Effect of inhaled corticosteroids on symptom severity and sputum mediator levels in chronic persistent cough

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DECLARATION

I am the sole author of this thesis and I have personally consulted all the references listed. The work was undertaken by me in the Department of Respiratory Medicine, Gartnavel General Hospital and the Western Infirmary, Glasgow. Laboratory processing of samples (cell counts and mediator levels) was performed by my colleague Kirsten Macleod in the Department of Immunology, Western Infirmary, Glasgow. Dr.Alex D. McMahon of the Robertson Centre for Biostatistics at the University of Glasgow, provided assistance with statistical analysis.

The thesis has not previously been submitted for a higher degree.

My family, Abhijit, Ma and Baba have been very tolerant and endured several cold dinners during the writing of this thesis! My children, Radhika and Abhishek have been exceptionally understanding and I now hope to fulfil their list of exciting plans made for "after mum's MD!!" My friends, Uma, Nora and Sujata have all been extremely kind during these last few difficult months and I cannot thank them enough for just 'being there!'

To my father, who has taught me that one should only work with sincerity and honesty and never for a reward or outcome: I hope I can live up to your example. Finally, I dedicate this thesis to my wonderful mother, who did not stay in this world long enough to see it completed. There are no words that can express my gratitude as if it wasn't for her, let alone this thesis, I would not be here today.

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PUBLICATIONS/PRESENTATIONS ARISING FROM THIS THESIS

Papers published

1. Journal of Allergy and Clinical Immunology.

Chaudhuri R, McMahon AD, Thomson LJ, MacLeod KJ, McSharry CP, Livingston E, McKay A, Thomson NC.

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Oral presentation at the American Thoracic Society Conference, Orlando, 2004

2. Chaudhuri R, Thomson L, MacLeod K, McSharry C, McKay A, Christie H, Thomson NC. The role of non-invasive investigations in predicting the response to inhaled steroids in chronic cough.
Oral presentation at the American Thoracic Society Conference, Atlanta 2002

3. Chaudhuri R, McMahon AD, Thomson L, McSharry C, MacLeod K, Livingston E, Thomson NC. Role of Neurotrophins in Chronic Persistent Cough. *Poster presentation at the American Thoracic Society Conference, Orlando, 2004*

4. Chaudhuri R, McMahon AD, Thomson L, Livingston E, McSharry C, Thomson NC. Change in Quality of Life following treatment of Chronic Cough *Poster presentation at the American Thoracic Society Conference, Orlando, 2004*

5. Chaudhuri R, Thomson L, MacLeod K, McSharrry C, Livingston E, McKay A, McMahon AD, Thomson NC. Effect of inhaled corticosteroids on induced sputum inflammatory mediator levels in chronic cough. *Poster presentation at the American Thoracic Society Conference, Seattle 2003.*

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7. Chaudhuri R, MacLeod K, McSharry C, Thomson L, McKay A, Christie H, Thomson NC. Induced sputum leukotriene concentrations in Chronic Cough. *Poster presentation at the British Thoracic Society, Winter Meeting, London 2001*

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10. Chaudhuri R, Thomson L, MacLeod K, McSharry C, McKay A, Christie H, Thomson NC. Non-invasive investigations as predictors of steroid responsiveness in chronic cough.
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LIST OF ABBREVIATIONS USED (in alphabetical order)

BDNF: brain derived neurotrophic factor

Bronchiec: bronchiectasis

CO: carbon monoxide

CVA: cough variant asthma

Cys-LT: cysteinyl leukotrienes

ECP: eosinophil cationic protein

eNO: exhaled nitric oxide

FEV_1 % pred: forced expiratory volume in one second, percentage of predicted value

FEV_1:   forced expiratory volume in one second

MIF_{50} :   maximum mid-inspiratory flow

GORD: gastro-oesophageal reflux disease

HADS: Hospital anxiety and depression scale

Idio: idiopathic cough

IL-8:   interleukin 8

IQR :   inter-quartile range

LAHR :   lower airway hyper-reactivity

LTB_4:   leukotriene B_4

MPO: myeloperoxidase

NGF :   nerve growth factor

NPV :   negative predictive value

NT-3 :   neurotrophin-3

PGE_2:   prostaglandin E_2

PNDS: postnasal drip syndrome

PPV :   positive predictive value

SGRQ: St. George's Respiratory Questionnaire

TNF-α: tumour necrosis factor-alpha

UAHR: upper airway hyper-reactivity

VAS: visual analogue scale

SUMMARY

Background: Cough often lasts for over one year and is associated with airway inflammation. The effect of inhaled corticosteroids on symptom severity and inflammatory mediator levels in these patients is unknown. The main objective of the thesis was to determine whether inhaled corticosteroids reduce cough severity and sputum mediator concentrations in patients with chronic persistent cough. We also endeavoured to find non-invasive markers of inflammation that would predict corticosteroid responsiveness.

The secondary aims of the thesis were to evaluate the role of neurotrophins in chronic cough, quality of life in cough and the effect of specific treatment, extrathoracic airway hyperreactivity and the value of clinical history in predicting the final diagnosis of cough.

Methods: We performed a double-blind, randomised, placebo-controlled, crossover study with inhaled fluticasone 500 mcg twice daily and placebo for 14 days in 88 patients with cough for over one year, with normal chest radiography and spirometry. Outcome measures were a daily cough visual analogue scale (VAS) and induced sputum concentrations of eosinophilic cationic protein (ECP), myeloperoxidase (MPO), leukotriene B_4 (LTB$_4$), leukotrienes $C_4/D_4/E_4$ (cys-LT), prostaglandin E_2 (PGE$_2$), interleukin-8 (IL-8) and tumor necrosis factor-alpha (TNF-α). Sputum cell counts, exhaled nitric oxide (eNO) and carbon monoxide (CO) were performed at all visits.

Neurotrophins were measured at baseline in serum and induced sputum by enzyme immumoassay. St. George's Respiratory Questionnaire (SGRQ) and the Hospital Anxiety and Depression Scale (HADS) were utilised to assess quality of life before and after specific treatment of cough. Extrathoracic airway hyperreactivity (EAHR) was measured using methacholine challenge with flow volume testing and recording a 25% drop in MIF_{50}. Symptoms asked in history at the baseline visit were analysed for their predictive value of the final diagnosis of cough.

Results: There was a significant improvement in the cough visual analogue scale following inhaled fluticasone compared to placebo [mean difference (95%CI) 1.0(0.4, 1.5), p<0.001].

There was evidence of airway inflammation in chronic persistent cough, with increased induced sputum cell counts, exhaled nitric oxide and inflammatory mediators (LTB_4, cys-LT, PGE_2, ECP, IL-8 and TNF-α). LTB_4, cys-LT and PGE_2 were elevated in all causes of cough. IL-8, MPO and TNF-α levels were elevated in bronchiectasis alone.

Sputum ECP, eNO and CO fell significantly following inhaled fluticasone. There was no change in sputum cell counts and other mediator concentrations. Exhaled nitric oxide was the best predictor of corticosteroid responsiveness in cough. This was followed by sputum eosinophil%, total IgE levels, sputum ECP and sputum cys-leukotriene levels.

There was no significant difference either in the levels of serum or sputum neurotrophins in chronic cough subjects compared to controls or between the commonest causes of cough: post-nasal drip syndrome (PNDS), gastro-oesophageal reflux disease (GORD), asthma and bronchiectasis. The median (inter-quartile range) for sputum NGF (pg/ml) was 516 (296-772) in healthy controls and 580 (312-880) in subjects with chronic cough (p=0.284). There was no correlation between neurotrophin levels and sputum cell counts. Sputum NGF levels correlated with duration of cough (r=0.34, p=0.002).

In the quality of life study, we found a marked impairment in all SGRQ domains and a significant improvement following specific treatment of cough. These findings were independent of the cause of cough. Clinical levels of anxiety and depression were not a feature of chronic cough, but there was a significant improvement in the scores after treatment.

Extrathoracic airway hyperreactivity was present in only 14% of subjects and was unrelated to any particular aetiology of cough. Airway inflammatory cells in induced sputum and mediator levels were similar in the group with EAHR compared to the group without, other than an increase in sputum LTB_4 levels. There was a marked predominance of females noted in the group with EAHR (11/12).

In the fifth experiment, the value of symptoms in making a final diagnosis was assessed. The diagnostic pattern was similar to other cough studies with PNDS [n=30, 34%], GORD [18, 20%], asthma [13, 15%], bronchiectasis [9, 10%] and eosinophilic bronchitis [5, 6%] being the commonest causes. In 10 subjects (11%) the cough was

termed idiopathic. General cough questions regarding the positional or diurnal variation in cough did not lead to any specific diagnosis. Positive predictive value of symptoms in diagnosing a specific cause of cough was low. However, we found that specific history for GORD had a 100% negative predictive value and for bronchiectasis, history of a dry cough had a 100% NPV. For asthma, the NPV of clinical history was 86%. Certain specific triggers like cat/dog dander and grass pollen had > 90 % specificity for asthma. Questions for PNDS had the lowest positive or negative predictive value and improving the specificity of questions with scoring systems for rhino-sinusitis did not improve this outcome.

Conclusion: Cough severity and sputum ECP levels are modestly reduced by inhaled corticosteroids in patients with chronic cough persisting for over one year. LTB_4, cys-LT, PGE_2, IL-8, MPO and TNF-α levels are unaltered by this therapy. This raises the possibility that drugs targeted to reduce the effects of these mediators may be of benefit in chronic persistent cough.

Measured levels of neurotrophins in serum and NGF in induced sputum were not elevated compared to controls. This implies that either neurotrophins are not responsible for the neurogenic inflammation in cough or that different methods of evaluating them, possibly at receptor level are required.

Extrathoracic airway hyperreactivity is not a common feature of chronic persistent cough and does not seem to add any clinically useful addition to the management of chronic cough.

Clinical history provided some useful questions with high negative predictive values for GORD and bronchiectasis, which will require evaluation in prospective cough studies.

The presence of airway inflammation and inflammatory mediators in all causes of cough presents future therapeutic targets in the management of chronic persistent cough.

CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

1.1 EPIDEMIOLOGY AND CAUSES OF CHRONIC COUGH

1.1.1 Definition and incidence of chronic cough

Cough is the commonest reason for patients to seek medical attention (1). At any given time, approximately 20% of the UK population have a troublesome cough and an indication of the size of the problem is the self-prescription of over the counter anti-tussives, estimated at 75 million doses per annum in the UK (2, 3). Sales of anti-tussives have been estimated to be over $600 million in the USA (4).

Cough is an explosive expiration which provides a means of clearing the tracheo-bronchial tree of secretions and foreign bodies. It also serves as a warning of severe lung disease and allows investigations to be performed in a timely manner. However, a chronic persistent cough is associated with several complications and severely disrupts quality of life.

Based on duration, two categories of cough are commonly described: acute, lasting less than three weeks; or chronic, lasting three weeks or more (5). This was based on the fact that most acute coughs are due to infections, which should resolve within three weeks. However some severe infections can persist longer and a cut-off period of eight weeks is now preferred (6).

Patients with persistent chronic cough account for 10-38% of the practice of a chest specialist (7-9) and recurrent cough has been reported in 22% of the general population (10).

1.1.2 Physiology of cough

A cough is an explosive burst of high velocity expiratory airflow, which clears secretions.

Coughing maybe initiated voluntarily or as a reflex.

The cough reflex has 5 components (11).

i.   the cough receptors

ii.   the afferent nerves

iii. a poorly defined medullary cough centre

iv.   efferent nerves

v.   effector muscles

Cough receptors

Receptors for cough belong to the general group of rapidly adapting irritant receptors (12). They have been demonstrated to exist in the lower airways and pharynx, and it is inferred that they must exist in other sites as mechanical stimulation of the external auditory canal, eardrums, paranasal sinuses, diaphragm, pleura, pericardium and stomach have all been reported to cause cough (5).

A variety of stimuli can initiate a cough reflex, possibly accounting for the multiplicity of causes of cough. Inflammation itself could stimulate the cough reflex. Mechanical receptors are sensitive to touch and displacement. They are found mainly in the larynx, trachea and carina and reduce in number more distally along the tracheo-bronchial tree (5). Chemical receptors are concentrated in the larynx and bronchi and are primarily sensitive to noxious gases and fumes (5). Changes in temperature can stimulate the cough response as well (5).

1.1.3   Causes and Complications of chronic cough

Cough can be caused be a variety of disorders, some of which are listed in table 1.1.1 (13) described by Madison, Irwin and colleagues. Some additional causes are listed in table 1.1.2. Acute cough is usually caused by infections, but can often be the only presenting symptom of more severe illnesses such as pulmonary embolism or aspiration.

Pathogenic triad of chronic cough

Amongst non-smokers, who are not being treated with ACE inhibitors, the cause of cough is usually post-nasal drip syndrome (PNDS), gastro-oesophageal reflux disease (GORD) or asthma; singly or in combination (figure 1.1.1). This has been termed the *pathogenic triad of chronic cough* (14). Although chronic bronchitis is a very common cause of cough, smokers tend to accept their cough and present infrequently for assessment of their cough.

Complications of Cough

The act of coughing can be associated with several complications, which can range from mild to severe and can severely impair the quality of life of sufferers (table 1.1.3).

Table 1.1.1: Causes of cough [*adapted from Madison et al (13)*]

Anatomic location	Causes
Extrathoracic	
central nervous system	Gilles de la Tourette's disease
head and neck	common cold
	ear conditions
	elongated uvula
	enlarged, infected tonsils
	laryngeal disorders
	nasal polyps
	neurilemmoma of vagus nerve
	osteophytes of cervical spine
	palatine artery aneurysm
	rhinitis/sinusitis
	rhinolith
	syngamus laryngeus infection (nematode infection)
	thyroiditis
Intrathoracic	
Airways	Aspiration
	asthma
	bronchial carcinoid
	bronchiectasis
	bronchogenic carcinoma
	broncholith
	chronic bronchitis
	foreign bodies
	endobronchial sarcoidosis
	endobronchial tuberculosis
	exposed endobronchial suture
	inhaled medications
	relapsing polychondritis
	Sjogren's syndrome
	tracheobronchitis (bacterial, viral or fungal)
	pertussis
	ulcerative colitis
	wegener's granulomatosis
Parenchyma of the lung	carcinoma-metastatic
	connective tissue disorders that involve the lung
	extrinsic allergic alveolitis
	idiopathic pulmonary fibrosis
	infectious pneumonias
	pulmonary vascular disorders

Anatomic location	Causes
Mediastinum	bronchogenic cyst hodgkin's disease intrathoracic goitre neural tumours thymoma teratoma
Cardiovascular	aberrant innominate artery aortic aneurysm enlarged left atrium left ventricular failure mitral stenosis pericardial stimulation by transvenous pacemaker pulmonary embolism treatment with angiotensin-converting enzyme inhibitors vascular ring
Upper gastrointestinal	oesophageal cyst gastro-oesophageal reflux disease pharyngeal swallowing disorders tracheobronchial fistula
Pleural space	effusion pneumothorax thoracentesis
Diaphragm	stimulation by transvenous pacemaker

Table 1.1.2   Additional causes of cough (Studies and Case Reports)

Cause of Cough	Reference
Abacavir as treatment for HIV	(15)
Aberrant right subclavian artery syndrome	(16)
Bronchomalacia	(17)
Chronic bronchitis in pigeon fanciers	(18)
Coeliac disease	(19)
Exposure to fungus Humicola fuscoatra at home	(20)
Holmes Adie Syndrome	(21)
Holmes Adie Syndrome with Thr124Met mutation in	
peripheral myelin protein zero (MPZ gene)	(22)
Impacted cerumen in the ear, hair lying against tympanic membrane	(23)

Occupational Causes

Cough in hot pepper workers (chilli peppers)	(24)
Latex-induced eosinophilic bronchitis	(25)
Tropical pulmonary eosinophilia	(26)
Working on a mushroom farm (67% of workers reported cough)	(27)
World Trade Centre Cough (fire-fighters after September 11, 2001)	(28)
Cough in glass bottle workers	(29)

Figure 1.1.1: Commonest causes of chronic cough in specialist clinics
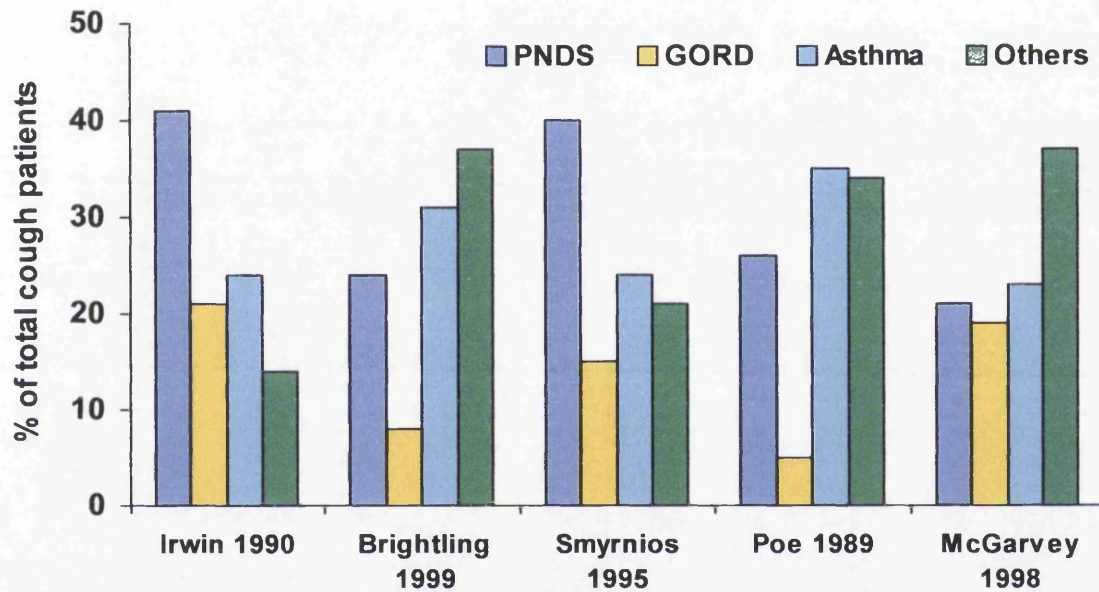
Sources: Irwin 1990 (8), Brightling 1999 (30), Smyrnios 1995 (31), Poe 1989 (32) and McGarvey 1998 (33).

Table 1.1.3: Complications of cough *[adapted from Irwin et al (5)]*

Cardiovascular	Arterial hypotension Loss of consciousness Rupture of subconjunctival, nasal and anal veins Dislodgement of intravascular catheters Bradyarrhythmias, tachyarrhythmias
Neurologic	Cough syncope Headache Cerebral air embolism CSF fluid rhinorrhoea Acute cervical radiculopathy Malfunctioning ventriculoatrial shunts Seizures Stroke due to vertebral artery dissection
Gastro-intestinal	Gastro-oesophageal reflux events Hydrothorax in peritoneal dialysis Splenic rupture Inguinal hernia
Genitourinary	Urinary incontinence
Musculoskeletal	Rupture of rectus abdominus muscles Rib fractures
Respiratory	Pulmonary interstitial emphysema, with risk of pneumomediastinum, pneumoperitoneum, subcutaneous emphysema, pneumothorax Laryngeal/tracheobronchial trauma Exacerbation of asthma Intercostal lung herniation
Miscellaneous	Petechiae and purpurae Disruption of surgical wounds Self-consciousness, hoarseness, dizziness

Decrease in quality of life

Difficulties in present diagnosis and treatment modalities

Although the causes of cough have been well studied, in the clinic setting it is often very difficult to arrive at a specific diagnosis.

Several algorithms have been developed for investigating the cause of chronic cough, involving a series of investigations and/or trials of empirical treatments (8, 33, 34). However, despite these attempts, in a significant proportion of patients (12-31%), the specific cause of the cough is not established (32, 33, 35).

Hence our current diagnostic and treatment modalities are inadequate, causing prolongation of the suffering of patients with chronic cough.

1.2   AIRWAY INFLAMMATION IN CHRONIC COUGH

1.2.1 Is airway inflammation present in chronic cough?

Patients with chronic cough have increased sensitivity to a variety of inhaled tussive stimuli including capsaicin and solutions with low chloride concentrations (36). Airway inflammation may contribute to the pathophysiology of cough as asthmatic subjects with cough and patients with cough following a viral infection also demonstrate a heightened response to inhaled capsaicin (37, 38).

Chronic cough is a multi-factorial condition. Airway inflammation could be a common unifying feature in all causes of cough. In cough due to asthma, rhino-sinusitis, gastro-oesophageal reflux disease and angiotensin-converting enzyme (ACE) inhibitors, inflammation of the airways has been demonstrated (39-42).

Studies utilising bronchial biopsies and broncho-alveolar lavage have shown increased number and activity of airway inflammatory cells (41, 43), suggesting an important role for airways inflammation in the pathophysiology of chronic cough.

Various studies in specific sub-groups of cough have shown the presence of elevated mediators of inflammation, such as interleukin-8, tumour necrosis factor-α (39), cysteinyl leukotrienes and eosinophilic cationic protein (44). Recently, Birring et al demonstrated elevated levels of mast cell derived mediators in chronic cough, where the main groups of subjects were cough variant asthma, eosinophilic bronchitis and idiopathic cough (45).

Inflammation of the airways does appear to be a significant finding in chronic cough. The pattern of inflammation has varied in different studies.

1.2.2 Eosinophilic inflammation

Airway inflammation with eosinophils is a characteristic feature of asthma. Eosinophilic inflammation has been described as the predominant cellular pattern in several cough studies. Carney and colleagues demonstrated high levels of eosinophils in induced sputum in 50% subjects with chronic cough compared to 19% in controls (46). In a comparative study, Niimi et al found the serum ECP level and percentage of eosinophils in bronchoalveolar lavage fluid and biopsy specimens in cough subjects were elevated, similar to patients with classic asthma associated with wheeze (40). In contrast, Hsu et al reported sputum eosinophilia in 28% of patients with chronic cough, (7/25 subjects); (of whom 5/7 responded to oral prednisolone) compared to 92% of asthmatics (47).

