary site of autoimmune inflammation or the result of an autoimmune process in the lung.

INTRODUCTION

Chronic cough is a common presenting symptom to both general practice and respiratory clinics (Irwin & Madison 2000). The cause of a persistent cough can be identified relatively simply in many cases with most due to one or more of asthma, eosinophilic bronchitis, gastro-oesophageal reflux and rhinitis with post-nasal drip (Brightling et al. 1999a; Irwin & Madison 2000). However in up to 20% of patients, the cough remains unexplained even after extensive investigation and treatment trials (McGarvey et al. 1998). These patients suffer considerable physical and psychological morbidity (French et al. 1998).

We have recently shown there is a marked female predominance and a strong association with organ specific autoimmune disease in patients with idiopathic chronic cough (section 3.1.1). A possible mechanism for the association between idiopathic chronic cough and organ specific autoimmune disease is homing of activated lymphocytes from the primary site of autoimmune inflammation to the lung. Previous small studies have found increased numbers of mononuclear cells in bronchial biopsies (Boulet et al. 1994; Lee et al. 2001) and a 50% excess of bronchoalveolar lavage (BAL) lymphocytes from patients with idiopathic chronic cough, findings that would be consistent with this mechanism (McGarvey et al. 1999). In this study we aimed to test the hypothesis that there is lymphocytic bronchoalveolar inflammation and to determine its characteristics in patients with idiopathic chronic cough by evaluating inflammatory cells numbers and phenotype in bronchoalveolar lavage and bronchial biopsy.

METHODS

Subjects

Nineteen patients with idiopathic chronic cough, 14 with explained chronic cough and 11 normal controls were recruited from Glenfield Hospital outpatient clinics and from healthy volunteers responding to local advertising. Patients with chronic cough were recruited from patients attending a specialised cough clinic between January 2000 and December 2001. The clinic receives referrals from primary and secondary care largely confined to a population of 970,000 within Leicestershire. Investigations were carried out according to a standardised

algorithm and the protocol for investigation and treatment, and criteria for accepting diagnosis were as previously described (Brightling et al. 1999a; Irwin et al. 1998) and figure 1.1. We defined idiopathic chronic cough as a cough lasting >3 weeks in association with normal clinical examination (including Ear Nose Throat), normal chest x-ray and high resolution CT scan, normal lung function tests, negative methacholine inhalation test (provocative concentration: PC₂₀ FEV₁ >8 mg/ml), normal peak expiratory flow variability, normal sputum eosinophil count (<2%) and no pathological gastro-oesophageal reflux or evidence of temporal association between cough and gastro-oesophageal reflux on 24 hour oesophageal pH monitoring. Patients had extensive negative treatment trials including trials of inhaled and systemic corticosteroids and trials of anti-reflux treatment as recommended (Irwin et al. 1998). Subjects with explained chronic cough were those in whom there was an identifiable cause for their cough and improvement in cough following specific treatment of this. Normal subjects were asymptomatic and had no evidence of variable airflow obstruction or airway hyperresponsiveness. Six normals and 9 patients with explained chronic cough recruited contemporaneously with another study were used for both studies (Brightling et al. 2002b). All subjects were non-smokers with a past smoking history of less than a 10-pack years. None had received corticosteroids or other specific treatment for the condition causing cough for at least six weeks 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.

Clinical measurements

Subjects attended on three occasions separated by 1-2 weeks. At the first visit, cough visual analogue score (0-100mm), total serum IgE, radioallergosorbent tests to timothy grass, dermatophagoides pteronissinus, cat fur and dog dander, a full autoantibody screen (including islet cell, adrenal, parietal, endomysial and thyroid peroxidase autoantibodies) and serum angiotensin converting enzyme (ACE) level were measured. We also measured exhaled nitric oxide, spirometry and methacholine airway responsiveness. Induced sputum was obtained for inflammatory cell differential counts using methods that have been described previously. (Green et al. 2002; Juniper, Cockcroft, & Hargreave 1994; Pavord et al.

1997) None of the subjects had taken bronchodilator therapy for at least 12 hours before spirometry and challenge testing. Subjects completed a questionnaire enquiring about the presence of autoimmune disease (hyperthyroidism, hypothyroidism, diabetes, pernicious anaemia, Addison's disease, alopecia, vitiligo, coeliac disease, Crohn's disease, ulcerative colitis, premature menopause and autoimmune hepatitis). Cough sensitivity was assessed on the second visit with capsaicin cough challenge test (Brightling et al. 2000b) using a dosimeter method standardised to limit inspiratory flow to 0.5 Litres/second (section 2.2.5).

Bronchoscopy

At the third visit, subjects underwent bronchoscopy using an Olympus fibre-optic bronchoscope (Olympus Company, Tokyo, Japan) in line with the most recent British Thoracic Society guidelines (2001). Subjects were pre-treated with nebulised 2.5mg salbutamol 20 minutes prior to bronchoscopy and had appropriate sedation as required of midazolam 0-5mg i.v. Lignocaine (1-4%) was used for local anaesthesia and continuous oxygen given via nasal cannulae throughout the procedure. A 20ml bronchial wash (BW) of pre-warmed normal saline into the bronchus intermedius was performed followed by a 180ml bronchoalveolar lavage (BAL) into the middle lobe in 60ml aliquots. Four to six bronchial mucosal biopsies were taken from the right middle and lower lobe carinae using size 20-22 cupped biopsy forceps.

BW and BAL cells were filtered and centrifuged without DTT, with the supernatant stored at -80°C and the cell pellet resuspended to produce total cell counts, cell viability and stained cytopins as per the sputum protocol (section 2.2.4). Differential cell counts were obtained from BW and BAL cytopins by two experienced blinded observers. The remaining BAL cells were assessed by flow cytometry.

Peripheral blood mononuclear cells (PBMC)

20mls of venous blood were taken from the subjects undergoing bronchoscopy and the mononuclear cell fraction was obtained by centrifugation 1300rpm for 30 mins at room temperature on Ficoll (Histopaque 1077). The mononuclear cells

were then washed twice with phosphate buffered saline (PBS) and 0.5% bovine serum albumin (BSA) before immunostaining for flow cytometry.

Flow cytometry (T-cell phenotype and activation markers and chemokine receptors)

Unlabelled first antibodies at concentrations stated in table 3.5 were added to 100µl BAL and PBMC cells ($1-5 \times 10^6$ cells/ml) per tube in PBS, 0.5% bovine serum albumin (BSA) depending on the number of cells available and incubated on ice for 30min. The cells were washed with 1ml PBS, 0.5% BSA per tube and centrifuged at 1200rpm for 8min at 4°C. The supernatant was discarded and cells resuspended and labelled with 100µl of 5% FITC-labelled Rabbit anti-Mouse Ig antibody in buffer. Following incubation for 15-30min on ice the cells were washed, centrifuged and resuspended with 10% mouse serum (Sigma, Poole, Dorset, UK) in PBS, 0.5% BSA per tube and incubated for a further 15min on ice to block non-specific antibody binding. Directly labelled anti-CD3-RPE (5µl) and anti-CD4-PerCP (10µl) was added to each tube for 30min on ice. Cells underwent a final wash and were resuspended in 300µl PBS, 0.5% BSA. Control tubes included an unlabelled isotypic control and cells labelled with a single colour FITC, RPE or Per-CP for every experiment. Three-colour flow cytometry was performed using a FACScan and CellQuest software version 3.1 (BD Biosciences). Lymphocytes were gated for CD3 expression and then further subdivided by CD4 or CD8 expression.

Flow cytometry (T-cell intracellular cytokines)

The remaining unused blood and BAL cell samples, prepared as above were resuspended at 1×10^6 cells/ml in culture medium RPMI, 10% Foetal Calf Serum (FCS), Hepes, Glutamax. The cell suspension was divided into two and half were stimulated with the remaining cells unstimulated or resting in culture medium alone. Cells were stimulated with phorbol 12-myristate 13-acetate (PMA) (5ng/ml) (Sigma, Poole, Dorset, UK), calcium ionophore (250ng/ml) (Sigma) and Brefeldin A (10µg/ml) (Sigma) was used to prevent release of intracellular cytokines (final concentrations) and all cells were incubated in a tissue culture plate for 4hr at 37°C in a CO₂ incubator.

Table 3.5 Antibody concentration used for flow cytometry (surface markers)

Antibody	Concentration	Source
MoIgG	1:10	(Dako)
CD8	1:20	(Pharmingen)
CCR3 (7D11) (1:10dil)	1:10	(Millenium)
CCR5	1:20	(Pharmingen)
CD103	1:10	(Dako)
CD25	1:10	(Dako)
CD49a	1:20	(Serotec)
HLA-DR	1:10	(Dako)
CXCR3	1:50	(R+D)
CCR6	1:50	(R+D)
MoIgG-RPE	1:50	(Dako)
CD3-RPE	1:20	(Dako)
CD4-PerCP	1:10	(Becton Dickenson)
Rabbit anti-Mouse Ig-FITC	1:10	(DAKO)

Following incubation harvested cells were washed with PBS, 0.5% BSA and centrifuged at 1200rpm for 10min at 4°C. The cell pellet was resuspended in 4% paraformaldehyde (Sigma); PBS and the cells were fixed for 15 min on ice. After washing the cells a further two times and centrifuging at 1400rpm the cells were stored overnight at 4°C in PBS, BSA.

The following day the cells were resuspended in fresh staining buffer and incubated with directly labelled CD3-FITC/RPE (Dako, Ltd.) and CD8-PerCP (Beckton Dickenson, BD) or isotypic controls for 15min on ice. CD8 was used to subdivide CD3 cells as CD4 becomes internalised after fixation. After a further wash the cells were fixed and permeabilised in 4% paraformaldehyde, 0.1% saponin for 15 min on ice. The cells underwent two further washes with PBS, 0.5% BSA, 0.1% saponin. Following incubation of the cells with PBS, BSA, 10% mouse serum for 15 minutes to block non-specific binding, direct-labelled IL-4-RPE (BD) and IFN- γ -FITC (BD) or isotypic controls were added for 45min at 4°C. The cells were washed twice with PBS, 0.5% BSA, 0.1% saponin and resuspended in a final volume of 300 μ l of PBS, 0.5%BSA ready for analysis by flow cytometry. Three-colour flow cytometry was performed using a FACScan as for the surface receptor expression. Control experiments showed that DMSO, fixation or overnight storage did not alter expression of intracellular cytokines.

Immunohistochemistry

a) Glycomethacrylate blocks

Bronchial biopsies were immediately transferred into ice-cooled acetone containing the protease inhibitors protease inhibitors iodoacetamide (20mM) and PMSF (2mM) for fixation, stored at -20°C for 24h. The fixative was replaced with acetone followed by methyl benzoate at room temperature for 15 minutes each. The biopsies were infiltrated with 5% methyl benzoate in glycol methacrylate (GMA solution A, Polysciences, Northampton, UK) at 4°C, 3x2 hours and then embedded in a solution of GMA solution A 10mls: GMA solution B 250µls: Benzoyl peroxide 45mg (which acts the catalyst for polymerisation). The blocks were polymerised at 4°C overnight and kept in dry airtight boxes at -20°C.

b) Buffers and solutions for immunohistochemistry

Tris Buffered Saline pH 7.6

Sodium chloride	80g
Tris	6.05g
1M hydrochloric acid	38mls
Distilled water	10L

Mix buffer salts and acid in 1L of distilled water, adjust pH to 7.65 and add to remaining 9L of water to give a final pH of 7.6

Blocking medium

Dulbecco’s modified Eagles medium	80mls
Fetal calf serum	20mls
Bovine serum albumin	1g

Tris HCl Buffer pH 7.6

0.2M Tris	12mls
0.1M hydrochloric acid	19mls
Distilled water	19mls

Mix all reagents together, adjust pH to 7.6

c) *Immunohistochemistry protocol*

Two-micrometer sections were cut, floated on 0.2% ammonia solution in water for 1 min and dried at room temperature for 1-4h. Slides were pretreated with a solution of 0.1% sodium azide and 0.3% hydrogen peroxide to inhibit endogenous peroxide. After 2X 5min washes in TBS pH 7.6, blocking medium consisting of Dulbecco's MEM, 10% FCS and 1% Bovine Serum Albumin (BSA) was applied for 30 min. Sections were then incubated with the primary antibody for 16-20h overnight at room temperature at appropriate concentrations (Table 3.6). Bound antibodies were labelled with biotinylated rabbit anti-mouse Fab fragments (Dako Ltd., Ely, Cambridgeshire, UK) during a 2h incubation, and demonstrated using the streptavidin-biotin-peroxidase detection system (Dako Ltd). Aminoethylcarbazole (AEC) was applied as the chromogen, which gives a red reaction product. Sections were counterstained with Mayers haematoxylin. Appropriate control sections were similarly treated either with the primary mAb omitted or in the presence of an unrelated antibody of the same isotype (IgG1 Dako, Ltd) (Britten, Howarth, & Roche 1993).

d) *Assessment and quantification of immunohistochemical staining*

Sub-epithelial mucosa was identified morphologically and the area calculated using a computer analysis system (Scion Image, Maryland, USA). Nucleated immunostained cells present in coded sections were enumerated in the submucosa and numbers of cells expressed as the number/mm² of submucosal by a blinded observer.

Table 3.6 Antibody concentrations used for immunohistochemistry

Antibody	Clone	Epitope Stained	Source	Dilution
CD3	UCHT1	T lymphocytes	DAKO	1:1000
CD4	Leu 3a/3b	T helper cells	BD	1:10
CD8	DK25	T suppressor cells	DAKO	1:100
MBP		MBP	Gift	1:25
EG2		ECP	PHARMACIA	1:200
Tryptase	AA1	Mast cell tryptase	DAKO	1:1000
NE	NP57	Neutrophils	DAKO	1:1000
CD14	TUK4	Macrophages	DAKO	1:8
CD45	2B11+P D7/26	Leucocyte common antigen	DAKO	1:150
CD56		NK cells	DAKO	1:100
IL-4	3H4	IL-4	AMS	1:50
IL-5	MAB7	IL-5	Glaxo (gift)	1:100
IFN γ		IFN γ	R+D	1:25
IgG1		Isotope control Ig	SIGMA	1:60
Biot-rab antiMo		Second stage antibody	DAKO	1:300
StABC-HRP		Third stage antibody	DAKO	1+1:200

Statistical Analysis

Subject characteristics were described using descriptive statistics and expressed as means (standard error). Methacholine PC₂₀ and concentration of capsaicin that causes 2 and 5 coughs (C₂ and C₅ μ mol/L) were calculated by linear interpolation of the log dose-response curves and described as geometric mean (logSEM). The primary outcome variable, BAL lymphocyte differential cell count was expressed as median (range). Other differential cell counts and subepithelial cell counts were expressed as medians (range) and flow cytometry data as means (SEM). 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. Chi-squared tests were used to make comparisons between groups in the prevalence of autoimmune disease. A value of $p < 0.05$ was taken as being statistically significant.

RESULTS

The subject characteristics were as shown (Table 3.7). The mean duration of cough for patients with idiopathic and explained chronic cough was 7 (0.5-50) and 8 (0.5-6.3) years respectively. Patients with cough were recruited at random from 236 patients where the primary causes of cough were cough variant asthma 39(17%), gastro-oesophageal reflux 35(15%), rhinitis 29(12%), eosinophilic bronchitis 17(7%), idiopathic 54(23%), post-viral 17(7%), bronchiectasis 14(6%), chronic bronchitis 10(4%), enlarged tonsils 8 (3%), pulmonary fibrosis 7 (3%), angiotensin converting enzyme inhibitor cough 4(2%), sarcoidosis 1 (0.5%) and bronchial tumour 1 (0.5%). The causes of cough in the explained chronic cough studied were: cough variant asthma (n=4), eosinophilic bronchitis (5), gastro-oesophageal reflux (2), rhinitis (2) and chronic bronchitis (1). All subjects had a normal serum ACE level. Sixteen patients out of the total population of 54 patients (30%) with idiopathic chronic cough had an organ specific autoimmune disease. Six (32%) of the 19 patients with idiopathic chronic cough included in this study had clinically overt organ-specific autoimmune disease (hypothyroidism 3, hypothyroidism / pernicious anaemia / vitiligo 1, celiac disease 1 and vitiligo 1) and a further 2 had positive organ specific autoantibodies (parietal and islet cell) with no clinical evidence of disease. One normal control and one patient with explained cough had positive autoantibodies (islet cell and islet cell/adrenal respectively); none had clinical evidence of autoimmune disease. The prevalence of organ specific autoimmune disease or positive autoantibody was significantly higher in idiopathic chronic cough than normals or explained chronic cough (Figure 3.2; $p=0.01$). Both cough groups had heightened capsaicin cough reflex sensitivity (Table 3.7).

Sputum, BW and BAL differential inflammatory cell counts were as shown in Table 3.8. Median BAL differential lymphocyte counts were significantly higher in patients with idiopathic cough (10.0%) than normals (6.3%; 95% confidence

interval of difference 1.5 to 11.9%, $p=0.01$) and patients with explained cough (5.2%; 95% CI of difference 2.0 to 10.4%, $p=0.001$; Figure 3.2). An example cytospin of a BAL lymphocytosis is shown Figure 3.3. Median BAL eosinophil counts were significantly higher in the explained cough group than normals and idiopathic cough (Table 3.8). There were no differences in the other differential cell counts between groups. There was adequate submucosa with good morphology, suitable for counting cells in 9 normals, 10 patients with idiopathic cough and 13 patient with explained cough. There was an insufficient number of biopsies with intact epithelium to compare epithelial cell counts across groups. There were no significant differences in T cell (CD3, CD4 and CD8) subepithelial cell counts in biopsies between groups (Table 3.9, Figure 3.4). The median CD56+ cells/mm² of subepithelium for idiopathic chronic cough (0.3) was significantly lower than normals (5.3; 95% confidence interval of difference 0.5 to 7.7; $p=0.01$) but not significantly different from explained cough. The median IFN γ + cells/mm² for idiopathic cough (0) was significantly lower than explained cough (3.7; 95% confidence interval of difference 0.4 to 3.8; $p=0.03$) but did not differ from normals (4.5; 95% confidence interval of difference 0 to 7.7; $p=0.06$). EG2+, MBP+ and 3H4+(IL-4) cells/mm² of subepithelium were significantly higher in explained cough than normals and idiopathic cough but were no significant differences in other cell counts between groups (Table 3.9). The intraclass correlation coefficients for cell counts between two blinded observers were: BAL lymphocytes 0.90, bronchial biopsy subepithelial cell counts for extracellular stains 0.97 and 0.86 for intracellular stains.

The mean(SEM) proportion of CD3+ PBMC expressing CD4 was significantly higher in normals than patients with idiopathic cough (69(3)% vs 58(3)%, mean difference 11%, 95% CI of difference 2, 20%, $p<0.02$) and approached significance when compared with explained cough (69(3)% vs 63(2)%, mean difference 7%, 95% CI of difference -1, 15%, $p=0.08$). The mean(SEM) PBMC CD4/CD8 ratio was significantly higher in normals than idiopathic cough patients (2.5(0.4) vs 1.6(0.2), mean difference 0.9, 95% CI 0.1, 1.8, $p<0.05$) but not explained cough (1.8(0.2), $p=0.09$). There were no significant differences between groups in CD3+ BAL expressing CD4 (mean(SEM): normal 58(5)%, idiopathic cough 59(3)%, explained

cough 55(4)%) or CD4/CD8 ratio (normal 1.8(0.5), idiopathic cough 1.6(0.2), explained cough 1.5(0.2)). No differences were seen in the proportion of CD3+CD4+ or CD3+CD8+ (CD3+CD4-) PBMC and BAL cells expressing activation, chemokine receptors and intracellular cytokines between groups (Tables 3.10 and 3.11). Examples of flow cytometry dot-plots for surface markers and intracellular cytokines are given in Figure 3.5 and 3.6.

Table 3.7 Subject characteristics.

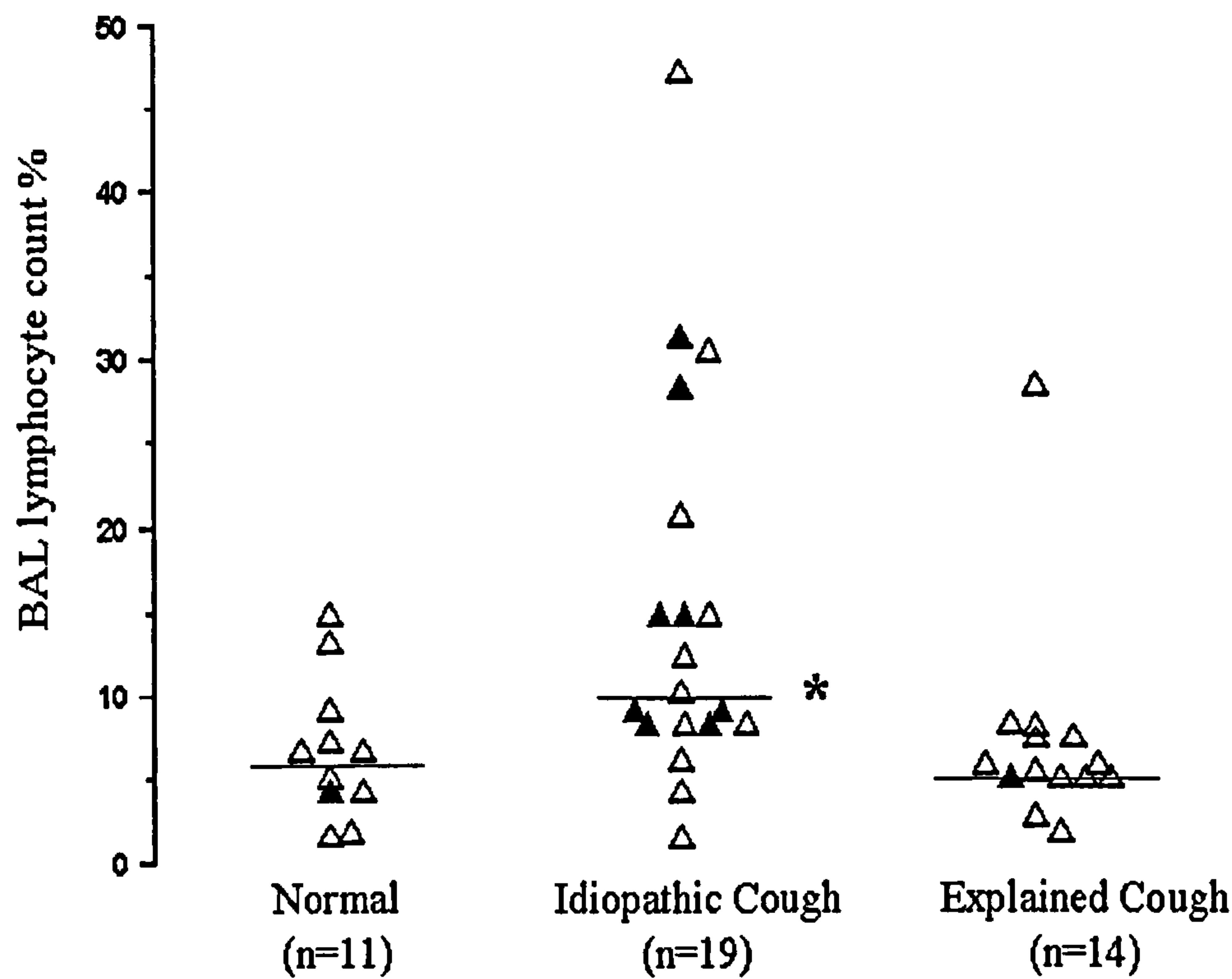
	Normal	Idiopathic Cough	Explained Cough
Number	11	19	14
Age (years)	52 (5)	54 (2)	55 (4)
Male (%)	3 (27)	4 (21)	5 (36)
Cough VAS (mm)	0	57 (4)	51 (5)
Age of onset of cough (y)		47 (3)	52 (4)
Cough Duration (years)	0	7 (3)	8 (6)
FEV ₁ % predicted	111 (5)	104 (4)	101 (3)
FEV ₁ /FVC %	80 (2)	80 (1)	77 (2)
PC ₂₀ FEV ₁ n(%)	11 (100)	19 (100)	10 (71)
>8mg/ml			
C ₂ (μmol/L) [#]	47.9 (0.2)	2.2 (0.2)*	2.2 (0.2)*
C ₅ (μmol/L) [#]	416.9 (0.1)	6.9 (0.2)*	11.5 (0.3)*
Nitric oxide (ppb) [#]	1.8 (0.2)	2.2 (0.1)	5.4 (0.1)
Positive allergen specific IgE n(%)	1 (9)	3 (16)	4 (29)
Blood lymphocytes	2.1 (0.2)	1.8 (0.1)	2.2 (0.1)

Data expressed as mean (SEM) except where shown. [#]Geometric mean (logSEM); VAS: visual analogue score (0-100mm- worst symptom); C₂ and C₅: concentration of capsaicin that causes 2 and 5 coughs; *p<0.001 (ANOVA).

Table 3.8 Median (range) differential cell counts in sputum, bronchial wash and BAL (%). Kruskal-Wallis Test: *p<0.05, **p<0.01

	Normal	Idiopathic cough	Explained cough
Induced sputum			
Lymphocytes	0.5 (0-9.8)	0.7 (0-5.8)	0.1 (0-1.3)
Eosinophil	0.5 (0-2.0)	0.2 (0-0.8)	3.8 (0-68)
Neutrophil	56 (38-84)	50 (15-81)	56 (11-88)
Macrophage	42 (12-51)	47 (17-82)	27 (1-86)
Epithelial cell	2.5 (0.8-15.1)	1.8 (0-19.8)	3.4 (0-10.8)
Squamous contamination	4 (0-15)	8 (0-35)	6 (0-16)
Viability	55 (20-81)	65 (23-87)	52 (34-83)
Total cell count x10 ⁶	1.6 (0.3-3.6)	0.9 (0.2-3.8)	1.4 (0.2-12)
Bronchial wash			
Lymphocytes	0.5 (0-1.3)	1.0 (0-25)	0.5 (0-5.0)
Eosinophil	0 (0-0.3)	0.2 (0-4.8)	0.8 (0-40.5)**
Neutrophil	41 (6-74)	24 (3-71)	31 (6-72)
Macrophage	26 (7-54)	13 (2-81)	22 (5-75)
Epithelial cell	32 (2-84)	41 (5-93)	39 (0-81)
Viability	19 (12-69)	28 (2-92)	22 (8-85)
Recovery (%)	33 (20-50)	35 (8-68)	26 (9-44)
Total cell count x10 ⁶	0.3 (0.1-1.0)	0.4 (0.2-1.8)	0.3 (0.1-1.0)
BAL			
Lymphocytes	6.3 (1.4-14.7)	10.0 (1.3-47)**	5.2 (2.2-28.2)
Total lymphocyte cell count (x10 ⁶)	0.5 (0-1.9)	1.5 (0.2-7.1)**	0.4 (0.1-2.2)
Eosinophil	0.3 (0-2.5)	0.2 (0-1.8)	1.5 (0-13)*
Neutrophil	9 (0-24)	5 (0-33)	5 (0-19)
Macrophage	77 (43-91)	78 (47-88)	81 (38-89)
Epithelial cell	7 (2-26)	5 (1-17)	5 (2-33)
Viability	71 (45-85)	69 (19-88)	65 (25-95)
Recovery (%)	24 (8-38)	28 (15-39)	26 (19-40)
Total cell count x10 ⁶	7.0 (0.8-48)	7.5 (3.1-22.9)	5.4 (4.0-8.5)

Figure 3.2 BAL lymphocyte differential cell count (%).