Gibson and colleagues first described the presence of eosinophils in induced sputum samples of subjects with cough and no evidence of asthma. They suggested the terminology 'eosinophilic bronchitis' (48) for this group of corticosteroid responsive subjects with cough. More recently, Fujimura and colleagues have proposed a corticosteroid sensitive disease entity known as 'atopic cough' which is associated with eosinophilic tracheo-bronchitis and atopy, without bronchial hyperreactivity (49).

The spectrum of diseases with eosinophilic inflammation of the airways has now broadened to include cough variant asthma, atopic cough, eosinophilic bronchitis, allergic rhinitis and COPD (50).

1.2.3 Neutrophilic inflammation

In contrast to the above studies reporting predominant eosinophilia, Jatakanon and colleagues found a two-fold increase in neutrophils in induced sputum in cough subjects compared to controls, when they studied subjects with non-asthmatic cough (51). This was associated with an increase in cytokines associated with neutrophil chemotaxis (IL-8 and TNF-α) and was in agreement with the study on non-asthmatic cough subjects by Pizzichini et al reporting that 59% of subjects had neutrophil elevation in sputum with increased IL-8 levels (52). A generalised neutrophilia in cough subjects was noted in a study from Japan (53), but response to asthma medications was seen only in those with evidence of eosinophilia.

A direct effect of acid in GOR or inhaled sino-nasal fluid in PNDS could induce neutrophilic inflammation in the airways. Corren et al demonstrated in an animal model that sinusitis can cause hyperreactivity of the lower airways by post-nasal dripping of cells or cell products into the lower airways (54). It has been speculated that the act of repeated coughing may contribute to inflammation (51). Epithelial damage induced by coughing may induce the release of inflammatory cytokines such as IL-8 and TNF-α, which are elevated in chronic cough (51). Both IL-8 and TNF-alpha can cause neutrophilia (55, 56).

Moodley and colleagues considered the possibility that hypertonic saline itself was causing neutrophilia in induced sputum and compared samples of bronchial lavage fluid collected at different sites in the airways in healthy individuals. Their conclusion was that the relatively high neutrophil count in sputum arose from the proximal airways and was not a response to hypertonic saline (57).

1.2.4 Role of mast cells and lymphocytes

There has been recent interest in the role of mast cells and lymphocytes in cough (45, 58, 59). Broncho-alveolar lavage in chronic non-productive cough subjects demonstrated an increase in eosinophils along with mast cells (41). Gibson et al studied bronchial brushings in chronic cough and found intraepithelial mast cells were elevated in subjects with inhaled corticosteroid responsive cough (60). Mast cell derived mediators, such as PGD_2 and histamine were increased in all causes of cough (45), suggesting a role for mast cells in the complex inflammation associated with cough. Lymphocytes were found to be elevated specifically in subjects with idiopathic cough (45, 59) with a possibility raised that idiopathic cough was related to auto-immune diseases.

1.2.5   Can coughing cause airway inflammation?

It is possible that inflammation itself may perpetuate cough by enhancing the cough reflex through the release of inflammatory mediators and cytokines. But can coughing exacerbate the inflammation, setting up a vicious cycle of events?

There is no real evidence at present to answer this question. During the procedure of sputum induction, changes in sputum composition were detectable after 24 hours (61). There maybe

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an influx of neutrophils into the airways (61), although it does not appear to be rapid as no increase is detectable over 30 minutes of sputum induction (61) nor is a prolonged effect likely as the sputum neutrophil count is repeatable after one or two weeks (62).

1.2.6 Effect of anti-inflammatory treatment in cough

Corticosteroids, oral and inhaled, can reduce cough when associated with eosinophilic inflammation, especially in conditions such as cough variant asthma, eosinophilic bronchitis and atopic cough. The role of corticosteroids as general anti-inflammatory agents in all causes of cough remains to be determined.

Leukotriene antagonists maybe useful in certain cases of cough variant asthma, even when resistant to corticosteroids (63). Newer, and more specific anti-inflammatory agents are being assessed, for example, a Th2 cytokine inhibitor, suplatast tosilate, used in 20 patients with cough variant asthma in a double blind, randomised placebo-controlled study reduced the initially elevated sputum ECP and eosinophils in induced sputum compared to placebo (64).

Conclusion

Chronic cough is associated with airway inflammation. The predominant cell type could be eosinophils, neutrophils, lymphocytes or mast cells. The type of airway inflammatory cell predominant in an individual patient presenting with chronic cough, may help to predict the response to corticosteroids (60), as occurs in patients with asthma (65) and COPD (66).

Information on cellular patterns in different causes of cough is limited. Their role in predicting corticosteroid responsiveness in patients with chronic cough due to all common causes has not been elucidated.

1.3 NON-INVASVIVE TESTS TO ASSESS AIRWAY INFLAMMATION AND CORTICOSTEROID RESPONSIVENESS

Bronchoscopy with biopsy and lavage is the 'gold standard' to assess inflammation in the airways in conditions such as asthma and COPD. However, it is an invasive technique associated with risks. Less invasive techniques, such as induced sputum and measurement of markers in exhaled air have been evaluated in several respiratory conditions, including chronic cough.

1.3.1 Induced sputum

Sputum has been a useful tool to evaluate many respiratory diseases. The difficulty in obtaining spontaneous samples lead to the development of techniques to stimulate the production of sputum. Ultrasonically nebulised hypertonic saline successfully induces sputum from normal as well as asthmatic subjects (67) with cellular and biochemical content similar to spontaneously produced samples (62). The advantages of induced sputum are that it is non-invasive, simple (62), safe (68) and responsive (69).

Methodology

Different approaches to sputum induction have been described in the literature, with fixed or increasing concentrations of hypertonic saline over varying time periods (70, 71). Although sputum cell counts do not appear to be affected by the tonicity of saline used (72), cellular and biochemical composition may change over the induction period (61) and it would be appropriate to standardise the duration of the procedure. The technique has evolved over

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several years and can now provide meaningful information about inflammation of the lower airways (67).

Role as a non-invasive marker of inflammation

The use of induced sputum to assess inflammation of the airways has lead to an improved understanding of the different phenotypes of asthma and COPD. Sputum eosinophil count can be used to predict the response to oral prednisolone in these conditions (66, 73). Severe asthma appears to be more commonly associated with a neutrophil predominance (74).

Role in cough

Induced sputum has been utilised as a tool to study the presence of inflammation in chronic cough (46, 51). Lack of sputum eosinophils predicted lack of responsiveness to corticosteroids in subjects with non-asthmatic chronic cough (52). Gibson and colleagues demonstrated that there was evidence of sputum eosinophilia in patients with chronic cough without variable obstruction and airway hyperreactivity typical of asthma (75, 76) and called this 'eosinophilic bronchitis'. This condition has now been diagnosed in up to 15% of cases of chronic cough in specialist centres (30) and induced sputum is now recommended as an additional test in the algorithm of cough management (6).

1.3.2: Exhaled nitric oxide

Nitric oxide (N0), previously known as endothelium derived relaxant factor (EDRF), is a highly reactive molecule formed from L-arginine by the action of the stereo-specific enzyme

nitric oxide synthase (NOS). At least three forms of NOS exist; two are constitutive and are activated by a rise in intracellular calcium ions, producing small amounts of NO involved in local regulatory functions and cell signalling. The final form of NO synthase known as iNOS or inducible nitric oxide synthase, is expressed following induction by inflammatory cytokines and endotoxin and produces large amounts of NO after activation.

The anatomical and cellular source of nitric oxide in the airways is unclear. Although majority of exhaled NO in normal subjects probably originates in the upper airways (77, 78), NO can be detected in the lower airways via bronchoscopic sampling in normal individuals (79, 80). Nitric oxide can be measured in exhaled air by the process of chemiluminescence, with detection of NO as a result of its photochemical reaction with ozone. The quantity of light is proportional to the concentration of NO in the exhaled gas. Most recent analysers are able to detect to < 1 part per billion.

The guidelines produced by the European Respiratory Task Force cover several technical factors to be considered during measurement (81). Slow expiration against a resistance elevates the soft palate and produces levels of exhaled NO identical to those samples directly from the lower airways at bronchoscopy (79, 80).

Role as a non-invasive marker of inflammation

Values of exhaled nitric oxide can be elevated in asthma (77, 79, 82) and viral respiratory infections (83). In asthma, exhaled NO has been advocated as a non-invasive marker of airway inflammation (84-86).

Exhaled NO levels decrease with corticosteroid treatment in asthma (87, 88) and in bronchiectasis (89), possibly because glucocorticoids suppress airway inflammation and prevent induction of iNOS (90).

Role in cough

Exhaled nitric oxide has been suggested as a method of differentiating 'asthmatic' from 'non-asthmatic' cough with a positive and negative predictive value of 60 and 93% respectively (91). In this study by Chatkin and colleagues all subjects with a positive methacholine challenge test were diagnosed to have an asthmatic cough, which is usually accurate in approximately 88% of cases (33). Most investigators would diagnose an asthmatic cough on the basis of a positive methacholine test with response to specific asthma treatment (5, 6).

Exhaled NO levels were elevated in subjects with eosinophilic bronchitis (92). In bronchiectasis, Kharitonov and colleagues reported high levels of eNO (89), but this was not supported by Ho et al (93).

Further work on the value of exhaled NO in diagnosing of the cause of cough and predicting corticosteroid responsiveness is indicated.

1.3.3 Carbon monoxide

Carbon monoxide (CO), a gas detectable in exhaled air, is produced from three major sources: enzymatic degradation of heme, non-heme related release (lipid peroxidation, bacterial) and

exogenous CO. Approximately 85% of CO in the body is from degradation of haemoglobin by the enzyme heme oxygenase (HO) and approximately 15% from degradation of myoglobin, catalase, NO synthase and cytochromes (94). HO exists in 3 isoforms - HO-1,2 and 3. HO-1 is a stress protein which can be induced by a variety of stimuli including pro-inflammatory cytokines, bacterial toxins and nitric oxide.

Exhaled CO is easily performed and usually detectable in all subjects. Measurements are reproducible and ambient air concentrations (0-2 ppm) do not have much effect on readings (95).

Role in inflammation and as non-invasive marker of inflammation

Levels of exhaled CO are elevated in asthma, upper respiratory tract infections, lower respiratory tract infections, seasonal allergic rhinitis and cystic fibrosis (95-99). Treatment with inhaled or oral corticosteroid has been shown to reduce exhaled CO in asthmatics (96, 100). Exhaled CO is related to the severity of asthma (101) and has been advocated as a non-invasive marker of airway inflammation (101-103).

Carbon monoxide levels are elevated in bronchiectasis (104). The value of this measurement in all causes of chronic cough and as a predictor of response to inhaled corticosteroid has not been evaluated.

1.3.4 Nasal nitric oxide

Elevated nasal nitric oxide can be measured in allergic and perennial rhinitis (105, 106) which is reduced by treatment with nasal glucocorticoids (105). Differences of nasal NO in subjects with rhinitis compared to normals are not very high and this reduces the value of nasal NO as a non-invasive test for monitoring rhinitis (107).

1.3.5 Exhaled breath condensate

Exhaled breath condensate is a new technique to measure inflammatory parameters in subjects with asthma and COPD (108, 109) . Recently, Niimi and colleagues demonstrated reduced pH in exhaled breath of patients with chronic cough (110) suggesting that endogenous acidity could contribute to heightened cough reflex sensitivity. Reduced pH in exhaled breath condensate has been associated with neutrophilic airway inflammation (111). Hydrogen peroxide levels were elevated in bronchiectasis (112) and correlated with disease severity. There have been no reports on measurement of inflammatory mediators in breath condensate of patients with chronic cough.

1.4   MEDIATORS INVOLVED IN CHRONIC COUGH

1.4.1 Inflammatory mediators in airway inflammation

Inflammation of the airways appears to be controlled and perpetuated through large extra-cellular signalling peptide mediators (113). These cytokines can be pro-inflammatory or anti-inflammatory. Various papers have shown that inflammatory mediators can be measured in the induced sputum cell-free supernatant repeatably (44, 62, 114). Induced sputum mediator levels, such as ECP and eicosanoids, can be higher than levels in broncho-alveolar lavage fluid (115, 116). There is a complex interaction between inflammatory cells and mediators released, which serves to perpetuate airway inflammation.

1.4.2 Specific mediators possibly involved in chronic cough

a. Leukotrienes (B_4 and cysteinyl leukotrienes)

Leukotrienes have been known of since the 1930's when they were collectively termed as slow-reacting substance of anaphylaxis (SRS-A). They were purified and individually characterized in the 1980's. Leukotrienes are formed during the breakdown of arachidonic acid by the enzyme 5-lipoxygenase (5-LO) and consist of LTA_4, LTB_4, LTC_4, LTD_4 and LTE_4. These mediators are produced by various cell types, especially mast cells and eosinophils as well as basophils, macrophages and monocytes (117). They are elevated in patients with asthma (118) and in nasal secretions of subjects with symptomatic rhinitis (119).

Cysteinyl leukotrienes

Leukotrienes C_4, D_4 and E_4 each contain a cysteine residue, hence the name cysteinyl leukotrienes. Cysteinyl-leukotrienes can cause bronchoconstriction (120), increase vascular permeability (121), increase mucous production (122) and may directly increase eosinophilic airway inflammation.

Leukotriene B_4

Leukotriene B_4 does not have a cysteine residue. Its biological activity includes a chemoattractant stimulus for inflammatory cells, bringing them from the circulation into the focus of inflammation (117). LTB_4 exerts its strongest chemoattractant effect on neutrophils (123), but it can also attract eosinophils and monocytes (124). It is released by activated mast cells and macrophages.

In subjects with cough, cys-LT was increased in the sputum supernatant of subjects with eosinophilic bronchitis and cough variant asthma (44, 45). LTB_4 levels have not been measured in chronic cough.

In patients with cough due to chronic bronchitis, the cys-LT receptor antagonist, pranlukast, had no significant effect (125). However, montelukast reduced cough in infants with reactive airway disease following respiratory syncytial virus (RSV) bronchiolitis (126).

b. Prostaglandins

Prostaglandins are small lipid molecules that regulate several processes in the body including immune function (127). In response to inflammatory stimuli, membrane phospholipids liberate arachidonic acid by the action of phospholipase A_2. Arachidonic acid is converted to PGH_2 by the cyclooxygenase enzymes COX-1 and COX-2 to a series of prostaglandins including PGI_2, $PGF_2\alpha$, PGD_2 and PGE_2 (127).

PGE_2 is usually considered to have an anti-inflammatory role in asthma (128, 129) but is pro-inflammatory in periodontal disease (127). PGE_2 could have a pro-inflammatory role in cough. Choudry et al (130) demonstrated that inhalation of PGE_2 significantly increased the cough response to capsaicin in normal volunteers and increased the respiratory resistance after capsaicin. These effects were not seen with inhalation of bradykinin, histamine or citric acid. Their results suggested that the cough reflex is increased in the presence of PGE_2 in the airways. Birring and colleagues demonstrated increased PGE_2 levels in induced sputum in all groups of patients with cough (45).

Brightling et al reported high levels of PGD_2 in induced sputum of subjects with eosinophilic bronchitis (44). Subsequently, Birring and colleagues demonstrated that PGD_2 was elevated in all causes of cough (45).

c. Eosinophilic cationic protein (ECP)

Eosinophils in an activated state contain granule proteins, one of which is ECP. It is a potent tissue damaging protein. It can release histamine from mast cells. Its destructive effect on

airway epithelium and surrounding tissues may expose underlying sensory nerves and cough receptors to various other environmental insults (41).

ECP levels are elevated in induced sputum of subjects with cough due to eosinophilic bronchitis (44, 131, 132). High levels of ECP in induced sputum have been measured in non-eosinophilic asthma (133) and in other non-eosinophilic conditions like COPD (134) and bronchiectasis (135); suggesting that neutrophils, as well as eosinophils, may contain ECP, as demonstrated by Sur et al (136).

d. Myeloperoxidase (MPO)

Neutrophils contain cytotoxic enzymes which help their role in host defence. One such cytotoxic system is provided by myeloperoxidase, a haemoprotein within azurophilic granules (137). Stimulation of neutrophils leads to rapid secretion of MPO which can be detected extracellularly.

Elevated levels of MPO in broncho-alveolar lavage has been demonstrated in asthma (138) and COPD and correlated with neutrophil numbers as well as IL-8 (139). Levels in chronic cough have not been measured.

e. Interleukin-8 (IL-8)

Interleukin-8 is a pro-inflammatory cytokine. It is a potent neutrophil chemoattractant and activator (140) released from a variety of inflammatory airway cells, including macrophages, eosinophils and epithelial cells (141-143). It may also have chemotactic activity for eosinophils (144, 145).

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In persistent asthma associated with neutrophilia in sputum, Il-8 levels were increased and correlated with neutrophils, ECP and MPO (133). IL-8 appears to be marker of severe asthma (146).

Jatakanon and colleagues demonstrated that non-asthmatic cough was associated with sputum neutrophils and found elevated IL-8 levels in induced sputum in these subjects (51). In patients with chronic cough reported by Birring and colleagues, sputum neutrophil count and IL-8 were within normal limits (45). However, the predominance of subjects in their study had a final diagnosis of asthma, eosinophilic bronchitis and idiopathic cough.

f. Tumour necrosis factor-α (TNF-α)

TNF-α is a potent pro-inflammatory cytokine. It is a peptide, not constitutively present in the lungs, but secreted rapidly upon stimulation from macrophages, T and B cells, mast cells and epithelial cells. It stimulates a generalised immune reaction.

Elevated levels of TNF-α are found in the airways of asthmatics using broncho-alveolar lavage (147). Inhalation of TNF-α results in increased neutrophil counts in induced sputum of normal subjects and mild asthmatics (55, 148). Jatakanon et al studied induced sputum samples of subjects with non-asthmatic cough and found significantly increased levels of TNF-α which did not correlate with the neutrophil count (51).

g. Other mediators

Histamine was elevated in induced sputum of certain sub-groups of cough (45). Bradykinin and related kinins are peptide hormones, formed in tissues and fluids during inflammation. It has been proposed that coughing during treatment with ACE inhibitors is linked to the action of kinins, since ACE is able to degrade kinins and the effects of ACE inhibitors are reduced by kinin antagonists (149).

1.4.3   Effect of corticosteroid on mediator levels

Corticosteroids are powerful anti-inflammatory agents. Serum ECP levels decline with inhaled corticosteroid treatment in asthmatics (150, 151).

The results for other mediators are less encouraging. Leukotriene levels did not respond to corticosteroid treatment and studies with ICS failed to detect any effect in urinary LTE_4 excretion (152). In vitro there was a 30% reduction of IL-8 release from epithelium and smooth muscle with corticosteroid treatment (153) and in patients with persistent asthma, IL-8 levels were high despite the use of inhaled corticosteroids (133).

Fujimoto and colleagues studied the effect of glucocorticoids on inflammation in patients with COPD and measured ECP and IL-8 in induced sputum (154). They found elevated levels of both at baseline, but after corticosteroids, only ECP showed a significant reduction.

1.4.4 Are inflammatory mediators involved in chronic cough?

Airway inflammation has been demonstrated in several studies studying the pathophysiology of chronic cough with neutrophilic, eosinophilic or mast cell associated inflammation. Elevated mediator levels in induced sputum were present in patients with non-asthmatic dry cough (51) and eosinophilic bronchitis (155). Birring and colleagues recently demonstrated that mast cell derived mediators (histamine, PGD_2) and PGE_2 were elevated in all subjects studied with cough (45).

Cough response to capsaicin can be enhanced by substances released during inflammation such as prostaglandins and bradykinin (156, 157). Choudry et al demonstrated inhalation of prostaglandin E_2 (PGE_2) in humans enhanced the cough reflex (130).

It is also possible that chemical mediators affect the electrophysiological properties of the afferent nerve membrane without causing a generator potential and activating the nerve. They could modulate nerve excitability. An example of PGE_2 and nerve modulation, described by Kollarik and Undem (158) is depicted in figure 1.4.1.

Summation of this information strongly suggests a pathogenic role for inflammatory mediators in chronic cough. The precise mediators involved in various causes of cough have not been confirmed and their response to anti-inflammatory agents remains to be investigated.

Figure 1.4.1: Examples of the action of activator and modulator of nerve excitability in vagal afferent fibres of guinea pig trachea [Kollarik and Undem, (158).

Activation of airway nociceptive afferent afferent nerve fibre by bradykinin [activator] and the sensitising effect of prostaglandin E_2 (PGE_2); [modulator].

Upper trace: transient administration of bradykinin directly over the receptive field evoked a short delayed burst of action potential discharge.

Middle trace: The tissue was incubated with PGE_2 that caused by itself no activation.

Lower trace: PGE_2 enhanced response to subsequent challenge with bradykinin.

Vagal afferent fibre in guinea pig trachea
(conduction velocity = 3.8 m/s)
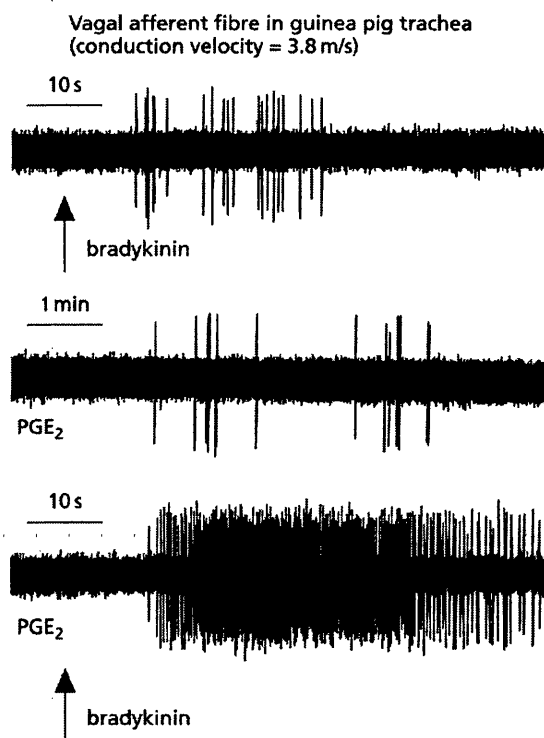
1.5 NEUROTROPHINS AND COUGH

1.5.1 Biology of neurotrophins and their receptors:

Neurotrophins are a family of structurally related growth factors which include nerve growth

factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), NT-4/5 and

the recently discovered neurotrophin-6 (NT-6) (159). Their potency to regulate the

development, differentiation, survival and function of distinct neuronal subsets is well

established. They derived their name from the term 'neuron' related to nerves and 'trophe'

meaning nutrient. Recently there is growing evidence for their involvement in the process of

inflammation. T-cells, B-cells, macrophages and mast cells are sources of neurotrophic factors

during the process of inflammation.

The biological effects of neurotrophins are mediated by binding either to high affinity

$(Kd-10^{-11})$ tyrosine kinase receptors (trkA, trkB, trkC) or the low affinity $(Kd-10^{-9})$ pan-

neurotrophin receptor $(p75^{NTR})$. Substantial biological effects of neurotrophins are mediated

through the high affinity tyrosine kinase receptors. TrkA is the high affinity receptor for NGF,

trkB for BDNF and NT-4, and trkC for NT-3. Trk receptors and p75NTR are widely

expressed in neurons of the peripheral and central nervous systems as well as on non-neuronal

cells such as immune cells, muscle cells and epithelial cells (160).

Functions of neurotrophins in the mature immune system

Since NGF is the best characterised neurotrophin, most of the available data deals with this

neurotrophin. The main functions of NGF (161) can be summarised as

- Stimulation of rapid degranulation of mast cells and basophils

- Promotion of differentiation, activation and cytokine production of mast cells, granulocytes and macrophages

- Activation of eosinophils

- Promotion of proliferation of B- and T-cell subsets

- Enhances Th2cytokine production and IgE synthesis

- Induces differentiation of activated B-cells in Ig-secreting plasma cells

1.5.2 Neurotrophins and asthma

Work in mouse models of allergic inflammation has shown that neurotrophins alter sensory innervation, enhance neuropeptide production and induce airway hyper-responsiveness (162, 163).

Patients with severe allergic bronchial asthma demonstrate high serum levels of NGF (164). Virchow and colleagues investigated the presence of NGF, BDNF and NT-3 in broncho-alveolar lavage after segmental allergen provocation in mild asthmatic patients (165) and found an increase in neurotrophins in allergen-exposed lung segments, but only during the late-phase response. Elevated NGF and BDNF levels have been demonstrated in serum of steroid-naïve asthmatic (166) with a reduction in levels following inhaled corticosteroids. NGF levels are also increased in other allergic conditions such as rhinoconjunctivitis and urticaria-angio-oedema (164). The role of NGF and BDNF in asthma is better established than that of NT-3 and NT-4 (167).

Neurotrophins could participate in the pathogenesis of asthma in different ways:

- as nerve growth factors, including neuropeptide synthesis and neuronal hyper-reactivity

- as cytokine-like factors influencing the allergic immune response and inflammation and

- as growth factors directly controlling smooth muscle function.

1.5.3 Neurogenic inflammation in cough

Tachykinins are a family of neuropeptides released on stimulation with mechanical, thermal, chemical (capsaicin, nicotine) or inflammatory stimuli (bradykinin, histamine, prostaglandins) (168). Substance P and neurokinin A are members the tachykinin family. These neuropeptides are transferred via axonal transport to pre-synaptic axon endings in the spinal cord and to peripheral nerve endings.

There are two principle cough receptors, rapidly adapting receptors (RAR's) which are innervated by A fibres and unmyelinated C-fibres (169, 170). Tachykinins have been shown to be present in unmyelinated sensory fibres using immunohistochemical techniques (171, 172). C-fibres antidromically release CGRP, SP and NKA when stimulated by irritants such as capsaicin (172, 173). Endobronchial biopsy specimens obtained from subjects with idiopathic cough showed an increase in airway calcitonin gene-related peptide (CGRP) and vasoactive intestinal peptide-immunoreactive nerves (174).

Hope-Gill and colleagues studied the cough reflex in patients with idiopathic pulmonary fibrosis and healthy controls (175). They found that substance P and bradykinin induced cough and oral prednisolone significantly reduced the cough. In induced sputum, elevated levels of nerve growth factor and brain derived neurotrophic factor were present (175).

1.5.4 Possible role of neurotrophins in chronic cough

In pulmonary tissue, there is expression of NGF, BDNF and NT-3, as revealed by microdensitometric analysis of bands of immunoreactivity (176).

Neurotrophins are signalling molecules, locally upregulated in allergic inflammatory processes. Sensory C-fibres contain neuropeptides in their peripheral and central terminals. Inflammation within the airway wall sends signals to the distant cell body in the relevant sensory ganglia to induce the transcription on neuropeptide synthesizing enzymes. The mechanism of this is unknown, but a likely mechanism could involve the action of neurotrophins (158).

In inflammatory processes, increased synthesis of tachykinins leads to inflammatory hypersensitivity. These effects can be mediated by NGF (177-179).

NGF and BDNF cause increased capsaicin sensitivity in sensory neurons (160, 178). In vitro BDNF gene transcription directly regulates capsaicin sensitivity in vagal sensory afferents (180).

Endogenous neuronal lipid mediator anandamide, which can be synthesised in the lung, is a ligand of cannabinoid (CB) and vanilloid receptors (VR). Anandamide when given by aerosol, can induce cough in conscious guinea pigs (181), possibly through activation of vanilloid (VR1) receptors. Recently, the vanilloid receptor, originally known as the capsaicin receptor, has been proposed as a receptor for cough in humans (182). It has been speculated that NGF affects airway nerves through the vanilloid receptor (VR1) in allergic asthma (183).

Taken together, this evidence suggests that neurotrophins may be involved in the inflammation in chronic cough.

1.6 QUALITY OF LIFE IN CHRONIC PERSISTENT COUGH

Chronic cough is one of the commonest causes of presentation to a general practitioner. It is associated with considerable morbidity and social disability (10, 184, 185). The diagnosis and management of chronic cough is often difficult. Cough often persists for several years (33, 35, 110) and patients are usually resigned to suffering with it. 23% of patients self-presenting to a cough clinic had been specifically told by their physician that they would have to learn to live with their cough (8).

1.6.1 Health related quality of life

Quality of life is an outcome related to a fundamental aim of health care (186). Health related quality of life measurement is a means of quantifying, in a standardised and objective manner, the impact of disease on patients' daily life, health and wellbeing. It is a process that is similar to a highly structured clinical history, where the end product is an objective measurement that can be used for scientific purposes (187). Health status questionnaires address the emotional/psychological effects of an illness along with the physical effects. The majority of items in these questionnaires are concerned with practical aspects of disturbances to daily life and aim to summarise the wide effects of a disease into an overall score.

1.6.2 Cough specific quality of life assessment

Until recently, the quality of life of people with chronic cough had never been studied. French and colleagues used the Sickness Impact Profile (SIP) and Adverse Cough Outcome Survey (ACOS) developed by themselves and demonstrated, in a prospective study, that chronic

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cough was associated with adverse psychosocial and physical effects on the quality of life (188). Some of the common complaints recorded are depicted in table 1.6.1.

French et al introduced a cough specific quality of life questionnaire (the CQLQ) in 2002 (189), followed by Birring and colleagues with the Leicester Cough Questionnaire (LCQ) in 2003 (190). Both are recent additions to outcome measures in cough studies which could prove very useful, but will benefit with further validation in larger and varied populations.

Table 1.6.1: Spectrum and frequency of cough-associated adverse occurrences before and after specific treatment to patients (n=28) cured of their coughs.

[Adapted from: French et al. Arch Intern Med 1998 (188)]

Adverse Occurrence	Frequency, %		
	Before treatment	After treatment	p
Needs reassurance nothing is serious	75	19	<0.001
Concerned something is wrong	68	11	<0.001
Frequent retching	57	8	<0.001
Exhaustion	61	15	<0.001
Others think something is wrong with me	46	19	0.03
Embarrassment	46	11	<0.004
Self-consciousness	46	14	0.01
Difficulty speaking on the phone	43	22	0.01
Hoarseness	46	15	0.01
Had to change lifestyle	36	11	0.03
Cannot sleep at night	43	15	0.02
Can no longer sing in church	29	8	<0.05
Spouse cannot tolerate cough	27	8	0.08
Wetting pants	32	7	0.02
Dizziness	29	7	0.04
Excessive sweating	29	7	0.04

1.6.3 Effect of treatment on quality of life

French and colleagues (188) studied 28 subjects before and after treatment of their cough and found a resolution of the deterioration in quality of life following successful treatment of the cough. The Sickness Impact profile (SIPS) scores significantly decreased towards normal and the ACOS items decreased from 8.6 to 1.9 (p<0.001). A substantial proportion of their patients' health related dysfunction in the ACOS was explained by cough inducing concern of cancer, exhaustion, fear of AIDS or TB and hoarseness. Birring and colleagues used the LCQ to assess the effect of specific therapy in 9 subjects (190) and found a highly significant correlation for improvement in VAS scores and LCQ scores (p=0.007). The change in quality of life following specific treatment of cough has not been assessed in a large study.

1.6.4 Comparison of quality of life across different respiratory conditions

The cough specific questionnaires will be helpful in cough specific studies, but will not be useful to compare the quality of life across different respiratory conditions. Quality of life is widely used as an outcome measure in studies in asthma and COPD with respiratory disease specific questionnaires such as the St. George's Respiratory Questionnaire (SGRQ) and in various medical disorders with the Hospital Anxiety and Depression scale. The SGRQ was designed so the scores would have an 'absolute' property and be independent of patient and study (191). It has 76 items divided into 3 sections: Symptoms, Activity and Impacts (which covers aspects concerned with social functioning and psychological disturbances resulting from airway disease) (191). A comparison of the effect of cough on quality of life compared with other medical conditions has not been done.

Objective measures of cough such as portable cough counters of good quality will eventually be the best method to assess effect of therapy on cough. However, at present, subjective measures such as health related quality of life instruments are likely to be the ones that best reflect the cough severity integrating the cough frequency, intensity and its effect on daily life.

1.7 BRONCHIAL AND EXTRATHORACIC AIRWAY HYPERREACTIVITY IN CHRONIC COUGH

1.7.1 Bronchial hyperreactivity (BHR) in chronic cough

Airway hyperresponsiveness or hyperreactivity is an increased ability of the airways to narrow after exposure to constrictor agonists. Airway hyperresponsiveness is believed to be affected by genetic as well as environmental factors (192, 193). The association of airway hyperresponsiveness and inflammation is controversial. Acute allergen exposure causes an increase of airway hyperresponsiveness that is consistently associated with an influx of inflammatory cells in the airways (194), which suggests a possible causal relation between airway inflammation and hyperresponsiveness (84, 195-197). Recently several studies have questioned this association. (198, 199). In chronic cough, Carney and colleagues found BHR in 23% subjects and sputum eosinophils in 50%, showing the dissociation between lower airway hyperreactivity and eosinophilic inflammation of the airways. Fujimura and colleagues found that capsaicin cough response was not related to airway hyperreactivity (200).

Methacholine, a direct stimulus of bronchoconstriction, is currently the gold standard for the assessment of bronchial hyper-reactivity (201, 202). The presence of bronchial hyperreactivity is used to diagnose cough variant asthma along with response to specific treatment. For a diagnosis of eosinophilic bronchitis, a negative challenge test is required along with eosinophils in sputum and response to corticosteroids. McGarvey and colleagues evaluated investigations in chronic cough and found the histamine challenge test had a positive

predictive value of 88% for asthma, implying that not all patients with cough and bronchial hyperresponsiveness have asthma (33) similar to work by Irwin et al (203). A negative methacholine challenge test is very useful in cough management to exclude the diagnosis of asthma (5, 6).

1.7.2 Extrathoracic airway hyperreactivity (EAHR) in chronic cough

Cough can be initiated anatomically by conditions that are outside the lower airways and hence measuring lower airway hyper-reactivity alone may not reveal the entire picture in cough. Asthma like symptoms, especially cough, could be associated with extrathoracic airway dysfunction.

Extrathoracic airway narrowing can be demonstrated during lung function testing using flow volume curves. Bucca and Rolla matched flow-volume loops with fibreoptic laryngoscopy during histamine challenge inhalation and found that the maximal mid-inspiratory flow (MIF_{50}) best reflected changes in mid-inspiratory glottis area (204). They used this index to study patients with upper respiratory disorders and found extrathoracic airway hyper-responsiveness in subjects with sinusitis, laryngitis and pharyngitis (204, 205). This was associated with damage to the epithelium and proliferation of submucosal fibres (206). It is unknown whether the hyperreactivity is a cause or effect of the nasal condition.

In patients with gastro-oesophageal reflux, those subjects with cough compared to those without, had a significantly lower $PC_{25}MIF_{50}$, indicative of extrathoracic airway

hyperreactivity (207). When patients with asthma like symptoms were studied, extrathoracic airway hyper-reactivity was found in 67% and was the sole abnormality in 27% (208). In 51% the diagnosis was considered as asthma. Patients presenting with cough alone had the highest probability of having extrathoracic airway responsiveness and the lowest probability of having bronchial responsiveness (208). MIF_{50} can be influenced by anxiety or defensive glottic closure and a positive test requires a decrease in a dose-dependent manner (208).

Carney and colleagues considered the possibility that upper airway hyperreactivity could be a unifying mechanism in cough, but found it present in only 12/30, 40% of subjects with chronic cough persistent for longer than 4 weeks (46). The mechanism of EAHR is unknown. It could be triggered by stimulation of pharyngo-laryngeal receptors (208), or simply a reflex narrowing of the glottis(209).

Extrathoracic airway hyperreactivity has not been studied in a large group of subjects with chronic persistent cough and its association with airway inflammation is unknown.

1.8   SPECIFIC DIAGNOSIS OF THE AETIOLOGY OF COUGH

Chronic cough is a formidable challenge to the physician, both in diagnosis and therapy. Anti-tussive therapy is classified as specific therapy when aimed at the established or presumed diagnosis of cough and non-specific therapy when the goal is to suppress the cough reflex (5).

In patients with chronic cough who are non-smokers, not on ACE-inhibitor treatment and have a normal chest radiograph – the commonest causes of cough are asthma, post-nasal drip syndrome and gastro-oesophageal reflux disease. Table 1.8.1 depicts the commonest causes of cough in patients investigated in specialist clinics (6).

1.8.1 Importance of establishing a specific diagnosis of the cough

In general respiratory clinics chronic cough is considered one of the most difficult cases to treat and success rates are low(7), however, specialised cough clinics report high success rates of therapy (8, 9, 32). The chief cause of this difference is a failure to consider the potential origin of the cough as being outside the lower respiratory tract (210).

Morice and committee members of the ERS Task Force describe the key to successful management is to establish a diagnosis and then treat the specific cause of cough (6). Chronic cough can often be due to more than one condition and treatment failures often result when this fact is not appreciated. In multiple prospective studies, it has been shown that chronic cough may be due to multiple causes from 18-62% of the time (8, 14, 31, 34).

1.8.2 Role of clinical history

It was generally believed that a carefully taken history, with detailed questioning about the character and timing of the cough would help in diagnosing the cause of cough. Mello and colleagues set up a study to specifically answer this question and found that the variability of character, timing or complications of cough could not lead to a specific diagnosis (211).

Specific questions to achieve a diagnosis of cough have a poor positive predictive value [52% for PNDS, 56% for asthma and 40% for GORD] (33).

1.8.3 Role of investigations

Chest X-ray and spirometry are primary tests for patients presenting with cough to a clinic. Specialised tests for chronic cough in the absence of any abnormality in chest radiography and lung functions include bronchial provocation testing, oesophageal pH monitoring, CT scan or X-ray of sinuses, bronchoscopy and high resolution CT scan of the chest. It is common to consider a response to specific therapy as an essential requirement for a diagnosis of cough, hence the value of performing these tests is in question.

Methacholine/histamine challenge test should be positive along with a response to specific therapy for a primary diagnosis of asthma (5). McGarvey and colleagues found that histamine challenge testing had a positive predictive value (PPV) of 88% for a diagnosis of CVA (33).

24 hour pH monitoring has a high negative predictive value for GORD as a cause of cough. PPV in a study by McGarvey and colleagues was 68%. Palombini et al demonstrated a PPV of 84% and negative predictive value (NPV) of 100% (14).

PNDS is a general term for any cause of rhinitis or sinusitis causing cough. Clinical examination has a PPV of 63% (33), radiological tests are not particularly sensitive (33) and treatment with sedative anti-histamines not very specific (6). Hence it is more difficult to make a precise diagnosis of PNDS as a cause of cough. Sinus CT scans in one study showed a PPV of 80% and NPV of 100% for this condition (14).

Flexible bronchoscopy adds little to the diagnosis of the cause of chronic cough with normal radiography (212). High resolution CT scans are useful in the diagnosis of bronchiectasis and should be performed when the cough does not respond to treatment for common conditions.

1.8.4 Management of the patient with chronic cough

Final diagnosis or diagnoses of the cause of the cough are usually based on defined pre-treatment criteria (5) and accepted only when the cough significantly improves or disappears with specific therapy (8).

The American College of Chest Physicians (5) published an algorithm to help the management of patients with chronic cough (Figure 1.8.1) and recently, the European Respiratory Task Force (6) have published their recommendations (Figure 1.8.2).

Diagnosing the cause of cough and treating it effectively can involve several investigations that are expensive and long treatment durations to achieve a cure. Balancing the cost with time to treatment success is a major challenge (6). A cost-effective analysis revealed that empirical treatment is the cheapest option but performing all investigations reduced the time to response (213). An appropriate combination of clinical history, investigations and therapeutic trials will be required. The choice ultimately depends on a combination of experience, facilities and resources.

Table 1.8.1 Commonest causes of cough in patients investigated in specialist clinics

Adapted from Table 1, ERS Task Force Report (6).

Reference	Patients improved%	Diagnosis % of total			
		Asthma syndrome	Oesophageal disease	Rhinitis	Most common other
Poe etal 1989 (32)	88	35	5	26	Idiopathic 12
Irwin et al 1990 (8)	99	24	21	41	Chronic bronchitis 5
O'Connell et al 1994 (35)	68	6	10	13	Idiopathic 22
Smyrnios et al 1995 (31)	97	24	15	40	Chronic bronchitis 11
McGarvey et al 1998 (33)	82	23	19	21	Idiopathic 18
Brightling et al 1999 (30)	93	31	8	24	Post-viral 13