Medians; Blocked triangles: history of organ-specific autoimmune disease or presence of organ-specific autoantibodies; Open triangles: no autoimmune disease or autoantibodies; * $p=0.002$ (Kruskal-Wallis test)

Figure 3.3 An example of a BAL lymphocytosis

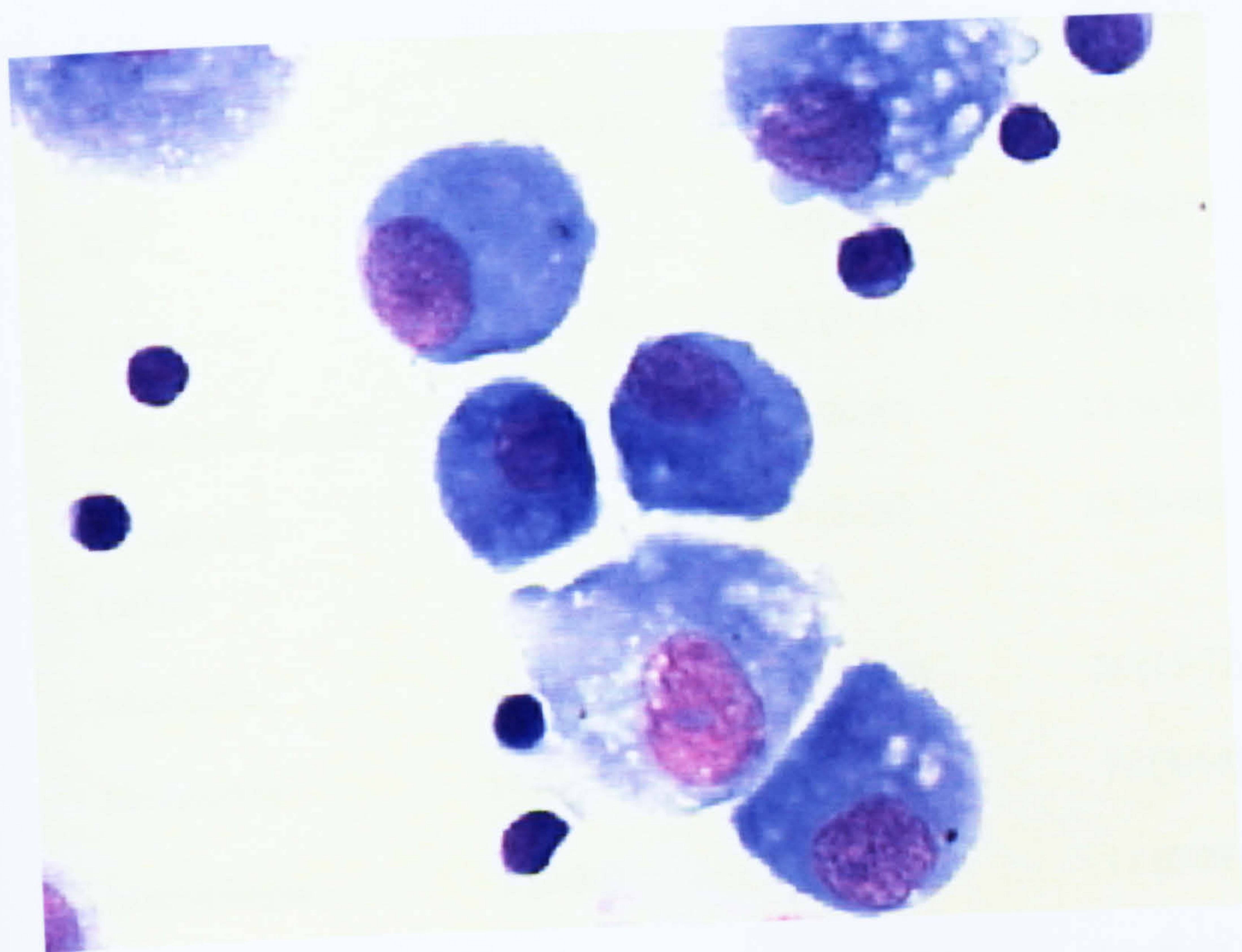


Table 3.9 Median (range) bronchial biopsy subepithelial cell counts per mm².

	Normal (n= 9)	Idiopathic cough (n=10)	Explained cough (n=13)
CD3 (T cells)	38 (8-69)	22 (6-105)	57 (19-125)
CD4	12 (0-51)	27 (4-119)	33 (12-95)
CD8	16 (0-33)	9 (1-27)	17 (0-73)
IFN γ	4.6 (0-10.7)	0 (0-7.1)*	3.7 (0-10.7)
IL5	5 (0-15)	3 (0-17)	7 (0-21)
IL4	1.9 (0-13.6)	0.3 (0-7.4)	5.3 (0-11.4)*
Eosinophils (EG2)	1 (0-12)	2 (0-29)	11 (0-58)*
Eosinophils (MBP)	5 (0-19)	7 (2-24)	16 (3-90)*
Mast cells	15 (7-50)	19 (6-60)	26 (15-72)
Neutrophils	14 (0-50)	24 (7-45)	15 (4-64)
Macrophages	8 (0-36)	5 (0-18)	11 (0-28)
NK cells	5.3 (0-15.6)	0.3 (0-4.8)*	2.4 (0-13.8)
Leukocytes (CD45)	53 (6-127)	40 (19-90)	56 (18-168)

Data expressed as medians (range). Kruskal-Wallis Test: *p<0.05

Figure 3.4 An example of positive submucosal staining for lymphocytes (CD3)

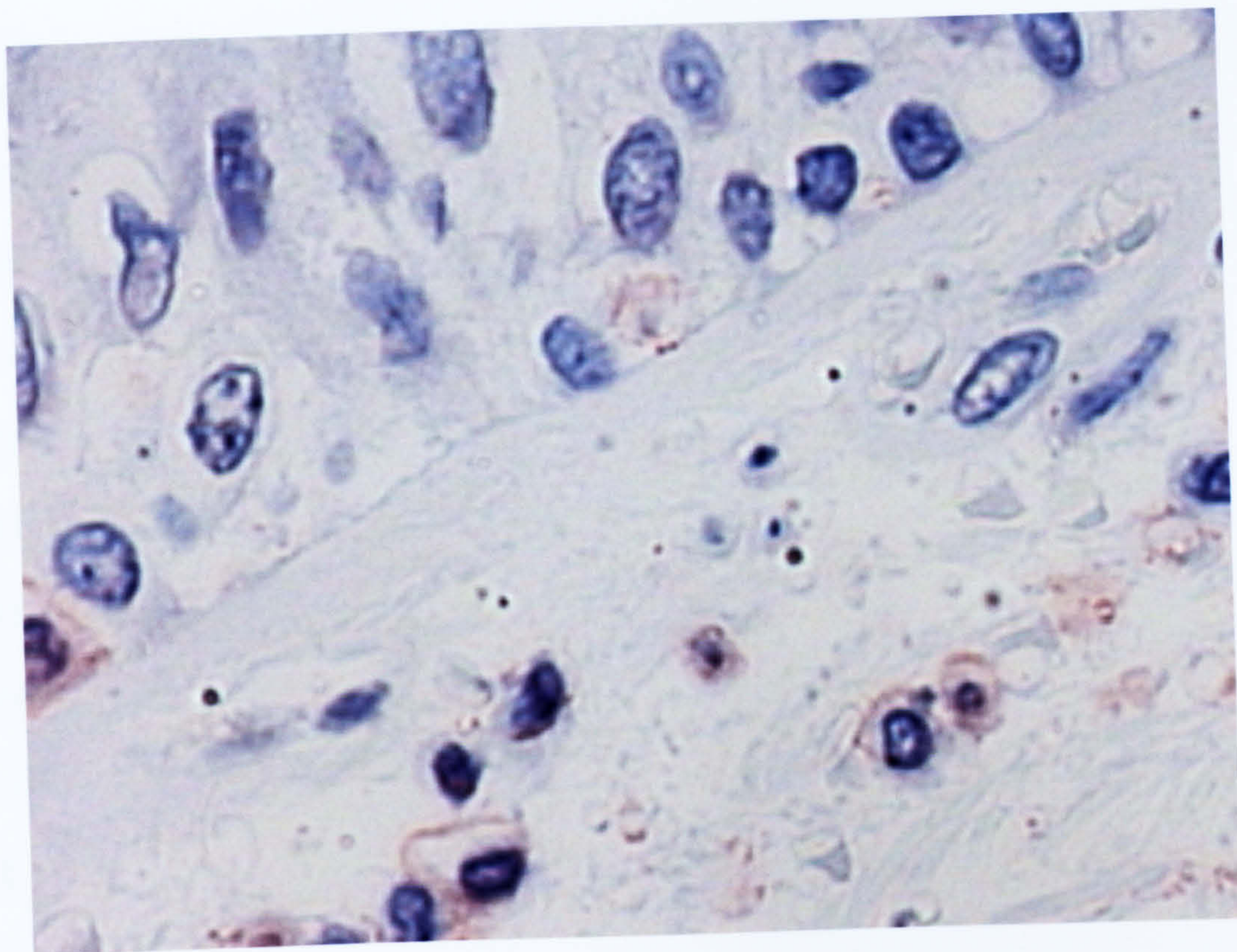


Table 3.10. Mean (SEM) of the proportion (%) of CD4+ (CD3+CD4+) and CD8+ (CD3+CD4-) T-cells that express chemokine receptors and activation markers in peripheral blood mononuclear cells (PBMC) and bronchoalveolar lavage (BAL) in subjects with idiopathic chronic cough, explained chronic cough normal individuals.

	Normal		Idiopathic cough		Explained cough	
PBMC	CD4	CD8	CD4	CD8	CD4	CD8
CD25	50 (5)	11 (3)	46 (3)	9 (2)	54 (4)	15 (5)
CD49a	7 (1)	8 (2)	11 (2)	6 (1)	11 (2)	12 (5)
HLA-DR	20 (4)	58 (6)	22 (4)	65 (5)	22 (4)	56 (8)
CD103	11 (7)	3 (0)	5 (1)	2 (0)	6 (5)	3 (1)
CCR3	0.7 (0.3)	5.5 (4.3)	2.7 (1.0)	3.3 (1.6)	1.7 (0.8)	4.1 (2.8)
CCR5	16 (3)	55 (5)	26 (4)	59 (4)	27 (4)	56 (7)
CCR6	21 (4)	20 (5)	26 (3)	13 (3)	24 (5)	18 (6)
C-X-CR3	29 (6)	76 (10)	40 (3)	78 (3)	38 (4)	87 (2)
BAL						
CD25	36 (4)	20 (4)	28 (4)	15 (4)	29 (3)	14 (2)
CD49a	49 (4)	65 (4)	52 (3)	67 (5)	55 (6)	68 (5)
HLA-DR	80 (2)	70 (4)	80 (3)	70 (5)	82 (3)	69 (4)
CD103	31 (3)	54 (4)	33 (3)	54 (4)	39 (6)	59 (4)
CCR3	1.4 (0.5)	3.6 (0.7)	2.1 (0.7)	2.6 (1.2)	6.8 (4.1)	10 (5)
CCR5	81 (2)	79 (3)	78 (5)	81 (3)	81 (4)	80 (3)
CCR6	57 (6)	25 (4)	61 (4)	30 (6)	44 (5)	23 (3)
C-X-CR3	76 (6)	82 (3)	86 (2)	84 (3)	79 (5)	80 (4)

Table 3.11 Mean (SEM) of the proportion (%) of CD4+ (CD3+CD8-) and CD8+ (CD3+CD8+) PBMC and BAL T-lymphocytes cells expressing intracellular cytokine staining. R-resting cells, S-stimulated cells, IL-4 interleukin 4, IFN γ –interferon gamma.

	Normal		Idiopathic cough		Explained cough	
PBMC	CD4+	CD8+	CD4+	CD8+	CD4+	CD8+
IL-4 R	2.3 (0.6)	2.9 (1.1)	4.9 (0.7)	2.7 (0.5)	3.2 (0.8)	3.1 (1.0)
IL-4 S	4.8 (2.8)	5.2 (3.5)	5.7 (2.0)	4.4 (1.8)	4.4 (1.9)	4.1 (1.8)
IFN- γ R	0.7 (0.1)	1.2 (0.2)	1.1 (0.3)	2.3 (0.8)	1.4 (0.4)	1.8 (0.5)
IFN- γ S	13.9 (7.6)	21.8 (8.0)	12.9 (3.4)	25.5 (5.8)	13.2 (3.4)	27.7 (7.8)
BAL						
IL-4 R	5.7 (1.3)	4.3 (0.7)	8.6 (1.4)	5.9 (0.7)	5.9 (1.2)	5.1 (1.3)
IL-4 S	12.5 (4.3)	7.4 (3.4)	9.6 (1.4)	7.8 (1.0)	8.9 (1.3)	8.9 (2.1)
IFN- γ R	2.3 (0.7)	2.4 (1.2)	2.3 (0.5)	1.7 (0.4)	4.2 (1.4)	6.2 (2.2)
IFN- γ S	19.6 (4.1)	32.3 (8.0)	24.4 (6.0)	29.2 (5.2)	26.3 (6.3)	41.9 (6.4)

Figure 3.5 Example FACS dot-plots from a normal control, a patient with idiopathic chronic cough and explained chronic cough for chemokine receptor CCR5 staining and isotypic control in BAL T-lymphocytes.

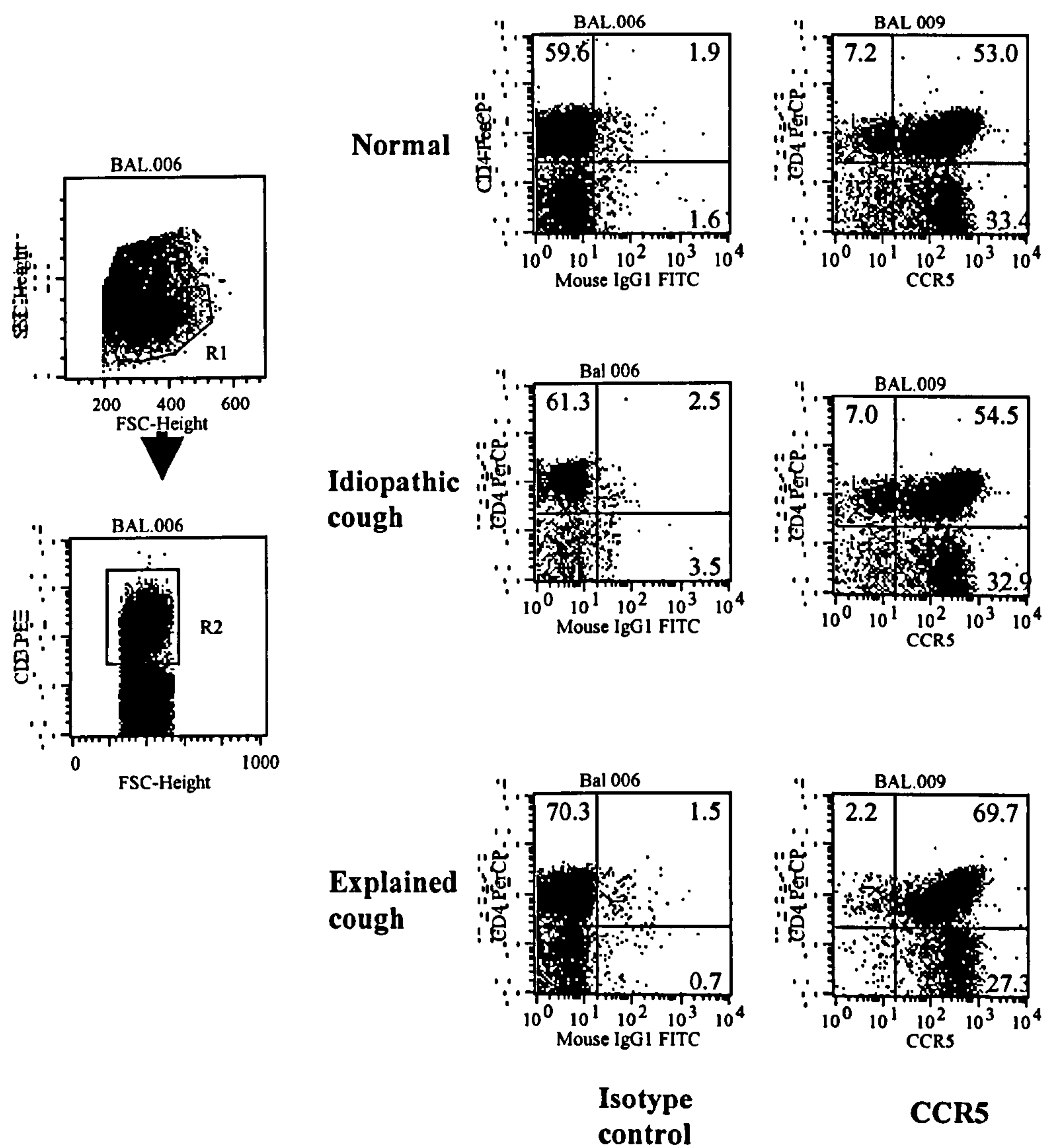
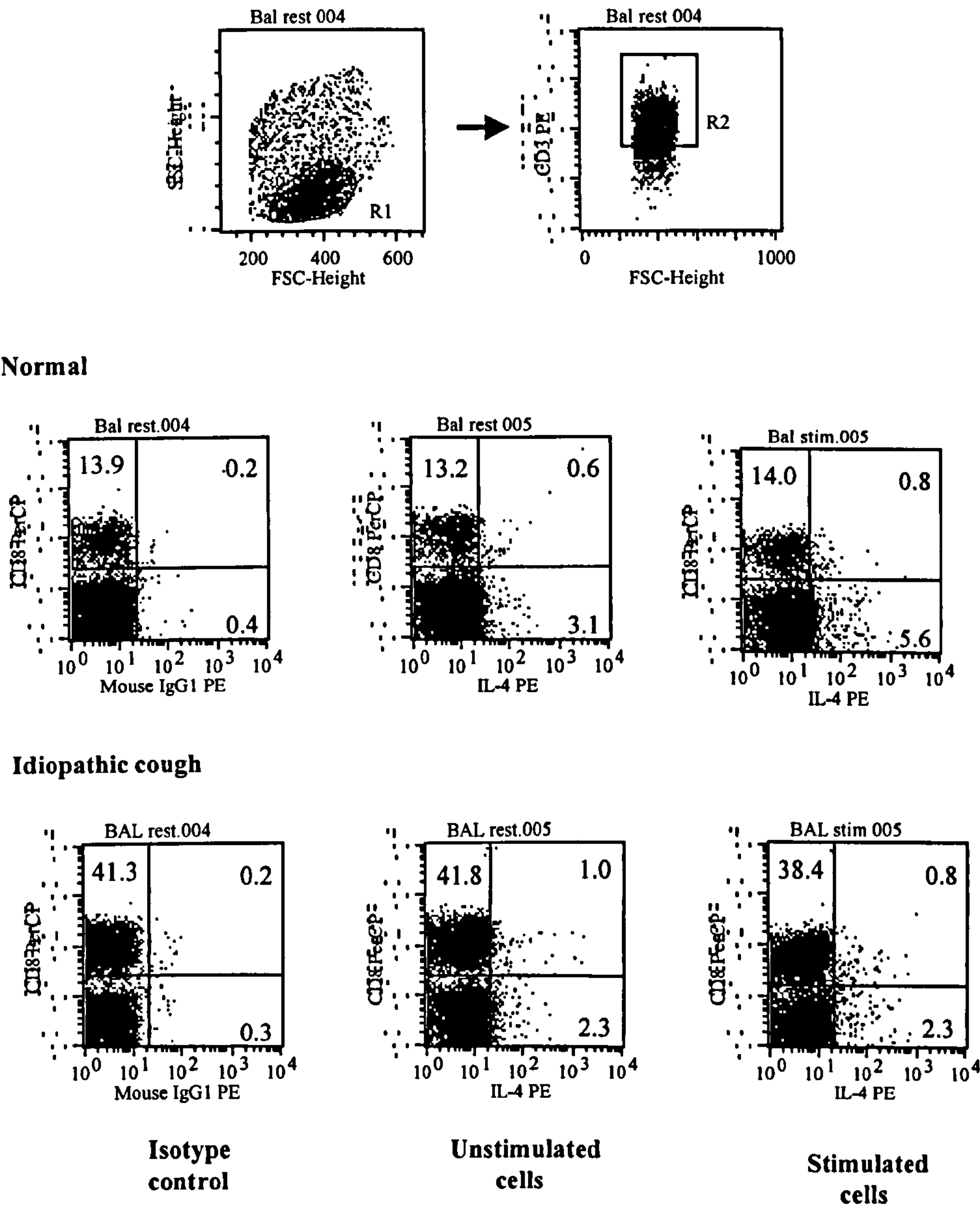


Figure 3.6 Example FACS dot-plots from an individual normal control and a patient with idiopathic chronic cough for IL-4 intracellular cytokine staining and isotypic control in resting and stimulated PBMC T-lymphocytes.



DISCUSSION

This is the first study to address in detail the lower airway immunopathology in idiopathic chronic cough. As noted previously, patients with idiopathic chronic cough were predominantly female (section 3.1.1), had heightened cough reflex sensitivity (Prudon et al. 2004) and had a high prevalence of organ specific autoimmune disease and organ specific autoantibodies (section 3.1.1). The prevalence was greater than expected from general population surveys (3-8%) and significantly higher than that seen in normal controls or patients with explained chronic cough (Mackay 2000; Rose & McKay 1998). The new observation was that patients with idiopathic chronic cough had a significant BAL lymphocytosis. The BAL lymphocytosis is unlikely to be a feature of cough per se since this was not seen in patients with explained chronic cough of similar severity. Our findings are consistent with the view that there is lymphocytic bronchoalveolar inflammation in idiopathic chronic cough.

McGarvey et al (McGarvey et al. 1999) have reported a relative increase of BAL lymphocytosis of a similar magnitude in 6 patients with idiopathic chronic cough compared to healthy controls although in this study the difference was not statistically significant. By contrast, Boulet et al (Boulet et al. 1994) found no differences in BAL lymphocyte count in 4 patients with chronic cough not due to rhinitis or gastro-oesophageal reflux compared to controls. The differences between these studies and ours may relate to power or the precision of diagnosis. A BAL lymphocytosis was not a consistent feature in our study and another possible explanation for the inconsistent findings is that idiopathic chronic cough is a heterogeneous collection of conditions with distinct pathophysiologies and that a BAL lymphocytosis is confined to a subgroup.

The increase in lymphocyte count was seen in BAL but not in induced sputum or bronchial biopsies suggesting that inflammation might be confined to distal airways and the alveolar compartment. However, the absence of an increase in lymphocyte count in induced sputum cannot necessarily be taken as evidence against larger airway involvement since the proportion of lymphocytes in these samples is low so they may not reflect lymphocytic airway inflammation well

(Pavord et al. 1997). Our findings in bronchial biopsies contrast with other studies where increased numbers of mononuclear cells have been reported in patients with idiopathic chronic cough (Boulet et al. 1994; Lee et al. 2001). We found reduced numbers of IFN γ ⁺ and NK cells in idiopathic chronic cough and minor changes in peripheral blood CD4 T cell phenotypes. The significance of these findings is unclear and they could have arisen by chance because they were not *a priori* hypotheses. However, NK cells are a subset of mononuclear cells that play an immunoregulatory role in the prevention of autoimmune diseases, mediating some of their effects through IFN γ secretion (Baxter & Smyth 2002) and they have been shown to be both functionally and numerically deficient in individuals at risk of developing organ-specific autoimmune disease, (Sharif et al. 2002) so these findings are potentially relevant to our hypothesis. One important caveat to interpretation of the bronchial biopsy data is that because of technical difficulties, perhaps related to our attempt to do both BAL and bronchial biopsies in patients who have a troublesome cough, adequate submucosa for analysis was only available in just over half of the subjects with idiopathic chronic cough so we cannot exclude the possibility that features were missed due to lack of power or bias. Another consideration is that we have studied a wide array of secondary outcome measures, so there is a potential for differences to emerge because of multiple comparisons. As this is the first study to investigate the lower airway immunopathology of idiopathic chronic cough in detail and so little is known about the pathogenesis, we feel that a wide-ranging descriptive approach is justified. Nevertheless our findings should be regarded as hypotheses generating rather than definitive and it is important that further confirmatory studies are done. Future studies should ideally incorporate larger biopsies and transbronchial biopsies so that better localisation and characterisation of lower airway inflammatory response is possible.

Bronchoalveolar lymphocytosis is also seen in sarcoidosis, (Inui et al. 2001) extrinsic allergic alveolitis, (Wahlstrom et al. 1997) and pulmonary involvement in rheumatoid arthritis (Gabbay et al. 1997) and Sjogren's disease (Wallaert et al. 1987). None of the patients with idiopathic chronic cough had clinical features, radiological findings or laboratory test results suggesting these diagnoses. BAL

lymphocytosis has also been associated with obliterative bronchiolitis in rheumatoid arthritis, Sjogren's disease, lung transplantation and inflammatory bowel disease (Tiroke, Bewig, & Haverich 1999; Wright et al. 1992). It is interesting to speculate that a similar, albeit lower grade process is operating in our patients, particularly since we have recently reported an association between organ specific autoimmune disease and unexplained fixed airflow obstruction in a population of predominantly older females, many of whom had a long history of dry cough at presentation (Birring et al. 2002).

The lungs and many of the organs involved in organ-specific autoimmune disorders share common embryological origins as foregut derivatives and a possible mechanism for the cough and BAL lymphocytosis is homing of activated T cells into the pulmonary compartment from the primary site of autoimmune inflammation. Similar mechanisms are thought to be responsible for the lymphocytic airway inflammation seen in inflammatory bowel disease, (Wallaert et al. 1985) and it is notable that patients with treated hypothyroidism and inflammatory bowel disease have increased prevalence of respiratory symptoms compared to controls, and that the profile of symptoms reported is remarkably similar (section 3.1.2). The concept that aberrant homing of activated lymphocytes occurs in the lung and can result in an isolated cough without physiological and radiological changes is supported by a recent case report of an otherwise unexplained chronic cough associated with BAL lymphocytosis which resolved following treatment of coeliac disease (Brightling et al. 2002c). The BAL lymphocytosis seen in the current study was not confined to the patients with overt organ specific autoimmune disease perhaps suggesting that the association was not directly related to the presence of lymphocytic inflammation elsewhere. An alternative and intriguing mechanism is that the cough might be due to a hitherto unrecognised autoimmune bronchitis, bronchiolitis or subtle interstitial process and that the association with other diseases simply reflects the well recognised association between different organ specific autoimmune diseases. One question is why corticosteroid therapy was not helpful in our patients? This may be because autoimmune hypothyroidism, pernicious anaemia, insulin dependent diabetes mellitus and other organ specific autoimmune

disorders differ from systemic autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis in that they do not respond well to corticosteroids (Rose & McKay 1998). Moreover, corticosteroid therapy is disappointing in pulmonary complications of some conditions associated with a BAL lymphocytosis such rheumatoid arthritis and lung transplantation (Wright et al. 1992).