Figure 1.8.1: Guidelines from the American College of Chest Physicians for Management of Chronic Cough (5)
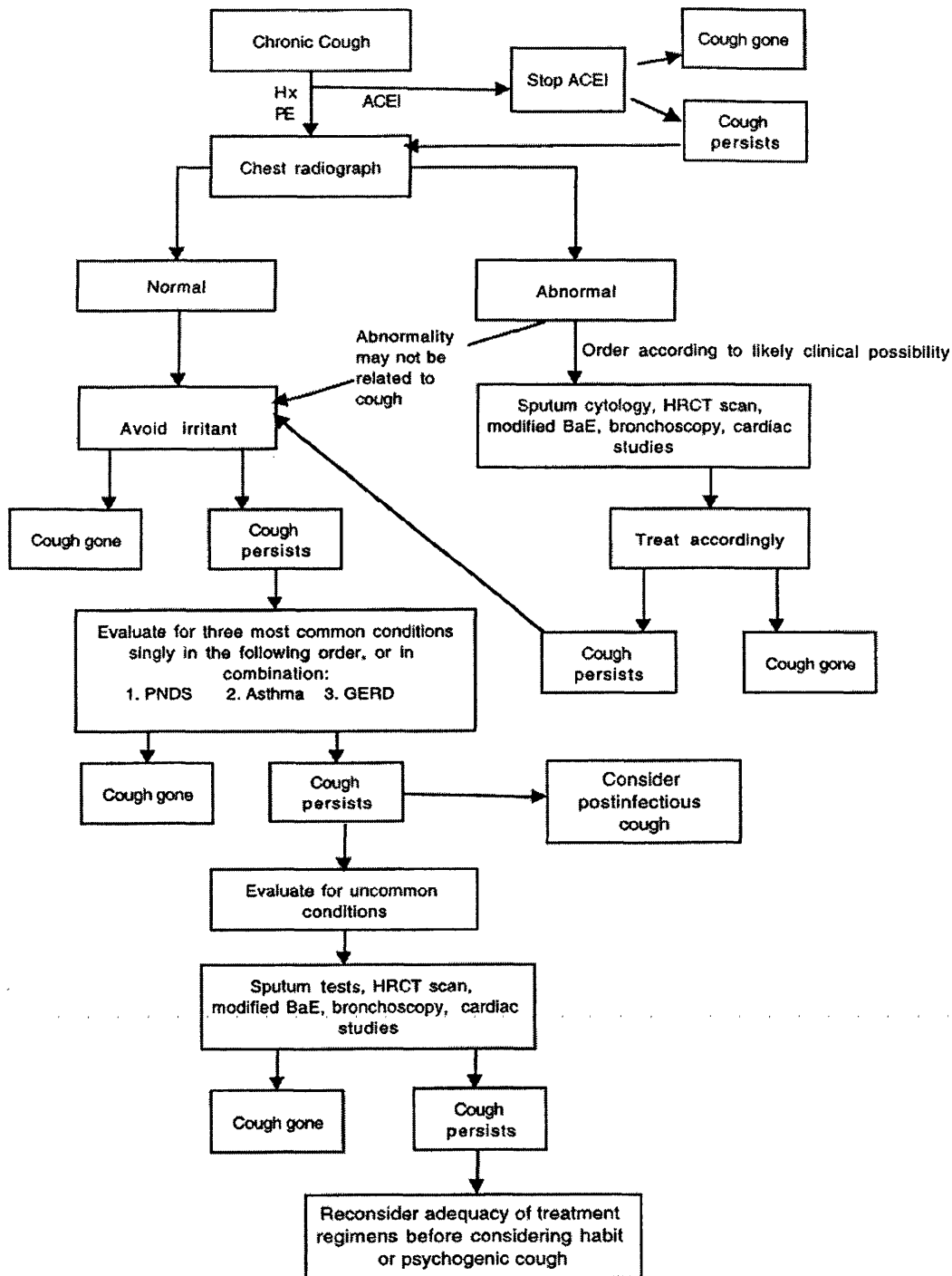
Figure 1.8.2: Guidelines from the ERS Task Force for management of chronic cough (6)
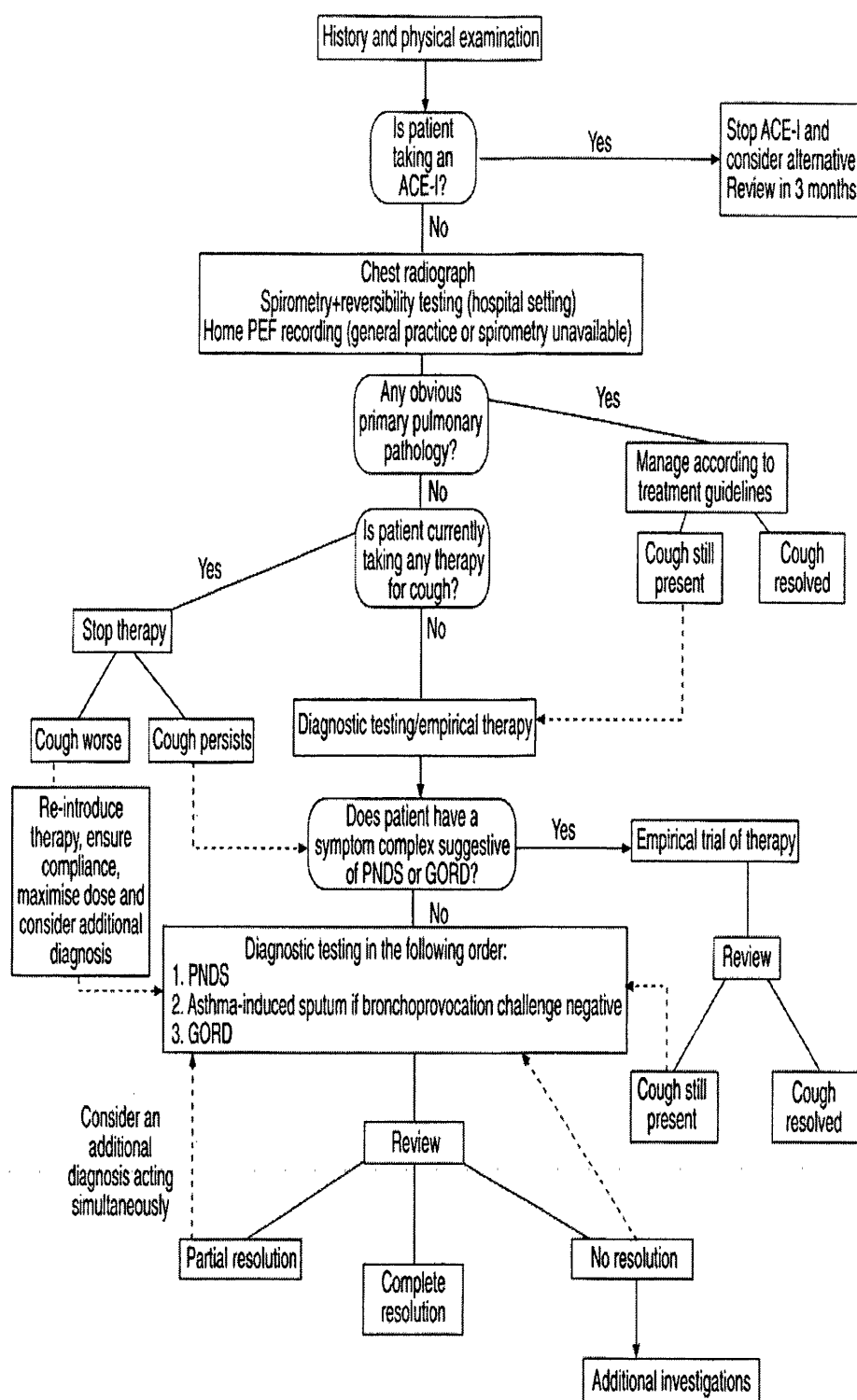
CHAPTER 2

HYPOTHESES AND AIMS OF THE STUDY

HYPOTHESES OF THE STUDY

The hypotheses of the study were

- Inhaled corticosteroids are effective in reducing symptoms in chronic persistent cough compared with a placebo inhaler.

- Non-invasive tests of airway inflammation can predict steroid responsiveness in chronic cough.

- There is airway inflammation in chronic cough with increased levels of inflammatory mediators which decreases following inhaled corticosteroid treatment.

- The quality of life is reduced in subjects with chronic persistent cough and improves following specific treatment of the cough.

- Extrathoracic airway hyperreactivity is present in the majority of patients with cough

- Clinical history will be useful in making a final diagnosis in chronic cough.

AIMS OF THE STUDY

The aims of this study were:

- To determine the efficacy of inhaled corticosteroids in patients with chronic cough that has persisted for over one year

- To assess the effect of this treatment on induced sputum cell counts, exhaled nitric oxide and carbon monoxide levels

- To assess the role of non-invasive tests of airway inflammation (induced sputum cell counts and exhaled gases) as predictors of a successful response to inhaled corticosteroid treatment in chronic persistent cough

- To measure inflammatory mediators in chronic cough (leukotriene B_4, cysteinyl leukotrienes $C_4D_4E_4$, interleukin-8, myeloperoxidase, eosinophilic cationic protein, prostaglandin E_2 and tumour necrosis factor-α) and the effect of inhaled steroids on these mediators

- To assess the role of neurotrophins (nerve growth factor, neurotrophin-3, brain derived neurotrophic factor) in chronic cough

- To assess the quality of life before and after specific therapy in patients with chronic cough

- To study the factors associated with extrathoracic hyperreactivity in chronic cough

- To judge the role of a clinical history in diagnosing the specific cause of chronic cough.

CHAPTER 3

GENERAL METHODS AND MEASUREMENTS

3.1: ETHICAL APPROVAL AND STUDY DESIGN

Ethical approval

The study was submitted to the Ethics Committee of the West Glasgow Hospital NHS Trust and full approval was received. The study was discussed in detail with all subjects and each patient signed an informed consent form prior to participating in the study.

Study design

All other treatment for cough was omitted during the course of the study. The subjects entered a double-blind, randomised, placebo-controlled, crossover study where they received a two-week course of treatment with either an inhaled steroid (fluticasone accuhaler 500 mcg bd) or a matching placebo inhaler. This was followed by a two-week washout phase where no treatment was administered and then a further 2 weeks of the inhaler not used initially. A diary was maintained at home on a daily basis where the severity of the cough was charted for the entire period of six weeks.

There were five visits for the study to complete the randomised crossover trial with inhaled corticosteroids versus placebo. Thereafter subjects were treated for their cough clinically, with two monthly visits as required.

Visit schedule and tests performed at each visit:

Visit 1: Written informed consent, eligibility check, clinical history and examination; chest radiograph, flow volume loops and reversibility to inhaled salbutamol, cough severity score as in the cough diary; blood tests for IgE, blood counts and neurotrophin mediator levels, sputum induction, exhaled CO, eNO, nasal NO, quality of life questionnaires, diary given to patients for peak expiratory flow measurements and cough severity scale.

Visit 2 (1 week later): Methacholine provocation test for bronchial and extrathoracic hyperreactivity; checked the cough diary; commenced placebo controlled cross-over trial of inhaled steroids.

Visit 3 (after 2 weeks): Cough diary checked; induced sputum, eNO and CO repeated; the washout period was initiated.

Visit 4 (after 2 weeks): Cough diary checked; induced sputum, CO and eNO repeated; the crossover inhaler started.

Visit 5 (end of steroid trial): Induced sputum, CO and eNO repeated; the cough symptom scores assessed.

Further follow-up

All subjects were offered trials of specific therapy for their cough depending on the likely clinical diagnosis as well as further investigations as indicated. Quality of life questionnaires were repeated at the end of the study.

3.2: SUBJECTS

Subjects with chronic cough (cough persisting longer than one year) were identified and recruited from general respiratory clinics of the hospital (Gartnavel General Hospital, Glasgow). Further patients were recruited through contact with local general practitioners and by advertisement in a local newspaper.

Inclusion criteria:

1. History of chronic cough for at least one year.

2. Never smoker or ex-smoker for at least one year.

3. No known chronic respiratory condition to account for the cough

4. Clinical examination of the respiratory system, chest radiograph and spirometry not indicative of any lung disease

5. No history suggestive of an upper respiratory tract infection in the preceding six weeks

6. No treatment with oral or inhaled corticosteroids for at least three weeks prior to inclusion

7. Willing to sign the informed consent form.

Exclusion Criteria:

1. Pregnancy or lactation

2. Age below 18 years

3. Patients receiving angiotensin-converting enzyme inhibitors.

3.3: LUNG FUNCTION MEASUREMENT

FLOW VOLUME LOOPS

Flow volume loops were performed in all subjects using an electronic pneumotachograph spirometer (Vitalograph-Compact, Vitalograph Ltd, Buckinghamshire, UK), which achieves standards set by the American Thoracic Society (214) and European Respiratory Society (215). The spirometer was calibrated regularly using a fixed volume syringe, with correction for ambient temperature, and serviced once a year by the company engineers.

The definition of a maximal flow volume curve is the graphical presentation of the flow versus volume signal recorded from a maximal forced expiration, starting from full inspiration, which is immediately followed by a maximal inspiration. Subjects were requested to wear a nose-clip and breathe in to full inspiration through the mouth. They were then asked to blow out forcefully through the mouth-piece to full expiration and then inhale to full inspiration once again through the mouth piece to complete the expiratory and inspiratory arms of the flow volume loop (216). Once the subject had learnt the technique, three recordings were made and the best of three measurements was recorded for forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), maximum mid-expiratory flow or forced expiratory flow (FEF_{25-75} or MMEF) and maximum mid-inspiratory flow (MIF_{50}).

Values were expressed as a percentage of predicted value, adjusted for age and height (217).

For the forced inspiratory flow predicted values, normal ranges are available only for males of different ages. For females, predicted values used are age-grouped as the normal data has not been accumulated in any large study (information on enquiry to Vitalograph, UK). For this reason, only the actual MIF values have been used for analysis in this study and not % predicted values.

Reversibility of FEV_1 to salbutamol was assessed using 2.5 mg nebulised salbutamol and repeating the flow volume loop in 15 minutes. The change in FEV_1 was calculated as a percentage of the pre-bronchodilator value using the formula:

[(post-salbutamol FEV_1 – pre-salbutamol FEV_1 ÷ pre-salbutamol FEV_1) X 100].

PEAK EXPIRATORY FLOW (PEF)

All patients were asked to maintain a peak flow diary (appendix 1) for a week to record their morning and night PEF. Patients were asked to stand or sit upright and blow forcefully into the Mini-Wright's peak flow meter (Clement Clarke, Harlow, UK) and the best of three readings was recorded in the diary card. PEF was expressed as litres/minute. Percentage lability was calculated using the formula (218):

[(maximum PEF of the day – minimum PEF of the day ÷ mean PEF) X 100]

Dividing by the mean value helps to normalise the amplitude for differences in body size (height, gender and age) and for the degree of baseline obstruction. The upper limit of peak flow lability is 19% for adults (219), hence a variability of 20% was considered positive in this study.

3.4: BRONCHIAL PROVOCATION TESTS

BRONCHIAL HYPERRESPONSIVENESS (BHR)

Bronchial hyperresponsiveness was measured using the methacholine challenge test similar to the technique described by Cockcroft et al (220). Histamine or methacholine administered by nebulisation using incremental doubling doses with measurement of lung function at each dose allows a dose-response curve to be established and a threshold response can be identified. This allows quantification of the response, expressed as the provoking concentration (PC) of the bronchoconstrictor substance required to produce a given change in lung function and is calculated by linear interpolation.

We used methacholine (an acetylcholine analogue) as the challenging agent in concentrations of 1, 2, 4 and 8 mg methacholine diluted in phosphate buffered saline [acetyl-β methyl choline chloride; Sigma Chemical Company, Poole, Dorset, UK; made up in phosphate buffered saline to pH 7.4. by the Sterile Pharmacy, Western Infirmary]

Aerosols were generated using a Wright nebuliser and subjects inhaled this aerosol by normal tidal breathing via the mouth. The nebuliser contained 3 ml of nebulisate and was driven by a continuous airflow of 9 l/m from a compressed air source of 50 lbs/inch (345 kPa), calibrated to deliver an output of 0.13 ml/min (221, 222)

A flow volume loop was measured as previously described. Subjects then inhaled phosphate buffered saline for two minutes with FEV_1 recorded at 30, 90 and 180 seconds following

inhalation. The lowest post-saline value for FEV_1 was then used as the baseline from which subsequent falls are calculated. At five-minute intervals the subjects received two-minute nebulised inhalations of doubling doses of methacholine, starting at 1 mg/ml. Inhalations were continued until the FEV_1 fell by more than 20% and the $PC_{20}FEV_1$ was then calculated by linear interpolation (223). If any subject had bronchoconstriction, they received inhaled salbutamol at the end of the procedure to reverse this effect.

A fall in FEV_1 of $\geq$ 20% at a methacholine concentration of ≤ 8 mg/ml was accepted as bronchial hyperresponsiveness.

EXTRATHORACIC AIRWAY HYPERRESPONSIVENESS (EAHR)

This was an additional measurement during the methacholine challenge test to assess upper airway hyper-reactivity. The maximum mid-inspiratory flow (MIF_{50}) was recorded during each of the flow volume loops performed for the methacholine challenge test. As MIF_{50} can be influenced by anxiety or defensive glottic closure, a positive test required a decrease in a dose-dependent manner.

Extra thoracic airway threshold was expressed as the methacholine concentration causing a 25% drop in MIF_{50} ($PC_{25}MIF_{50}$) and the concentration of 8 mg/ml was expressed as the cut-off to define extrathoracic airway hyperresponsiveness (207, 224).

3.5: MEASUREMENT OF EXHALED NITRIC OXIDE, CARBON MONOXIDE AND NASAL NITRIC OXIDE

Exhaled nitric oxide was measured using a Logan Research Analyser (LR2000, Logan Research Ltd, Rochester, Kent, UK) which utilises the principle of chemiluminescence. The equipment has a detection range from < 1 to 5000 parts per billion (ppb), accuracy of ±2 ppb and a response time of < 2 seconds. The sampling rate was 250 ml/min. The measurements are repeatable within a coefficient of variation ≤ 10%. The equipment was calibrated regularly against a standard gas mixture containing 96 ppb of nitric oxide (BOC Special Gases, Surrey Research Park, Guildford, UK). The equipment was fitted with bacteriological and system protection filters which were changed frequently to prevent contamination.

The technique was based on the recommendation of the European Respiratory Society Task Force (81). The measurement of exhaled NO was achieved by performing an exhalation manoeuvre (wearing a nose clip) from total lung capacity for 15 to 20 seconds with a constant flow (5 to 6 L/min) and exhalation pressure (3 ± 0.4 mmHg) [figure 3.5.1]. This closes the soft palate and prevents nasal contamination of the sample. The value corresponding to the plateau at the end of expiration was recorded. All readings were performed in triplicate and mean values were calculated.

Nitric oxide within the exhaled air reacts with ozone produced within the machine, with the production of nitrogen dioxide in an excited state. In returning to a stable base state, electromagnetic radiation with a wavelength of 600-3000 nm in released and detected by a

photomultiplier tube. There is a direct relationship between nitric oxide concentration and radiation detected. A continuous reading is produced, with all parameters measured every 40 milliseconds, and the concentration of nitric oxide recorded when at a stable level in late expiration. Expired carbon dioxide and flow rates are monitored to ensure a satisfactory manoeuvre and that the reading is taken from exhaled alveolar air rather than dead space gas.
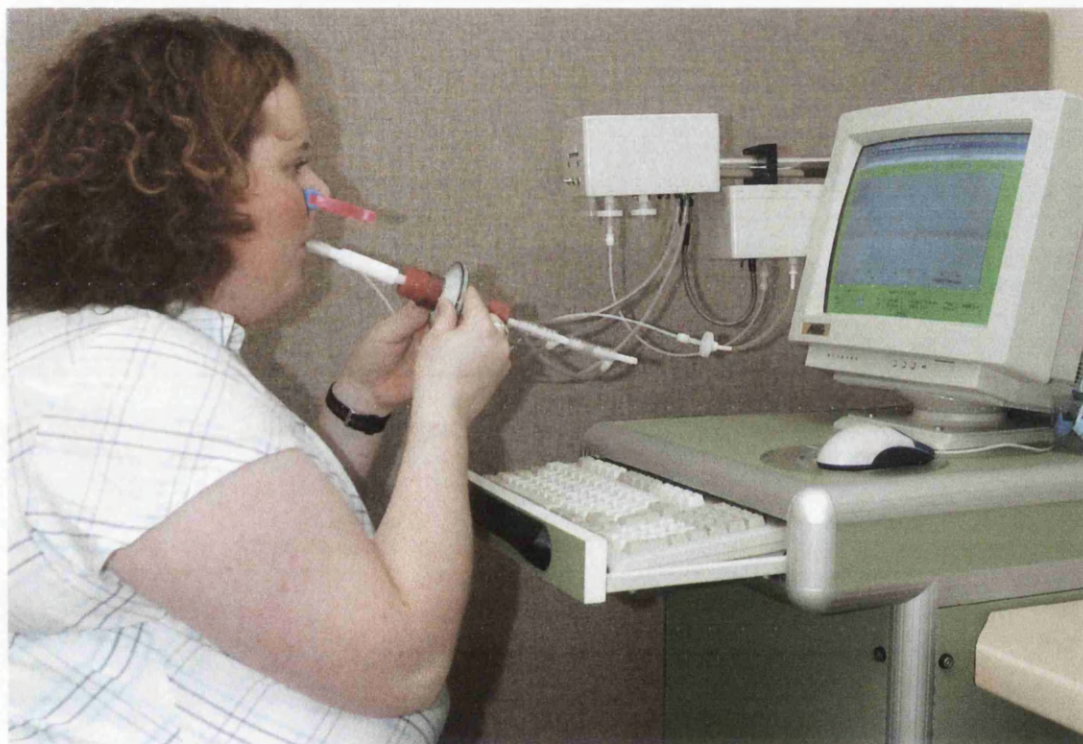
Figure 3.5.1: Procedure for measurement of exhaled nitric oxide

CARBON MONOXIDE

Exhaled carbon monoxide (CO) was measured simultaneously using a modified electrochemical cell with a device integrated into the chemiluminescence analyser described above for measurement of exhaled nitric oxide (CO option, LR2000, Logan Research Ltd., Rochester, Kent, UK). It was sensitive to CO from 0 to 50 parts per million (ppm), adapted for online recording of CO concentration and also equipped for measurement of exhaled volume, sample pressure, NO and CO_2. Subjects wore a nose clip and were asked to exhale from total lung capacity without breath holding into a Teflon tube at a constant flow of 5 L/min. A point measurement of CO at 75% of exhaled volume was used as a measure of lower respiratory tract CO. All readings were performed in triplicate and mean values were calculated. The methods used to measure exhaled NO and CO complied with the ERS Task Force guidelines.

3.6 NASAL NITRIC OXIDE

This was measured by the same machine used for eNO (LR2000, Logan Research Ltd., Rochester, Kent) with a teflon tube inserted into one of the nares during breath holding after a maximal inhalation. This manoeuvre achieved soft palate closure and allowed analysis of the local NO concentration with free flow of ambient air from one nostril to the other and subsequent direction into the analyser. Nasal NO was recorded as the plateau for NO concentration during this manoeuvre. All readings were performed in triplicate and mean values were calculated.

3.7: INDUCED SPUTUM TESTING

Procedure of sputum induction:

Sputum was induced using a modification of the method of Pin et al (71). Subjects were pre-treated with 200 μg salbutamol administered through a metered dose inhaler and large volume spacer device to minimise bronchoconstriction. Sputum was then induced using 3% hypertonic saline administered through a nebuliser (Sonix 2000, Medix Ltd, Harlow, Essex,UK) over a period of 20 minutes (nebuliser output 0.9 ml/min; mass median diameter 5.5 micrometer) [figure 3.7.1]. Subjects were encouraged to expectorate into a sterile container at any time during the procedure. Spirometry was performed every 5 minutes to confirm there was no adverse effect on lung functions. If FEV_1 fell by 20% or more, or if troublesome symptoms occurred, the nebulisation was stopped. The sample was transferred to the laboratory on ice as soon as possible, and in all cases in < 2 hours.

Figure 3.7.1 : Procedure of sputum induction

Processing of sputum samples

The procedure of processing sputum samples was similar to that described by Popov et al (225). In the laboratory, the whole sample of sputum and saliva was transferred to a petri dish and the macroscopic characteristics recorded. Sputum plugs from samples were selected out, if necessary using an inverted microscope, to minimise salivary contamination. The selected portion was weighed and treated with 4 times the volume of fresh dithiothreitol (DTT) ('Sputolysin', Calbiochem-Novabiochem (UK) Ltd, Nottingham, UK) in a balanced salt solution (Sputolysin 10%; Calbiochem-Novabiochem (UK) Ltd, Nottingham, UK) diluted to 0.1% with distilled water.

The sample was briefly vortexed (15s) and agitated on a bench rocker for 15minutes. A further 4 times the initial volume of dulbeccos phosphate buffered saline was added to give a final concentration of 0.05% DTT. The sample was filtered through a 50micron nytex mesh (R. Cadoch & Sons, London, UK) to remove residual clumps and a total cell count was done using a Neubauer haemocytometer and a trypan blue exclusion test for cell viability were carried out. An aliquot was removed and a $1x10^6$ cells/ml suspension prepared to make two cytospin slides using a Shandon III cytocentrifuge (Shandon, Cheshire, UK) at 450 rpm for 6 minutes.