In summary, we have found a BAL lymphocytosis in some patients with idiopathic chronic cough. These patients are predominantly middle-aged females and there is a high prevalence of organ specific autoimmune disease raising the possibility of a link between autoimmune inflammation and lymphocytic inflammation in the lung and cough. Further studies are required to investigate the mechanism of BAL lymphocytosis and how it relates to cough.

3.2.2 Induced sputum inflammatory mediator concentrations in chronic cough

ABSTRACT

Previous studies have shown evidence of airway inflammation in patients with chronic cough and have suggested that the cough may be due to release of tussive mediators and activation of afferent sensory nerve endings. We measured the concentration of various proinflammatory and tussive mediators in induced sputum supernatants in 20 patients with cough variant asthma or eosinophilic bronchitis, 20 patients with non-asthmatic chronic cough, 22 patients with idiopathic chronic cough and 18 healthy controls. We measured histamine, cysteinyl-leukotrienes, prostanoids (prostaglandin D₂, prostaglandin E₂) and interleukin-8 by enzyme immunoassay. The median sputum histamine concentrations were significantly higher in patients with idiopathic chronic cough (8.0ng/ml) and cough variant asthma / eosinophilic bronchitis (10.2ng/ml) than normals (2.6ng/ml; 95% confidence interval of difference from idiopathic chronic cough 25.8 to 0.8; p=0.009 and 95% confidence interval of difference from cough variant asthma / eosinophilic bronchitis 1.1 to 20.1; p=0.01). Median sputum prostaglandin D₂ and prostaglandin E₂ concentrations were significantly higher in all categories of chronic cough. Our findings support the view that there is release of inflammatory and tussive mediators in patients with chronic cough and suggest that there might be similarities in the mechanism of cough in a diverse range of conditions.

INTRODUCTION

Cough is a common presenting symptom in both general practice and respiratory clinics. Most cases are mild and self-limiting but a small proportion have persistent troublesome cough and require specialist review. Persistent cough is thought to be due to one or more of asthma, eosinophilic bronchitis, gastro-oesophageal reflux and rhinitis with post-nasal drip in the majority of cases (Brightling et al. 1999a;Irwin & Madison 2000). However in up to 20% of patients, the cough remains unexplained even after extensive investigation and treatment trials (McGarvey et al. 1998).

Several studies employing different techniques to assess airway inflammation have shown that airway inflammation is present in patients with chronic cough. Various abnormalities have been detected depending on the category of patient and method used to assess airway inflammation, including a sputum neutrophilia and increased concentration of interleukin-8 in induced sputum (Jatakanon et al. 1999), a bronchoalveolar lavage and bronchial biopsy lymphocytosis in patients with idiopathic chronic cough (Boulet et al. 1994;Lee et al. 2001;McGarvey et al. 1999) and a sputum, bronchoalveolar lavage and bronchial biopsy eosinophilia in asthma and eosinophilic bronchitis (Brightling et al. 1999a;Brightling et al. 2002a;Brightling et al. 2002b). The mechanism of cough associated with these different patterns of airway inflammation is unclear, but one possibility is release of pro-tussive mediators and activation of sensory nerve endings (Choudry, Fuller, & Pride 1989). Mast cell derived mediators may be particularly important since there is evidence of increased mast cell numbers in the superficial airway of idiopathic chronic cough and eosinophilic bronchitis (Gibson et al. 1998;McGarvey et al. 1999).

In this study we tested the hypothesis that chronic cough syndromes are associated with increased concentration of mast cell derived tussive mediators by measuring induced sputum histamine, prostaglandin D₂ (PGD₂) concentrations in patients with cough variant asthma or eosinophilic bronchitis, non-asthmatic explained chronic cough, idiopathic chronic cough and in healthy subjects. We also measured induced sputum concentrations of other potentially relevant

inflammatory mediators: PGE₂, cysteinyl leukotrienes (cystLTs; LTC₄, LTD₄ and LTE₄) and interleukin 8 (IL-8).

METHODS

Subjects

Twenty-two patients with idiopathic chronic cough (ICC), 20 with cough variant asthma or eosinophilic bronchitis (CVA: 12 / EB: 8), 20 with nonasthmatic explained chronic cough (NACC) and 18 normal controls were recruited from Glenfield Hospital outpatients clinics and from healthy volunteers responding to local advertising. Patients with chronic cough were randomly recruited from a population seen in the cough clinics in 2000 and 2001. The clinic receives referrals from primary and secondary care largely confined to a population of 970,000 within Leicestershire. The participants represented all eligible patients who consented to participate during the study period (June-December 2001). Investigations were carried out according to a standardised algorithm (Brightling et al. 1999a) and figure 1.1. The protocol for investigation and treatment, and criteria for accepting diagnosis were as previously described (Brightling et al. 1999a; Irwin et al. 1998) and figure 1.1. We defined idiopathic chronic cough as a cough lasting >3 weeks in association with normal clinical examination (including Ear Nose Throat), normal chest x-ray and CT scan, normal lung function tests, negative methacholine inhalation test (provocative concentration: PC₂₀ FEV₁ >8 mg/ml), normal peak expiratory flow variability, normal sputum eosinophil count and no pathological gastro-oesophageal reflux or evidence of temporal association between cough and gastro-oesophageal reflux on 24 hour oesophageal pH monitoring. Patients had extensive negative treatment trials including trials of inhaled and systemic corticosteroids and treatment for gastro-oesophageal reflux according to current guidelines (Irwin et al. 1998). Subjects with eosinophilic bronchitis had an isolated chronic cough, no symptoms suggesting variable airflow obstruction, normal spirometric values, normal peak expiratory flow (PEF) variability, a methacholine PC₂₀ > 16mg/ml, normal chest radiograph, and a sputum eosinophilia (>3% nonsquamous cell). Subjects with cough variant asthma had an isolated cough and objective evidence of variable airflow obstruction, as

indicated by one of the following: (1) methacholine airway hyperresponsiveness ($PC_{20} < 8\text{mg/ml}$); (2) $>15\%$ improvement in FEV_1 10 minutes after $200\mu\text{g}$ albuterol; or (3) PEF ($>20\%$ maximum within-day amplitude from twice daily PEF measurements over 14 days). Subjects with NACC were those in whom there was an identifiable cause for their cough (other than asthma or eosinophilic bronchitis) and improvement in cough following specific treatment of this. The protocol for investigation and treatment, and criteria for accepting diagnosis were as previously described (Brightling et al. 1999a; Irwin et al. 1998). Normal subjects were asymptomatic and had normal spirometry and methacholine PC_{20} $FEV_1 >16\text{mg/ml}$. No subjects had received corticosteroids or other specific treatment for the condition causing cough for at least six weeks prior to the study. The current study overlapped with another study (Birring et al. 2003a) and 5 normals, 12 patients with idiopathic chronic cough and 4 patients with explained chronic cough who met the criteria for both studies participated in these studies after giving separate informed consent. 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 measured spirometry and completed a questionnaire enquiring about the presence of autoimmune disease. At the second visit we measured cough visual analogue score (0-100mm worst cough) followed by exhaled nitric oxide measurement, methacholine inhalation test and sputum induction using methods that have been described in section 2.2). We obtained a differential cell count and the cell-free sputum supernatant was stored at -80°C until mediator analysis. Cell counting was performed by an experienced observer blind to the subjects clinical characteristics.

Mediator measurements

The concentration of mediators were determined within 6 months of obtaining samples in sputum supernatant by commercial competitive enzyme immunoassays for Histamine (Immunotech, Marseille, France), PGD_2 (Cayman Chemical, Ann Arbor, MI), PGE_2 (R&D systems, Oxon, UK), cystLTs (Cayman Chemical, Ann Arbor, MI) and sandwich enzyme linked immunosorbent assay for IL-8

(Pharmingen, San Diego). Because PGD₂ is a relatively unstable compound, we measured PGD₂-methoxime (PGD₂-MOX), a stable derivative of PGD₂. The standard curve was unaffected by the addition of DTT. The sensitivity of the assays were: Histamine: 0.5nM, PGD₂: 3.2pg/ml, PGE₂: 8.25pg/ml, cystLTs: 13pg/ml and IL-8: 0.8pg/ml. The intra-assay coefficient of variability of the assays was 5 to 10% and the interassay coefficient of variability was 3-15% across the range of concentrations of mediators measured.

Analysis

Subject characteristics were described using descriptive statistics and expressed as means (standard error). Methacholine PC₂₀ was calculated by linear interpolation of the log dose-response curves and described as geometric mean (logSEM). Comparisons across the four 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). Chi-squared tests were used to make comparisons between groups in the prevalence of autoimmune disease. A value of $p < 0.05$ was taken as being statistically significant.

RESULTS

The subject characteristics are as shown (Table 3.12). All subjects were non-smokers. Patients with cough were recruited at random from a population (n=236) where the primary causes of cough were cough variant asthma 39(17%), gastro-oesophageal reflux 35(15%), rhinitis 29(12%), eosinophilic bronchitis 17(7%), idiopathic 54(23%), post-viral 17(7%), bronchiectasis 14(6%), chronic bronchitis 10(4%), enlarged tonsils 8 (3%), pulmonary fibrosis 7 (3%), angiotensin converting enzyme inhibitor cough 4(2%), sarcoidosis 1 (0.5%) and bronchial tumour 1 (0.5%). The causes of cough in the non-asthmatic chronic cough group were: gastro-oesophageal reflux (4), rhinitis (5), post-viral (5), chronic bronchitis (3), angiotensin converting enzyme inhibitor cough (1), pulmonary fibrosis (1), and bronchiectasis (1). Patients with idiopathic chronic cough had a significantly higher prevalence of organ specific autoimmune disease than normals and patients

with explained cough (Table 3.12). The sputum eosinophil counts and exhaled nitric oxide concentrations were significantly higher in patients with CVA/EB than ICC, NACC and normals. There were no other differences in sputum leukocyte differential cell counts between groups (Table 3.13).

The cell-free sputum supernatant mediator concentrations were as shown in Table 3.14 and Figure 3.7. There were significant differences in sputum histamine concentrations between the 4 groups ($p=0.017$; Table 3.14, Figure 3.7). The median sputum histamine concentrations were significantly higher in patients with idiopathic chronic cough (8.0ng/ml) than normals (2.6ng/ml; 95% confidence interval of difference 0.8 to 25.8; $p=0.009$) and approached significance when compared to NACC (3.3ng/ml; 95% CI of difference -0.5 to 19.6; $p=0.07$). Median sputum histamine concentration was also significantly higher in patients with CVA/EB (10.2 ng/ml) compared to normals (95% confidence interval of difference 1.1 to 20.5; $p=0.01$). There were significant differences in PGD_2 , PGE_2 and cysteinyl leukotrienes, but not IL-8 between the four groups ($p=0.008$, $p<0.001$, and $p=0.01$ respectively; Table 3.14, Figure 3.7). Median sputum PGD_2 and PGE_2 concentrations were significantly higher in all cough groups compared to normals (Table 3.14, Figure 3.7). Median sputum Cysteinyl-Leukotriene concentration was significantly higher in patients with CVA/EB than normals (Table 3.14, Figure 3.7). Within the NACC group, there were no important differences in sputum mediator profile between different diagnostic groups (Figure 3.7).

Table 3.12 Subject characteristics

	Normal	Idiopathic cough	CVA/EB	NACC
Number	18	22	20	20
Age (years)	48 (4)	55 (2)	54 (4)	53 (4)
Male (%)	6 (33)	3 (14)	6 (30)	8 (40)
Cough VAS (mm)	0	59 (4)	56 (6)	51 (7)
Cough Duration (years)	-	5 (2)	3 (1)	2 (1)
Autoimmune disease (%)	0	9 (41)*	2 (10)	0
FEV ₁ % predicted	104 (3)	105 (3)	98 (4)	108 (4)
FEV ₁ /FVC %	82 (2)	80 (1)	78 (2)	79 (2)
Methacholine PC ₂₀ FEV ₁ (mg/ml) [#]	>16	>16	EB >16 CVA 1.2 (0.2)	>16

Data expressed as mean (SEM) except where indicated; [#]Geometric mean (log SEM); CVA: cough variant asthma; EB: Eosinophilic bronchitis; NACC: non-asthmatic chronic cough; VAS: visual analogue score; Autoimmune disease: hypothyroidism 5, hyperthyroidism 3, vitiligo 1; FEV₁: Forced expiratory volume in 1 second; FVC: Forced vital capacity; *p<0.001 (chi square test)

Table 3.13 Sputum cells counts and exhaled nitric oxide levels.

	Normal	Idiopathic cough	CVA/EB	NACC
Nitric Oxide (ppb) [#]	1.5 (0.2)	2.7 (0.1)	5.6 (0.1)*	1.7 (0.1)
Total cell count x 10 ⁶ /ml	1.2 (0.3)	1.4 (0.3)	1.3 (0.3)	2.4 (0.7)
Squamous cell contamination (%)	10 (3)	9 (2)	9 (3)	12 (4)
Viability (%)	63 (7)	58 (4)	69 (4)	67 (5)
Eosinophils (%) [#]	0.3 (0.1)	0.3 (0.1)	4.2 (0.2)**	0.3 (0.1)
Macrophages (%)	38.7 (6.0)	42.8 (3.8)	26.9 (3.7)	33.3 (6.1)
Neutrophils (%)	58.6 (5.9)	47.9 (3.7)	57.5 (4.7)	61.1 (6.9)
Lymphocytes (%)	1.1 (0.3)	1.2 (0.5)	0.9 (0.3)	1.4 (0.4)
Epithelial cells (%)	1.8 (0.4)	6.3 (1.6)	3.0 (1.2)	3.4 (0.9)

Data expressed as mean (SEM) except where indicated; [#]Geometric mean (log SEM); ppb: parts per billion; *p<0.05; **p<0.001 ANOVA

Table 3.14 Sputum mediator concentrations.

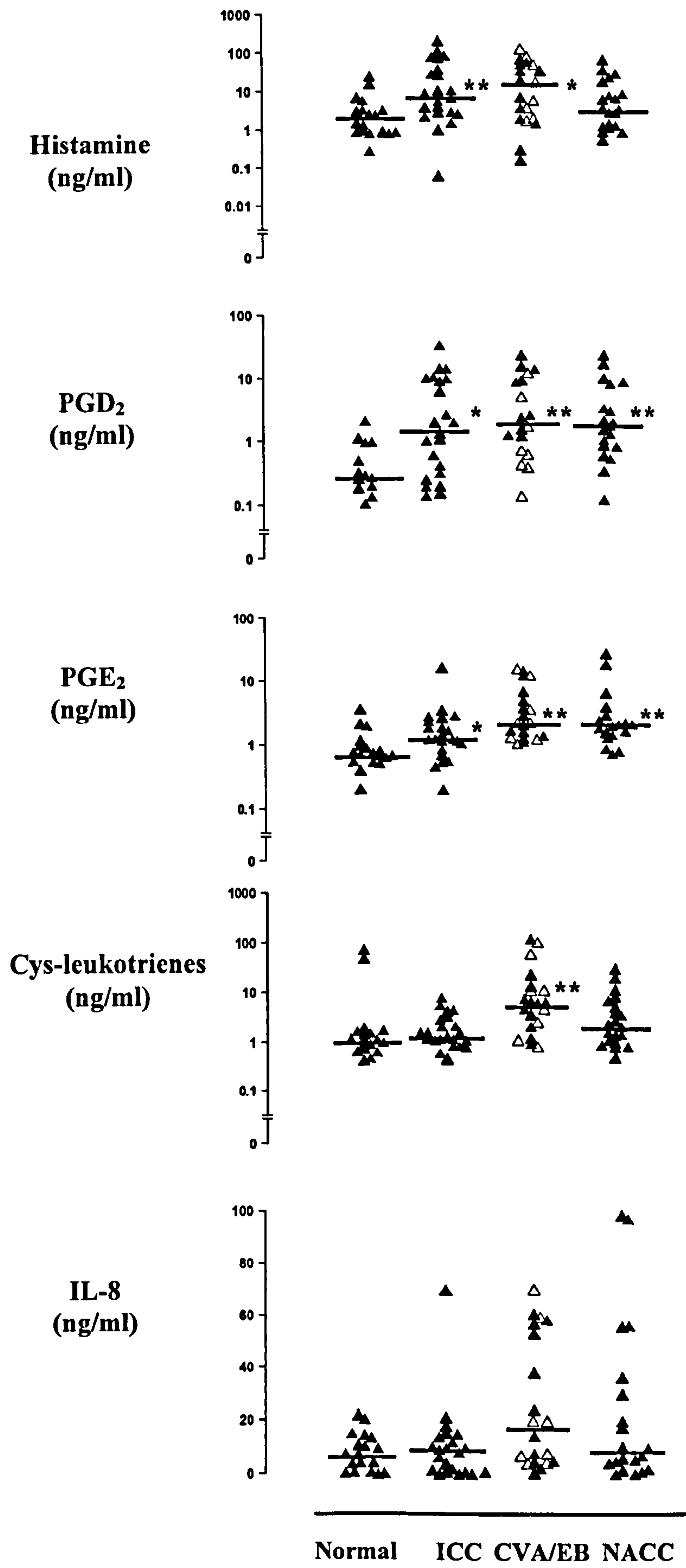
	Normal	Idiopathic cough	CVA/EB	NACC
Histamine (ng/ml)	2.6 (5.3)	8.0 (45.1)**	10.2 (44.9)*	3.3 (15.2)
PGE ₂ (ng/ml)	0.7 (0.49)	1.3 (1.4)*	2.1 (3.4)**	2.1 (2.3)**
PGD ₂ (ng/ml)	0.3 (0.8)	1.2 (10.1)*	1.7 (9.6)**	1.5 (7.7)**
Cysteinyl-Leukotrienes (ng/ml)	1.1 (1.0)	1.3 (2.5)	5.3 (11.3)**	2.2 (5.4)
Interleukin-8 (ng/ml)	4.6 (12.9)	7.5 (12.7)	18.2 (51.5)	6.9 (32.5)

Data expressed as median (interquartile range); PG: Prostaglandin; *p<0.05;
**p<0.01 vs normals (Mann Whitney test)

Figure 3.7

Median concentrations of Histamine, prostaglandin D₂ (PGD₂), prostaglandin E₂ (PGE₂), cysteinyl-Leukotrienes (cys-Leukotrienes) and interleukin-8 (IL-8) in each group of subjects (closed triangles). Subjects with eosinophilic bronchitis (EB): open triangles. ICC: idiopathic chronic cough; CVA: cough variant asthma; NACC: non-asthmatic chronic cough. *p<0.05; **p<0.01 vs normals (Mann Whitney test).

Figure 3.7



DISCUSSION

This is the first study to investigate the concentrations of pro-tussive mediators in induced sputum from patients with chronic cough. We found increased histamine concentration in idiopathic chronic cough and cough variant asthma/eosinophilic bronchitis compared to normal subjects. Sputum PGD₂ and PGE₂ concentrations were raised in all subjects with chronic cough. Our findings are consistent with the hypothesis that chronic cough is associated with abnormal release of inflammatory and potentially pro-tussive mediators within the airway.

We chose to estimate airway mediator production using induced sputum supernatant since this is a non-invasive, simple (Pavord et al. 1999b), safe (Hunter et al. 1999), and responsive (Pizzichini et al. 1997), and previous studies have shown that sputum mediator concentrations are significantly higher than in bronchoalveolar lavage (Liu et al. 1990;Sladek et al. 1994) and can be measured repeatably (Pavord et al. 1999b;Pizzichini et al. 1996). Our primary focus was to investigate the inflammatory mediator release in explained chronic cough and idiopathic chronic cough. Since cough variant asthma and eosinophilic bronchitis have characteristic and largely similar inflammatory cell profiles (Brightling et al. 2000c) we further subdivided the explained chronic cough group into cough variant asthma or eosinophilic bronchitis and non-asthmatic explained chronic cough. We have measured a wide spectrum of mediators with the potential to activate the cough reflex by a variety of mechanisms with a bias towards mast cell derived mediators, because of evidence particularly implying this cell in the genesis of chronic cough.

We chose to investigate mast cell activation using sputum supernatant histamine and PGD₂ assays since unlike alternative measures such as sputum mast cell counts and markers of mast cell degranulation, these are well validated techniques for doing this (Pavord et al. 1999b). Furthermore, we reasoned that mediator concentrations would better reflect the presence and activation of mast cells within superficial airway structures than mast cell numbers in sputum. Histamine and PGD₂ produced by mast cells are smooth muscle contractile agonists (Liu et al. 1990) and along with PGE₂, may increase cough reflex sensitivity by a direct

effect on cough sensory receptors (Choudry, Fuller, & Pride 1989). Cysteinyl-leukotrienes produced by eosinophils and mast cells cause bronchoconstriction and increase mucus production and vascular permeability (Laitinen et al. 1993). We also measured interleukin-8, a cytokine associated with neutrophilic inflammation, since an earlier study showed that sputum interleukin-8 concentration is increased in patients with non asthmatic chronic cough (Jatakanon et al. 1999).

We found increased sputum histamine concentration in idiopathic chronic cough, to a degree similar to that seen in patients with cough variant asthma and eosinophilic bronchitis. The elevation in histamine concentration in combination with PGD₂ is highly suggestive of mast cell activation since basophils, which also produce histamine, do not produce PGD₂ (Schulman et al. 1983). Thus mast cell activation with release of mediators appears to be a feature of both idiopathic chronic cough and cough due to asthma/eosinophilic bronchitis. In support of this view, increased mast cell numbers have been reported in bronchoalveolar lavage fluid from patients with idiopathic chronic cough (McGarvey et al. 1999), and in bronchial brushings from patients with eosinophilic bronchitis (Beasley et al. 1989) compared to normal subjects. An important role for histamine and other mast cell derived mediators in the pathogenesis of cough is also supported by studies showing that high dose antihistamines improve distilled water induced cough in patients with idiopathic chronic cough (Tanaka et al. 1996) and cause a marked decrease in cough due to seasonal atopic asthma (Rafferty et al. 1990). Our findings of raised sputum histamine and PGD₂ concentrations in patients with cough variant asthma contrast to previous findings in non cough predominant asthma (Brightling et al. 2000c). A plausible explanation for this discrepancy that is worth further investigation is that there are differences in the site of release of histamine, with localisation of mast cells within the airway smooth muscle important in the genesis of bronchoconstriction and airway hyperresponsiveness (Brightling et al. 2002a), and localisation to sensory nerve endings important in cough (Brightling et al. 2000c). Thus there may be parallels between mechanism of cough and the mechanism of itch, where a role for mast cells localising to

sensory nerve endings in the skin is well established (Yosipovitch, Greaves, & Schmelz 2003).

Sputum PGE₂ concentrations were elevated in all patients with cough. Whilst we cannot exclude the possibility that this is due to the mechanical effects of coughing per se since it was seen in all cough groups, it is possible that this mediator release is related to the enhanced cough reflex sensitivity seen in these subjects since inhaled PGE₂ results in heightened cough reflex sensitivity to capsaicin (Choudry, Fuller, & Pride 1989). Our findings do not confirm previous reports of increased sputum neutrophil cell numbers or sputum IL-8 concentrations in idiopathic chronic cough (Jatakanon et al. 1999). This difference seen in the earlier study may have been due to a young control population since recent data suggests that sputum neutrophil cell count increases with age (Thomas RA et al. 2001). We did find increased exhaled nitric oxide levels in patients with cough variant asthma/eosinophilic bronchitis, findings that are in keeping with Chatkin et al (Chatkin et al. 1999) who showed that exhaled nitric oxide levels were significantly higher in patients with chronic cough due to asthma than those due to causes other than asthma. Recent bronchoscopy studies have shown evidence of lymphocytic airway and perhaps alveolar inflammation in patients with idiopathic chronic cough (Birring et al. 2003a; Lee et al. 2001). The absence of an increase in lymphocyte count in induced sputum cannot necessarily be taken as evidence against these earlier findings since the proportion of lymphocytes in these samples is low so they may not reflect lymphocytic airway inflammation well (Pavord et al. 1997).

The NACC group contained patients with various conditions which may be associated with different patterns and degrees of airway inflammation. There were no differences in mediator profile suggesting perhaps that there is a common final pathway in the genesis of chronic cough in these diverse conditions although our power to identify differences in mediators was low. Further studies are required to address this question in more detail.

A significant proportion (23%) of the patients in the population with chronic cough referred to our clinic had idiopathic chronic cough. Our findings provide some insight into the mechanisms of cough and airway inflammation. In common with earlier reports and case control studies (Birring S.S. et al. 2002) (and section 3.1.1) patients with idiopathic chronic cough were predominantly female and there was a high incidence of organ specific autoimmune disease. The association with organ specific autoimmune disease and bronchoalveolar lymphocytosis in patients with idiopathic chronic cough has lead us to speculate that cough is due to homing of activated lymphocytes into the pulmonary compartment from primary sites of autoimmune inflammation (Birring et al. 2003a). The current study supports the view that idiopathic chronic cough is associated with airway inflammation and organ-specific autoimmune disease. It provides new evidence that the final mechanism of cough might have similarities to cough in better defined conditions such as asthma and eosinophilic bronchitis, with involvement of mast cell derived mediators. Further work is required to determine whether other pro-tussive mediators are involved and to determine the mechanism of the associated cough.

3.3 Unexplained airflow obstruction - a possible link with idiopathic chronic cough?

3.3.1 Clinical, radiological and induced sputum features of chronic obstructive pulmonary disease in non-smokers. A descriptive study.

ABSTRACT

Epidemiological studies show that 5-12% of subjects with Chronic Obstructive Pulmonary Disease (COPD) are non-smokers. Little is known about the pathophysiology of the fixed airflow obstruction in these subjects. We have prospectively identified 25 patients with COPD who never smoked or had a <5 pack years smoking history and present the clinical, radiological and induced sputum features. Our population represented 5.7% of total referrals with fixed airflow obstruction over 2 years. Patients had a mean age of 70 years, were predominantly female (86%), with a mean duration of respiratory symptoms of 7 years. The mean Forced Expiratory Volume in one second (FEV₁)% predicted was 58%, and FEV₁ /Forced Vital Capacity 55%. Features on High Resolution Computed Tomographic scanning were non-specific and considered typical of a wider population with COPD. Induced sputum differential inflammatory cell count suggested the presence of 2 distinct groups. 9 had significant sputum eosinophilia (mean 8.1%: normal <1.9%) and the remaining 13 had a normal sputum eosinophil and tended to have a raised sputum neutrophil count (mean 70.1%: normal <65%). Organ specific autoimmune disease was present in 7 of the 22 patients (32%) and was particularly prevalent in those without sputum eosinophilia (6 of 13). In conclusion, COPD in non-smokers predominantly affect females and has at least 2 pathological subgroups, one of which may be associated with organ specific autoimmune disease. Further investigation of this group may disclose novel mechanisms of fixed airflow obstruction.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is largely attributable to smoking – the major known environmental risk factor. However, only 15% of smokers develop significant airflow obstruction and COPD (Barnes 2000), suggesting other factors are involved. Epidemiological studies show that 5-12% of patients with a diagnosis of COPD have never smoked and there is evidence of increasing incidence with increasing age (Coultas et al. 2001). These subjects are predominantly female, and there is an association with lower income (Whittemore, Perlin, & DiCiccio 1995) otherwise little else is known about the clinical, radiological, physiological and pathological features of COPD in this group. A clearer understanding of the mechanism of airflow obstruction might identify novel pathogenic mechanisms relevant to a wider population of patients. We set out to characterise the clinical, radiological, physiological and induced sputum features of COPD in non-smokers attending our clinic over a two-year period in a prospective descriptive study.