The slides were air dried and stained with Giemsa stain to allow a cell differential count to be performed. This was expressed after exclusion of squamous epithelial cells that are taken to represent salivary contamination. The criterion for rejection of samples was if the viability was < 40% or squamous cell contamination was > 20%. (No samples were rejected on these criteria). Viability was typically >70% and squamous cell count usually <10%. The bronchial epithelial cell count was very low in all samples, typically <2%). The remaining sputum suspension was centrifuged at 4000 rpm for 5 minutes and the resulting supernatant stored at -70°C prior to mediator assays. Figure 3.7.2 describes this procedure graphically.

Normal values for the induced sputum cell counts in experiment 1 were based on reference values obtained from large cohorts of healthy individuals (81, 226) and confirmed to be similar to our laboratory values by measuring them in 50 healthy controls prior to this study. Cut-off values at the upper limits of normality were: total sputum cell count 8 $x10^6$, sputum eosinophils 2%, neutrophils 53% and lymphocytes 3%.

Figure 3.7.2. Laboratory processing of samples of induced sputum

(adapted from Pavord et al, 1997 (67)

1. Select viscid portion of sputum (using inverted microscope if necessary)

2. Weigh and incubate with 4x volume of 0.1% dithiothreitol (DTT)

3. Gently aspirate with pasteur pipette, vortex for 15 seconds

4. Rock on bench rocker for 15 minutes

5. Mix with equal volume (to DTT) of Dulbecco's phosphate Buffered saline (D-PBS)

6. Rock for 5 minutes

8. Filter using 48µm nylon gauze

9. Centrifuge for 10 minutes

10. Store supernatant to measure fluid phase component

11. Total cell count and assess viability; discard if low viability (<40%)

12. Resuspend cell pellet in D-PBS

13. Adjust cell suspension to 1 x 10^6 cells per ml

14. Prepare cytospins for differential cell counts

3.8: MEASUREMENT OF INFLAMMATORY MEDIATORS

Inflammatory cytokines from the sputum supernatant were measured using commercially available kits. All tests were performed according to the manufacturer's instructions.

Mediators measured using radio-immunoassay were ECP and MPO (Pharmacia UK Ltd, Milton Keynes, UK). The other mediators were measured by their immunoreactivity in sandwich enzyme immunoassay: IL-8 and TNF-α (IDS, Boldon, Tyne and Wear, UK), PGE_2 and LTB_4 (R&D Systems, Abingdon, UK)] and cysteinyl leukotrienes ($LTC_4/D_4/E_4$) (Amersham Pharmacia Biotech, Buckinghamshire, UK).

Typical sensitivities of the assays were for IL-8 (25pg/ml), TNF-α (10pg/ml), PGE_2 (36.2 pg/ml), LTB_4 (19.5 pg/ml), ECP (2 μg/ml), MPO (< 8 μg/ml) and Cys-LT (10 pg/ml). The precision of the assays was described by the within-plate and between-plate coefficients of variation, which across the concentration ranges of the study were, for TNF-α (< 1.0% and < 3.1%), IL-8 (< 2.1% and < 6.2%), Cys-LT (both < 8.6%), LTB_4 (<6.9% and <16.7%), ECP (< 11% and <9.2%), MPO (< 8.4% and 12.4%) and PGE_2 (< 5.2% and < 17.6%) respectively. The cross-reactivity of the prostanoid assays was as follows. Cys-LT assay: for LTC_4 (100%), LTD_4 (115%), and for LTE_4 62.7%; the cross-reactivity with the other main prostanoids was < 1.2%. LTB_4 assay: for 6-trans-12-epi-LTB_4 [5.5%], and for 6-trans-LBT_4 [4.9%]; the cross-reactivity with the other main prostanoids was <0.2%. PGE_2 assay: for PGE_1 [70%], and for PGE_3 [16.3%], the cross-reactivity with the other main prostanoids was <1.0%.

Nerve growth factor (NGF) was measured with an enzyme immunoassay (Promega, UK). It was measured using an antibody sandwich ELISA. The mouse anti-human NGF antibody was used to coat flat-bottomed 96 well plates (Immunol 4). Any NGF present in the samples under analysis was captured during incubation on the immobilised anti human NGF. Following washing to remove any unbound NGF, the addition of biotinylated goat anti-human NGF bound the captured NGF. After washing, streptavidin conjugated to Horseradish peroxidase (HRP) bound the sandwich complex during incubation. A coloured substrate, TMB, was added and the amount of specifically bound NGF present in the samples under analysis was proportional to the resulting colour. This was quantified against a standard curve of known amounts of NGF. The reaction was stopped with 1N HCL and the absorbance recorded at 450 nm on a plate reader. The sensitivity of the kit was15.6 pg/ml.

Neurotrophin-3 (NT-3) and brain derived neurotrophic factor (BDNF) were measured using a similar technique with an ELISA kit (Promega, UK). Sensitivity of the kit for NT3 was 10 pg/ml and 15.6 pg/ml for BDNF.

For nerve growth factor the performance characteristics of the assay were: intra-assay co-efficient of variation less than 4.3%, sensitivity 15.6 pg/ml, and less than 3% cross-reactivity with other neurotrophic factors. For BDNF the performance characteristics were: intra-assay CV less than 8.9%, sensitivity 15.6 pg/ml, and less than 3% cross-reactivity with other neurotrophic factors. For NT-3 the performance characteristics were: intra-assay CV less than 5.3%, sensitivity 10 pg/ml, and less than 3% cross-reactivity with other neurotrophins.

3.9: BLOOD TESTS:

Serum IgE Levels:

Total serum IgE and IgE to common allergens (house dust mite, grass pollen and cat dander) were measured by enzyme immunoassay (Unicap System, Pharmacia UK Ltd, Milton Keynes, UK). Total IgE > 120 IU/L and specific IgE >0.35 IU/L were considered positive.

Cell counts:

The total and differential white blood cell counts were measured by an automated cell counter (SE9500, Sysmex UK Ltd, Milton Keynes, UK), with our standard laboratory values used as control values.

3.10: COUGH SEVERITY VISUAL ANALOGUE SCALE

Subjects were asked to enter the severity of cough on a 10 cm visual analogue scale, with 0 being the best cough and 10 the most severe. This scale was incorporated in their daily diary card (appendix 2) for the 6 weeks of the cross-over study.

3.11: QUALITY OF LIFE QUESTIONNAIRES

All subjects were asked to complete 2 sets of questionnaires, the St. George's Respiratory Questionnaire and the Hospital Anxiety and Depression Scale at baseline and after final treatment of cough.

St. George's Respiratory Questionnaire (SGRQ) [appendix 3]: has 76 weighted items divided into 3 sections: Symptoms (distress caused by respiratory symptoms), Activity (physical activities that cause or are limited by breathlessness) and Impact (aspects concerned with social functioning and psychological disturbances resulting from airway disease) (191). A score is calculated for each section as well as a total score derived from all items. The scoring uses a 0-100 scale, with the highest scores indicating the poorest level of health. It takes approximately ten minutes to complete. The SGRQ was designed so the scores would have an 'absolute' property and be independent of patient and study (191).

Hospital Anxiety and Depression Scale (HADS) [appendix 4]: is a self-administered questionnaire consisting of 14 items, with 7 items each for the sub-scales of anxiety and depression, using a 0-3 scale for each question. The range of scores for each sub-scale is 0-21, with higher scores indicating greater emotional distress. Scores of 7 or less represent non-cases, 8-10 indicate possible cases and scores of 11 or more definite cases (227).

3.12: FINAL DIAGNOSIS OF COUGH

On completion of the cross-over study, to achieve a final diagnosis for the cause of the cough, all subjects were offered sequential trials of specific therapy depending on the likely clinical diagnoses for their cough. Final diagnosis or diagnoses of the cause of the cough were based on defined pre-treatment criteria (5) and accepted only when the cough significantly improved or disappeared with specific therapy (8).

Pre-treatment diagnostic criteria:

Certain pre-treatment criteria were set to aid the preliminary diagnoses of cough.

Asthma: History of cough with or without paroxysmal dyspnoea and wheeze or evidence of reversible airflow obstruction (15% improvement in FEV_1 following salbutamol) and/or $>/=$ 20% lability on peak flow readings. The PC_{20} on methacholine challenge testing was $\leq$ 8 mg/ml.

Post nasal drip syndrome: Any one of the following on history or examination: sensation of something dripping down the throat, nasal discharge, need to clear the throat frequently, mucoid/mucopurulent nasal secretions on examination or cobblestone appearance of the oro-pharyngeal mucosa.

Gastro-oesophageal reflux disease: History of heartburn and/or acid regurgitation.

Bronchiectasis: History of cough with large volumes of sputum production.

Eosinophilic bronchitis was considered when sputum eosinophilia > 2%, normal PEF variability, spirometry and methacholine challenge test.

Final diagnosis

The final diagnosis was based on the above criteria and a response to appropriate treatment. When the cause of cough was unknown, it was termed '*Idiopathic cough*'.

Treatment plan:

All patients were treated with medication for their cough, starting with the most likely diagnosis based on their history and baseline tests as above. As cough is known to be

frequently due to multiple causes, for each patient a plan was drawn up with a list of potential diagnoses and the order in which to treat them.

Treatment trials were done sequentially, for two months each, with the clinical response noted to each treatment. This was based on the patient's view of the effectiveness of the treatment in reducing their cough compared to their baseline level prior to that treatment trial.

The treatment responses were noted as

Very good response: if the improvement was between 75-100%

Good response: if the improvement was between 50-75%

Minimal response: if the improvement was between 25-50%

No response: if the improvement was < 25%.

A final diagnosis was made when there was a 'very good' response to treatment.

If the response was 'good', then patients were asked to continue with further treatment trials, until the cough resolved. If subjects declined further treatment, then the final diagnosis of the cause of cough would be accepted based on a 'good response' to treatment. If a subject had good responses to two treatment lines, then a combined diagnosis was made with the primary diagnosis being the one for which there was a better therapeutic response.

If there was 'minimal' or 'no response' to treatment, patients were requested to continue with further treatment trials using alternative medication. If they declined further treatment at any stage, the cause of cough was considered idiopathic.

A high resolution CT scan was performed prior to confirming a diagnosis of bronchiectasis.

Therapy used for the common causes of cough:

For each of the common conditions, a plan of treatment was made. First line therapy was used initially, if possible, in all subjects. Second line therapy was used when the first line treatment did not work or was not suitable for an individual patient based on adverse events or contra-indications due to other medical conditions. Third line therapy was very occasionally used to tailor to the requirements of an individual patient and consisted of a combination of first and second line treatment.

a. Gastro-oesophageal reflux disease:

First line therapy for GORD ($GORD_1$):Specific first line therapy used for GORD was oral omeprazole 20 mg bid for a month, followed by 20 mg od for a second month along with advice on life-style alterations to reduce acidity, including elevation of the head end of the bed and alterations in diet.

Second line therapy for GORD ($GORD_2$):

This was a combination of metoclopramide and ranitidine for two months along with advice on life-style alterations to reduce acidity.

b. Cough variant asthma and eosinophilic bronchitis

First line therapy for CVA and EB (CVA_1 and EB_1):

Inhaled corticosteroid (budesonide turbohaler) was prescribed at a dose of 400 mcg bid for two months along with a short acting $beta_2$ agonist as required for asthma.

Second line therapy for CVA (CVA_2):

Inhaled corticosteroid dose was 800 mcg bid for two months along with a short acting beta$_2$ agonist as required for asthma.

c. Post nasal drip syndrome

First line therapy for PNDS (PNDS$_1$):

A combination of a local nasal decongestant (pseudo-ephedrine drops, 2-3 drops to each nostril, tid) for a week, along with two months of intra-nasal corticosteroid (mometasone, 50 mcg/day, 2 sprays od) and an oral non-sedating anti-histamine (loratadine 10 mg od).

Second line therapy for PNDS (PNDS$_2$):

This comprised of three weeks of an oral decongestant (pseudo-ephedrine, 60 mg tid) and a first generation antihistamine (brompheniramine maleate, 4 mg tid).

Subjects were treated with first and second line treatment for each of the common causes of cough. A high resolution CT scan of the chest, CT scan of the sinuses and an oesophageal pH monitoring test were performed if the patient was agreeable, to look for a specific diagnosis for the cough.

3.13: DATA HANDLING AND STATISTICAL ANALYSIS

The baseline characteristics of the cough patients were compared with either a normal comparator group or cut-off values, using appropriate tests. Correlation amongst the baseline factors was examined by Spearman-rank correlation coefficients. The response to inhaled steroids was calculated for each variable in turn by first removing the effect of baseline from

the post treatment and post placebo periods. The differences of these differences were then calculated by subtracting the placebo effect from the treatment effect. The responses were analyzed by one-sample t-tests, and the corresponding 95% confidence intervals were calculated. This method was used because many of the underlying variables were not suitable for ANOVA analyses due to non-normality.

The study was adequately powered, as with a sample size of 68, the study would have 90% power to detect a within-subject change of one unit on the VAS scale, assuming a standard deviation of 2.5, and using a two-tailed test at the five percent significance level.

The treatment difference for the cough VAS scale was modelled by regression analyses to see if any of the baseline factors could predict steroid responsiveness.

The analysis of the study was conducted with Dr. Alex McMahon of the Robertson Centre for Bio-statistics at the University of Glasgow, using a computerised statistical package, SAS version 8.02.

CHAPTER 4

INDIVIDUAL EXPERIMENTS, RESULTS AND DISCUSSION

"When you can measure what you are speaking about and express it in numbers, you know something about it. But, when you cannot- your knowledge is of a meagre and unsatisfactory kind".

Lord Kelvin, British physicist.

Chapter 4.1

EFFECT OF INHALED CORTICOSTEROIDS ON SYMPTOM SEVERITY AND SPUTUM MEDIATOR LEVELS IN CHRONIC PERSISTENT COUGH

INTRODUCTION

Chronic cough has a reported prevalence of between 14-23%, and is associated with considerable morbidity including sleep loss, impaired performance of daily activities and considerable social disability (10, 184, 185). The diagnosis and management of chronic cough is often unsatisfactory. In non-smokers with a normal chest radiograph, the most frequent causes identified are postnasal drip syndrome (PNDS), gastro-oesophageal reflux disease (GORD) and cough variant asthma (CVA) (8, 32, 34). Several algorithms have been developed for investigating the cause of chronic cough, involving a series of investigations and/or trials of empirical treatments (8, 33, 34). However, despite these attempts, in a significant proportion of patients (12-40%), the specific cause of the cough is not established (32, 33, 35, 110). Patients presenting with chronic cough have often had symptoms for many years (33, 35, 110). In clinical practice, inhaled corticosteroids are often used empirically when patients first present with a chronic cough. The value of inhaled corticosteroids in patients with chronic cough that has persisted for over one year is not known.

Airway inflammation is usually a common underlying feature of all the main causes of cough, such as asthma, rhino-sinusitis, gastro-oesophageal reflux disease or angiotensin-converting enzyme (ACE) inhibitor induced cough (40-42, 51). Eosinophil and/or neutrophil numbers in the airways can be elevated in patients with chronic cough (40, 41). The type of airway inflammatory cell present in an individual patient presenting with chronic cough, may help to predict the response to corticosteroids (60), as occurs in patients with asthma (65) and COPD (66). Inflammatory mediators within the airways are elevated in specific groups of patients with cough, such as IL-8 and TNF-α in non-asthmatic patients with chronic dry cough (51), cys-LT and ECP in eosinophilic bronchitis (44). Recently, Birring et al demonstrated elevated levels of mast cell derived mediators in all causes of cough, where the main groups of subjects were cough variant asthma, eosinophilic bronchitis and idiopathic cough (45). The effect of inhaled corticosteroids on pro-inflammatory mediators in cough is unknown. Exhaled nitric oxide (eNO) and exhaled carbon monoxide (CO) have been used as markers of airway inflammation in various respiratory conditions, especially asthma, and have been shown to be responsive to corticosteroids (88, 96). Their value as predictors of corticosteroid responsiveness in chronic cough has not been determined.

The aims of this study were to determine the efficacy of inhaled corticosteroids in patients with chronic cough that has persisted for over one year and to assess the effect of this treatment on induced sputum inflammatory mediators, cell counts, exhaled nitric oxide and carbon monoxide levels. The secondary aims were to assess the role of non-invasive tests, such as induced sputum cell counts and exhaled gases as predictors of a successful response to inhaled corticosteroid treatment in chronic persistent cough.

METHODS

Study design

Adults with a cough persisting for longer than one year were recruited from hospital respiratory clinics and by advertisement in a local newspaper. No subject had evidence of any other lung disease based on history, clinical examination, chest radiography and spirometry. Details of study design are included in the general methods section, chapter 3.

This was a double-blind, randomised, placebo-controlled, crossover study where subjects were treated with inhaled fluticasone accuhaler 500 mcg or matching placebo, twice daily, for 14 days, with a 2-week washout phase in between. All other treatment for cough was omitted. A daily cough diary was maintained by the patient to chart the cough severity. A computer generated randomisation sequence was used with random numbers allocated consecutively. The randomisation code was withheld from the investigators until completion of the study. The study medication was packed by the central pharmacy according to the randomisation code and was identical in appearance and taste.

On completion of the cross-over study, to achieve a final diagnosis for the cause of the cough, all subjects were offered sequential trials of specific therapy depending on the likely clinical diagnoses for their cough. Final diagnosis or diagnoses of the cause of the cough (described in chapter 3) were based on defined pre-treatment criteria (5) and accepted only when the cough significantly improved or disappeared with specific therapy (8).

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Measurements

The primary outcome measure for improvement in the cough was a cough severity visual analogue scale (a linear scale with 0 cm = no cough to 10 cm = worst cough). Patients were asked to enter the number of puffs administered of the study inhalers into their daily diary card (as 1 or 0), morning and night. The number of inhalations left in the accuhaler device used for fluticasone and placebo were recorded as an indicator of adherence to medication.

Details of measurement techniques are included in the general methodology section, chapter 3. Investigations performed were:

- Pre-and post inhaled salbutamol FEV_1

- Total serum IgE and IgE to common allergens

- Total and differential white blood cell counts

- Bronchial challenge testing with methacholine

- Sputum induction with 3% hypertonic saline (at 4 visits). The supernatant was frozen at $-70°$ C for subsequent mediator measurements. Normal values for the induced sputum cell counts and exhaled gases were based on reference values obtained from large cohorts of healthy individuals (81, 226) and confirmed to be similar to our laboratory values by measuring them in 50 healthy controls prior to this study. Cut-off values at the upper limits of normal were: total sputum cell count 8 $x10^6$, sputum eosinophils 2%, neutrophils 53% and lymphocytes 3%.

- Exhaled nitric oxide (eNO) and exhaled carbon monoxide (CO) were measured at all visits. Exhaled NO was considered elevated above 9 ppb and CO above 4 ppm.

- Mediators in induced sputum:

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- ECP and MPO were measured using radio-immunoassay

- IL-8, TNF-α, PGE$_2$, LTB$_4$ and cysteinyl leukotrienes (LTC$_4$/D$_4$/E$_4$) were measured by their immunoreactivity in sandwich enzyme immunoassay:

Healthy volunteers were recruited from hospital staff to perform induced sputum to obtain control levels for the mediator measurements.

Statistical Methods

The baseline characteristics of the cough patients were compared with either a normal comparator group or cut-off values, using appropriate tests. Correlation amongst the baseline factors was examined by Spearman-rank correlation coefficients. The response to inhaled steroids was calculated for each variable in turn by first removing the effect of baseline from the post treatment and post placebo periods. The differences of these differences were then calculated by subtracting the placebo effect from the treatment effect. The responses were analysed by one-sample t-tests, and the corresponding 95% confidence intervals were calculated. This method was used because many of the underlying variables were not suitable for ANOVA analyses due to non-normality. The study was adequately powered, as with a sample size of 68, the study would have 90% power to detect a within-subject change of one unit on the VAS scale, assuming a standard deviation of 2.5, and using a two-tailed test at the five percent significance level. The treatment difference for the cough VAS scale was modelled by regression analyses to see if any of the baseline factors could predict steroid responsiveness. All analyses were carried out using SAS version 8.02.

RESULTS

Baseline subject characteristics and investigations

We screened 120 subjects with cough, 93 of whom met all the inclusion criteria and were randomised; 88 completed all study visits. Of these, 52 were recruited from the community (GP referrals and advertisement in a newspaper) and 36 were referred from hospital clinics. Five subjects dropped out due to inability to attend for all study visits.

A final diagnosis for the cough was obtained in 78/88 (89%) of subjects; 74% had a single diagnosis, 24% had 2 diagnoses and 2% had 3 diagnoses. The pattern of primary diagnoses (figure 4.1.1) was PNDS (30), GORD (18), CVA (13), bronchiectasis (9), eosinophilic bronchitis (5), habitual cough (2) and bronchitis (1). In 10 subjects, the cough was termed idiopathic. Baseline characteristics of the patients are shown in table 4.1.1. Individual data for the smaller diagnostic sub-groups are not depicted because the numbers were too small for meaningful analysis.

An adequate sample of sputum for differential cell counts was obtained in 70 chronic cough subjects (80%). All were able to perform the eNO and CO tests adequately. The results of the non-invasive tests are depicted in Table 4.1.2. The total sputum cell count, sputum neutrophil proportion and eNO were elevated in subjects with chronic cough, compared to cut-off values. The sputum eosinophil proportion correlated significantly with the eNO levels ($r=0.43$, $p<0.001$) and the total cell count correlated with the neutrophil proportion in sputum ($r=0.48$, $p<0.001$). There was no significant correlation between neutrophils in induced sputum and

age of the patient (r=0.249, p=0.225) or duration of cough (r = -0.013, p=0.916). The total and differential blood cell counts were within the normal range (table 4.1.3).

Mediators at baseline were measurable in 61/88 subjects and 12/17 controls (table 4.1.4). Mean age (SD) of controls was 41 (11) years. LTB_4, cys-LT and PGE_2 were significantly elevated in all causes of cough. IL-8, MPO and TNF-α were elevated only in bronchiectasis (Figures 4.1.2 – 4.1.8).