METHODS

Subjects

We recruited patients who had symptoms of chronic airflow obstruction and who fulfilled lung function criteria as set out by the NHLI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines (Pauwels et al. 2001), from our respiratory outpatient clinics. All patients had post-bronchodilator $FEV_1/FVC < 70\%$ and no substantial improvement in FEV_1 after taking 2.5mg nebulised salbutamol ($< 15\%$ or, if $FEV_1 < 1.2$ L, < 200 mL improvement) and following a corticosteroid trial (2-week course of 30mg prednisolone daily, or 2 months high dose inhaled corticosteroids, with reassessment of symptoms and spirometry). We excluded patients if they had a clinical diagnosis of asthma, variability of symptoms not associated with infections, or a history of acute wheeze, breathlessness, or deterioration associated with allergens. Other exclusion criteria were a history of childhood respiratory disorders (Johnston, Strachan, & Anderson 1998), significant bronchiectasis (Nogrady, Evans, & Davies 1978), inflammatory bowel disease (Camus et al. 1993), rheumatoid arthritis (Vergnenegre et al. 1997) and chest wall deformity (Phillips et al. 1987) because

of their association with fixed airflow obstruction. All subjects were life-long non-smokers or had a smoking history of less than five pack years. The smoking status was validated by exhaled carbon monoxide monitoring or urine cotinine levels and by review of hospital and General Practitioner records. The study was approved by the Leicestershire Research Ethics Committee.

Data collection

Clinical data was collected by an operator led standardised questionnaire designed to obtain a symptom history, detailed occupation history, and details of current (last 12 months) and past passive smoking history at home (at least one household smoker), at work and during childhood (0-16 years old) was ascertained together with a four generation family history. All patients were asked to have a venous blood sample to measure peripheral blood eosinophil and lymphocyte count, Immunoglobulin levels (IgG,A,M), total IgE and radioallergosorbent tests (timothy grass, cat epithelium, dog dander, *Dermatophagoides pteronissinus*), serum angiotensin converting enzyme (ACE) level, α_1 -antitrypsin level, and an autoantibody screen (section 2.1.1). All patients had a chest radiograph and high resolution computed tomographic scanning (HRCT) with both inspiration and expiration phases. An estimate of traffic derived particle exposure was obtained from the Leicester City Council as the Annual Average Daily Traffic flows (AADT – vehicles per day on nearest main road). A road effect is considered to be present if the residence was less than a 100 metres from the main road (>10,000 vehicles per day), otherwise emissions were considered to be background (Hoek et al. 2001; Venn et al. 2001).

Pulmonary function tests

Spirometry was done with a Vitalograph spirometer before and 15 minutes after nebulised 2.5mg salbutamol (section 2.2.1). Lung-function tests were done with a benchmark (P K Morgan, Chatham, UK) and lung volumes assessed by the helium dilution method.

Sputum was induced and processed in all patients as described in section 2.2.4. We have shown this technique is safe and effective in subjects with moderate to

severe COPD (Brightling et al. 2001)(14). Sputum induction was done at least 2 months after an exacerbation and the last use of corticosteroids.

Data analysis

All results are expressed as mean (standard error) or geometric mean (log standard error). Pulmonary function test results are expressed as a percentage of predicted values (Quanjer et al. 1993).

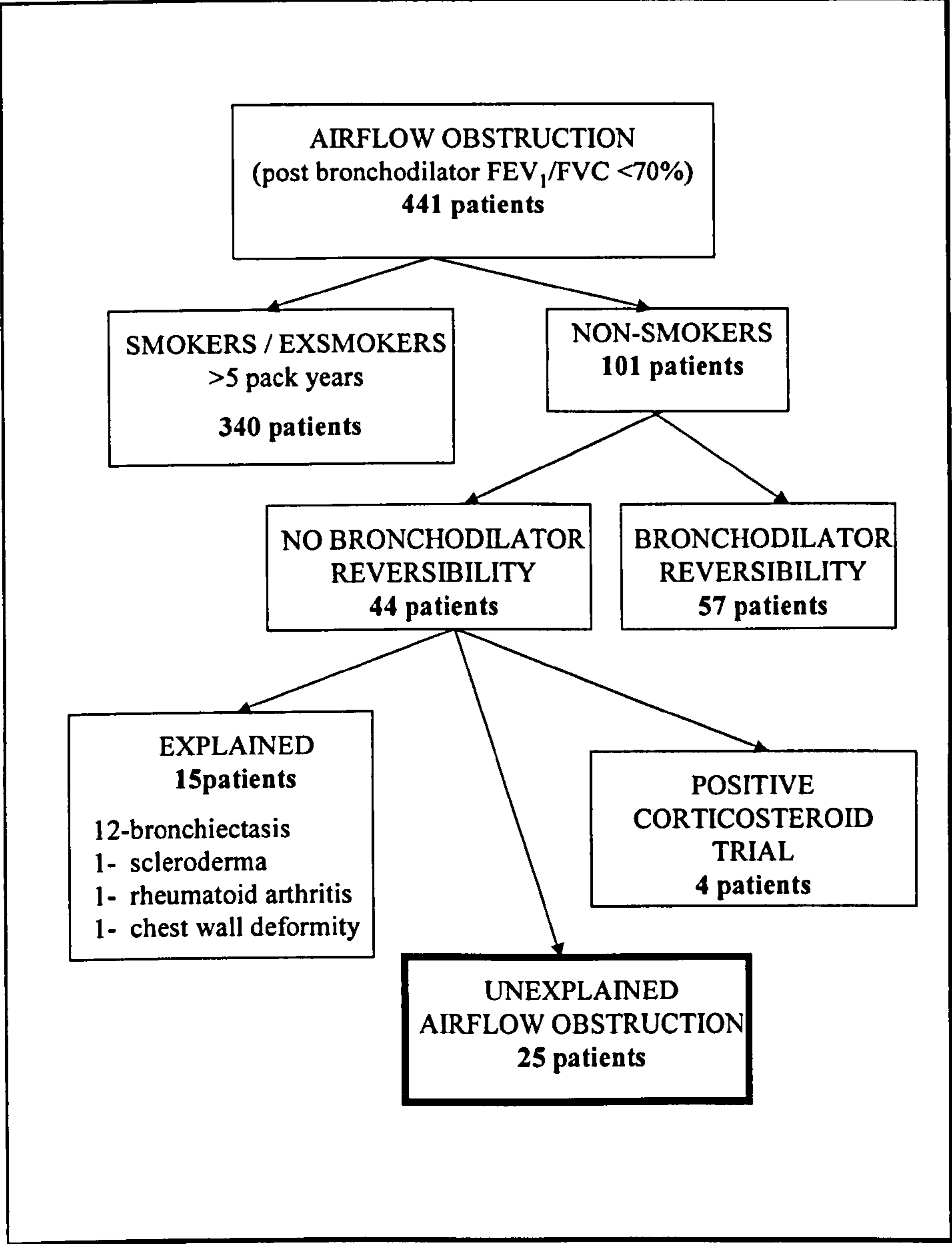
RESULTS

We identified twenty-five non-smoking patients with COPD, which represented 5.7% of total referrals with COPD over 2 years (Figure 3.8). Sixteen were life-long non-smokers. One patient had significant bronchiectasis on HRCT scan despite unremarkable physical examination and chest radiograph. Investigation of a further patient revealed a carcinoid tumour obstructing the right upper lobe and a third patient was found to have severe alpha-1 antitrypsin (ZZ) deficiency. Characteristics of the remaining patients are given in Tables 3.15 and 3.16. Patients had a mean(SE) age of 70(2) years, were predominantly female (n=20, 83%), with a mean(SE) duration of symptoms 7.3(1.5) years. No patients were current passive smokers (last 12 months). The mean(SE) Body Mass Index for subjects was 24(2) kg/m². All had normal serum ACE and immunoglobulin levels. Features on HRCT scan were non-specific and considered typical of a wider population with COPD (Table 3.17: emphysema n=2, bronchial wall thickening n=4, air trapping n=4, bronchial dilation n=5, bulla n=1, mosaic oligoemia=0, normal n=8).

Patient's lung function results are presented in Table 3.17 and Figure 3.9. Patients had a mean(SE) FEV₁ of 1.2(0.1)L, and mean(SE) % predicted of: FEV₁ 58(5)%, FEV₁/FVC 55(2)%, Residual Volume (RV) 117(7)%, Total Lung Capacity (TLC) 93(3), RV/TLC 53(2)%, Diffusing Capacity for Carbon Monoxide (DLco) 81(5)% and Carbon Monoxide Diffusion Coefficient (Kco) 107(6)%. Induced sputum inflammatory cell count suggested the presence of two subgroups (table 3.17). Nine patients (patients 1-9) had a significant sputum eosinophilia (geometric mean differential cell count (logSE): 8.1(0.1)%, normal

<1.9%) and the remaining 13 (patients 10-24) had a normal sputum eosinophil and raised mean(SE) sputum neutrophil differential cell count 70.1(6.3)% (normal <65%). The mean(SE) sputum neutrophil differential cell count for the eosinophilic group was 64.5(5.8)%. There was no significant difference in sputum neutrophil counts between the two groups. Six (27%) of the patients (2 in the sputum eosinophilia group) had positive allergen-specific IgE to one or more allergen. Organ specific autoimmune disease was present in 7 of the 22 patients (32%) and was particularly prevalent in those without sputum eosinophilia (6 of 13: Table 3.16). Similarly, there was a high prevalence of autoantibodies in the non-eosinophilic group (n=4: 31%; 4 thyroid). There was one patient with diabetes in the eosinophilic group but none had positive autoantibodies. No patients had positive anti-nuclear antibody or rheumatoid factor. Six (46%) non-eosinophilic patients had a low peripheral blood lymphocyte count (0.7-1.3; our laboratory normal range $1.5-4.0 \times 10^9$ cells/L) compared to 2(22%) eosinophilic patients. There were no significant differences between the 2 subgroups with reference to age, symptom scores and lung function (Tables 3.15-7, Figure 3.9).

Figure 3.8 Patients with airflow obstruction seen in a respiratory out-patient clinic over 2 years.



FEV₁/FVC: forced expiratory volume in 1 second / forced vital capacity; Reversibility to bronchodilator (2.5mg nebulised albuterol) or corticosteroid (2-week course of 30mg prednisolone daily, or 2 months high dose inhaled corticosteroids): >15% improvement in FEV₁ or, if FEV₁ <1.2 L, >200mL improvement; no non-smoking patients with fixed AFO declined to take part in the study.

Table 3.15 Patient characteristics I.

Patient Number	Age (years)	Sex	Symptom VAS	Sputum	Occupation	Family History	Passive Smoking	Past smoking (py)
1	64	F	96	0	Secretary	0	0 (+H20y)	0
2	64	M	40	+	Sales rep	0	0 (+C)	0
3	82	F	74	+	Nurse	0	0	5
4	69	F	-	+	Housewife	0	0	0
5	67	F	76	0	Waitress	0	0 (++CW20y)	0
6	80	F	95	+	Office worker	0	0 (+H20y)	0
7	67	F	67	0	Accountant	0	0 (+H20y)	0
8	77	F	57	+	Care assistant	son/aunt-asthma	0 (+W20y)	5
9	73	F	50	0	Housewife	0	0 (+H20y)	0
10	77	F	56	0	Hosiery worker	0	0 (+H10y)	5
11	67	F	70	+	Office worker	0	0	0
12	70	M	72	+	Iron foundry	0	0 (+H20y)	5
13	68	F	65	+	Retail manager	0	0 (+H20y)	0
14	54	F	50	+	Cleaner	F(s)-bronchitis	0	0
15	68	F	72	0	Housewife	0	0 (+H10y)	0
16	55	M	42	0	Builder	0	0	0
17	78	F	87	0	Teacher	0	0 (+H30y)	0
18	77	F	59	0	Hosiery worker	B(s)-bronchitis	0	0
19	55	F	20	0	Secretary	son-asthma	0 (+H30y)	5
20	75	F	17	+	Cook	GF(s)-bronchitis	0	0
21	77	F	52	0	Hosiery worker	0	0	3
22	82	F	70	0	Shoe machinist	0	0 (++H20y)	0

F: female; M: male; VAS: visual analogue score for most severe symptom out of breathlessness, cough and wheeze (0=no symptoms, 100=worst ever symptom); current passive smoking (past passive smoking; + <4 hours per day, ++ >4 hours per day, C=childhood, W=work, H=household, y=years of exposure); F Father; GF Grandfather; B Brother; (s) smoker; py: pack years; Sputum: + <5ml day and/or occasional, ++ >5ml per day; 0:none

Table 3.16 Patient characteristics II.

Patient Number	Autoimmune Disease	Alpha-1	AADT
		antitrypsin g/L	
1	0	1.4 MM	na
2	0	1.4 MM	3,020
3	0	nd	17,235
4	0	nd	6,555
5	0	2.0 MM	11,065
6	0	1.7MM	14,915
7	Diabetes	1.3 MM	na
8	0	1.6MM	na
9	0	1.8 MM	6,820
10	Hyperthyroid	1.7 MM	6,495
11	Hypothyroid (tpo 1:100)	1.5 MM	3,500
12	Hypothyroid	1.4 MM	10,995
13	0	1.6 MM	na
14	0	1.4 MM	6835+
15	Hypothyroid	1.4 MM	9,485
16	0 (tpo 1:163)	1.1 MM	na
17	0	1.3 SS	5,490
18	Hyperthyroid (tpo 1:1275)	1.8 MM	na
19	0	2.0 MM	4,985
20	0	1.3 MS	11,645
21	0 (tpo 1:320)	1.6 MM	1,915
22	Coeliac disease	1.1 MZ	685

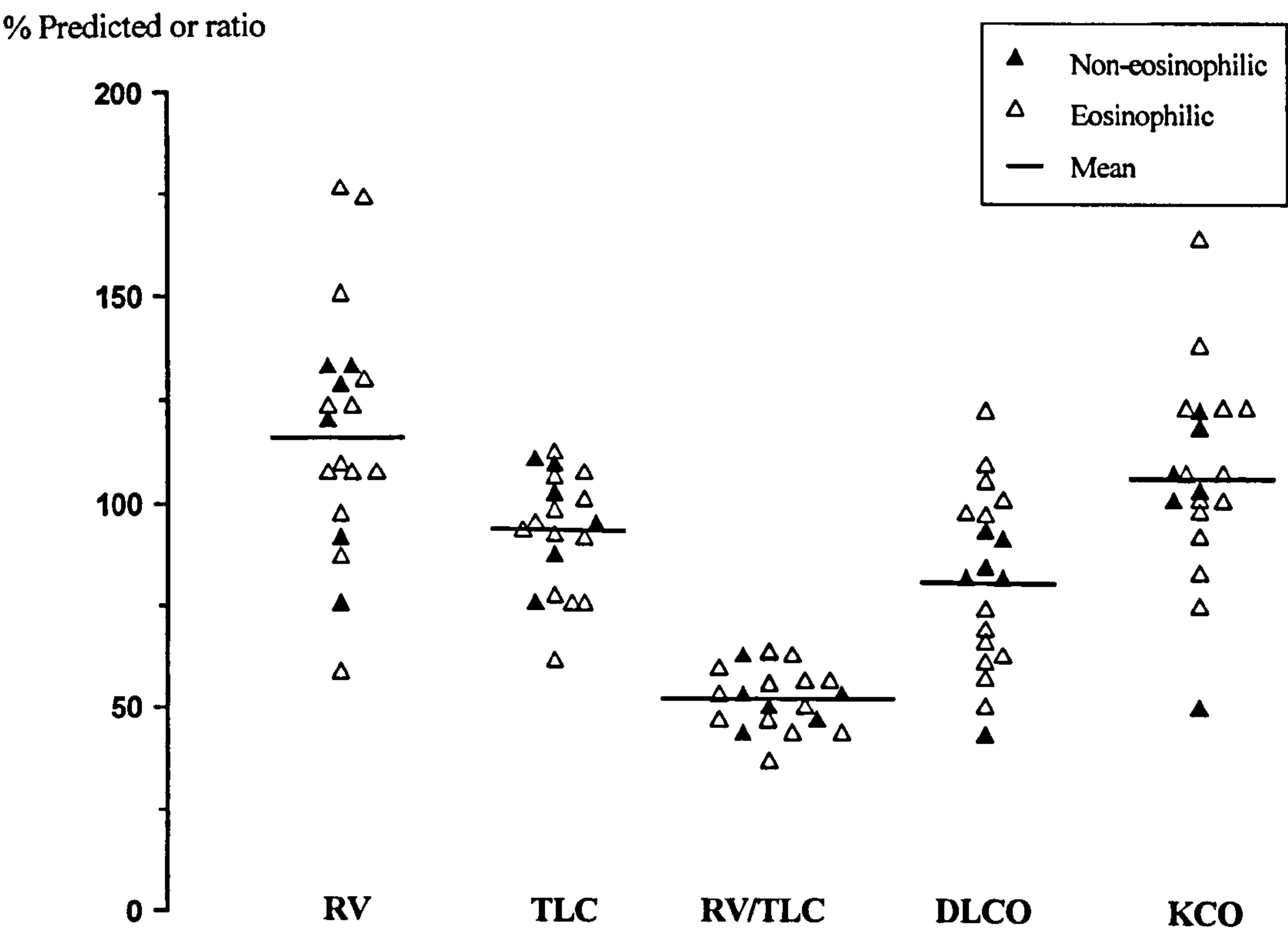
Diabetes: juvenile onset-insulin dependent; tpo: thyroid peroxidase antibody titre where positive (strong positive 100-2000 IU/ml); alpha-1 antitrypsin normal range 1.1-2.4 g/L (Pi phenotype); AADT: average annual daily traffic flows (vehicles per day on nearest main road; range: <10000 Low, 10000-30000 Medium, >30000 High); +road effect; nd: not done; na: not available

Table 3.17 Lung function, induced sputum differential cell counts, and high resolution computed tomography scanning (HRCT) findings.

Patient Number	FEV ₁ pre-albuterol (L)	FEV ₁ post-albuterol (L)	FEV ₁ /FVC %	Sputum Eosinophil count (%)	Sputum Neutrophil count (%)	HRCT findings
1	1.79	1.88	63	14.3	67.5	nd
2	3.55	3.60	62	8.5	71.8	normal
3	0.84	0.97	53	11.5	61.5	nd
4	0.71	0.75	50	27.5	47.0	normal
5	0.77	0.84	35	3.8	86.5	emphysema
6	1.41	1.53	69	5.5	89.5	BWT, BD
7	1.30	1.46	58	4.6	63.0	normal
8	1.06	1.25	61	11.0	59.0	AT
9	1.25	1.34	58	3.8	35.0	nd
10	1.35	1.35	69	0	88.5	normal
11	1.23	1.41	55	1.1	26.6	normal
12	0.94	1.02	49	0.3	75.5	AT, minor BD & PS
13	0.96	1.09	47	1.8	80.5	BD, minor PS
14	0.51	0.61	29	0.2	98.2	minor PS & BD
15	1.45	1.60	69	0.3	41.0	AT, BWT
16	0.92	1.11	35	1.2	77.8	emphysema, BWT
17	0.75	0.83	56	0	64.0	normal
18	1.78	1.82	65	0.3	87.8	normal
19	2.00	1.92	59	1.0	37.5	small bulla
20	0.90	0.95	43	0.5	74.2	AT, minor atelectasis
21	0.84	1.02	54	1.3	64.3	normal
22	0.75	0.85	61	0.7	95.0	BWT, BD

Subjects 1-9 are eosinophilic group (normal eosinophil count < 1.9%, normal neutrophil count <65%), FEV₁: Forced expiratory volume in 1 second, FVC: Forced vital capacity, AT: air trapping, BWT: bronchial wall thickening, BD: minor bronchial dilatation (<1.5 times the internal diameter of adjacent artery without bronchial wall thickening); PS: focal parenchymal scarring, nd: not done

Figure 3.9 Detailed lung function.



Data shown is % of predicted values (15). RV:residual value, TLC:total lung capacity, KCO: diffusion coefficient.

DISCUSSION

This is the first study to address the clinical, radiological and induced sputum features of non-smoking patients with COPD. We chose to study subjects who had never smoked, or whose smoking history was trivial and very unlikely to be important in the development of respiratory disease. Smoking histories were validated by review of medical records and objective measurements. We found that 5.7% of the COPD population met our inclusion criteria. These patients were predominantly female, consistent with epidemiological evidence (Coultas et al. 2001;Whittemore, Perlin, & DiCiccio 1995). Induced sputum analysis suggested the presence of at least two subgroups, one of which was associated with a high prevalence of organ specific autoimmune disease.

Our demonstration of heterogeneity of induced sputum features in non-smokers with COPD is consistent with evidence in smoking related COPD, where both eosinophilic and neutrophilic patterns have been demonstrated (Balzano et al. 1999;Brightling et al. 2000a). Although the long term stability of the sputum phenotype in these subjects is unclear, repeatability studies in COPD suggest that these measures are stable over two weeks (Brightling et al. 2001). The most obvious explanation for the fixed airflow obstruction in the eosinophilic inflammation subgroup is that it is the end result of airway remodelling secondary to long standing asthma. However our patients had a relatively short history of symptoms and none gave a history suggesting asthma. In addition, there was no evidence of reversibility following nebulised bronchodilators or a two week course of oral prednisolone at the time of assessment. It is also possible that eosinophilic COPD may complicate eosinophilic bronchitis, a common cause of isolated chronic cough in middle age (Brightling et al. 1999a). We have recently reported a case of eosinophilic bronchitis in a non-smoker where COPD developed over two to three years in association with poorly controlled eosinophilic airway inflammation (Brightling et al. 1999b). Corticosteroid responsive eosinophilic bronchiolitis has also been described in a patient with fixed airflow obstruction with HRCT findings resembling diffuse panbronchiolitis but none of our patients had radiological features supporting this condition

(Takayanagi et al. 2001). Further study of the natural history of eosinophilic bronchitis is warranted as it is a potentially preventable cause of COPD.

The other larger subgroup had no sputum evidence of eosinophilic inflammation and tended to have a neutrophilia. This group had a high prevalence of organ specific autoimmune disease and autoantibodies, in particular thyroid disease. The prevalence was much higher than the sex and age adjusted prevalence of autoimmune disease in the general population (6-8%) (Rose & McKay 1998) and the prevalence of 12% in the healthy control subjects in a recent case-control study (section 3.1.1). The significance of this finding is unclear and it needs to be verified in a case-control study. However a causal association between organ specific autoimmune disease and airflow obstruction is plausible. The lungs and many organs involved in autoimmune disorders share common embryological origins as foregut derivatives and it is possible that homing of activated inflammatory cells into the pulmonary compartment as well as the primary site of autoimmune inflammation may cause airway wall inflammation and destruction leading to airflow obstruction. An alternative and intriguing mechanism is that the airflow obstruction might be consequence of a hitherto unrecognised autoimmune bronchitis and that the association with other diseases simply reflects the well recognised association between different organ specific autoimmune diseases. There is some recent evidence that thyroid disease, the most common organ specific autoimmune disorder, may be associated with excess respiratory disease independently of thyroid hormonal status since the odds ratio of death from respiratory disease was 2.8 in a recent large case series of subjects with normal thyroxine level, but suppressed thyrotropin levels (Parle et al. 2001). Moreover in a recent case control study, we have reported a marked excess of cases of organ specific autoimmunity in a population of middle aged patients with unexplained chronic cough (section 3.1.1). Further studies should investigate the possibility of a link between unexplained chronic cough, COPD and organ specific autoimmunity.

Our view that some cases of COPD and chronic cough are due to immune dysregulation is supported by the association of cough and fixed airflow

obstruction with other disorders with an immunological basis such as inflammatory bowel disease (Camus et al. 1993), Sjogren's disease (Newball & Brahim 1977), rheumatoid arthritis (Vergnenegre et al. 1997), graft versus host disease (Yousem 1993) and more directly by the raised number of mononuclear cells seen in bronchial biopsies of non-smoking patients with chronic cough (Boulet et al. 1994). The significance of lymphopenia noted in just under half of our patients is unclear but it could reflect pulmonary sequestration of activated lymphocytes, similar to that seen in sarcoidosis (Wilsher, Hallows, & Birchall 1995). We doubt that these patients had sarcoidosis since there were no features of this on HRCT scanning and serum ACE levels were normal.

It is also possible that other causes of chronic foregut inflammation might be relevant to airway diseases. Kanazawa et al (Kanazawa, Hirata, & Yoshikawa 2003) have reported an accelerated decline of lung function in COPD patients with concomitant hepatitis C infection that was reduced with interferon-alpha therapy. They suggest that the airway disease may be related to the underlying chronic inflammatory disorder. One important difference between the primary sites of inflammation in chronic hepatitis C infection and autoimmune thyroid disease is that the former is treatable. Kanazawa's findings with interferon therapy (Kanazawa, Hirata, & Yoshikawa 2003) raise the interesting possibility that treatment may modify the airway consequences of chronic inflammation of the foregut. The search is on for other treatable causes of chronic foregut inflammation that might be relevant to airway diseases. COPD is associated with peptic ulcer disease (Monson 1970) so one possibility worth investigating is that chronic gastric inflammation secondary to helicobacter pylori infection is a potentially modifiable factor underlying the amplified immune response to cigarette smoking and other pollutants that characterises COPD.

One limitation of our study is the absence of pathological material in the form of open or thoracoscopic lung biopsy. We found it difficult to justify invasive investigation with a low potential to alter management in an elderly population with slowly progressive disease. Previous studies where biopsy has been performed in patients with fixed airflow obstruction of obscure aetiology has

identified discrete entities that may be relevant to our non-smoking population. Kraft et al., (Kraft et al. 1993) found pathological evidence of constrictive bronchiolitis in 4 non-smoking patients (2 with airflow obstruction) and Turton et al (Turton, Williams, & Green 1981) have suggested similar pathophysiology in a subgroup of 10 non-smoking patients with fixed airflow obstruction on the basis of a strong association with rheumatoid arthritis. None of our patients had clinical features suggesting rheumatoid arthritis or positive rheumatoid factor and, whilst we cannot exclude the possibility that constrictive bronchiolitis antedated onset of joint symptoms, such a sequence of events is unusual and we doubt this is an important explanation (Wright et al. 1992).

It is possible that our patients had idiopathic constrictive bronchiolitis. Transplant mediated constrictive bronchiolitis has been associated with a sputum neutrophilia (Beeh et al. 2001) and the physiological changes seen in our patients such as raised residual volume and preserved KCO are similar to those seen in the patients reported by Turton et al. Although characteristic radiological features of constrictive bronchiolitis have been defined, the sensitivity and specificity of these findings in mild disease is unclear (Hansell 2001). Diffuse panbronchiolitis seems unlikely in our population since patients were predominantly Caucasian, did not report prominent sinusitis, and had no radiological evidence of fine nodularity or 'tree in bud' shadowing on HRCT (Homma et al. 1983). Aguayo et al., (Aguayo et al. 1992) have described a group of patients with fixed airflow obstruction, pathological evidence of neuroendocrine cell hyperplasia and presence of pulmonary carcinoid tumour. This condition is unlikely in our patients because of the absence of reticulo-nodular infiltrates on imaging. We were particularly interested in whether this condition was present in our patient with fixed airflow obstruction and carcinoid tumour, although further pathological examination of the resected lobe did not reveal the presence of neuroendocrine cell hyperplasia. Finally, the MZ genotype of the alpha1-antitrypsin gene has been associated with a more rapid decline in FEV₁ in smokers (Sandford et al. 2001). Alpha-1 antitrypsin testing identified one patient with the MZ genotype and one with severe disease (ZZ genotype) suggesting that testing should be considered a

routine investigation in patients with non-smoking fixed airflow obstruction as well as in smokers with COPD.

The use of a structured questionnaire provided us with an opportunity to explore the relationship between disease and other risk factors for COPD. A positive family history occurred with an incidence that was similar to that expected in smoking COPD patients (Tager et al. 1978). Similarly, although some patients had a possibly relevant occupational history, this was not common. The proportion of our patients exposed to passive smoking (work 4%, parental 8%, last 12 months at home 0%) was less than that found in a recent United Kingdom/European general population survey (work 11%, parental 68%, home 32%) (Janson et al. 2001b). Most patients had a low to medium particle exposure as reflected by AADT. Although this is not life long exposure, there is little variation in these figures over time (Hoek et al. 2001; Venn et al. 2001) and it provides a reasonably robust estimate. Thus no striking potential causal factors have been identified although this is a small study with no control group so our ability to identify such relationships is limited.

HRCT scan changes included air trapping, bronchial wall thickening and dilatation, emphysema and bullae. These features are not dissimilar to what would be expected in a wider population of subjects with smoking related COPD (O'Brien et al. 2000). However we identified one subject with significant bronchiectasis and another with an obstructing carcinoid tumour, neither of which were expected after detailed clinical review and plain chest X-ray. HRCT therefore had a reasonable pick-up of abnormalities that might potentially alter management and should be considered in the investigation of subjects with unexplained airflow obstruction.

In summary, we have shown that COPD in non-smokers predominantly affects elderly females and has at least two subgroups, one of which may be associated with organ specific autoimmune disease. This is a preliminary observation of a poorly understood group and suggests some potentially novel pathogenic mechanisms. Further studies are required to investigate the relationship between

organ-specific autoimmunity and airflow obstruction in more detail, characterise the nature of lower airway inflammation and investigate the mechanisms involved.

3.4 Health status in patients with chronic cough

3.4.1 Development of a symptom specific health status measure for patients with chronic cough: Leicester Cough Questionnaire (LCQ)

ABSTRACT

Chronic cough is a common condition that has a significant impact on quality of life. Assessment and management are hampered by the absence of well-validated outcome measures. We present the development and validation of the Leicester Cough Questionnaire (LCQ), a self-completed health related quality of life measure of chronic cough. Patients with chronic cough were recruited from outpatient clinics. The development of the LCQ consisted of three phases: Phase 1: item generation; Phase 2: item reduction, allocation of items to domains and validation of questionnaire; Phase 3: repeatability and responsiveness testing of final version of questionnaire.

Phase 1: Literature review, multidisciplinary team meeting and 15 structured interviews with chronic cough patients generated 44 items (LCQ1) with a 7-point Likert response scale. Phase2: 104 chronic cough outpatients completed the LCQ1 along with an importance rating for each item. The clinical impact factor method was used for item reduction to 19 items (LCQ2: final version). These items were divided into 3 domains (physical, psychological and social) following expert opinion. Internal reliability, as assessed using Cronbach's alpha coefficients, varied between 0.79-0.89. Concurrent validity was high when the LCQ2 (n=56) was compared to a cough visual analogue score ($r=-0.72$). There was a moderate relationship with response to the St George's Respiratory Questionnaire ($r=-0.54$) and SF36 total score ($r=0.46$). Phase3: Two week repeatability (n=24) was high, with intraclass correlation coefficients for domains varying between 0.88 and 0.96. Responsiveness in 9 patients who had a successful treatment of their cough varied within domains from an effect size of 0.84 to 1.75

In conclusion, the LCQ is a 19 item self completed quality of life in chronic cough measure divided into 3 domains (physical, psychological and social) with a 7-point Likert response scale. The LCQ is valid, repeatable and responsive to change. It should be a useful tool in clinical trials and longitudinal studies.

INTRODUCTION

Chronic cough is one of the commonest causes of presentation to general practice. At any one time 20% of the UK population have a troublesome cough and sufferers consume 75 million doses of over the counter anti-tussive medication annually (Fuller & Jackson 1990). Most cases are acute and self-limiting although a significant minority are referred for a specialist opinion with an isolated persistent chronic cough (Irwin & Madison 2000). These patients suffer considerable physical and psychological morbidity (French et al. 1998).

The assessment of health status is increasingly important in respiratory disease and has been extensively studied in asthma and chronic obstructive pulmonary disease by development of disease specific questionnaires (Jones et al. 1992;Juniper et al. 1993). Very little is known about the effects of chronic cough on health status because of the lack of such validated questionnaires. Indeed, there is a striking paucity of objective and well validated outcome measures in chronic cough. Our aims were to develop a health related quality of life questionnaire specifically for chronic cough that is brief, simple to administer and score, suitable to monitor individual patients, assess different aspects of health affected in patients, be sensitive enough to detect changes in health status within an adult chronic cough population and be an outcome measure in clinical trials of new antitussive agents. This study describes the development and validation of the Leicester Cough Questionnaire (LCQ), a self-completed health related quality of life measure of chronic cough.

METHODS

Patients with chronic cough were recruited from an adult respiratory outpatient clinic. Chronic cough was defined as a cough lasting greater than three weeks that remained unexplained after assessment by the primary care physician. Patients were investigated and treated using a diagnostic protocol described previously (Brightling et al. 1999a) and Figure 1.1. All patients with an isolated chronic cough were identified prior to the outpatient session, during which they were approached by another investigator who had not read the case notes. Patients were asked for their consent to participate in the development of the questionnaire and

the protocol was approved by the Leicestershire Ethics Committee. The LCQ was developed using a multi-step method (Fayers & Machin D 2000;Guyatt, Bombardier, & Tugwell 1986) divided into three phases; Phase 1: item generation; Phase 2: item reduction, allocation of items to domains and validation of the questionnaire; Phase 3: repeatability and responsiveness testing of final version of the questionnaire. The item reduction was based on questionnaire responses from 104 patients and concurrent validity was assessed in a separate 56 patients, 27 of whom had a cough sensitivity measurement.

Phase 1: item generation

Phase 1 consisted of item generation for a preliminary questionnaire LCQ1 (appendix III) by the following processes: (1) critical review of health related quality of life (HRQOL) literature, (2) review of existing generic and respiratory specific HRQOL questionnaires, (3) multidisciplinary team meeting to generate items for the questionnaire which included respiratory and non-specialist doctors, nurse, asthma nurse specialist, physiotherapist, pharmacist and a rehabilitation expert involved in delivering care to patients with chronic cough. A layperson was also present at the meeting, (4) Semi-structured interviews with 15 patients with chronic cough to outline areas of concern to them.

Phase2: item reduction, allocation of items to domains and validation of the questionnaire

Item Reduction

During Phase 2, 104 patients with chronic cough were asked to answer each item of the LCQ1 and in addition, rate the importance of each item to them on a 5-point scale (1=not important, 5=extremely important). Data obtained from the LCQ1 was used to reduce items by the clinical impact factor method (Juniper et al. 1997), which selects items that are most frequently perceived as important by patients. The mean impact score for each item was calculated as the product of frequency of the item occurring (0-1.00) and mean importance rating of the item. Items were ranked based on their impact scores and an impact score threshold of 1.5 was used to eliminate low ranking items. The following criteria were used to

further eliminate items from the questionnaire; (1) high ceiling effect: items with >60% of responses falling into the two lowest categories “none of the time” and “hardly any of the time,” (2) the lower impact score items of those with correlation coefficient >0.8, (3) items that were similar or ambiguously worded by consensus opinion. The remaining items formed the LCQ2, the final version (appendix IV).

Allocation of items to domains

Domains for the LCQ were predefined on; (1) World Health Definition of health (World Health Organisation 1947), (2) semi-structured interviews held with patients for item selection process and (3) expert opinion. Based on this, domains were physical, psychological and social. Each item was allocated to one of the predefined domains using the following criteria; (1) each items face validity was determined by an expert panel, (2) each item was correlated with the domain score to ensure that the item was in the most appropriate scale. Internal reliability of each domain was assessed using Cronbach’s alpha coefficients which indicate the extent to which the items are interrelated. Internal reliability is generally acceptable for domains with a Cronbach’s alpha coefficient of 0.7 or above (Fayers & Machin D 2000).

Concurrent Validity

Concurrent validity, which is the assessment of an instrument against other standards that provide an indication of the true value for the measurements was assessed by correlating scores of LCQ2 with three health outcome measures completed at the same time in 56 patients; (1) Cough Visual Analogue Score (VAS) (Brightling et al. 2000b) which has a scale from 0-100mm with 100mm being the worst imaginable cough, (2) St Georges Respiratory Questionnaire (SGRQ) (Jones et al. 1992) which includes cough related items and (3) Short Form 36 item (SF36) health status questionnaire (Brazier et al. 1992) which is a well tested and validated generic health status measure. The time scale over which symptoms or events being questioned in the SGRQ or SF36 were adjusted to two weeks to be consistent with the LCQ2. Twenty-seven patients also had a cough reflex sensitivity measurement using protocol described in section 2.2.5. Patients

were also asked which of the three questionnaires they found easiest to complete and relevant to them and the time taken for completion was measured.

Response Scale

A seven-point Likert scale was used throughout the development of the LCQ ranging from 1= all of the time to 7= none of the time. A higher score indicated better health status. Domains were scored out of 7 (total score from items in domain / number of items in domain). The overall score for the LCQ for each patient was calculated by adding the individual domain scores. (Appendix V)

Phase 3: Repeatability and responsiveness testing

The test-retest procedure measured the stability of scores in the LCQ2 over time in patients who had a stable chronic cough. The LCQ2 and cough VAS was administered in the outpatient clinic. The repeat questionnaire was mailed to the participants in time for them to complete it two weeks after the first questionnaire. Patients were asked to what extent their cough had changed since the completion of the first questionnaire.

Responsiveness of LCQ2 and cough VAS was tested in the outpatient clinic before and two months after a treatment had been commenced. Criteria for starting therapy and individual treatments used were as outlined in section 1.1. Patients were asked if their cough had improved.

Statistical analysis

SPSS version 10 was used for data analysis. Data was described as mean and standard error means (SEM) or ranges. Both Pearson correlation coefficients and Spearman rank correlation coefficients were used to determine relationships within and between different outcome measures according to the distribution of the variables. Item to domain correlations were adjusted for over-fitting by removing the score from the item being considered from the total domain score. Agreement between domain and total scores for the first and second completion of the LCQ during repeatability testing were assessed using intraclass correlation coefficients. 95% Limit of agreement was calculated as: $1.96 \times \text{standard deviation}$

(SD) of within subject differences. Effect size for total LCQ score was determined by the difference in mean total LCQ score pre- and post- intervention / SD of LCQ total scores pre- intervention.

RESULTS

Phase 1: item generation

Review of existing HRQOL questionnaires and literature and multidisciplinary team meeting generated an initial pool of 38 items. A further 6 items were added after patient interviews. Therefore a preliminary questionnaire (LCQ1; appendix III) comprising of 44 items was obtained.

Phase2: item reduction, allocation of items to domains and validation of the questionnaire

104 patients completed the LCQ1. Patients had a mean (range): age 57 (19-78) years; cough duration 5 (0.2-50) years; cough VAS 48 (3-96)mm and 39 (38%) were male.

Item Reduction

25 of the 44 items were omitted for the reasons given in Table 3.18. This resulted in a 19-item questionnaire.

Allocation of items to domains

8 items were assigned to the physical domain, 7 to psychological and 4 to social by the expert panel. All items correlated well with their domains (Pearson correlations adjusted for over-fitting from 0.4 – 0.8, all $p < 0.01$). Internal consistency was high for all domains as well as the total score (Table 3.19).

Concurrent Validity

56 patients completed the LCQ2 and other outcome measures. Patients had a mean (range): age 55 (22-85) years; cough duration 5 (0-7) years; cough VAS 48 (4-100) mm; and 21 (38%) were male. Correlations between the 19-item LCQ2 and other outcome measures are presented in Table 3.20. All were highly significant ($p < 0.001$). Correlations did not change significantly when items

common to both scales were excluded. Correlations between LCQ and SF36 domains were; physical/role limitation due to physical problems $\rho=0.46$, psychological/mental health $\rho=0.59$ and social/social functioning $\rho=0.46$. Pearson's correlations between LCQ total score and cough sensitivity (logC₂ and logC₅) were 0.14 and 0.18 respectively ($p=0.5$ and 0.4 respectively, $n=27$). There were no significant correlations between cough sensitivity and LCQ domains or cough VAS. When asked which questionnaire they found easiest to complete, 68% of patients indicated they preferred the LCQ, SF36: 16%, SGRQ: 8% and no preference: 8%. The mean time to complete the LCQ was 5 minutes.

Phase 3: Repeatability and responsiveness testing

Repeatability was tested in 24 patients. Intraclass correlation coefficients for the LCQ domains were; physical 0.93, psychological 0.90, social 0.88 and total score 0.96. A Bland – Altman plot of the LCQ total score is shown in Figure 3.10. The mean difference (SD) between the first and second LCQ total score was 0.73 (0.94). The intraclass correlation coefficient for the cough VAS was 0.84.

Responsiveness was tested in nine patients who had a specific therapeutic intervention and stated their cough had improved. The causes of cough were: gastro-oesophageal reflux ($n=3$), cough variant asthma ($n=2$), chronic bronchitis / bronchiectasis ($n=2$), rhinitis ($n=1$), enlarged tonsils ($n=1$). Interventions were proton pump inhibitor therapy ($n=3$), inhaled corticosteroids ($n=3$), nasal corticosteroid ($n=1$), tonsillectomy ($n=1$) and postural drainage ($n=1$). Changes in cough VAS score were used as a surrogate marker for response to treatment. The mean(SEM) change in cough VAS was $-42.3(8.6)$ mm. The effect sizes for change in domain and total LCQ scores are presented in Table 3.21. A t-test comparing the change in LCQ total score after treatment was highly significant ($p=0.007$).

Table 3.18 LCQ1 item reduction reasons.

	Number of items (%)
Low ranking items (impact factor score <1.5)	15 (34)
High ceiling effect	1 (2)
Correlation >80% with higher ranking items	5 (11)
Similar wording	3 (7)
Ambiguous wording	1 (2)
Total	25 (57)

Table 3.19 Cronbach’s alpha coefficients for each domain and total score for Leicester Cough Questionnaire.

DOMAIN	Cronbach’s alpha coefficient
Physical	0.79
Psychological	0.89
Social	0.85
TOTAL	0.92

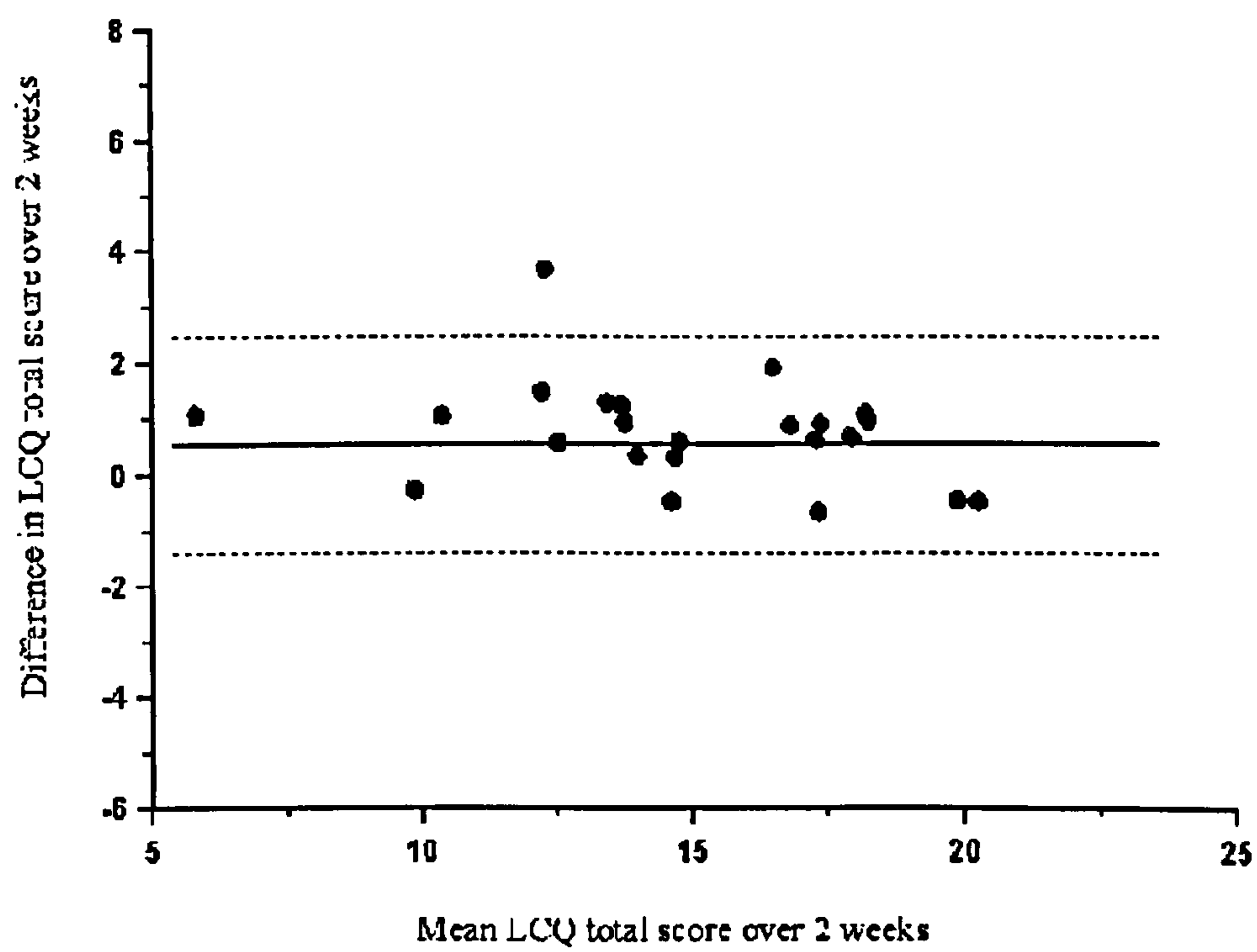
Table 3.20 Spearman rank correlations between different health outcome measures and LCQ2.

	Correlation with LCQ2 total score
Cough VAS	-0.72
SGRQ	-0.54
SF36 – total	0.46

Table 3.21 LCQ responsiveness: effect sizes of LCQ domains and cough VAS after treatment.

	Effect sizes (n=9)
Physical	1.00
Psychological	1.75
Social	0.84
LCQ total	1.68
Cough VAS	3.19

Figure 3.10 Bland –Altman plot of LCQ total score repeated over two weeks in patients whose cough remained unchanged. Solid line represents mean difference and dashed line represents 95% limit of agreement.



DISCUSSION

The LCQ is a valid and reliable health status measure for adults with chronic cough. The final version contains 19 items with a 7 point Likert response scale. It is designed for self-administration and takes less than 5 minutes for completion. 68% of our patients found our questionnaire easier to complete and more relevant than two other widely used HRQOL measures. This may be because it is a patient derived questionnaire and hence has items, domains and response scales that are more meaningful to them. The LCQ was highly repeatable and responsive to change, suggesting it might be a particularly useful outcome measure in assessing the response to treatment in the clinic and in trials.

Item reduction was performed using the clinical impact factor method instead of traditional psychometric techniques such as factor analysis. The impact factor chooses items, which patients label as a problem and ranks the importance that they associate with them. Items are categorised into domains using clinical sensibility. The factor analysis approach is based largely on the structure of correlations between items and investigators must also make a number of subjective decisions throughout the process (Juniper 1997). Factor analysis does not take into account the perception of clinical relevance of items by the intended population. Instead, item reduction is performed predominantly on complex correlations between items. Many items chosen during questionnaire development will be similar using both techniques but there are important differences (Juniper et al. 1997). We share the views of Juniper et al, that all items of functional impairment that are important to patients should be included in a disease-specific quality of life health status measure, irrespective of their association with each other.

One limitation of the study was our limited ability to explore the relationship between the LCQ and other objective markers of cough severity. This is due to the lack of well validated outcome measures in chronic cough. However, the LCQ correlated well with the cough VAS and less well with cough sensitivity suggesting that, as in asthma, relationship between symptoms, quality of life and physiological impairment is complex (Moy et al. 2001). An absence of correlation

between cough symptoms and cough sensitivity has also been noted in patients with cryptogenic fibrosing alveolitis (Doherty et al. 2000a) and chronic obstructive pulmonary disease (Doherty et al. 2000b). As expected LCQ correlated moderately with SGRQ, which has four specific cough items out of many respiratory items and less so with SF36 which focuses on general health alone, providing evidence of construct validity.

The LCQ was shown to be highly repeatable over two weeks. This was better than repeatability of other measures of chronic cough such as cough VAS and cough sensitivity (Chang et al. 1996). Our data indicates that a change in total LCQ score greater than 1.8 is likely to be significant since this lies outside the 95% limit of agreement. We have also shown that the LCQ was responsive to change after treatment although the effect sizes were less than that seen with the cough VAS suggesting the latter might be the outcome measure of choice in clinical trials. Effect sizes were comparable to that seen with cough sensitivity measurement in a study where inhaled corticosteroids were given for cough due to eosinophilic bronchitis (effect sizes for C₂ and C₅: 1.72 and 1.17 respectively) (Brightling et al. 2000b). These characteristics suggest that the LCQ can detect changes in health status as a result of successful treatment.

Since our manuscript was submitted, French et al (French et al. 2002) have described the validation of another cough specific quality of life questionnaire. The LCQ is a briefer questionnaire with fewer domains than the cough specific quality of life questionnaire (CQLQ), which comprises 28 items and 6 domains. The latter questionnaire also differs from ours in that it used factor analysis for allocation of items to domains, subjective criteria for item reduction and provided more limited information on concurrent validity against other measures. Further work is necessary to compare the LCQ and CQLQ in the assessment of chronic cough in European and North American populations.

This study suggests that the LCQ will be suitable for a number of applications. Firstly, it would be useful in describing longitudinal changes that take place in patients with chronic cough. It can be used to identify aspects of health affected

by cough and how these change over time. Finally, it can be used in clinical trials evaluating new treatments for cough and their effect on health related quality of life. In summary, the LCQ is a brief, easy to administer and well validated chronic cough HRQOL questionnaire. It represents an advance in the management of chronic cough where there is lack of objective measures to guide the clinician and scientist.

4 CONCLUSIONS

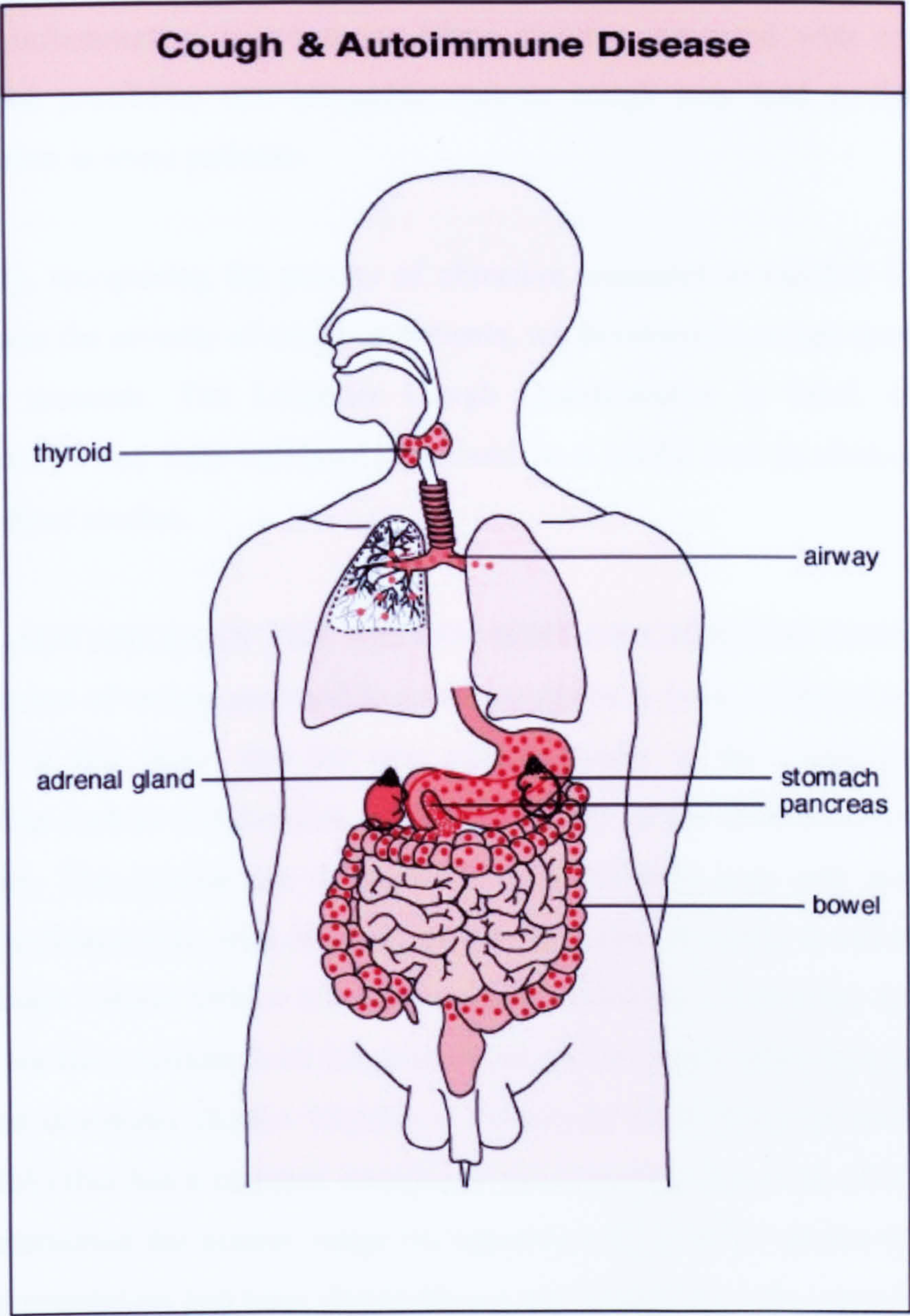
4.1 SUMMARY OF FINDINGS

This thesis is the first detailed study to address the clinical, physiological and immunopathological features of idiopathic chronic cough. We have shown that patients with idiopathic chronic cough are predominantly female, have an onset of cough in middle age and have heightened cough reflex sensitivity. We have also found a striking association between idiopathic chronic cough and organ specific autoimmune disease.