Response to inhaled corticosteroids

There was a significant difference in the cough VAS scales pre- and post- fluticasone compared to pre- and post- placebo [difference of differences (95%CI) 1.0 (0.4, 1.5), p<0.001] (Figure 4.1.9). The mean (95%CI) % change in VAS was 22.3% (-3.5, 48.2). There was no carry-over effect of active treatment. Compliance was >90% with fluticasone and placebo inhalers. Sputum ECP levels, eNO and CO showed a significant reduction following inhaled corticosteroids. The mean difference (CI), p value was [–2.1 (-3.56,-0.64), p=0.005] for eNO, [-0.34 (-0.6, 0.04), p=0.025] for CO and [–396 (-753, 39), p=0.031] for ECP (figure 4.1.10). There was no significant difference in the other mediators following treatment (figures 4.1.11 – 4.1.16). For the individual causes of cough, there was no significant change in sputum cell counts, cys-LT, LTB_4, PGE_2, IL-8, TNF-α and MPO following treatment (tables 4.1.5, 4.1.6), except for a reduction in sputum eosinophils in CVA and IL-8 levels in bronchiectasis.

Predictors of inhaled corticosteroid responsiveness

Exhaled NO (R^2 0.152, p < 0.001), sputum eosinophils (R^2 0.08, p=0.019), sputum ECP (R^2 0.064, p=0.05), cys-LT (R^2 0.07, p=0.039) and total IgE levels (R^2 0.07, p=0.022) were significant predictors of corticosteroid responsiveness (table 4.1.7, figure 4.1.17) . The other mediators and cell counts had no predictive value for this response. Factors unrelated to corticosteroid responsiveness were age, gender, duration of cough, baseline cough VAS level, positive specific IgE levels, FEV_1% predicted, previous smoking history and whether the patient entered the study through the community or hospital clinics. Only one subject had received a course of oral corticosteroids in the past. Forty-eight subjects had previously received inhaled corticosteroids in varying doses and durations of treatment. There was no significant difference in response to fluticasone in those who had received inhaled corticosteroids in the past compared to those who had never received this treatment earlier (R^2= 4%, p = 0.069).

Figure 4.1.1: Patterns of Primary Diagnosis in Chronic Cough

PNDS=post-nasal drip syndrome, GORD=gastro-oesophageal reflux disease, Bronch=bronchiectasis,
Eos bronch = eosinophilic bronchitis, Idio=idiopathic cough

Table 4.1.1: Baseline characteristics of chronic cough subjects.

	All causes	PNDS	GORD	CVA	Bronchiec	Idio
Number	88	30	18	13	9	10
Age, years*	59.0 (12.7)	58.8 (12.4)	67.7 (4.4)	48.5 (17.3)	64.0 (9.6)	57.7 (8.7)
Gender, male/female	32/57	12/18	8/10	2/11	3/6	4/6
Duration of cough, years	16.2 (16.1)	18.6 (17.4)	14.9 (15.8)	13.6 (9)	17.8 (19.4)	13.9 (18.6)
Baseline cough VAS, cm.	4.1 (2.0)	3.9 (2.3)	3.9 (1.9)	4.7 (2.1)	4.3 (1.7)	4.4 (2.2)
Ex-smokers: number	35	13	8	4	4	4
Years quit smoking	19.7 (12.9)	20.7 (13.7)	21.6 (13.9)	7.1 (8.9)	20.3 (6.9)	25.8 (16.4)
Pack years smoked	14.8 (15.8)	10.0 (13.1)	24.7 (19.9)	14.6 (24.4)	20.3 (8.7)	7.4 (7.0)
Previous inhaled corticosteroids, n (%)	48 (55)	15 (50)	8 (44)	6 (46)	6 (67)	6 (60)
FEV$_1$, L	2.6 (0.8)	2.7 (0.8)	2.4 (0.6)	2.4 (1.0)	2.1 (0.8)	2.9 (0.9)
FEV$_1$ % predicted	94 (16)	96 (12)	94 (15)	86 (24)	88 (12)	101 (13)

*Data listed as mean (SD), unless stated otherwise., *ANOVA test p<0.001 between the sub-groups.*

Table 4.1.2.: Baseline values of exhaled gases and sputum cell counts in subjects with chronic cough.

	Cut-off value	All causes	PNDS	GORD	CVA	Bronchiec	Idio
Number		88	30	18	13	9	10
Exhaled NO, ppb	9	10.4* (5.7)	8.9 (3.5)	10.0 (3.8)	12.6 (7.6)	11.2 (3.7)	8.6 (4.3)
CO, ppm	4	3.7 (1.1)	3.6 (1.0)	3.7 (1.4)	3.4 (0.9)	3.5 (1.2)	4.1 (0.9)
Induced sputum							
Total cell count, x10^6	8	10.4* (9.1)	10.1 (7.0)	6.1 (5.6)	7.9 (4.9)	21.3* (12.1)	14.6 (12.9)
Neutrophils,%	53	62 (22.9) †	60.4 (25)	58.1 (20.6)	68.1* (21.2)	81.2 (13) †	62.7 (26.9)
Eosinophils, %	2	1.0 (2.3)	0.3 (0.4)	0.4 (0.5)	2.4 (4.3)	1.4 (3.3)	0.3 (0.8)
Lymphocytes,%	3	0.2 (0.4)	0.3 (0.6)	0.2 (0.4)	0.2 (0.5)	0.1 (0.2)	0.1 (0.2)

*Data depicted as mean (SD), unless stated otherwise; * = $p<0.05$, † = $p \leq 0.001$ compared to healthy control values.*

Table 4.1.3.: Baseline values of blood cells and serum IgE in subjects with chronic cough.

	Normal/Cut-off	All causes	PNDS	GORD	CVA	Bronchiec	Idio
Number		88	30	18	13	9	10
Blood cell counts x10⁹/L							
Total white cells	4.0-11.0	7.9 (1.7)	8.1 (1.4)	7.7 (1.5)	7.9 (2.3)	8.5 (2.2)	7.5 (1.7)
Neutrophils	2.0-7.5	3.97 (1.3)	3.98 (1.0)	4.2 (1.1)	3.6 (1.2)	4.6 (2.0)	3.8 (1.7)
Eosinophils	0.04-0.4	0.09 (0.1)	0.09 (0.1)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.07 (0.1)
Lymphocytes	1.5-4.0	3.43 (1.01)	3.7 (0.9)	3.1 (0.9)	3.8 (1.4)	3.3 (1.1)	3.3 (1.1)
Serum IgE levels							
Total serum IgE, IU/l	>120	96 (195)	61 (72)	110 (162)	32 (35)	75 (99)	168 (419)
Specific IgE positive, n (%) >0.35		18 (21)	4 (13)	4 (22)	4 (33)	1 (14)	4 (13)

*Data listed as mean (SD), unless stated otherwise., *ANOVA test p<0.001 between the sub-groups.*

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Table 4.1.4: Baseline mediator levels in induced sputum in different causes of cough

	Controls	All causes	PNDS	GORD	CVA	Bronchiec	Idio
Number	12	70	18	12	9	9	6
ECP, ng/ml	363 (249)	1082 (891) *	1055 (931)	1182* (966)	895 (904)	1479* (812)	661 (305)
PGE_2, ng/ml	0.1 (0.2)	16.0 (10.7) †	10.2 (6.5) †	21.0 (14.2) †	11.5 (7.1) †	17.5 (8.0) †	27.9 (10.7) †
LTB_4, ng/ml	4.58 (5.4)	28.2 (12.7) †	35.3 (13.7) †	25.3 (14.3) †	25.9 (10.7) †	32.7 (8.4) †	33.3 (6.6) †
Cys-LT, ng/ml	0.3 (0.8)	1.4 (1.0) †	1.3 (0.9) *	1.4 (1.3) *	1.5 (1.2) *	1.2 (0.6) *	1.7 (1.6) *
IL-8, ng/ml	4.8 (2.1)	29.7 (65.4)*	9.0 (6.9)	25.1(47)	14.6 (23.1)	142 (126) †	12.9 (18.6)
MPO, µg/ml	5.2 (4.1)	17.8 (24.6)	11.6 (19.0)	16.6 (23.0)	14.7 (15.8)	57 (32.2) †	8.8 (6.0)
TNF-α, ng/ml	0.02 (0.02)	1.3 (4.1)	0.04 (0.1)	1.0 (2.8)	0.05 (0.1)	6.0 (8.1)*	0.01 (0.0)

Data depicted as mean (SD), unless stated otherwise;   * = $p<0.05$, † = $p \leq 0.001$ compared to healthy control values.

Table 4.1.5. Change in cough VAS scale, exhaled gases and sputum cells after inhaled corticosteroids compared to placebo.

Data depicted as mean difference (95% confidence intervals).

Number	All causes 88	PNDS 30	GORD 18	CVA 13	Bronchiec 9	Idio 10
Δ cough VAS,cm	1.0†	0.9*	1.2	1.4*	0.7	0.5
	(0.4,1.5)	(-0.0,1.8)	(0.0,2.3)	(-0.0,2.7)	(-0.9,2.4)	(-1.0,2.1)
Δ eNO,ppb	-2.1*	0.0	-3.1*	-3.3*	-0.9	-1.4
	(-3.6,-0.6)	(-2.0,2.0)	(-5.8,-0.5)	(-6.5,-0.2)	(-4.5,2.7)	(-4.8,2.1)
Δ eCO,ppm	-0.3*	-0.1	-0.3	-0.3	-0.7	-0.1
	(-0.6,-0.0)	(-0.6,0.4)	(-1.0,0.4)	(-1.1,0.5)	(-1.6,0.2)	(-1.0,0.7)
Induced sputum						
Δ total cells x10^6	-1.0	-1.5	-4.6	3.4	5.1	-2.8
	(-7.4,7.7)	(-11.9,9.0)	(-12.3,3.1)	(-7.0,13.9)	(-6.3,16.4)	(-15.2,9.6)
Δ neutrophils %	1.1	-1.2	-13.8	0.9	0.6	5.3
	(-7.5,9.7)	(-18.6,16.2)	(-29.8,2.1)	(-19.9,21.7)	(-20.2,21.4)	(-14.2,24.8)
Δ eosinophils %	-0.7	-0.0	-0.1	-4.6†	1.5	0.1
	(-1.8,0.3)	(-2.0,2.1)	(-2.0,1.8)	(-7.1,-2.1)	(-1.0,3.9)	(-2.2,2.4)
Δ lymphocytes %	-0.1	0.0	0.1	-0.3	-0.1	-0.1
	(-0.2,0.1)	(-0.3,0.4)	(-0.3,0.4)	(-0.7,0.1)	(-0.5,0.3)	(-0.5,0.3)

*=p<0.05,†=P<0.001

Table 4.1.6. Change in induced sputum mediators after inhaled corticosteroids compared to placebo.

Data depicted as mean difference (95% CI).

	All causes	PNDS	GORD	CVA	Bronchiec	Idio
Δ ECP, ng/ml	-396 *	-506	62	80	-142	-248
	(-753,39)	(-1160,148)	(-554,679)	(-1227,1387)	(-969,684)	(-1316,820)
Δ MPO, μg/ml	10.7	-34.0	-17.7	70.0	133.5 *	9.1
	(-29.5,51.1)	(-113.3,45.3)	(-97.1,61.6)	(-98.3,238.3)	(27.0,239.9)	(-128.3,146.5)
Δ PGE$_2$, ng/ml	-1.9	4.9	-4.7	12.1	-11.6	-9.8
	(-9.0,5.3)	(-10.3,20.1)	(-20.9,11.4)	(-10.7,34.9)	(-30.2,7.1)	(-32.7,13.0)
Δ LTB$_4$, ng/ml	2.3	5.1	1.7	25.6	-15.7	-9.2
	(-13.2,17.8)	(-32.8,43.1)	(-36.3,39.7)	(-18.3,69.4)	(-49.7,18.2)	(-53.1,34.6)
Δ Cys-LT, ng/ml	-0.4	0.1	-0.2	-0.6	-1.4	0.3
	(-1.2,0.4)	(-1.5,1.7)	(-1.8,1.5)	(-2.9,1.6)	(-3.3,0.4)	(-2.3,2.9)
Δ IL-8, ng/ml	-21.5	-3.5	-27.0	1.5	-74.7*	-20.9
	(-48.3,5.2)	(-61.9,55.0)	(-88.9,35.0)	(-86.1,89.1)	(-146.3,-3.1)	(-122.1,80.3)
Δ TNF-α, ng/ml	0.3	-3.9	3.6	0.0	0.4	nd
	(-4.9,5.4)	(-17.7,9.9)	(-8.4,15.6)	(-13.8,13.8)	(-10.3,11.1)	(0,0)

Δ = change in value following inhaled fluticasone compared to placebo, nd=not detectable;   * = p < 0.05, †= $p \leq$ 0.001

Figure 4.1.2: LTB$_4$ levels [mean (SD)] in induced sputum in different causes
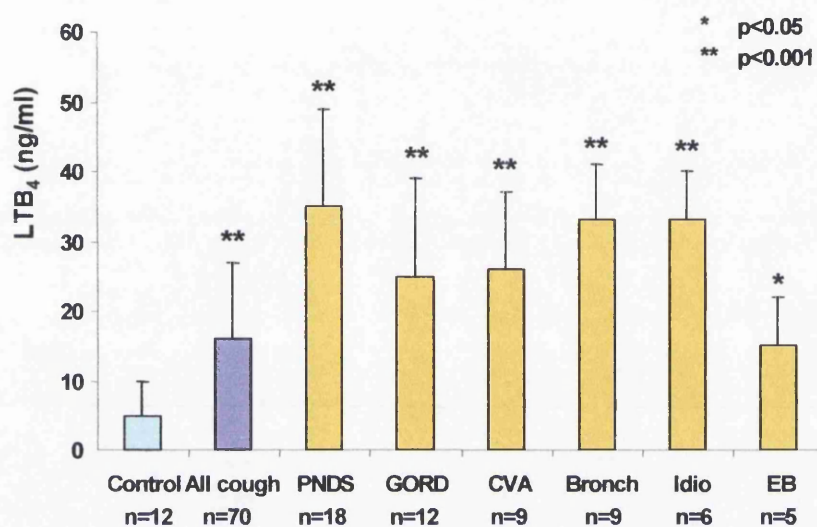
of chronic cough

Figure 4.1.3: Cysteinyl leukotriene levels [mean (SD)] in induced sputum in different

causes of chronic cough
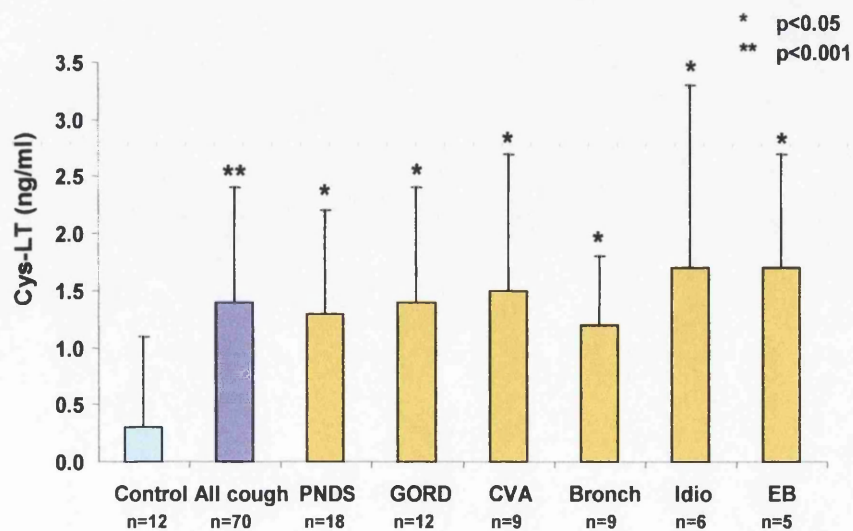
Figure 4.1.4: Prostaglandin E_2 levels [mean (SD)] in induced sputum in different causes of chronic cough
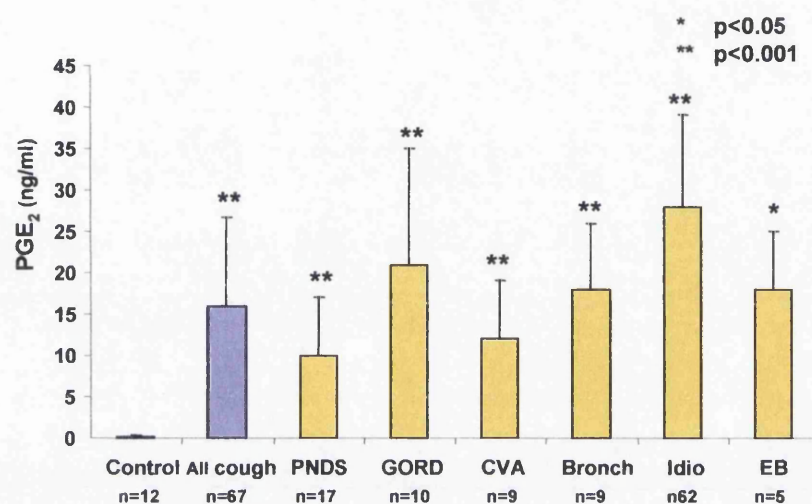
Figure 4.1.5: Eosinophilic cationic protein levels [mean (SD)] in induced sputum in different causes of chronic cough
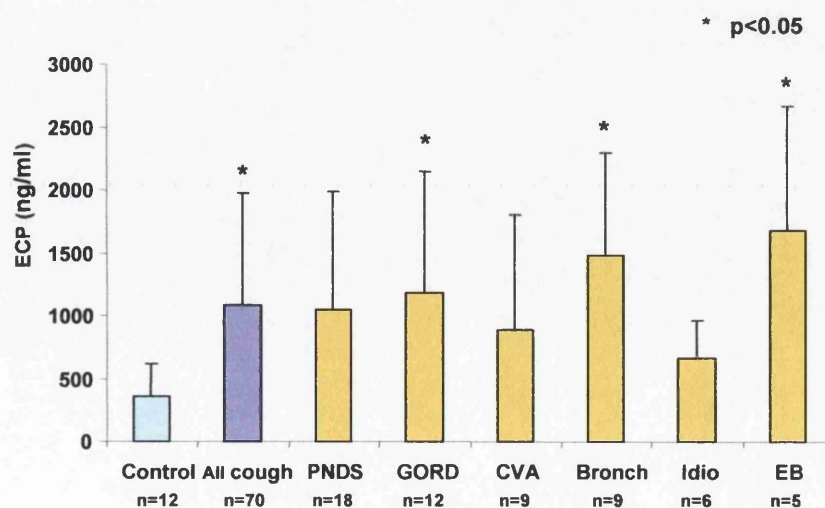
Figure 4.1.6: Myeloperoxidase levels [mean (SD)] in induced sputum in different causes of chronic cough
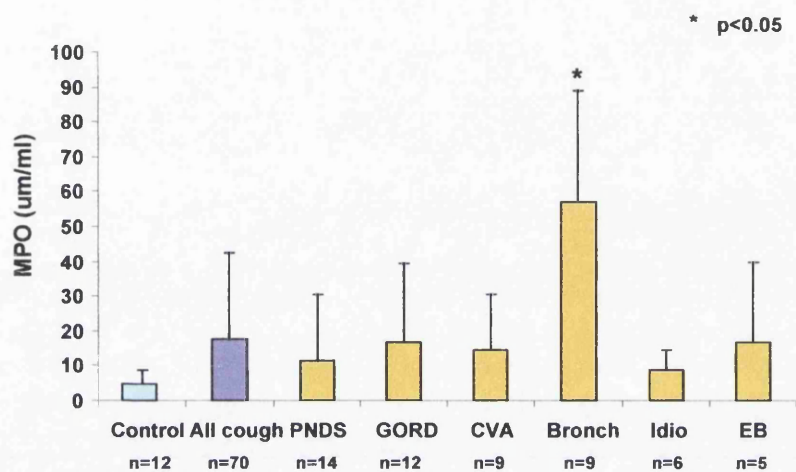
Figure 4.1.7: Tumour necrosis factor-α levels [mean (SD)] in induced sputum in different causes of chronic cough
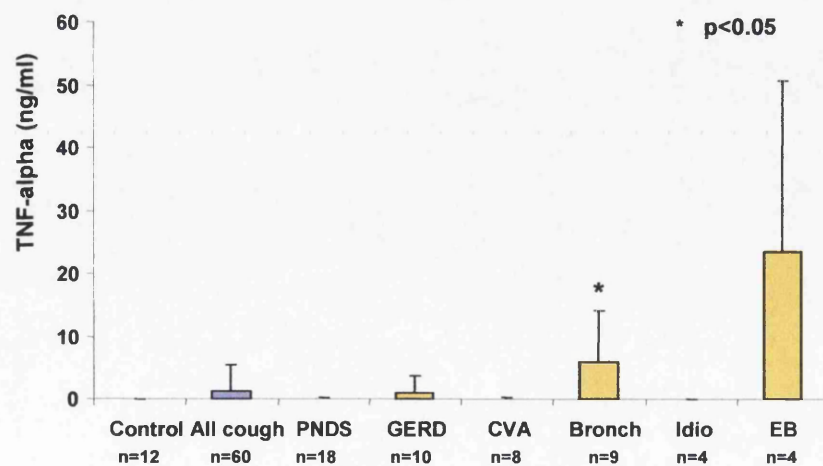
Figure 4.1.8: Interleukin-8 levels [mean (SD)] in induced sputum in different causes of chronic cough
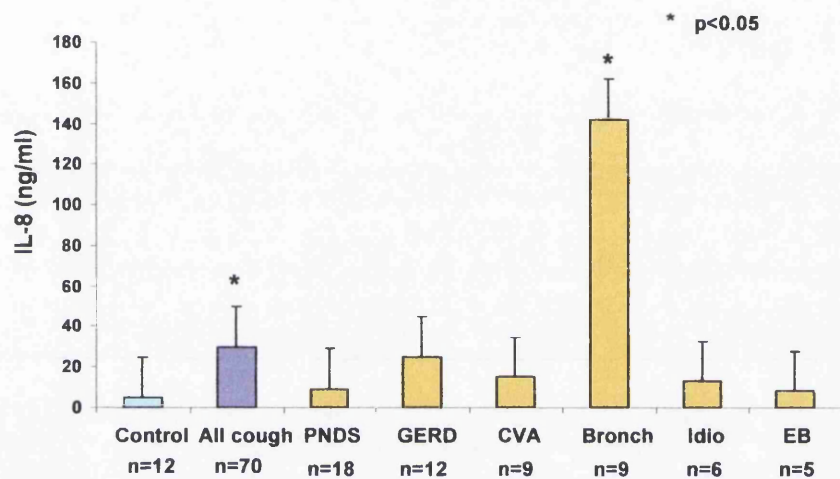
Figure 4.1.9: Change in cough visual analogue scale following fluticasone compared to placebo
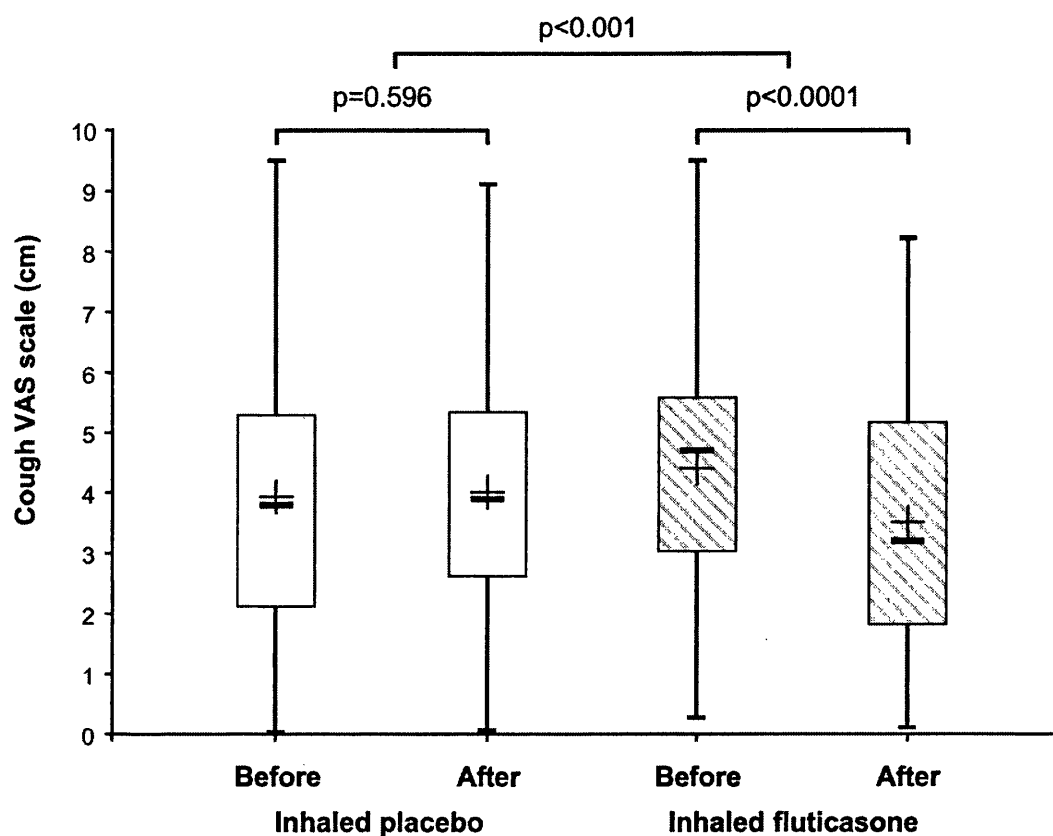
Mean visual analogue scale (VAS) for cough severity measured on a 10 cm scale, following inhaled fluticasone (hatched box) compared to placebo (clear box).