We have demonstrated a BAL lymphocytosis in patients with idiopathic chronic cough which was not present in patients with explained chronic cough. We have suggested that one possible mechanism for the cough and BAL lymphocytosis is homing of activated T cells to embryologically related structures such as the airways from the primary site of autoimmune inflammation (Figure 4.1). Similar mechanisms are thought to be responsible for the lymphocytic airway inflammation seen in inflammatory bowel disease, and it is notable that we found that patients with treated hypothyroidism and inflammatory bowel disease have increased prevalence of respiratory symptoms compared to controls, and that the profile of symptoms reported is remarkably similar. An alternative and intriguing mechanism is that the cough might be due to a hitherto unrecognised autoimmune bronchitis, bronchiolitis or interstitial process and that the association with other diseases simply reflects the well recognised association between different organ specific autoimmune diseases.

We have shown for the first time that there is increased concentrations of histamine, PGD₂ and PGE₂ in airway secretions of patients with idiopathic chronic cough confirming the presence of active inflammation in the airways. Raised histamine levels in idiopathic chronic cough suggest that antihistamines may have a therapeutic role. Further studies need to characterise the source of these mediators and their effector functions in cough.

Figure 4.1 Lymphocyte homing in idiopathic chronic cough



Red circles represent lymphocytes

We have studied the clinical characteristics of a series of patients with COPD who are non-smokers and found that they are predominantly elderly females with a high prevalence of organ specific autoimmune disease, particularly hypothyroidism. It is possible that homing of lymphocytes from the primary site of autoimmune inflammation to the airways over a prolonged period has lead to airway inflammation and damage. Many patients presented with cough which raises the possibility that idiopathic chronic cough may lead to fixed airflow obstruction in some patients.

Finally, recognising the paucity of objective measures to validate the presence and assess the severity of cough in patients, we developed a cough specific quality of life measure. The Leicester Cough Questionnaire is brief, simple, self administered and fully validated. It should be a useful tool in clinical trials and longitudinal studies.

4.2 ADVANCES IN THE ASSESSMENT OF CHRONIC COUGH

A number of techniques used to assess cough have been validated or developed as part of this thesis that are now used routinely in the cough clinic at our institution such as cough reflex sensitivity, quality of life measurement and cough monitors. The routine use of methacholine challenge tests and assessment of airway inflammation with induced sputum has made it easier to identify patients with cough variant asthma and eosinophilic bronchitis. During the course of this thesis, we have standardised the assessment of the cough reflex using a computer operated dosimeter (KoKo Digidoser, Pulmonary Data Services Instrumentation Inc, USA) that has a constant inspiratory flow rate (Prudon et al. 2004). We have also determined the normal range for capsaicin cough reflex sensitivity in a large healthy population and have demonstrated this procedure to be repeatable in both health and disease (Prudon et al. 2004) and Table 4.1. The Leicester Cough Questionnaire, which is a cough specific quality of life measure represents an advance in assessment of health status for a group of patients that suffer considerable physical and psychological morbidity. Finally, we are developing a digital ambulatory cough monitor that determines cough frequency from recorded sound. Preliminary data suggests that this tool can clearly discriminate between

health and disease, is repeatable and an acceptable form of monitoring to patients (Matos et al. 2003).

Table 4.1 Repeatability and responsiveness of outcome measures used to assess patients with chronic cough.

OUTCOME MEASURE	REPEATABILITY		RESPONSIVENESS
	Within subject SD	Between subject SD	Effect Size
LCQ (range 3-21)	0.9	3.4	1.7
CQLQ (range 28-112)	-	13.9	2.4
Cough VAS (0-100mm)	7.8mm	26.5mm	3.2
C2 (doubling dose)	0.5 dd	1.5 dd	1.7
C5 (doubling dose)	1.7 dd	3.1 dd	1.2
Cough monitor(cough/hr)	32	38	NK

SD: standard deviation; Effect size: difference in mean measurement pre- and post- intervention / SD of measurement pre- intervention (effect size>0.4 indicates responsive instrument); LCQ: total Leicester Cough Questionnaire score; CQLQ: total Cough specific quality of life questionnaire score; cough VAS: visual analogue score (worst cough: 100mm); C2: concentration of capsaicin that causes 2 coughs; C5: concentration of capsaicin that causes 5 coughs; Cough monitor (Matos et al. 2003); NK: not known

4.3 PROBLEMS ENCOUNTERED AND CRITICISMS

Some potential problems encountered and criticisms of the work presented in this thesis are addressed in the discussion of each chapter. However, some more general issues deserve particular attention. It is possible that selection and reporting bias may be partly responsible for the high prevalence of organ specific autoimmune disease seen in patients with idiopathic chronic cough. However, the increased prevalence of organ specific autoantibodies provides objective evidence of autoimmunity and these findings were consistent across three studies, suggesting the association with organ specific autoimmune disease is real.

Our studies of the immunopathology of idiopathic chronic cough were all cross-sectional in design. The longitudinal nature of broncho-alveolar inflammation in idiopathic chronic cough is not known. However, our findings are consistent with those from others that suggest the presence of lymphocytic airway inflammation and a role for histamine and other protussive mediators in the pathogenesis of idiopathic chronic cough. We did not identify any differences in the BAL lymphocyte phenotype, activation status and chemokine receptor expression in patients with idiopathic chronic cough compared with controls. These findings are similar to those in asthma and eosinophilic bronchitis and suggest that the T-cell markers studied are not important in determining the disease phenotype. The T-cell chemokine receptors involved in lymphocyte homing to the lung remain unidentified. One problem encountered in studying the immunopathology of idiopathic chronic cough was that because of technical difficulties, perhaps related to our attempt to do both BAL and bronchial biopsies in patients who have a troublesome cough, adequate submucosa for analysis was only available in just over half of the subjects with idiopathic chronic cough so we cannot exclude the possibility that features were missed due to lack of power or bias. Future studies should ideally incorporate larger biopsies and transbronchial biopsies so that better localisation and characterisation of lower airway inflammatory response is possible.

Another potential weakness of this thesis is that we have not investigated other explanations for idiopathic chronic cough such as viruses, allergens and non-

organic causes. There was no history of a preceding viral illness in patients with idiopathic chronic cough and in most, the cough duration was too long but it is difficult to exclude occult infections with organisms such as *Bordetella Pertussis*. There was no excess of atopy in patients with idiopathic chronic cough and no evidence of allergic inflammation in blood or BAL, suggesting allergic causes are unlikely. We feel that psychogenic cough was not present in our population with idiopathic chronic cough since there was objective evidence of abnormality such as heightened cough reflex sensitivity, impaired quality of life and high cough frequency when assessed with digital cough monitors (Matos et al. 2003).

4.4 FUTURE STUDIES

This thesis has suggested that idiopathic chronic cough may have an autoimmune basis in some patients. Further studies are needed to identify the chemokines and their receptors involved in the homing of inflammatory cells to the lung. Due to time constraints, this thesis has not explored the possibility of systemic or airway autoantibodies that might be involved in the pathogenesis of idiopathic chronic cough. These experiments would involve incubating patients and control serum and BAL with cryostat sections of normal bronchi and lung parenchyma surrounding resection specimens from lung cancer surgery. Autoantibodies can then be detected using indirect immunofluorescence and titres determined by serial dilution. HLA typing of patients may also be helpful in substantiating an autoimmune basis for the cough in patients with idiopathic chronic cough. The possibility that viral infections may be important in the development of an autoimmune process in the lungs of patients with idiopathic chronic cough would be difficult to confirm. However, the presence of latent viruses could be studied from bronchial biopsies, perhaps using molecular techniques such as PCR.

This thesis suggests a role for antihistamines in the therapy of idiopathic chronic cough. A randomised controlled trial of antihistamines is indicated using objective markers of cough assessment described in this thesis. We have noted that the cough in patients with idiopathic chronic cough is not responsive to corticosteroids, so one possibility for therapy is to use immuno-modulating drugs

such as thalidomide, which may have an impact on the underlying bronchoalveolar lymphocytic inflammation. Another possibility for therapy is to modify the cough reflex using antagonists to the recently cloned capsaicin receptor (vanilloid receptor 1).

There is a clear need to define the natural history of idiopathic chronic cough. We have suggested that some patients with idiopathic chronic cough develop fixed airflow obstruction, which may be a consequence of homing of inflammatory cells from the primary site of organ specific autoimmune inflammation to the lung. An immunopathological study of unexplained COPD in non-smokers is warranted since it may lead to novel pathogenic mechanisms that are relevant to both smoking and non-smoking related COPD.

Despite being one of the most common reasons for referral to a respiratory outpatient clinic, chronic cough remains a poorly understood and under researched area. This thesis should lead to questioning of current practices in the management of chronic cough that is often based on anecdotal evidence or expert opinion. I hope that some of the novel mechanisms identified for the pathogenesis of idiopathic chronic cough will lead to further studies, both laboratory based and clinical and the identification of new therapeutic targets.

APPENDIX I

CHRONIC COUGH QUESTIONNAIRE

Please fill in the following information. This will take you approximately 10 minutes to complete.

Part 1

Name

Address

DOB

Sex M/F

Telephone

Ethnic origin

Date

Part 2

Have you ever suffered from the following illnesses?

Please answer the questions by circling yes (Y) or no (N)

Overactive thyroid	Y / N
Underactive thyroid	Y / N
Enlarged thyroid (goitre)	Y / N
Diabetes	Y / N
Pernicious anaemia (requiring injections)	Y / N
Addison's disease	Y / N
Alopecia (baldness of skin affecting eyebrows and/or beard)	Y / N
Vitilgo (patches of skin that remain white and do not tan)	Y / N
Coeliac disease (wheat allergy)	Y / N
Crohn's disease	Y / N
Ulcerative colitis	Y / N
Premature menopause (before age 40)	Y / N
Chronic hepatitis	Y / N

If you answered no to all the above illnesses, please go straight to part 4.

Part 3

If you answered yes to any of the above questions please answer the following questions:

Please state how old you were at the time of diagnosis?

Have you been given any treatment or tablets for this condition? Y / N

If yes, please list:

Part 4

Have you had any other major illnesses during your life? Y / N

If yes please list illnesses, how old you were at the onset of the illness and any treatment you received.

Illness

Age it started

Treatment received

Part 5

Please list any medications you take regularly?

Have you ever taken any anti-inflammatories, for example aspirin, diclofenac (volterol), naproxen, ibuprofen? Y / N

If yes please list, and how long you took the tablets for

Part 6

Would you be able to help us with our research by donating a small blood sample? Y / N

Please return in SAE. Thank you for your time and cooperation.

Appendix II Respiratory symptom questionnaire

Respiratory Questionnaire

University Hospitals of Leicester NHS Trust
Glenfield Hospital, Leicester

Patient Number

Your date of birth

Male/Female

I AM GOING TO ASK YOU SOME QUESTIONS. THESE WILL BE MOSTLY ABOUT YOUR BREATHING. WHEREVER POSSIBLE, I WANT YOU TO ANSWER YES OR NO BY CIRCLING THE APPROPRIATE ANSWER.

Yes **No**

WHEEZE AND TIGHTNESS IN THE CHEST

1. Have you had wheezing or whistling in your chest at any time in the last 12 months?

Yes **No**
2. Have you woken up with a feeling of tightness in your chest at any time in the last 12 months?

Yes **No**

SHORTNESS OF BREATH

3. Have you had an attack of shortness of breath that came on during the day when you were at rest at any time in the last 12 months?

Yes **No**
4. Have you been woken by an attack of shortness of breath at any time in the last 12 months?

Yes **No**
5. Have you had an attack of shortness of breath that came on FOLLOWING strenuous activity at any time in the last 12 months?

Yes **No**

COUGH

6. Have you ever been woken by an attack of coughing at any time in the last 12 months?

Yes **No**

7. Have you had a head cold, sore throat, 'flu or chest infection in the last week? Yes No

- IF 'YES' TO QUESTION 7, GO TO QUESTION 9

8. Have you had a head cold, sore throat, 'flu or chest infection in the last 4 weeks? Yes No

COUGH & PHLEGM FROM THE CHEST

9. Do you USUALLY cough first thing in the morning in the winter? Yes No

10. Do you USUALLY cough during the day, or at night, in the winter? Yes No

- IF 'NO' TO QUESTIONS 9 & 10, GO TO QUESTION 12

11. Do you cough like this most days for as much as three months each year? Yes No

12. Do you USUALLY bring up any phlegm from your chest first thing in the morning in the winter? Yes No

13. Do you USUALLY bring up any phlegm from your chest during the day, or at night, in the winter? Yes No

- IF 'NO' TO QUESTIONS 12 AND 13, GO TO QUESTION 15

14. Do you bring up phlegm like this on most days for as much as 3 months each year? Yes No

BREATHING

15. Do you ever have trouble with breathing? Yes No

- IF 'NO' TO QUESTION 15, GO TO QUESTION 17

16. Do you have this trouble: Tick box ✓

1. Continuously, so that your breathing is never quite right?

2. Repeatedly, but it always gets completely better?

3. Only rarely?
- ☐
☐
☐

17. Are you disabled from walking by a condition OTHER THAN heart or lung disease? Yes No

If 'YES', please state condition.....

- IF 'YES', GO TO QUESTION 21

18. Are you troubled by shortness of breath when hurrying on level ground or walking up a slight hill? **Yes No**

19. Do you get short of breath walking with other people of your own age on level ground? **Yes No**

- IF 'NO', GO TO QUESTION 21

20. Do you have to stop for breath when walking at your own pace on level ground? **Yes No**

YOUR FAMILY

21. Were you born in the United Kingdom? **Yes No**

- IF 'YES' TO QUESTION 21, GO TO QUESTION 23

22. How many years have you lived in the UK? **Years**

YOUR HOME

23. How many years have you lived in your present home?
.....

24. How many years have you lived in the same town?

SMOKING HABIT

25. Have you ever smoked for as long as a year? **Yes No**
(Enter Yes if at least 1 cigarette/day or 1 cigar/week for 1 year)

- IF 'NO' TO QUESTION 25, GO TO QUESTION 32

26. How old were you when you started smoking?

27. Do you smoke now (as of ONE MONTH AGO)? **Yes No**

- IF 'NO' TO QUESTION 27, GO TO QUESTION 29

28. How much do you now smoke on average?
(For own-rolled cigarettes, 28g (1oz) tobacco/wk = 4 cigarettes/day)

Number of cigarettes per day
Number of cigarillos per day
Number of cigars per week
Amount of pipe tobacco per weekg

29. Have you stopped or cut down smoking Yes No

- IF 'NO' TO QUESTION 29, GO TO QUESTION 32

30. How old were you when you stopped or cut down smoking?

31. ON AVERAGE, of the entire time you smoked (before you stopped or cut down) how much did you smoke?

(For own-rolled cigarettes, 28g (1oz) tobacco/wk = 4 cigarettes/day)

Number of cigarettes per day
Number of cigarillos per day
Number of cigars per week
Amount of pipe tobacco per weekg

32. Have you been REGULARLY (i.e., on most days or nights) exposed to other people's tobacco smoke in the last 12 months? Yes No

- IF 'NO' TO QUESTION 32, GO TO QUESTION 34

33. How many hours per day are you exposed to OTHER PEOPLE'S tobacco smoke?hours

ALL SUBJECTS

34. If you have a cough, how long have you had it?months.....years

35. When were you diagnosed with your thyroid disorder?.....(approximately)

36. Do you have a goitre? (thyroid swelling that can be felt) Yes No

37. Do you take carbimazole tablets for your thyroid disorder? Yes No

38. .Do you take propylthiouracil tablets for your thyroid disorder? Yes No

39. What other treatments and medications do you take?
.....
.....

40. Have you had or are awaiting thyroid surgery? Yes No

41. Have you had or are awaiting radio – iodine treatment? Yes No

42. What is your occupation or previous occupation?.....

Appendix III LCQ version 1 (preliminary version)

Please fill in this page

NAME (or sticker)..... Date

DATE OF BIRTH.....

UNIT NUMBER.....

SEX M / F

Duration of cough YearsMonths

Visual analogue score

(please put a cross on the line to indicate where on the scale your cough has been in the last 2 weeks)

WORST COUGH EVER

NO COUGH

For doctor to fill in
DIAGNOSIS

1. 2. 3.

LEICESTER COUGH QUESTIONNAIRE 1

This questionnaire is designed to assess the impact of cough on various aspects of your life. Read each question carefully and answer by CIRCLING the response that best applies to you. You also need to answer the 'importance of each question' section for each item. The next page shows an example from a completed questionnaire. Please answer ALL questions, as honestly as you can. This questionnaire will remain confidential and your name will not be recorded on this document. Please return in the stamped addressed envelope provided.

No.....

DATE.....

© 2001. University Hospitals of Leicester NHS Trust, UK.

EXAMPLE PAGE

(Not to be completed)

1. As a result of your cough, have you suffered in the last 2 weeks from chest or stomach pains?

- 1. All of the time
- 2. Most of the time
- 3. A good bit of the time
- 4. Some of the time
- 5. A little of the time
- 6. Hardly any of the time
- 7. None of the time

Please answer
both sections

Please rate the importance of this question to you

- 1. Not important
- 2. Little importance
- 3. Some importance
- 4. Very important
- 5. Extremely important

2. In the last 2 weeks, my cough has made me feel frustrated

- 1. All of the time
- 2. Most of the time
- 3. A good bit of the time
- 4. Some of the time
- 5. A little of the time
- 6. Hardly any of the time
- 7. None of the time

Please rate the importance of this question to you

- 1. Not important
- 2. Little importance
- 3. Some importance
- 4. Very important
- 5. Extremely important

3. In the last 2 weeks, my cough has interrupted conversation or telephone calls

- 1. All of the time
- 2. Most of the time
- 3. A good bit of the time
- 4. Some of the time
- 5. A little of the time
- 6. Hardly any of the time
- 7. None of the time

Please rate the importance of this question to you

- 1. Not important
- 2. Little importance
- 3. Some importance
- 4. Very important
- 5. Extremely important

LEICESTER COUGH QUESTIONNAIRE 1

1. In the last 2 weeks, have you had chest or stomach pains as a result of your cough?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

2. In the last 2 weeks, have you produced sputum (phlegm) when you cough?

1. Every time
2. Most times
3. Several times
4. Some times
5. Occasionally
6. Rarely
7. Never

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

3. In the last 2 weeks, have you had sickness or vomiting as a result of your cough?

1. Every time
2. Most times
3. Several times
4. Some times
5. Occasionally
6. Rarely
7. Never

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

4. In the last 2 weeks, have you had headaches as a result of your cough?

1. Every time
2. Most times
3. Several times
4. Some times
5. Occasionally
6. Rarely
7. Never

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

5. In the last 2 weeks, have you had blackouts, faint or dizzy spells as a result of your cough?

1. Every time
2. Most times
3. Several times
4. Some times
5. Occasionally
6. Rarely
7. Never

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

6. In the last 2 weeks, have you been tired because of your cough?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

7. In the last 2 weeks, have you had stress incontinence (wetting your underwear) due to coughing?

1. Every time I cough
2. Most times when I cough
3. Several times when I cough
4. Some times when I cough
5. Occasionally when I cough
6. Rarely
7. Never

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

8. In the last 2 weeks, exposure to paints or fumes has made me cough:

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

9. In the last 2 weeks, has your cough disturbed your sleep?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

10. In the last 2 weeks, has your cough been associated with eating?

1. Every time I cough
2. Most times when I cough
3. Several times when I cough
4. Some times when I cough
5. Occasionally when I cough
6. Rarely
7. Never

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

11. In the last 2 weeks, have you suffered from a hoarse voice as a result of your cough?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

12. In the last 2 weeks, have you felt in control of your cough?

1. None of the time
2. Hardly any of the time
3. A little of the time
4. Some of the time
5. A good bit of the time
6. Most of the time
7. All of the time

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

13. Over the last 2 weeks, on how many days have you had coughing bouts?

1. Every day of each week
2. Six days of each week
3. Five days of each week
4. Four days of each week
5. Three days of each week
6. One or two days of each week
7. None

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

14. In the last two weeks, how many times a day have you had coughing bouts?

1. All the time (continuously)
2. Most times of during the day
3. Several times during the day
4. Some times during the day
5. Occasionally through the day
6. Rarely
7. None

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

15. How often during the last 2 weeks have you felt embarrassed by your coughing?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

16. In the last 2 weeks, my cough has made me feel anxious

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

17. In the last 2 weeks, my cough has made me feel frustrated

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

18. In the last 2 weeks, my cough has made me feel irritable

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

19. In the last 2 weeks, my cough has made me feel angry

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

20. In the last 2 weeks, my cough has made me feel fed up

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

21. In the last 2 weeks, my cough has made me feel guilty

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

22. In the last 2 weeks, my cough has made me feel tense

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

23. In the last 2 weeks, my cough has made me feel low and down in the dumps

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

24. In the last 2 weeks, have you had any sudden feelings of fear or panic?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

25. In the last 2 weeks, I felt that my cough interfered with the overall enjoyment of my life

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

26. In the last 2 weeks, I have had worrying thoughts because of my cough

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

27. In the last 2 weeks, have you felt full of life?

1. None of the time
2. Hardly any of the time
3. A little of the time
4. Some of the time
5. A good bit of the time
6. Most of the time
7. All of the time

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

28. In the last 2 weeks, have you had a lot of energy?

1. None of the time
2. Hardly any of the time
3. A little of the time
4. Some of the time
5. A good bit of the time
6. Most of the time
7. All of the time

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

29. In the last 2 weeks, I have thought that my health will get worse because of my cough

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

30. In the last 2 weeks, have you worried that your cough may indicate a serious illness?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

31. In the last 2 weeks, have you been concerned that other people think something is wrong with you, because of your cough?

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

32. In the last 2 weeks, has it concerned you that people don't take you seriously, because of your cough?

- 1. All of the time
- 2. Most of the time
- 3. A good bit of the time
- 4. Some of the time
- 5. A little of the time
- 6. Hardly any of the time
- 7. None of the time

Please rate the importance of this question to you

1. Not important

2. Little importance

3. Some importance

4. Very important

5. Extremely important

33. In the last 2 weeks, have you avoided going out and socialising because of your cough?

- 1. All of the time
- 2. Most of the time
- 3. A good bit of the time
- 4. Some of the time
- 5. A little of the time
- 6. Hardly any of the time
- 7. None of the time

Please rate the importance of this question to you

1. Not important

2. Little importance

3. Some importance

4. Very important

5. Extremely important

34. In the last 2 weeks, have you tended to avoid visiting friends because of your cough?

- 1. All of the time
- 2. Most of the time
- 3. A good bit of the time
- 4. Some of the time
- 5. A little of the time
- 6. Hardly any of the time
- 7. None of the time

Please rate the importance of this question to you

1. Not important

2. Little importance

3. Some importance

4. Very important

5. Extremely important

35. In the last 2 weeks, my cough has caused difficulties in my relationship with my partner

- 1. All of the time
- 2. Most of the time
- 3. A good bit of the time
- 4. Some of the time
- 5. A little of the time
- 6. Hardly any of the time
- 7. None of the time

Please rate the importance of this question to you

1. Not important

2. Little importance

3. Some importance

4. Very important

5. Extremely important

36. In the last 2 weeks, my cough has caused difficulties in my relationship with family and friends

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

37. In the last 2 weeks, I have sat near exits in public places in case I cough

1. Every time
2. Most times
3. Several times
4. Sometimes
5. Occasionally
6. Rarely
7. Never

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

38. In the last 2 weeks, my cough has stopped me getting out of the house

1. All of the time
2. Most times
3. Several times
4. Sometimes
5. Occasionally
6. Rarely
7. Never

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

39. In the last 2 weeks, my cough has interrupted conversation or telephone calls

1. Every time
2. Most times
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

40. In the last 2 weeks, my cough has disturbed my partner's sleep

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

41. In the last 2 weeks, I feel that my cough has annoyed my partner, family or friends

1. Every time I cough
2. Most times when I cough
3. Several times when I cough
4. Some times when I cough
5. Occasionally when I cough
6. Rarely
7. Never

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

42. In the last 2 weeks, my cough has interfered with my sex life

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

43. In the last 2 weeks, my cough has interfered with my job, or other daily tasks

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

44. In the last 2 weeks, I have had difficulty in performing my job

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

Please rate the importance of this question to you

1. Not important
2. Little importance
3. Some importance
4. Very important
5. Extremely important

PLEASE LIST any other important activities that your cough may stop you doing or make any other comments (continue overleaf if necessary):

THANK YOU FOR COMPLETING THE QUESTIONNAIRE. PLEASE RETURN IN THE STAMPED ADDRESSED ENVELOPE PROVIDED OR HAND IT TO THE RECEPTIONIST.

APPENDIX IV

FINAL VERSION OF LEICESTER COUGH QUESTIONNAIRE (LCQ)

This questionnaire is designed to assess the impact of cough on various aspects of your life. Read each question carefully and answer by CIRCling the response that best applies to you. Please answer ALL questions, as honestly as you can.

1. In the last 2 weeks, have you had chest or stomach pains as a result of your cough?

- | | | | | |
|-----------------------------|-----------------------|-----------------------------|-----------------------|---------------------------|
| 1
All of the time | 2
Most of the time | 3
A good bit of the time | 4
Some of the time | 5
A little of the time |
| 6
Hardly any of the time | 7
None of the time | | | |

2. In the last 2 weeks, have you been bothered by sputum (phlegm) production when you cough?

- | | | | | | |
|-----------------|-----------------|--------------------|-----------------|-------------------|-------------|
| 1
Every time | 2
Most times | 3
Several times | 4
Some times | 5
Occasionally | 6
Rarely |
| 7
Never | | | | | |

3. In the last 2 weeks, have you been tired because of your cough?

- | | | | | |
|-----------------------------|-----------------------|-----------------------------|-----------------------|---------------------------|
| 1
All of the time | 2
Most of the time | 3
A good bit of the time | 4
Some of the time | 5
A little of the time |
| 6
Hardly any of the time | 7
None of the time | | | |

4. In the last 2 weeks, have you felt in control of your cough?

- | | | | |
|-----------------------------|-----------------------------|---------------------------|-----------------------|
| 1
None of the time | 2
Hardly any of the time | 3
A little of the time | 4
Some of the time |
| 5
A good bit of the time | 6
Most of the time | 7
All of the time | |

5. How often during the last 2 weeks have you felt embarrassed by your coughing?

- | | | | | |
|-----------------------------|-----------------------|-----------------------------|-----------------------|---------------------------|
| 1
All of the time | 2
Most of the time | 3
A good bit of the time | 4
Some of the time | 5
A little of the time |
| 6
Hardly any of the time | 7
None of the time | | | |

6. In the last 2 weeks, my cough has made me feel anxious

- | | | | | |
|-----------------------------|-----------------------|-----------------------------|-----------------------|---------------------------|
| 1
All of the time | 2
Most of the time | 3
A good bit of the time | 4
Some of the time | 5
A little of the time |
| 6
Hardly any of the time | 7
None of the time | | | |

7. In the last 2 weeks, my cough has interfered with my job, or other daily tasks

- | | | | | |
|-----------------------------|-----------------------|-----------------------------|-----------------------|---------------------------|
| 1
All of the time | 2
Most of the time | 3
A good bit of the time | 4
Some of the time | 5
A little of the time |
| 6
Hardly any of the time | 7
None of the time | | | |

8. In the last 2 weeks, I felt that my cough interfered with the overall enjoyment of my life

- | | | | | |
|-----------------------------|-----------------------|-----------------------------|-----------------------|---------------------------|
| 1
All of the time | 2
Most of the time | 3
A good bit of the time | 4
Some of the time | 5
A little of the time |
| 6
Hardly any of the time | 7
None of the time | | | |

9. In the last 2 weeks, exposure to paints or fumes has made me cough

1	2	3	4	5
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time
6		7		
Hardly any of the time		None of the time		

10. In the last 2 weeks, has your cough disturbed your sleep?

1	2	3	4	5
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time
6		7		
Hardly any of the time		None of the time		

11. In the last two weeks, how many times a day have you had coughing bouts?

1	2	3		
All the time (continuously)	Most times of during the day	Several times during the day		
4	5	6	7	
Some times during the day	Occasionally through the day	Rarely	None	

12. In the last 2 weeks, my cough has made me feel frustrated

1	2	3	4	5
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time
6		7		
Hardly any of the time		None of the time		

13. In the last 2 weeks, my cough has made me feel fed up

1	2	3	4	5
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time
6		7		
Hardly any of the time		None of the time		

14. In the last 2 weeks, have you suffered from a hoarse voice as a result of your cough?

1	2	3	4	5
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time
6		7		
Hardly any of the time		None of the time		

15. In the last 2 weeks, have you had a lot of energy?

1	2	3	4	5
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time
6		7		
Hardly any of the time		None of the time		

16. In the last 2 weeks, have you worried that your cough may indicate a serious illness?

1	2	3	4	5
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time
6		7		
Hardly any of the time		None of the time		

17. In the last 2 weeks, have you been concerned that other people think something is wrong with you, because of your cough?