The boxes represent the inter-quartile range, with the extended lines representing the maximum and minimum values. The mean (+) and the median (-) values of the VAS are depicted in the figure. p<0.001 is the difference of differences for treatment with placebo compared to fluticasone.

Figure 4.1.10: Eosinophilic cationic protein in induced sputum following inhaled fluticasone compared to placebo
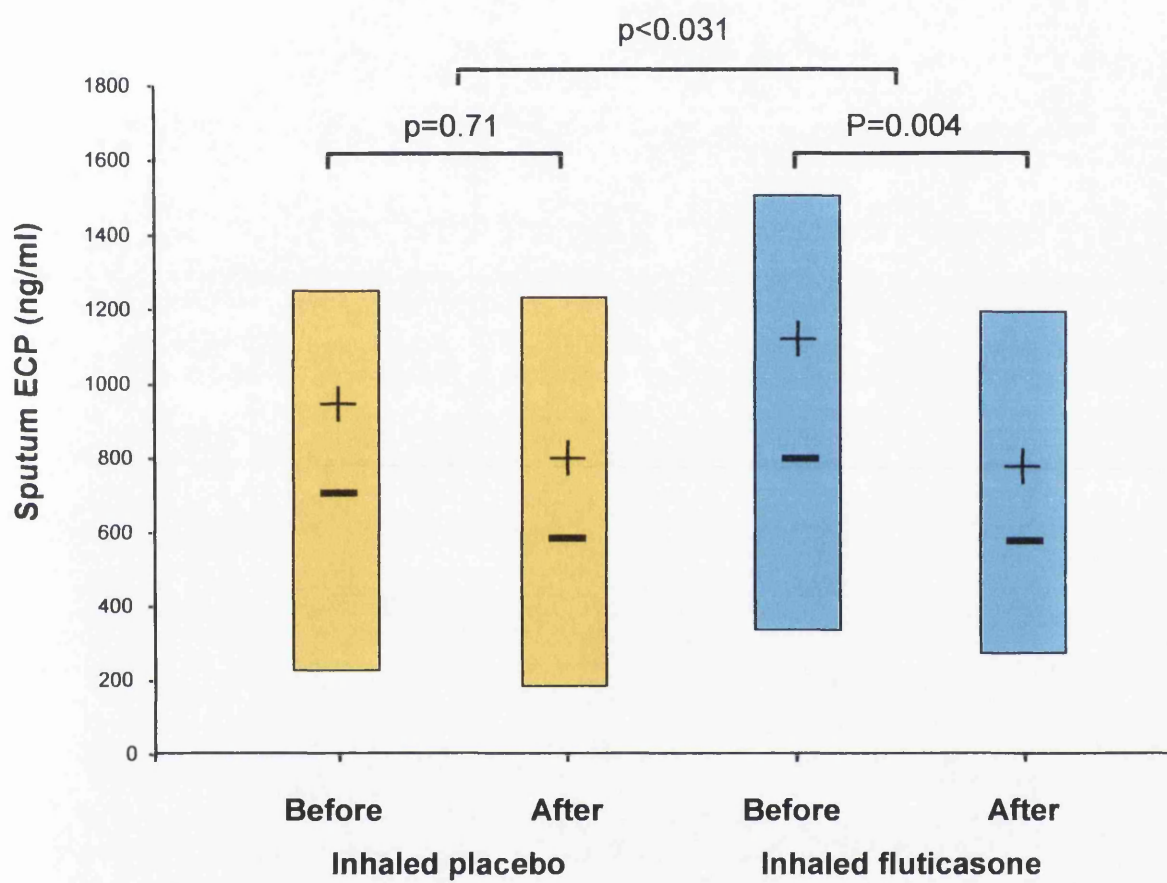
The box represents the interquartile range, the + the mean value and – the median value.

Figure 4.1.11: Cysteinyl leukotrienes in induced sputum following inhaled fluticasone
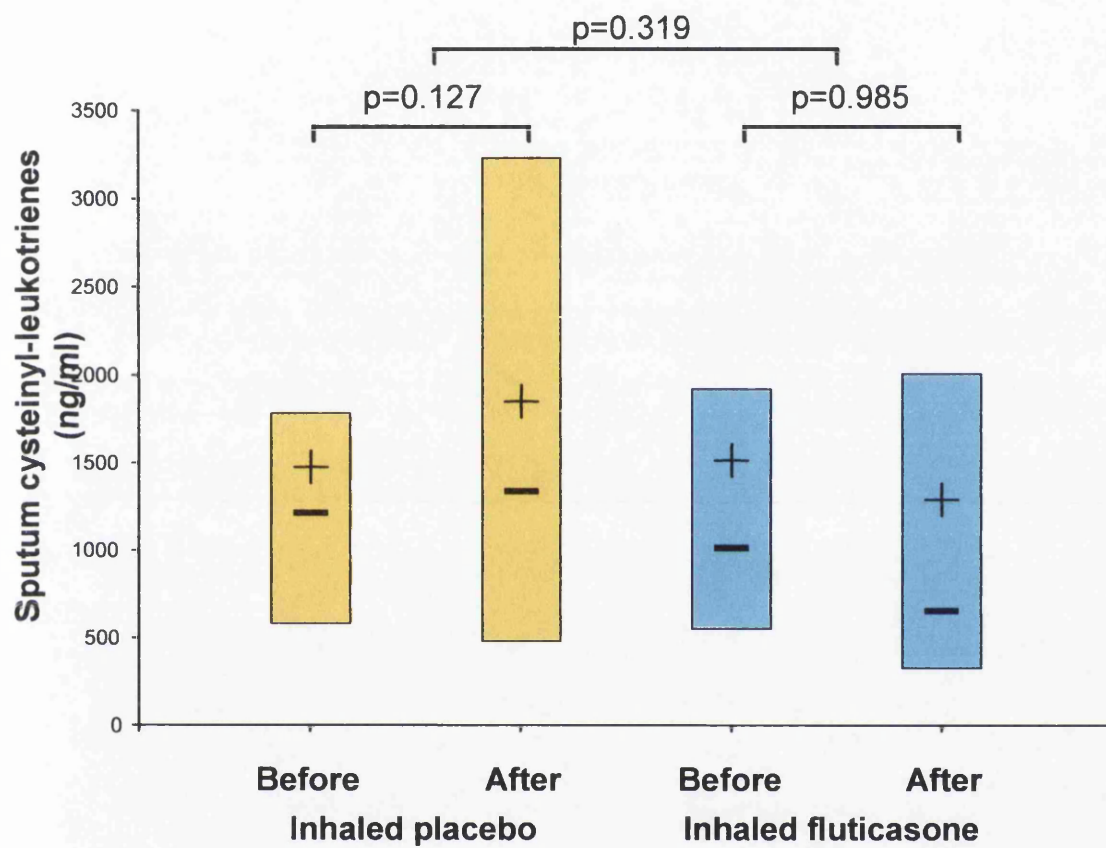
compared to placebo

The box represents the interquartile range, the + the mean value and – the median value.

Figure 4.1.12: Leukotriene B$_4$ in induced sputum following inhaled fluticasone compared to placebo
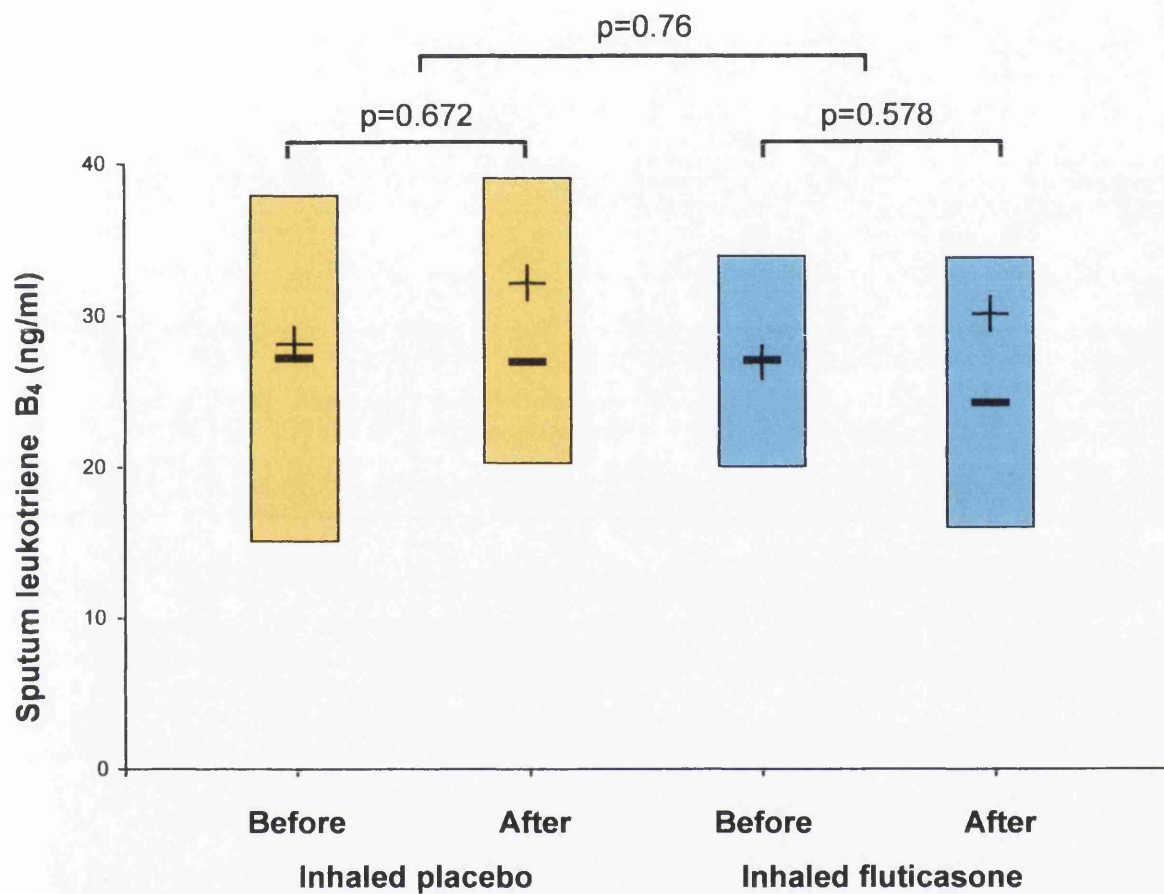
The box represents the interquartile range, the + the mean value and – the median value.

Figure 4.1.13: Prostaglandin E_2 in induced sputum following inhaled fluticasone compared to placebo
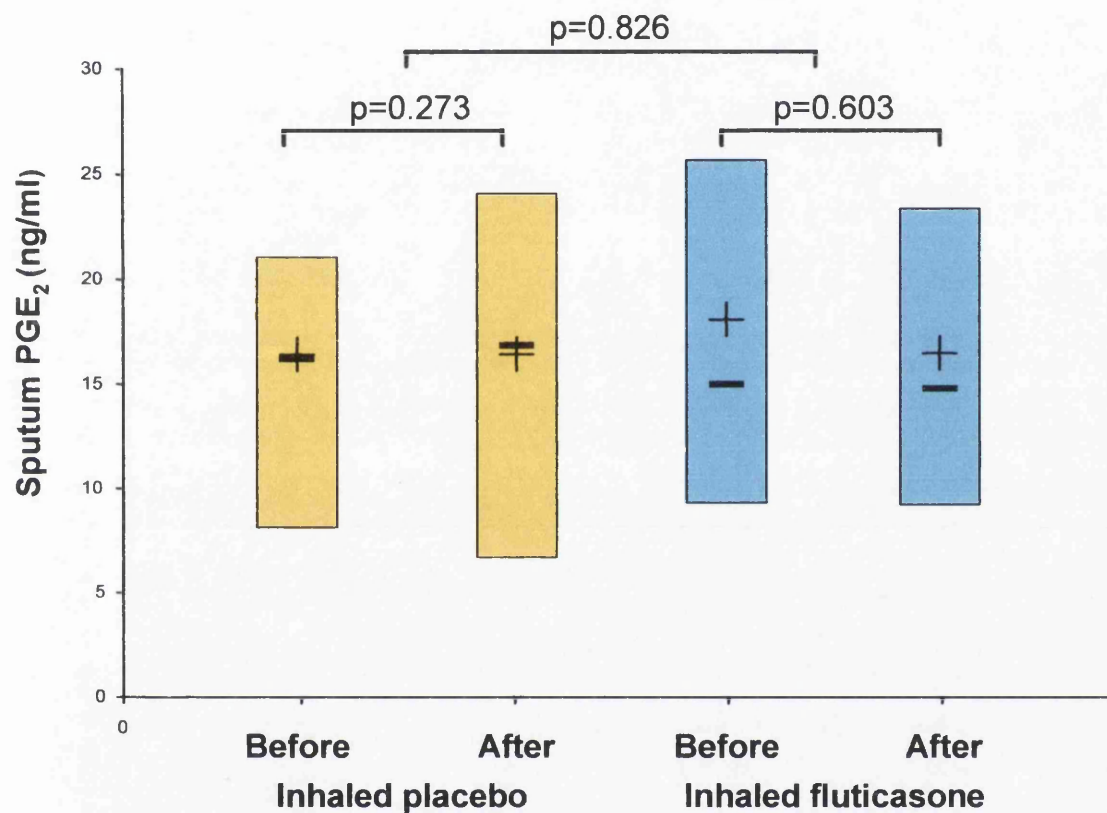
The box represents the interquartile range, the + the mean value and – the median value.

Figure 4.1.14: Myeloperoxidase in induced sputum following inhaled fluticasone compared to placebo
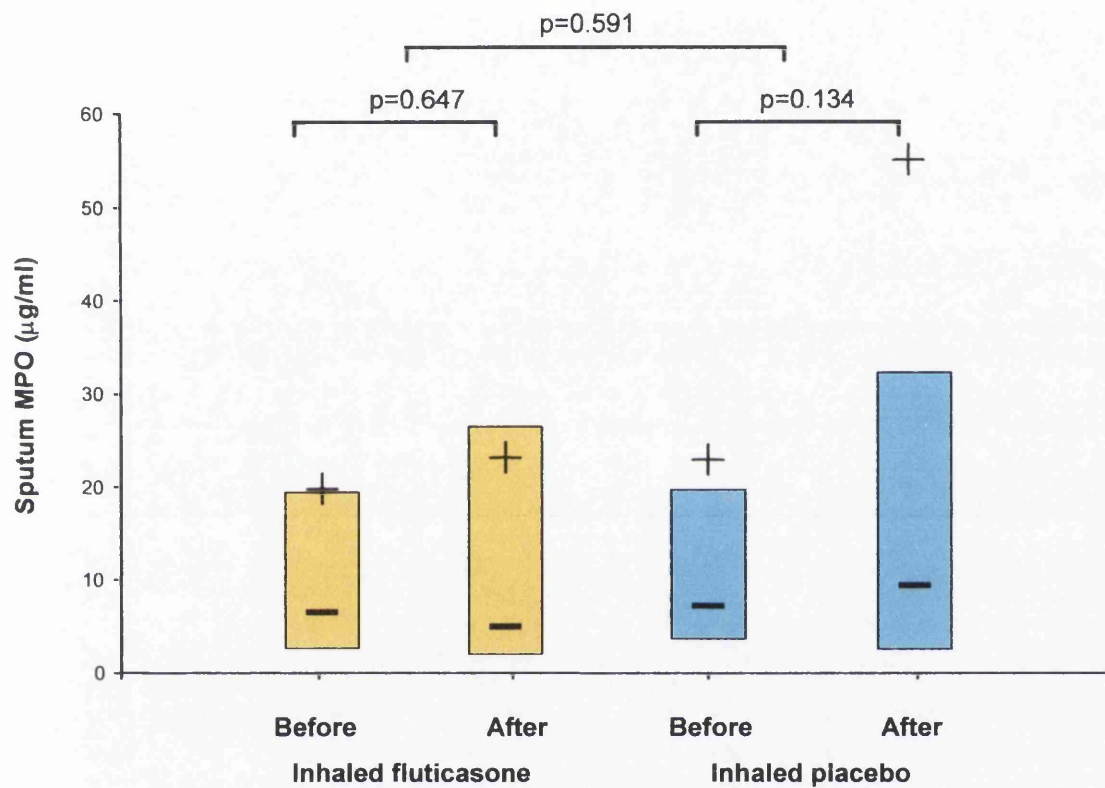
The box represents the interquartile range, the + the mean value and – the median value.

Figure 4.1.15: TNF-alpha in induced sputum following inhaled fluticasone compared to placebo
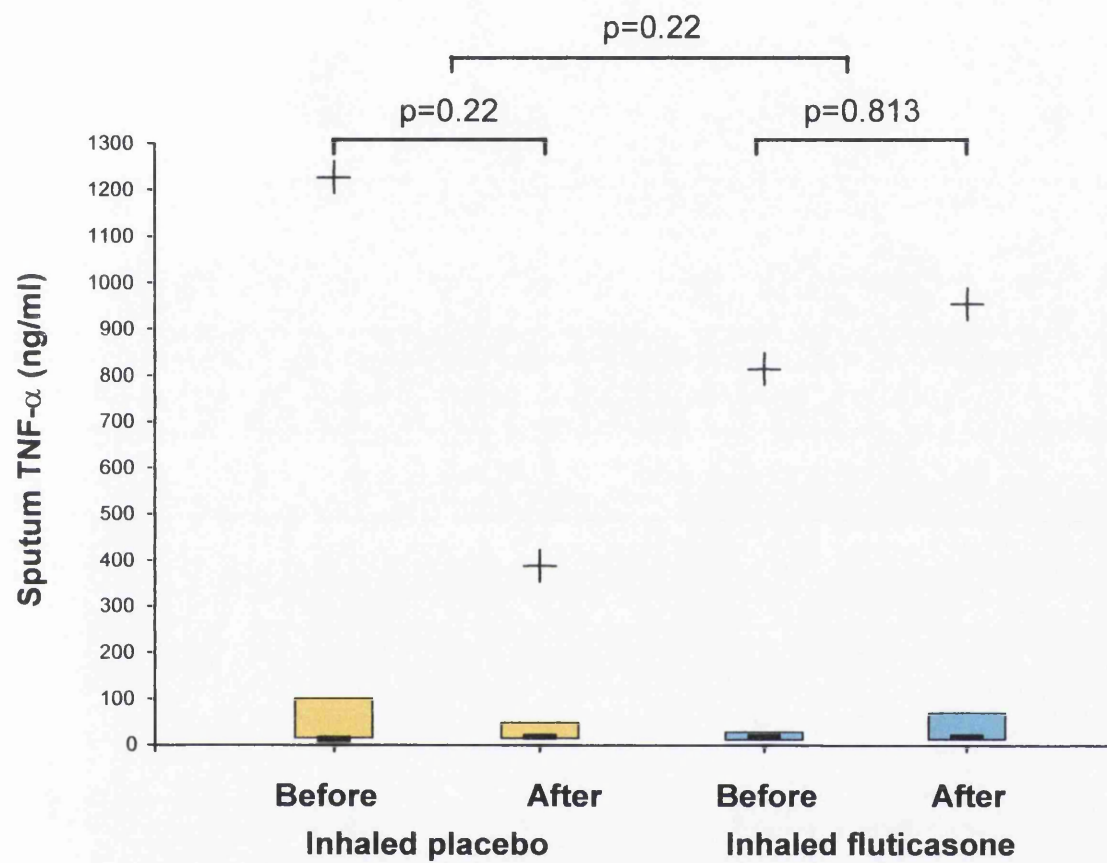
The box represents the interquartile range, the + the mean value and – the median value.