1	2	3	4	5
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time
6		7		
Hardly any of the time		None of the time		

18. In the last 2 weeks, my cough has interrupted conversation or telephone calls

1	2	3	4	5
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time
6		7		
Hardly any of the time		None of the time		

19. In the last 2 weeks, I feel that my cough has annoyed my partner, family or friends

- | | | | |
|---------------------------|-------------------------|----------------------------|-------------------------|
| 1 | 2 | 3 | 4 |
| Every time I cough | Most times when I cough | Several times when I cough | Some times when I cough |
| 5 | 6 | 7 | |
| Occasionally when I cough | Rarely | Never | |

Thank you for completing this questionnaire.

APPENDIX V: SCORING OF LCQ

- | | |
|-------------------------|---|
| 1. Domains (questions): | Physical: 1,2,3,9,10,11,14,15
Psychological 4,5,6,12,13,16,17
Social: 7,8,18,19 |
| 2. Domain Scores: | Total score from items in domain / number of items in domain (range 1-7) |
| 3. Total Scores: | Addition of domain scores (range 3-21) |

References

2001, "British Thoracic Society guidelines on diagnostic flexible bronchoscopy", *Thorax*, vol. 56 Suppl 1, pp. i1-21.

Aguayo, S. M., Miller, Y. E., Waldron, J. A., Jr., Bogin, R. M., Sunday, M. E., Staton, G. W., Jr., Beam, W. R., & King, T. E., Jr. 1992, "Brief report: idiopathic diffuse hyperplasia of pulmonary neuroendocrine cells and airways disease", *N.Engl.J Med*, vol. 327, no. 18, pp. 1285-1288.

Almansa-Pastor, A. 1996, "Treating refractory cough with aerosols of mepivacaine", *Chest*, vol. 110, no. 5, pp. 1374-1375.

Baggiolini, M. 1998, "Chemokines and leukocyte traffic", *Nature*, vol. 392, no. 6676, pp. 565-568.

Balzano, G., Stefanelli, F., Iorio, C., De Felice, A., Melillo, E. M., Martucci, M., & Melillo, G. 1999, "Eosinophilic inflammation in stable chronic obstructive pulmonary disease. Relationship with neutrophils and airway function", *Am J Respir Crit Care Med*, vol. 160, no. 5 Pt 1, pp. 1486-1492.

Barnes, P. J. 2000, "Chronic obstructive pulmonary disease", *N.Engl.J Med*, vol. 343, no. 4, pp. 269-280.

Baxter, A. G. & Smyth, M. J. 2002, "The role of NK cells in autoimmune disease", *Autoimmunity*, vol. 35, no. 1, pp. 1-14.

Beasley, R., Roche, W. R., Roberts, J. A., & Holgate, S. T. 1989, "Cellular events in the bronchi in mild asthma and after bronchial provocation", *Am.Rev.Respir.Dis.*, vol. 139, no. 3, pp. 806-817.

Beeh, K. M., Kornmann, O., Lill, J., & Buhl, R. 2001, "Induced sputum cell profiles in lung transplant recipients with or without chronic rejection: correlation with lung function", *Thorax*, vol. 56, no. 7, pp. 557-560.

Benini, L., Ferrari, M., Sembenini, C., Olivieri, M., Micciolo, R., Zuccali, V., Bulighin, G. M., Fiorino, F., Ederle, A., Cascio, V. L., & Vantini, I. 2000, "Cough threshold in reflux oesophagitis: influence of acid and of laryngeal and oesophageal damage", *Gut*, vol. 46, no. 6, pp. 762-767.

Berkin, K. E. & Ball, S. G. 1988, "Cough and angiotensin converting enzyme inhibition", *Br.Med J (Clin.Res.Ed)*, vol. 296, no. 6632, p. 1279.

Berkson J. Limitations of the application of fourfold table analysis to hospital data. *Biometrics* 2, 47-53. 1946.

Ref Type: Journal (Full)

Berlyne, G. S., Parameswaran, K., Kamada, D., Efthimiadis, A., & Hargreave, F. E. 2000, "A comparison of exhaled nitric oxide and induced sputum as markers of airway inflammation", *J Allergy Clin.Immunol.*, vol. 106, no. 4, pp. 638-644.

Berry, M. A., Brightling, C. E., Hargadon, B., McKenna, S., Wardlaw, A. J., & Pavord, I. D. 2003, "Observational study of the natural history of eosinophilic bronchitis", *Thorax*, vol. 58, p. iii46.

Birring S.S., Murphy, A. C., Scullion, J. E., Brightling, C. E., Browning, M., & Pavord, I. D. Idiopathic chronic cough and organ specific autoimmune diseases: a case-control study. *Respir Med* . 2004: 98: 242-246.

Birring S.S., Patel R.B., Prudon B, Singh, S. J., Morgan, M. D. L., & Pavord, I. D. 2003, "Quality of life in chronic cough", *Am J Respir Crit Care Med*, vol. 167, p. A135.

Birring, S. S., Brightling, C. E., Bradding, P., Entwisle, J. J., Vara, D. D., Grigg, J., Wardlaw, A. J., & Pavord, I. D. 2002, "Clinical, radiologic, and induced sputum features of chronic obstructive pulmonary disease in nonsmokers: a descriptive study", *Am.J.Respir.Crit Care Med.*, vol. 166, no. 8, pp. 1078-1083.

Birring, S. S., Brightling, C. E., Symon, F. A., Barlow, S. G., Wardlaw, A. J., & Pavord, I. D. 2003a, "Idiopathic chronic cough: association with organ specific autoimmune disease and bronchoalveolar lymphocytosis", *Thorax*, vol. 58, no. 12, pp. 1066-1070.

Birring, S. S., Parker, D., Brightling, C. E., Bradding, P., Wardlaw, A. J., & Pavord, I. D. 2003b, "Induced sputum mediator concentrations in chronic cough", *Am J Respir Crit Care Med*, vol. 169, pp. 15-19.

Bolser, D. C. & Davenport, P. W. 2002, "Functional organization of the central cough generation mechanism", *Pulm.Pharmacol.Ther.*, vol. 15, no. 3, pp. 221-225.

Bonniere, P., Wallaert, B., Cortot, A., Marchandise, X., Riou, Y., Tonnel, A. B., Colombel, J. F., Voisin, C., & Paris, J. C. 1986, "Latent pulmonary involvement in Crohn's disease: biological, functional, bronchoalveolar lavage and scintigraphic studies", *Gut*, vol. 27, no. 8, pp. 919-925.

Boulet, L. P., Milot, J., Boutet, M., St Georges, F., & Laviolette, M. 1994, "Airway inflammation in nonasthmatic subjects with chronic cough", *Am J Respir Crit Care Med*, vol. 149, no. 2 Pt 1, pp. 482-489.

Brazier, J. E., Harper, R., Jones, N. M., O'Cathain, A., Thomas, K. J., Usherwood, T., & Westlake, L. 1992, "Validating the SF-36 health survey questionnaire: new outcome measure for primary care", *BMJ*, vol. 305, no. 6846, pp. 160-164.

Brightling, C. E., Bradding, P., Symon, F. A., Holgate, S. T., Wardlaw, A. J., & Pavord, I. D. 2002a, "Mast-cell infiltration of airway smooth muscle in asthma", *N.Engl.J.Med.*, vol. 346, no. 22, pp. 1699-1705.

Brightling, C. E., Monteiro, W., Ward, R., Parker, D., Morgan, M. D., Wardlaw, A. J., & Pavord, I. D. 2000a, "Sputum eosinophilia and short-term response to prednisolone in chronic obstructive pulmonary disease: a randomised controlled trial", *Lancet*, vol. 356, no. 9240, pp. 1480-1485.

Brightling, C. E., Monterio, W., Green, R. H., Parker, D., Morgan, M. D., Wardlaw, A. J., & Pavord, D. 2001, "Induced sputum and other outcome measures in chronic obstructive pulmonary disease: safety and repeatability", *Respir.Med.*, vol. 95, no. 12, pp. 999-1002.

Brightling, C. E., Symon, F. A., Birring, S. S., Bradding, P., Pavord, I. D., & Wardlaw, A. J. 2002b, "TH2 cytokine expression in bronchoalveolar lavage fluid T lymphocytes and bronchial submucosa is a feature of asthma and eosinophilic bronchitis", *J.Allergy Clin.Immunol.*, vol. 110, no. 6, pp. 899-905.

Brightling, C. E., Symon, F. A., Birring, S. S., Bradding, P., Wardlaw, A. J., & Pavord, I. D. 2003, "Comparison of airway immunopathology of eosinophilic bronchitis and asthma", *Thorax*, vol. 58, no. 6, pp. 528-532.

Brightling, C. E., Symon, F. A., Birring, S. S., Wardlaw, A. J., Robinson, R., & Pavord, I. D. 2002c, "A case of cough, lymphocytic bronchoalveolitis and coeliac disease with improvement following a gluten free diet", *Thorax*, vol. 57, no. 1, pp. 91-92.

Brightling, C. E., Ward, R., Goh, K. L., Wardlaw, A. J., & Pavord, I. D. 1999a, "Eosinophilic bronchitis is an important cause of chronic cough", *Am J Respir Crit Care Med*, vol. 160, no. 2, pp. 406-410.

Brightling, C. E., Ward, R., Wardlaw, A. J., & Pavord, I. D. 2000b, "Airway inflammation, airway responsiveness and cough before and after inhaled budesonide in patients with eosinophilic bronchitis", *Eur.Respir J*, vol. 15, no. 4, pp. 682-686.

Brightling, C. E., Ward, R., Woltmann, G., Bradding, P., Sheller, J. R., Dworski, R., & Pavord, I. D. 2000c, "Induced sputum inflammatory mediator concentrations in eosinophilic bronchitis and asthma", *Am J Respir Crit Care Med*, vol. 162, no. 3 Pt 1, pp. 878-882.

Brightling, C. E., Woltmann, G., Wardlaw, A. J., & Pavord, I. D. 1999b, "Development of irreversible airflow obstruction in a patient with eosinophilic bronchitis without asthma", *Eur.Respir J*, vol. 14, no. 5, pp. 1228-1230.

Britten, K. M., Howarth, P. H., & Roche, W. R. 1993, "Immunohistochemistry on resin sections: a comparison of resin embedding techniques for small mucosal biopsies", *Biotech.Histochem.*, vol. 68, pp. 271-280.

Britton, J., Pavord, I., Richards, K., Wisniewski, A., Knox, A., Lewis, S., Tattersfield, A., & Weiss, S. 1994, "Dietary magnesium, lung function, wheezing, and airway hyperreactivity in a random adult population sample", *Lancet*, vol. 344, no. 8919, pp. 357-362.

Bronsky, E. A., Druce, H., Findlay, S. R., Hampel, F. C., Kaiser, H., Ratner, P., Valentine, M. D., & Wood, C. C. 1995, "A clinical trial of ipratropium bromide nasal spray in patients with perennial nonallergic rhinitis", *J Allergy Clin.Immunol.*, vol. 95, no. 5 Pt 2, pp. 1117-1122.

Burney, P. G., Laitinen, L. A., Perdrietz, S., Huckauf, H., Tattersfield, A. E., Chinn, S., Poisson, N., Heeren, A., Britton, J. R., & Jones, T. 1989, "Validity and repeatability of the IUATLD (1984) Bronchial Symptoms Questionnaire: an international comparison", *Eur.Respir J*, vol. 2, no. 10, pp. 940-945.

Camus, P. & Colby, T. V. 2000, "The lung in inflammatory bowel disease", *Eur.Respir J*, vol. 15, no. 1, pp. 5-10.

Camus, P., Piard, F., Ashcroft, T., Gal, A. A., & Colby, T. V. 1993, "The lung in inflammatory bowel disease", *Medicine (Baltimore)*, vol. 72, no. 3, pp. 151-183.

Carney, I. K., Gibson, P. G., Murree-Allen, K., Saltos, N., Olson, L. G., & Hensley, M. J. 1997, "A systematic evaluation of mechanisms in chronic cough", *Am J Respir Crit Care Med*, vol. 156, no. 1, pp. 211-216.

Chang, A. B., Phelan, P. D., Roberts, R. G., & Robertson, C. F. 1996, "Capsaicin cough receptor sensitivity test in children", *Eur.Respir.J*, vol. 9, no. 11, pp. 2220-2223.

Chatkin, J. M., Ansarin, K., Silkoff, P. E., McClean, P., Gutierrez, C., Zamel, N., & Chapman, K. R. 1999, "Exhaled nitric oxide as a noninvasive assessment of chronic cough", *Am.J Respir.Crit Care Med*, vol. 159, no. 6, pp. 1810-1813.

Cheriyian, S., Greenberger, P. A., & Patterson, R. 1994, "Outcome of cough variant asthma treated with inhaled steroids", *Ann.Allergy*, vol. 73, no. 6, pp. 478-480.

Choudry, N. B. & Fuller, R. W. 1992, "Sensitivity of the cough reflex in patients with chronic cough", *Eur.Respir J*, vol. 5, no. 3, pp. 296-300.

Choudry, N. B., Fuller, R. W., & Pride, N. B. 1989, "Sensitivity of the human cough reflex: effect of inflammatory mediators prostaglandin E2, bradykinin, and histamine", *Am Rev.Respir Dis.*, vol. 140, no. 1, pp. 137-141.

Chung, K. F. 2002, "Assessment and measurement of cough: the value of new tools", *Pulm.Pharmacol.Ther.*, vol. 15, no. 3, pp. 267-272.

Cockcroft, D. W., Jokic, R., Marciniuk, D. D., & Fitzpatrick, M. F. 1997, "The current dilemma with spirometric inclusion criteria for asthma drug trials", *Ann.Allergy Asthma Immunol.*, vol. 79, no. 3, pp. 226-228.

Corrao, W. M., Braman, S. S., & Irwin, R. S. 1979, "Chronic cough as the sole presenting manifestation of bronchial asthma", *N.Engl.J Med*, vol. 300, no. 12, pp. 633-637.

Coultas, D. B., Mapel, D., Gagnon, R., & Lydick, E. 2001, "The health impact of undiagnosed airflow obstruction in a national sample of United States adults", *Am J Respir Crit Care Med*, vol. 164, no. 3, pp. 372-377.

Cullinan, P. 1992, "Persistent cough and sputum: prevalence and clinical characteristics in south east England", *Respir.Med.*, vol. 86, no. 2, pp. 143-149.

Curley, F. J., Irwin, R. S., Pratter, M. R., Stivers, D. H., Doern, G. V., Vernaglia, P. A., Larkin, A. B., & Baker, S. P. 1988, "Cough and the common cold", *Am Rev.Respir Dis.*, vol. 138, no. 2, pp. 305-311.

Davis, S. F., Sutter, R. W., Strebel, P. M., Orton, C., Alexander, V., Sanden, G. N., Cassell, G. H., Thacker, W. L., & Cochi, S. L. 1995, "Concurrent outbreaks of pertussis and *Mycoplasma pneumoniae* infection: clinical and epidemiological characteristics of illnesses manifested by cough", *Clin.Infect.Dis.*, vol. 20, no. 3, pp. 621-628.

Dicpinigaitis, P. V., Dobkin, J. B., & Reichel, J. 2002, "Antitussive effect of the leukotriene receptor antagonist zafirlukast in subjects with cough-variant asthma", *J Asthma*, vol. 39, no. 4, pp. 291-297.

Doan, T., Patterson, R., & Greenberger, P. A. 1992, "Cough variant asthma: usefulness of a diagnostic-therapeutic trial with prednisone", *Ann.Allergy*, vol. 69, no. 6, pp. 505-509.

Doherty, M. J., Mister, R., Pearson, M. G., & Calverley, P. M. 2000a, "Capsaicin induced cough in cryptogenic fibrosing alveolitis", *Thorax*, vol. 55, no. 12, pp. 1028-1032.

Doherty, M. J., Mister, R., Pearson, M. G., & Calverley, P. M. 2000b, "Capsaicin responsiveness and cough in asthma and chronic obstructive pulmonary disease", *Thorax*, vol. 55, no. 8, pp. 643-649.

Eccles, R., Morris, S., & Jawad, M. 1992, "Lack of effect of codeine in the treatment of cough associated with acute upper respiratory tract infection", *J Clin.Pharm.Ther.*, vol. 17, no. 3, pp. 175-180.

Elliott, W. J. 1999, "Double-blind comparison of eprosartan and enalapril on cough and blood pressure in unselected hypertensive patients. Eprosartan Study Group", *J Hum.Hypertens.*, vol. 13, no. 6, pp. 413-417.

Fayers, P. M. & Machin D 2000, *Quality of life: assessment, analysis and interpretation.*, 1 edn, Wiley.

Ferrari, M., Olivieri, M., Sembenini, C., Benini, L., Zuccali, V., Bardelli, E., Bovo, P., Cavallini, G., Vantini, I., & Lo, C., V 1995, "Tussive effect of capsaicin in patients with gastroesophageal reflux without cough", *Am J Respir Crit Care Med*, vol. 151, no. 2 Pt 1, pp. 557-561.

Forsythe, P., McGarvey, L. P., Heaney, L. G., MacMahon, J., & Ennis, M. 2000, "Sensory neuropeptides induce histamine release from bronchoalveolar lavage

cells in both nonasthmatic coughers and cough variant asthmatics", *Clin.Exp.Allergy*, vol. 30, no. 2, pp. 225-232.

French, C. L., Irwin, R. S., Curley, F. J., & Krikorian, C. J. 1998, "Impact of chronic cough on quality of life", *Arch.Intern.Med*, vol. 158, no. 15, pp. 1657-1661.

French, C. T., Irwin, R. S., Fletcher, K. E., & Adams, T. M. 2002, "Evaluation of a cough-specific quality-of-life questionnaire", *Chest*, vol. 121, no. 4, pp. 1123-1131.

Fujimura, M., Kasahara, K., Kamio, Y., Naruse, M., Hashimoto, T., & Matsuda, T. 1996, "Female gender as a determinant of cough threshold to inhaled capsaicin", *Eur.Respir.J*, vol. 9, no. 8, pp. 1624-1626.

Fujimura, M., Ogawa, H., Nishizawa, Y., & Nishi, K. 2003, "Comparison of atopic cough with cough variant asthma: is atopic cough a precursor of asthma?", *Thorax*, vol. 58, no. 1, pp. 14-18.

Fujimura, M., Sakamoto, S., & Matsuda, T. 1992, "Bronchodilator-resistive cough in atopic patients: bronchial reversibility and hyperresponsiveness", *Intern.Med*, vol. 31, no. 4, pp. 447-452.

Fuller, R. W. & Jackson, D. M. 1990, "Physiology and treatment of cough", *Thorax*, vol. 45, no. 6, pp. 425-430.

Gabbay, E., Tarala, R., Will, R., Carroll, G., Adler, B., Cameron, D., & Lake, F. R. 1997, "Interstitial lung disease in recent onset rheumatoid arthritis", *Am.J.Respir.Crit Care Med.*, vol. 156, no. 2 Pt 1, pp. 528-535.

Geddes, D. M., Corrin, B., Brewerton, D. A., Davies, R. J., & Turner-Warwick, M. 1977, "Progressive airway obliteration in adults and its association with rheumatoid disease", *Q.J Med*, vol. 46, no. 184, pp. 427-444.

Gibson, P. G., Dolovich, J., Denburg, J., Ramsdale, E. H., & Hargreave, F. E. 1989, "Chronic cough: eosinophilic bronchitis without asthma", *Lancet*, vol. 1, no. 8651, pp. 1346-1348.

Gibson, P. G., Hargreave, F. E., Girgis-Gabardo, A., Morris, M., Denburg, J. A., & Dolovich, J. 1995, "Chronic cough with eosinophilic bronchitis: examination for variable airflow obstruction and response to corticosteroid", *Clin.Exp.Allergy*, vol. 25, no. 2, pp. 127-132.

Gibson, P. G., Zlatic, K., Scott, J., Sewell, W., Woolley, K., & Saltos, N. 1998, "Chronic cough resembles asthma with IL-5 and granulocyte-macrophage colony-stimulating factor gene expression in bronchoalveolar cells", *J Allergy Clin.Immunol.*, vol. 101, no. 3, pp. 320-326.

Glauser, F. L. 1972, "Variant asthma", *Ann.Allergy*, vol. 30, no. 8, pp. 457-459.

Green, R. H., Brightling, C. E., McKenna, S., Hargadon, B., Parker, D., Bradding, P., Wardlaw, A. J., & Pavord, I. D. 2002, "Asthma exacerbations and sputum

- eosinophil counts: a randomised controlled trial", *Lancet*, vol. 360, no. 9347, pp. 1715-1721.
- Guyatt, G. H., Bombardier, C., & Tugwell, P. X. 1986, "Measuring disease-specific quality of life in clinical trials", *CMAJ*, vol. 134, no. 8, pp. 889-895.
- Hancox, R. J., Leigh, R., Kelly, M. M., & Hargreave, F. E. 2001, "Eosinophilic bronchitis", *Lancet*, vol. 358, no. 9287, p. 1104.
- Hansell, D. M. 2001, "Small airways diseases: detection and insights with computed tomography", *Eur.Respir J*, vol. 17, no. 6, pp. 1294-1313.
- Harvey, R. F. & Bradshaw, J. M. 1980, "A simple index of Crohn's-disease activity", *Lancet*, vol. 1, no. 8167, p. 514.
- Higginson, I. J. & Carr, A. J. 2001, "Measuring quality of life: Using quality of life measures in the clinical setting", *BMJ*, vol. 322, no. 7297, pp. 1297-1300.
- Hoek, G., Fischer, P., Van Den, B. P., Goldbohm, S., & Brunekreef, B. 2001, "Estimation of long-term average exposure to outdoor air pollution for a cohort study on mortality", *J Expo.Anal.Environ.Epidemiol.*, vol. 11, no. 6, pp. 459-469.
- Homma, H., Yamanaka, A., Tanimoto, S., Tamura, M., Chijimatsu, Y., Kira, S., & Izumi, T. 1983, "Diffuse panbronchiolitis. A disease of the transitional zone of the lung", *Chest*, vol. 83, no. 1, pp. 63-69.
- Hsu, J. Y., Stone, R. A., Logan-Sinclair, R. B., Worsdell, M., Busst, C. M., & Chung, K. F. 1994, "Coughing frequency in patients with persistent cough: assessment using a 24 hour ambulatory recorder", *Eur.Respir J*, vol. 7, no. 7, pp. 1246-1253.
- Hubbard, R., Lewis, S., Richards, K., Johnston, I., & Britton, J. 1996, "Occupational exposure to metal or wood dust and aetiology of cryptogenic fibrosing alveolitis", *Lancet*, vol. 347, no. 8997, pp. 284-289.
- Hunter, C. J., Brightling, C. E., Woltmann, G., Wardlaw, A. J., & Pavord, I. D. 2002, "A comparison of the validity of different diagnostic tests in adults with asthma", *Chest*, vol. 121, no. 4, pp. 1051-1057.
- Hunter, C. J., Ward, R., Woltmann, G., Wardlaw, A. J., & Pavord, I. D. 1999, "The safety and success rate of sputum induction using a low output ultrasonic nebuliser", *Respir.Med.*, vol. 93, no. 5, pp. 345-348.
- Hwang, S. W. & Oh, U. 2002, "Hot channels in airways: pharmacology of the vanilloid receptor", *Curr.Opin.Pharmacol.*, vol. 2, no. 3, pp. 235-242.
- Ing, A. J., Ngu, M. C., & Breslin, A. B. 1992, "A randomised double-blind placebo controlled crossover study of ranitidine in patients with chronic persistent cough associated with gastroesophageal reflux.", *Am Rev.Respir Dis.*, vol. 145, p. A11.

Ing, A. J., Ngu, M. C., & Breslin, A. B. 1994, "Pathogenesis of chronic persistent cough associated with gastroesophageal reflux", *Am J Respir Crit Care Med*, vol. 149, no. 1, pp. 160-167.

Inman, W. H. 1986, "Enalapril-induced cough", *Lancet*, vol. 2, no. 8517, p. 1218.

Inui, N., Chida, K., Suda, T., & Nakamura, H. 2001, "TH1/TH2 and TC1/TC2 profiles in peripheral blood and bronchoalveolar lavage fluid cells in pulmonary sarcoidosis", *J.Allergy Clin.Immunol.*, vol. 107, no. 2, pp. 337-344.

Irwin, R. S., Boulet, L. P., Cloutier, M. M., Fuller, R., Gold, P. M., Hoffstein, V., Ing, A. J., McCool, F. D., O'Byrne, P., Poe, R. H., Prakash, U. B., Pratter, M. R., & Rubin, B. K. 1998, "Managing cough as a defense mechanism and as a symptom. A consensus panel report of the American College of Chest Physicians", *Chest*, vol. 114, no. 2 Suppl Managing, pp. 133S-181S.

Irwin, R. S., Corrao, W. M., & Pratter, M. R. 1981, "Chronic persistent cough in the adult: the spectrum and frequency of causes and successful outcome of specific therapy", *Am.Rev.Respir.Dis.*, vol. 123, no. 4 Pt 1, pp. 413-417.

Irwin, R. S., Curley, F. J., & French, C. L. 1990, "Chronic cough. The spectrum and frequency of causes, key components of the diagnostic evaluation, and outcome of specific therapy", *Am Rev.Respir Dis.*, vol. 141, no. 3, pp. 640-647.

Irwin, R. S., French, C. L., Curley, F. J., Zawacki, J. K., & Bennett, F. M. 1993, "Chronic cough due to gastroesophageal reflux. Clinical, diagnostic, and pathogenetic aspects", *Chest*, vol. 104, no. 5, pp. 1511-1517.

Irwin, R. S., French, C. T., Smyrniotis, N. A., & Curley, F. J. 1997, "Interpretation of positive results of a methacholine inhalation challenge and 1 week of inhaled bronchodilator use in diagnosing and treating cough-variant asthma", *Arch.Intern.Med*, vol. 157, no. 17, pp. 1981-1987.

Irwin, R. S. & Madison, J. M. 2000, "The diagnosis and treatment of cough", *N.Engl.J Med*, vol. 343, no. 23, pp. 1715-1721.

Irwin, R. S. & Madison, J. M. 2002, "Diagnosis and treatment of chronic cough due to gastro-esophageal reflux disease and postnasal drip syndrome", *Pulm.Pharmacol.Ther.*, vol. 15, no. 3, pp. 261-266.

Irwin, R. S., Pratter, M. R., & Hamolsky, M. W. 1982, "Chronic persistent cough: an uncommon presenting complaint of thyroiditis", *Chest*, vol. 81, no. 3, pp. 386-388.

Irwin, R. S., Zawacki, J. K., Curley, F. J., French, C. L., & Hoffman, P. J. 1989, "Chronic cough as the sole presenting manifestation of gastroesophageal reflux", *Am Rev.Respir Dis.*, vol. 140, no. 5, pp. 1294-1300.

Irwin, R. S., Zawacki, J. K., Wilson, M. M., French, C. T., & Callery, M. P. 2002, "Chronic cough due to gastroesophageal reflux disease: failure to resolve despite

total/near-total elimination of esophageal acid", *Chest*, vol. 121, no. 4, pp. 1132-1140.

Jack, C. I., Walshaw, M. J., Tran, J., Hind, C. R., & Evans, C. C. 1994, "Twenty-four-hour tracheal pH monitoring--a simple and non-hazardous investigation", *Respir Med*, vol. 88, no. 6, pp. 441-444.

Janson, C., Chinn, S., Jarvis, D., & Burney, P. 2001a, "Determinants of cough in young adults participating in the European Community Respiratory Health Survey", *Eur.Respir.J.*, vol. 18, no. 4, pp. 647-654.

Janson, C., Chinn, S., Jarvis, D., Zock, J. P., Toren, K., & Burney, P. 2001b, "Effect of passive smoking on respiratory symptoms, bronchial responsiveness, lung function, and total serum IgE in the European Community Respiratory Health Survey: a cross-sectional study", *Lancet*, vol. 358, no. 9299, pp. 2103-2109.

Jatakanon, A., Laloo, U. G., Lim, S., Chung, K. F., & Barnes, P. J. 1999, "Increased neutrophils and cytokines, TNF-alpha and IL-8, in induced sputum of non-asthmatic patients with chronic dry cough", *Thorax*, vol. 54, no. 3, pp. 234-237.

Johnson, D. & Osborn, L. M. 1991, "Cough variant asthma: a review of the clinical literature", *J Asthma*, vol. 28, no. 2, pp. 85-90.

Johnston, I. D., Strachan, D. P., & Anderson, H. R. 1998, "Effect of pneumonia and whooping cough in childhood on adult lung function", *N.Engl.J Med*, vol. 338, no. 9, pp. 581-587.

Jones, P. W., Quirk, F. H., Baveystock, C. M., & Littlejohns, P. 1992, "A self-complete measure of health status for chronic airflow limitation. The St. George's Respiratory Questionnaire", *Am Rev.Respir Dis.*, vol. 145, no. 6, pp. 1321-1327.

Juniper, E. F. 1997, "Health-related quality of life," in *Asthma*, 1 edn, P. J. Barnes et al., eds., Lippincott-Raven Publishers, Philadelphia, pp. 1487-1497.

Juniper, E. F., Cockcroft, D. W., & Hargreave, F. E. 1994, *Histamine and methacholine inhalation tests: a laboratory tidal breathing protocol.*, second edn, Astra Draco AB, Lund, Sweden.

Juniper, E. F., Guyatt, G. H., Ferrie, P. J., & Griffith, L. E. 1993, "Measuring quality of life in asthma", *Am Rev.Respir Dis.*, vol. 147, no. 4, pp. 832-838.

Juniper, E. F., Guyatt, G. H., Streiner, D. L., & King, D. R. 1997, "Clinical impact versus factor analysis for quality of life questionnaire construction", *J Clin.Epidemiol.*, vol. 50, no. 3, pp. 233-238.

Kanazawa, H., Hirata, K., & Yoshikawa, J. 2003, "Accelerated decline of lung function in COPD patients with chronic hepatitis C virus infection: a preliminary study based on small numbers of patients", *Chest*, vol. 123, no. 2, pp. 596-599.

Kastelik, J. A., Redington, A. E., Aziz, I., Buckton, G. K., Smith, C. M., Dakkak, M., & Morice, A. H. 2003, "Abnormal oesophageal motility in patients with chronic cough", *Thorax*, vol. 58, no. 8, pp. 699-702.

Kharitonov, S. A., Wells, A. U., O'Connor, B. J., Cole, P. J., Hansell, D. M., Logan-Sinclair, R. B., & Barnes, P. J. 1995, "Elevated levels of exhaled nitric oxide in bronchiectasis", *Am J Respir Crit Care Med*, vol. 151, no. 6, pp. 1889-1893.

Kharitonov, S. A., Yates, D., & Barnes, P. J. 1995, "Increased nitric oxide in exhaled air of normal human subjects with upper respiratory tract infections", *Eur. Respir J*, vol. 8, no. 2, pp. 295-297.

Kiljander, T. O., Salomaa, E. R., Hietanen, E. K., & Terho, E. O. 2000, "Chronic cough and gastro-oesophageal reflux: a double-blind placebo-controlled study with omeprazole", *Eur. Respir J*, vol. 16, no. 4, pp. 633-638.

Koch, T. R. 2001, "NSAID colitis--resolving a diagnostic dilemma?", *Am J Gastroenterol.*, vol. 96, no. 3, pp. 914-915.

Kraft, M., Mortenson, R. L., Colby, T. V., Newman, L., Waldron, J. A., Jr., & King, T. E., Jr. 1993, "Cryptogenic constrictive bronchiolitis. A clinicopathologic study", *Am Rev. Respir Dis.*, vol. 148, no. 4 Pt 1, pp. 1093-1101.

Laitinen, L. A., Laitinen, A., Haahtela, T., Vilkkä, V., Spur, B. W., & Lee, T. H. 1993, "Leukotriene E4 and granulocytic infiltration into asthmatic airways", *Lancet*, vol. 341, no. 8851, pp. 989-990.

Lalloo, U. G., Barnes, P. J., & Chung, K. F. 1996, "Pathophysiology and clinical presentations of cough", *J Allergy Clin. Immunol.*, vol. 98, no. 5 Pt 2, p. S91-S96.

Lee, S. Y., Cho, J. Y., Shim, J. J., Kim, H. K., Kang, K. H., Yoo, S. H., & In, K. H. 2001, "Airway inflammation as an assessment of chronic nonproductive cough", *Chest*, vol. 120, no. 4, pp. 1114-1120.

Liu, M. C., Bleecker, E. R., Lichtenstein, L. M., Kagey-Sobotka, A., Niv, Y., McLemore, T. L., Permutt, S., Proud, D., & Hubbard, W. C. 1990, "Evidence for elevated levels of histamine, prostaglandin D2, and other bronchoconstricting prostaglandins in the airways of subjects with mild asthma", *Am. Rev. Respir. Dis.*, vol. 142, no. 1, pp. 126-132.

Loudon, R. G. & Brown, L. C. 1967, "Cough frequency in patients with respiratory disease", *Am Rev. Respir Dis.*, vol. 96, no. 6, pp. 1137-1143.

Lunde, H., Hedner, T., Samuelsson, O., Lotvall, J., Andren, L., Lindholm, L., & Wiholm, B. E. 1994, "Dyspnoea, asthma, and bronchospasm in relation to treatment with angiotensin converting enzyme inhibitors", *BMJ*, vol. 308, no. 6920, pp. 18-21.

Mackay, I. R. 2000, "Science, medicine, and the future: Tolerance and autoimmunity", *BMJ*, vol. 321, no. 7253, pp. 93-96.

Matos, S., Birring, S. S., Patel, R. B., Evans, D. E., & Pavord, I. D. 2003, "Day time cough counts using the Leicester Cough Monitor (LCM) in patients with chronic cough and healthy controls", *Thorax*, vol. 58, p. iii48.

McEwan, J. R., Choudry, N., Street, R., & Fuller, R. W. 1989, "Change in cough reflex after treatment with enalapril and ramipril", *BMJ*, vol. 299, no. 6690, pp. 13-16.

McEwan, J. R., Choudry, N. B., & Fuller, R. W. 1990, "The effect of sulindac on the abnormal cough reflex associated with dry cough", *J Pharmacol.Exp.Ther.*, vol. 255, no. 1, pp. 161-164.

McGarvey, L. & Morice, A. H. 2003, "Atopic cough: little evidence to support a new clinical entity", *Thorax*, vol. 58, no. 8, pp. 736-737.

McGarvey, L. P., Forsythe, P., Heaney, L. G., MacMahon, J., & Ennis, M. 1999, "Bronchoalveolar lavage findings in patients with chronic nonproductive cough", *Eur.Respir J*, vol. 13, no. 1, pp. 59-65.

McGarvey, L. P., Heaney, L. G., Lawson, J. T., Johnston, B. T., Scally, C. M., Ennis, M., Shepherd, D. R., & MacMahon, J. 1998, "Evaluation and outcome of patients with chronic non-productive cough using a comprehensive diagnostic protocol", *Thorax*, vol. 53, no. 9, pp. 738-743.

McGarvey, L. P., Heaney, L. G., & MacMahon, J. 1998, "A retrospective survey of diagnosis and management of patients presenting with chronic cough to a general chest clinic", *Int.J Clin.Pract.*, vol. 52, no. 3, pp. 158-161.

McKeever, T. M., Scrivener, S., Broadfield, E., Jones, Z., Britton, J., & Lewis, S. A. 2002, "Prospective study of diet and decline in lung function in a general population", *Am J Respir Crit Care Med*, vol. 165, no. 9, pp. 1299-1303.

Mello, C. J., Irwin, R. S., & Curley, F. J. 1996, "Predictive values of the character, timing, and complications of chronic cough in diagnosing its cause", *Arch.Intern.Med*, vol. 156, no. 9, pp. 997-1003.

Monson, R. R. 1970, "Duodenal ulcer as a second disease", *Gastroenterology*, vol. 59, no. 5, pp. 712-716.

Morice, A. H. & Kastelik, J. A. 2003, "Cough * 1: Chronic cough in adults", *Thorax*, vol. 58, no. 10, pp. 901-907.

Morice, A. H., Kastelik, J. A., & Thompson, R. 2001, "Cough challenge in the assessment of cough reflex", *Br.J.Clin.Pharmacol.*, vol. 52, no. 4, pp. 365-375.

Morice, A. H., Lowry, R., Brown, M. J., & Higenbottam, T. 1987, "Angiotensin-converting enzyme and the cough reflex", *Lancet*, vol. 2, no. 8568, pp. 1116-1118.

Moy, M. L., Israel, E., Weiss, S. T., Juniper, E. F., Dube, L., & Drazen, J. M. 2001, "Clinical predictors of health-related quality of life depend on asthma severity", *Am J Respir Crit Care Med*, vol. 163, no. 4, pp. 924-929.

Mund, E., Christensson, B., Larsson, K., & Gronneberg, R. 2001, "Sex dependent differences in physiological ageing in the immune system of lower airways in healthy non-smoking volunteers: study of lymphocyte subsets in bronchoalveolar lavage fluid and blood", *Thorax*, vol. 56, no. 6, pp. 450-455.

Newball, H. H. & Brahim, S. A. 1977, "Chronic obstructive airway disease in patients with Sjogren's syndrome", *Am Rev.Respir Dis.*, vol. 115, no. 2, pp. 295-304.

Niimi, A., Amitani, R., Suzuki, K., Tanaka, E., Murayama, T., & Kuze, F. 1998, "Eosinophilic inflammation in cough variant asthma", *Eur.Respir J*, vol. 11, no. 5, pp. 1064-1069.

Niimi, A., Matsumoto, H., Minakuchi, M., Kitaichi, M., & Amitani, R. 2000, "Airway remodelling in cough-variant asthma", *Lancet*, vol. 356, no. 9229, pp. 564-565.

Nogrady, S. G., Evans, W. V., & Davies, B. H. 1978, "Reversibility of airways obstruction in bronchiectasis", *Thorax*, vol. 33, no. 5, pp. 635-637.

Novitsky, Y. W., Zawacki, J. K., Irwin, R. S., French, C. T., Hussey, V. M., & Callery, M. P. 2002, "Chronic cough due to gastroesophageal reflux disease: efficacy of antireflux surgery", *Surg.Endosc.*, vol. 16, no. 4, pp. 567-571.

O'Brien, C., Guest, P. J., Hill, S. L., & Stockley, R. A. 2000, "Physiological and radiological characterisation of patients diagnosed with chronic obstructive pulmonary disease in primary care", *Thorax*, vol. 55, no. 8, pp. 635-642.

O'Connell, F., Springall, D. R., Moradoghli-Haftvani, A., Krausz, T., Price, D., Fuller, R. W., Polak, J. M., & Pride, N. B. 1995, "Abnormal intraepithelial airway nerves in persistent unexplained cough?", *Am J Respir Crit Care Med*, vol. 152, no. 6 Pt 1, pp. 2068-2075.

O'Connell, F., Thomas, V. E., Pride, N. B., & Fuller, R. W. 1994, "Capsaicin cough sensitivity decreases with successful treatment of chronic cough", *Am J Respir Crit Care Med*, vol. 150, no. 2, pp. 374-380.

Orejas Garcia, C. & Pascual Pascual, T. 1998, "Cough variant asthma: Clinical and functional characteristics. Report of 63 cases.", *Arch bronchoneumol*, vol. 34, pp. 232-236.

Ours, T. M., Kavuru, M. S., Schilz, R. J., & Richter, J. E. 1999, "A prospective evaluation of esophageal testing and a double-blind, randomized study of omeprazole in a diagnostic and therapeutic algorithm for chronic cough", *Am J Gastroenterol.*, vol. 94, no. 11, pp. 3131-3138.

Palombini, B. C., Villanova, C. A., Araujo, E., Gastal, O. L., Alt, D. C., Stolz, D. P., & Palombini, C. O. 1999, "A pathogenic triad in chronic cough: asthma, postnasal drip syndrome, and gastroesophageal reflux disease", *Chest*, vol. 116, no. 2, pp. 279-284.

- Parameswaran, K., Anvari, M., Efthimiadis, A., Kamada, D., Hargreave, F. E., & Allen, C. J. 2000, "Lipid-laden macrophages in induced sputum are a marker of oropharyngeal reflux and possible gastric aspiration", *Eur.Respir J*, vol. 16, no. 6, pp. 1119-1122.
- Parle, J. V., Maisonneuve, P., Sheppard, M. C., Boyle, P., & Franklyn, J. A. 2001, "Prediction of all-cause and cardiovascular mortality in elderly people from one low serum thyrotropin result: a 10-year cohort study", *Lancet*, vol. 358, no. 9285, pp. 861-865.
- Pauwels, R. A., Buist, A. S., Calverley, P. M., Jenkins, C. R., & Hurd, S. S. 2001, "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.*, vol. 163, no. 5, pp. 1256-1276.
- Pavord, I. D., Brightling, C. E., Woltmann, G., & Wardlaw, A. J. 1999a, "Non-eosinophilic corticosteroid unresponsive asthma", *Lancet*, vol. 353, no. 9171, pp. 2213-2214.
- Pavord, I. D., Pizzichini, M. M., Pizzichini, E., & Hargreave, F. E. 1997, "The use of induced sputum to investigate airway inflammation", *Thorax*, vol. 52, no. 6, pp. 498-501.
- Pavord, I. D., Ward, R., Woltmann, G., Wardlaw, A. J., Sheller, J. R., & Dworski, R. 1999b, "Induced sputum eicosanoid concentrations in asthma", *Am.J.Respir.Crit Care Med.*, vol. 160, no. 6, pp. 1905-1909.
- Phillips, M. S., Miller, M. R., Kinnear, W. J., Gough, S. E., & Shneerson, J. M. 1987, "Importance of airflow obstruction after thoracoplasty", *Thorax*, vol. 42, no. 5, pp. 348-352.
- Pizzichini, E., Pizzichini, M. M., Efthimiadis, A., Evans, S., Morris, M. M., Squillace, D., Gleich, G. J., Dolovich, J., & Hargreave, F. E. 1996, "Indices of airway inflammation in induced sputum: reproducibility and validity of cell and fluid-phase measurements", *Am.J.Respir.Crit Care Med.*, vol. 154, no. 2 Pt 1, pp. 308-317.
- Pizzichini, E., Pizzichini, M. M., Gibson, P., Parameswaran, K., Gleich, G. J., Berman, L., Dolovich, J., & Hargreave, F. E. 1998, "Sputum eosinophilia predicts benefit from prednisone in smokers with chronic obstructive bronchitis", *Am J Respir Crit Care Med*, vol. 158, no. 5 Pt 1, pp. 1511-1517.
- Pizzichini, M. M., Pizzichini, E., Clelland, L., Efthimiadis, A., Mahony, J., Dolovich, J., & Hargreave, F. E. 1997, "Sputum in severe exacerbations of asthma: kinetics of inflammatory indices after prednisone treatment", *Am.J.Respir.Crit Care Med.*, vol. 155, no. 5, pp. 1501-1508.
- Poe, R. H., Harder, R. V., Israel, R. H., & Kallay, M. C. 1989, "Chronic persistent cough. Experience in diagnosis and outcome using an anatomic diagnostic protocol", *Chest*, vol. 95, no. 4, pp. 723-728.

Poe, R. H., Israel, R. H., Utell, M. J., & Hall, W. J. 1982, "Chronic cough: bronchoscopy or pulmonary function testing?", *Am Rev. Respir Dis.*, vol. 126, no. 1, pp. 160-162.

Pozzilli, P., Carotenuto, P., & Delitala, G. 1994, "Lymphocytic traffic and homing into target tissue and the generation of endocrine autoimmunity", *Clin. Endocrinol. (Oxf)*, vol. 41, no. 5, pp. 545-554.

Pratter, M. R., Bartter, T., Akers, S., & DuBois, J. 1993, "An algorithmic approach to chronic cough", *Ann. Intern. Med.*, vol. 119, no. 10, pp. 977-983.

Prime, F. J. 1961, "The assessment of antitussive drugs in man.", *BMJ*, vol. 22, pp. 1149-1151.

Prudon, B., Birring, S. S., Vara, D. D., Hall, A. P., Thompson, J. P., & Pavord, I. D. Cough and glottic stop reflex sensitivity in health and disease. *Chest* . 2004.
Ref Type: In Press

Quanjer, P. H., Tammeling, G. J., Cotes, J. E., Pedersen, O. F., Peslin, R., & Yernault, J. C. 1993, "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*, vol. 16, pp. 5-40.

Rafferty, P. 1990, "Antihistamines in the treatment of clinical asthma", *J. Allergy Clin. Immunol.*, vol. 86, no. 4 Pt 2, pp. 647-650.

Rafferty, P., Jackson, L., Smith, R., & Holgate, S. T. 1990, "Terfenadine, a potent histamine H1-receptor antagonist in the treatment of grass pollen sensitive asthma", *Br. J. Clin. Pharmacol.*, vol. 30, no. 2, pp. 229-235.

Rolla, G., Colagrande, P., Magnano, M., Debernardi, V., Dutto, L., Delpiano, L., Cassolino, P., & Bucca, C. 1998, "Extrathoracic airway dysfunction in cough associated with gastroesophageal reflux", *J Allergy Clin. Immunol.*, vol. 102, no. 2, pp. 204-209.

Rose, N. R. & McKay, I. R. 1998, *The autoimmune diseases*, 3 edn, Academic press, San Diego.

Rossi, D. & Zlotnik, A. 2000, "The biology of chemokines and their receptors", *Annu. Rev. Immunol.*, vol. 18, pp. 217-242.

Sandford, A. J., Chagani, T., Weir, T. D., Connett, J. E., Anthonisen, N. R., & Pare, P. D. 2001, "Susceptibility genes for rapid decline of lung function in the lung health study", *Am J Respir Crit Care Med*, vol. 163, no. 2, pp. 469-473.

Schulman, E. S., MacGlashan, D. W., Jr., Schleimer, R. P., Peters, S. P., Kagey-Sobotka, A., Newball, H. H., & Lichtenstein, L. M. 1983, "Purified human basophils and mast cells: current concepts of mediator release", *Eur. J. Respir. Dis. Suppl*, vol. 128 (Pt 1), pp. 53-61.

Sesoko, S. & Kaneko, Y. 1985, "Cough associated with the use of captopril", *Arch Intern. Med.*, vol. 145, no. 8, p. 1524.

Sevelius, H. & Colmore, J. P. 1966, "Objective assessment of antitussive agents in patients with chronic cough", *J New Drugs*, vol. 6, no. 4, pp. 216-223.

Sharif, S., Arreaza, G. A., Zucker, P., & Delovitch, T. L. 2002, "Regulatory natural killer T cells protect against spontaneous and recurrent type 1 diabetes", *Ann.N.Y.Acad.Sci.*, vol. 958, pp. 77-88.

Simpson, G. 1999, "Investigation and management of persistent dry cough", *Thorax*, vol. 54, no. 5, pp. 469-470.

Sladek, K., Dworski, R., Soja, J., Sheller, J. R., Nizankowska, E., Oates, J. A., & Szczeklik, A. 1994, "Eicosanoids in bronchoalveolar lavage fluid of aspirin-intolerant patients with asthma after aspirin challenge", *Am.J.Respir.Crit Care Med.*, vol. 149, no. 4 Pt 1, pp. 940-946.

Smyrnios, N. A., Irwin, R. S., & Curley, F. J. 1995, "Chronic cough with a history of excessive sputum production. The spectrum and frequency of causes, key components of the diagnostic evaluation, and outcome of specific therapy", *Chest*, vol. 108, no. 4, pp. 991-997.

Tager, I., Tishler, P. V., Rosner, B., Speizer, F. E., & Litt, M. 1978, "Studies of the familial aggregation of chronic bronchitis and obstructive airways disease", *Int.J.Epidemiol.*, vol. 7, no. 1, pp. 55-62.

Takayanagi, N., Kanazawa, M., Kawabata, Y., & Colby, T. V. 2001, "Chronic bronchiolitis with associated eosinophilic lung disease (eosinophilic bronchiolitis)", *Respiration*, vol. 68, no. 3, pp. 319-322.

Tanaka, S., Hirata, K., Kurihara, N., Yoshikawa, J., & Takeda, T. 1996, "Effect of loratadine, an H1 antihistamine, on induced cough in non-asthmatic patients with chronic cough", *Thorax*, vol. 51, no. 8, pp. 810-814.

Thomas RA, Green, R. H., Brightling, C. E., Birring S.S., & Pavord ID. The influence of age on induced sputum differential cell counts in normal subjects. *Thorax* 56, iii79. 2001.

Ref Type: Abstract

Tiroke, A. H., Bewig, B., & Haverich, A. 1999, "Bronchoalveolar lavage in lung transplantation. State of the art", *Clin.Transplant.*, vol. 13, no. 2, pp. 131-157.

Turton, C. W., Williams, G., & Green, M. 1981, "Cryptogenic obliterative bronchiolitis in adults", *Thorax*, vol. 36, no. 11, pp. 805-810.

Vaezi, M. F. & Richter, J. E. 1997, "Twenty-four-hour ambulatory esophageal pH monitoring in the diagnosis of acid reflux-related chronic cough", *South.Med J*, vol. 90, no. 3, pp. 305-311.

Venn, A. J., Lewis, S. A., Cooper, M., Hubbard, R., & Britton, J. 2001, "Living near a main road and the risk of wheezing illness in children", *Am J Respir Crit Care Med*, vol. 164, no. 12, pp. 2177-2180.

Vergnenegre, A., Pugnere, N., Antonini, M. T., Arnaud, M., Melloni, B., Treves, R., & Bonnaud, F. 1997, "Airway obstruction and rheumatoid arthritis", *Eur. Respir J*, vol. 10, no. 5, pp. 1072-1078.

von Andrian, U. H. & Mackay, C. R. 2000, "T-cell function and migration. Two sides of the same coin", *N. Engl. J Med*, vol. 343, no. 14, pp. 1020-1034.

Wahlstrom, J., Berlin, M., Lundgren, R., Olerup, O., Wigzell, H., Eklund, A., & Grunewald, J. 1997, "Lung and blood T-cell receptor repertoire in extrinsic allergic alveolitis", *Eur. Respir. J.*, vol. 10, no. 4, pp. 772-779.

Wallaert, B., Colombel, J. F., Tonnel, A. B., Bonniere, P., Cortot, A., Paris, J. C., & Voisin, C. 1985, "Evidence of lymphocyte alveolitis in Crohn's disease", *Chest*, vol. 87, no. 3, pp. 363-367.

Wallaert, B., Prin, L., Hatron, P. Y., Ramon, P., Tonnel, A. B., & Voisin, C. 1987, "Lymphocyte subpopulations in bronchoalveolar lavage in Sjogren's syndrome. Evidence for an expansion of cytotoxic/suppressor subset in patients with alveolar neutrophilia", *Chest*, vol. 92, no. 6, pp. 1025-1031.

Walmsley, R. S., Ayres, R. C., Pounder, R. E., & Allan, R. N. 1998, "A simple clinical colitis activity index", *Gut*, vol. 43, no. 1, pp. 29-32.

Waring, J. P., Lacayo, L., Hunter, J., Katz, E., & Suwak, B. 1995, "Chronic cough and hoarseness in patients with severe gastroesophageal reflux disease. Diagnosis and response to therapy", *Dig. Dis. Sci.*, vol. 40, no. 5, pp. 1093-1097.

Whittemore, A. S., Perlin, S. A., & DiCiccio, Y. 1995, "Chronic obstructive pulmonary disease in lifelong nonsmokers: results from NHANES", *Am J Public Health*, vol. 85, no. 5, pp. 702-706.

Widdicombe, J. G. 1995, "Neurophysiology of the cough reflex", *Eur. Respir J*, vol. 8, no. 7, pp. 1193-1202.

Wilsher, M. L., Hallows, M., & Birchall, N. M. 1995, "Gamma/delta T lymphocytes in the blood of patients with sarcoidosis", *Thorax*, vol. 50, no. 8, pp. 858-862.

Wolfe, J. E., Bone, R. C., & Ruth, W. E. 1976, "Diagnosis of gastric aspiration by fiberoptic bronchoscopy", *Chest*, vol. 70, no. 4, pp. 458-459.

World Health Organisation 1947, "Constitution of the World Health Organisation.", *Geneva, WHO*.

Wright, J. L., Cagle, P., Churg, A., Colby, T. V., & Myers, J. 1992, "Diseases of the small airways", *Am. Rev. Respir. Dis.*, vol. 146, no. 1, pp. 240-262.

Yeo, W. W., Foster, G., & Ramsay, L. E. 1991, "Prevalence of persistent cough during long-term enalapril treatment: controlled study versus nifedipine", *Q.J Med*, vol. 80, no. 293, pp. 763-770.

Yosipovitch, G., Greaves, M. W., & Schmelz, M. 2003, "Itch", *Lancet*, vol. 361, no. 9358, pp. 690-694.

Yousem, S. A. 1993, "Lymphocytic bronchitis/bronchiolitis in lung allograft recipients", *Am J Surg. Pathol.*, vol. 17, no. 5, pp. 491-496.