Figure 4.1.16: Interleukin-8 in induced sputum following inhaled fluticasone compared to placebo
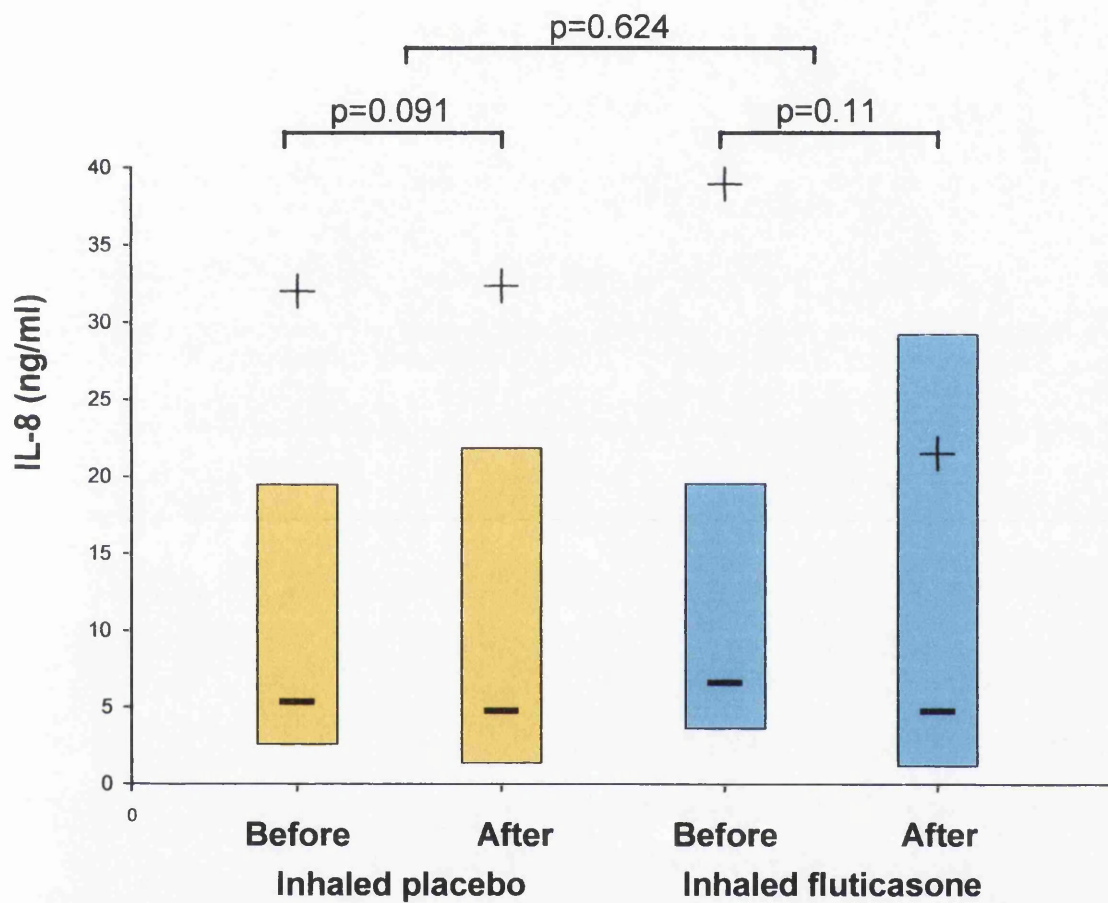
The box represents the interquartile range, the + the mean value and – the median value.

Table 4.1.7 Predictors of steroid responsiveness in chronic cough

a: Baseline demographic features as predictors of steroid responsiveness in chronic cough

	R^2	P value
Duration of cough, weeks	0.01	0.377
Age	0.01	0.510
IgE total	0.07	**0.022**
IgE specific	0.00	0.522
FEV_1 pre	0.04	0.080
FEV_1%pred	0.00	0.674
Baseline VAS	0.01	0.454
Gender	0.04	0.071
Ex-smoker	0.00	1.000
Source of patient (community or hospital)	0.00	0.971

Table 4.1.7 b: Non-invasive tests as predictors of steroid responsiveness in chronic cough

Test	R^2	P value
Exhaled NO	0.152	**< 0.001**
CO	0.018	0.209
Methacholine challenge test	0.01	0.403
Sputum total cell count	0.00	0.946
Sputum neutrophils %	0.00	0.604
Sputum eosinophils %	0.08	**0.019**
Sputum lymphocytes %	0.00	0.783
Sputum macrophages %	0.01	0.448
Combined exhaled NO and sputum eosinophils %	0.2527	**< 0. 001**

Table 4.1.7c: Mediator levels in induced sputum as predictors of steroid responsiveness in chronic cough

Mediator in induced sputum	R^2	P value
ECP	0.064	**0.050**
PGE$_2$	0.045	0.111
IL-8	0.000	0.940
LTB$_4$	0.004	0.625
Cys-LT	0.070	**0.039**
MPO	0.005	0.573
TNF-α	0.018	0.342

Figure 4.1.17: Predictors of steroid responsiveness

a. Correlation of exhaled nitric oxide and change in cough severity following inhaled fluticasone

$R^2 = 0.152$
$p<0.001$
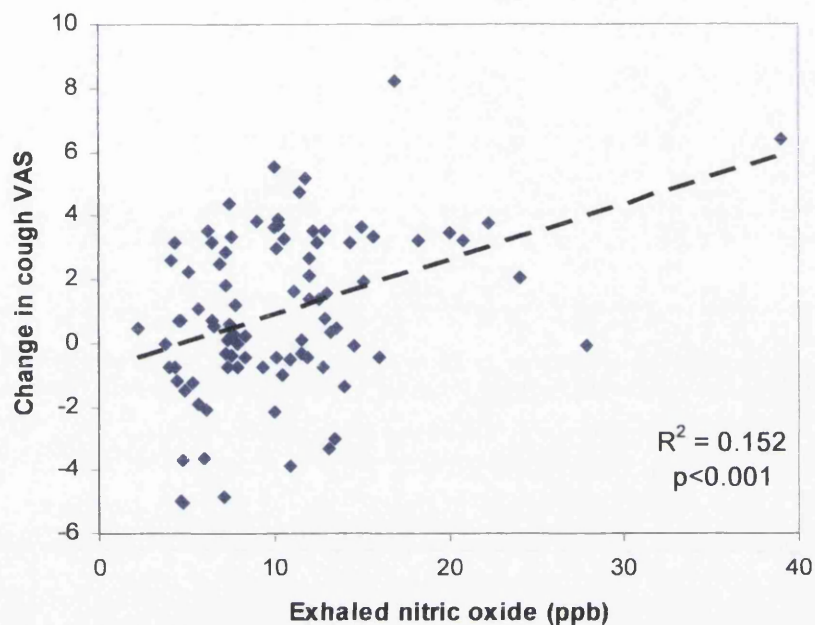
b. Correlation of sputum eosinophil % and change in cough severity following inhaled fluticasone

$R^2 = 0.08$
$p = 0.019$
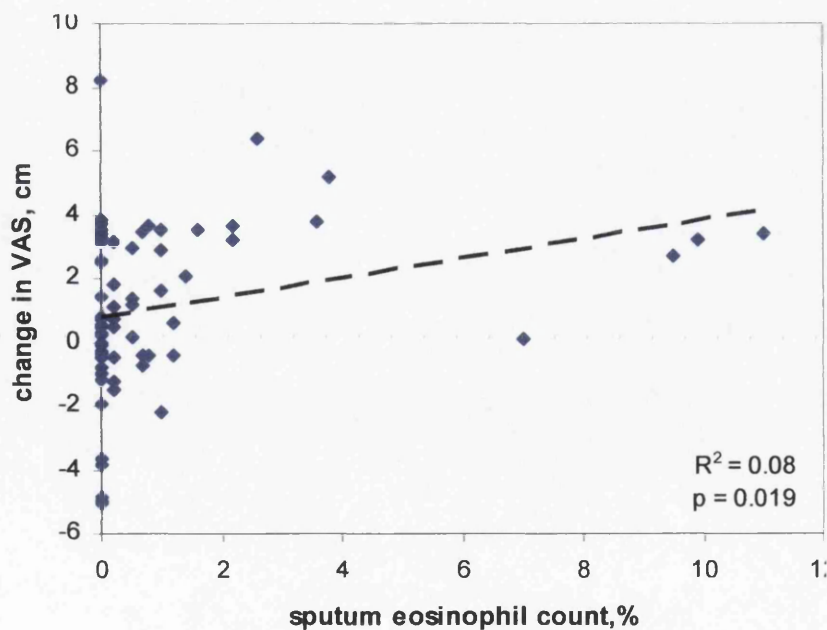
DISCUSSION

This is the first randomised, placebo-controlled study to demonstrate an improvement in cough severity following inhaled fluticasone compared to placebo in patients with chronic cough for over one year. The decrease in cough severity score was accompanied by a fall in sputum ECP, eNO and CO but there was no reduction in sputum mediator levels of LTB_4, cys-LT, PGE_2, IL-8, MPO and TNF-α. Exhaled NO levels were the best predictor of corticosteroid responsiveness.

Although there was a statistically significant decrease in the cough VAS scale, this corresponded to a mean (95%CI) % improvement of 22.3 (-3.5, 48.2) in the cough. There are several possible explanations for the modest improvement in cough severity score for most subjects in this study. Firstly, the baseline sputum samples demonstrated a predominant airway neutrophilia rather than eosinophilia. Neutrophilic inflammation of the airways is relatively insensitive to corticosteroid therapy (228). The association between airway eosinophilia and corticosteroid responsiveness has been demonstrated in patients with chronic cough (76). Corticosteroids are very effective at reducing eosinophils in the airways (229) and sputum eosinophilia has been shown to predict a clinical benefit from corticosteroids in asthma and COPD (65, 230). A study by Pizzichini et al (52), which included subjects with chronic cough where asthma, PNDS and GORD were excluded as possible causes, found none of the subjects had elevated sputum eosinophils and there was no response to a trial of inhaled corticosteroids. Secondly, we included subjects with a duration of cough greater than one year, as it represented the most problematic patients with cough in the community. It is

possible that cough of a shorter duration may be more corticosteroid responsive. Thirdly, it is possible that a longer duration of treatment with inhaled corticosteroids could increase the number of responders. In some studies response to inhaled corticosteroids was seen within two weeks (60, 75) and in others, extending treatment to four weeks did not improve the response rate (43, 52, 231). We believe that two weeks of high dose inhaled corticosteroids should have identified a change in cough severity in the vast majority of subjects who are likely to respond to this treatment.

In this study, patients had a history of cough for a mean duration of 16.2 years. Most of the subjects had previously received some form of treatment for chronic cough, details of which they were unable to accurately recall. As we had no record of response to previous therapy, we included all subjects provided they had not been taking inhaled or oral corticosteroids in the previous 3 weeks. There was no significant difference in response to inhaled fluticasone in those who had received a prior course of inhaled corticosteroid compared to those who had never received this treatment. Hence there was no bias towards selecting subjects who were unlikely to respond to inhaled corticosteroids.

We have shown that in cough persistent for over a year, regardless of aetiology, there is airway inflammation, with an increase in induced sputum neutrophils, airway mediators and exhaled nitric oxide. Jatakanon and colleagues selected patients with non-asthmatic chronic cough and demonstrated a marked neutrophilia in induced sputum (51). We have shown that this neutrophilia extends across the spectrum of causes of chronic persistent cough, with the highest levels in bronchiectasis. Although the reasons for this are not known, speculation has

included the effect of mediators such as TNF-alpha and IL-8 promoting the neutrophil influx, which may be perpetuated by the act of coughing itself (51). In contrast, Birring and colleagues noted elevated sputum eosinophils in a sub-group of patients and normal neutrophil counts (45). They included subjects with a duration of cough greater than three weeks as opposed to one year in our study and the mean duration of cough was about three years compared to 16 years in ours. Hence, although the trend of inflammation in induced sputum in persistent cough appears to be neutrophilic, further work on this subject is required.

As the inflammatory pattern can vary in cough, we chose to measure seven mediators to assess activation of different inflammatory cells. ECP and MPO are indirect markers of eosinophil and neutrophil activity respectively. IL-8 is a neutrophil chemo-attractant and activator and can also induce the release of histamine and cys-LTs from human blood basophils (232, 233). TNF-α is a pro-inflammatory cytokine produced by macrophages and mast cells and maybe an important mediator in initiating chronic inflammation by activating the secretion of cytokines from a variety of cells in the airways (234). Leukotrienes are produced by mast cells, eosinophils and neutrophils and are implicated in the inflammation of asthma and COPD. PGE_2 can increase cough sensitivity (130).

In our study we noted elevated levels of cysteinyl-LT, LTB_4, ECP, PGE_2 and IL-8. Following two weeks of inhaled fluticasone, 1000 mcg daily, exhaled NO, CO and ECP were significantly suppressed compared with placebo, with no change in sputum cell counts and other mediators. Responsiveness of ECP to corticosteroids has been noted in asthma (150, 235) as well as in COPD (154). Leukotrienes and IL-8 were elevated at baseline but did not

respond to corticosteroids, similar to findings in asthma and COPD (152, 154). Choudry et al demonstrated that the cough reflex is increased in the presence of PGE_2 in the airways (130) and Birring et al found elevated PGE_2 in induced sputum of subjects with cough (45). We have demonstrated that induced sputum levels of PGE_2, cys-LT and LTB_4 are significantly high in patients with all causes of chronic persistent cough and persist after inhaled corticosteriod treatment.

Exhaled NO has been shown to be increased in inflammatory lung conditions such as asthma and has been used as a predictor of oral corticosteroid responsiveness (73). In this study, eNO was mildly elevated in patients with chronic cough. We found a strong correlation between eNO and sputum eosinophils, similar to patients with asthma (84). Regression analysis revealed eNO to be the most useful predictor of corticosteroid responsiveness. Levels of exhaled CO are elevated in asthma, bronchiectasis, upper respiratory tract infections, lower respiratory tract infections, seasonal allergic rhinitis and cystic fibrosis (95-99, 104). Treatment with inhaled or oral corticosteroid has been shown to reduce exhaled CO in asthmatics (96, 100). The level of CO was not elevated at baseline in chronic persistent cough, but did reduce significantly after inhaled corticosteroid treatment.

In conclusion, chronic cough, persistent for over one year, partially responds to inhaled corticosteroids regardless of the aetiology of cough. Exhaled nitric oxide is the most useful predictor of this response. The inflammation is complex with sputum neutrophilia and elevated PGE_2, LTB_4 and cys-LT in all causes of cough. ECP was the only mediator to reduce following corticosteroid treatment. This result raises the possibility that alternative drugs

targeted to reduce the production/action of these pro-inflammatory mediators may be of therapeutic benefit in chronic cough.

4.2: SERUM AND SPUTUM NEUROTROPHIN LEVELS IN CHRONIC PERSISTENT COUGH

INTRODUCTION

Chronic cough is a common clinical problem with a reported prevalence of between 14-23%. It often persists for over a year (33, 35) and has adverse effects on quality of life. In non-smokers with a normal chest radiograph, the most frequent causes are post-nasal drip syndrome (PNDS), gastro-oesophageal reflux disease (GORD) and cough variant asthma (CVA) (6, 8, 32, 34). Although chronic cough can be caused by various different conditions, underlying airway inflammation appears to be a common factor (40-42, 51).

There is evidence that chronic cough is associated with neurogenic inflammation. The afferent sensory receptors and nerves (the myelinated rapidly adapting receptors and the unmyelinated C-fibres) are considered important components of the cough reflex (170). Up-regulation in sensory nerve function in the afferent portion of the cough reflex appears fundamental to the pathophysiology which underlies chronic cough (236). It is believed that activation of C-fibre receptors in the airway releases sensory neuropeptides, which cause neurogenic inflammation and may activate rapidly adapting receptors to cause cough (170). Inhaled substance P induces cough in humans with an upper respiratory tract infection or idiopathic pulmonary fibrosis and increased levels have been found in nasal lavage fluid from patients with chronic productive cough (237). The neuropeptide calcitonin gene-related peptide (CGRP) induces histamine release from broncho-alveolar lavage mast cells obtained from asthmatic and non-asthmatic subjects with chronic cough (236).

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Neurotrophins are a family of structurally related growth factors, which include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5). The physiological role of neurotrophins includes regulating the development, differentiation, survival and function of distinct neurons. In addition, there is growing evidence for their involvement in inflammation. T-cells, B-cells, macrophages and mast cells may be sources of neurotrophic factors during the process of inflammation (167).Mast cells are able to produce, store and release NGF. *In vitro* stimulation with NGF leads to degranulation with the release of pre-formed and newly generated mediators such as histamine, leukotrienes and cytokines, all of which are known to be increased in cough (45, 238). NGF is a known regulator of neuropeptide synthesis in sensory nerves, which triggers neurogenic inflammation (239). In a mouse model of allergic inflammation and in NGF transgenic mice, neurotrophins altered sensory innervation, enhanced neuropeptide production and induced airway hyper-responsiveness (162, 163). Elevated levels of NGF and BDNF have been demonstrated in plasma from patients with untreated asthma (164, 166), which were reduced by corticosteroid treatment (166) and in the sputum of patients with idiopathic pulmonary fibrosis (175).

The aim of the present study was to assess the role of neurotrophins in chronic persistent cough by measuring the levels of nerve growth factor, brain derived neurotrophic factor and neurotrophin-3 in serum/induced sputum of subjects with this condition.

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METHODS

Subjects

Adult subjects with a cough persistent for at least one year were recruited from hospital respiratory clinics and by advertisement in a local newspaper. No subject had evidence of any other lung disease based on history, clinical examination, spirometry and chest radiography. Healthy volunteers with no history of recent upper or lower respiratory tract infection, were recruited as controls. Details are included in Chapter 3 (General Methods).

Measurements

Pre- and post- inhaled salbutamol FEV_1 and bronchial challenge testing with methacholine were performed as per techniques described in Chapter 3. Total serum IgE and IgE to common allergens (house dust mite, grass pollen and cat dander) were measured by enzyme immunoassay to assess atopic status. Patients recorded their cough severity on a 10 cm linear visual analogue scale (0=no cough, 10=maximum cough).

Sputum induction with 3% hypertonic saline was performed using a modification of the method described by Pin et al (71, 240). The sputum was processed according to the technique described by Popov et al (225). The supernatant was frozen at $-70°$ C for NGF measurements.

Measurement of NGF, BDNF and NT-3

Serum NGF, BDNF and NT-3 immuno-reactivity was measured by enzyme immunoassay (EIA) according to the manufacturers' instructions (Promega UK Ltd., Southampton, UK),

described in the Methods section, Chapter 3. As the reducing agent dithiothreitol (DTT) used to homogenize the specimens can affect measurements, we performed an extra test for assay performance and found full recovery of NGF immunoreactivity in normal serum samples spiked with exogenous NGF (Sigma Ltd, Poole, UK).

Final Diagnosis of the cough

To achieve a final diagnosis for the cause of the cough, all subjects were offered sequential trials of specific therapy depending on the likely clinical diagnoses for their cough. Final diagnosis or diagnoses of the cause of the cough were based on defined pre-treatment criteria (5) and accepted only when the cough significantly improved or disappeared with specific therapy (8).

Statistical Methods

Associations between demographic variables, sputum cell counts and neurotrophins were assessed by spearman rank correlations. Differences between groups of patients were examined by Wilcoxon tests. A p value of <0.05 was considered significant. All analyses were carried out with SAS version 8.02 software.

RESULTS

Baseline subject characteristics

We screened 110 subjects with cough, 81 of whom met all the inclusion criteria. Of these, 49 were recruited from the community and 32 were referred from hospital clinics. A final diagnosis for the cough was obtained in 73/81 (90%) of subjects; 73% had a single diagnosis, 25% had 2 diagnoses and 3% had 3 diagnoses. The pattern of primary diagnoses was PNDS (29), GORD (16), CVA (12), bronchiectasis (9), eosinophilic bronchitis (5), habitual cough (1) and bronchitis (1). In 8 subjects, the cough was termed idiopathic. 31% of cough subjects were atopic (allergen specific IgE positive) and the median (IQR) total serum IgE levels were 28.5 (16.5-72.5).

Cough subjects were significantly older than the control group; median (IQR) age 63 (51.3-68.4) compared to 31 (26-32) for controls, $p < 0.001$. 30 cough subjects were male (51 female) and 16 controls were male (14 female). Median (IQR) FEV_1 % predicted was 96 (83-106) in cough subjects and 100 (89-105) in controls (p=0.314).

Neurotrophin levels

Serum NGF, BDNF and NT-3 were not elevated in persistent cough subjects (n=77) compared to healthy controls (n=30) (Table 4.2.1; figure 4.2.1). NGF was measured in induced sputum samples of 46 subjects with persistent cough and 17 healthy controls and there was no significant difference between the two groups (Table 4.2.1).

Induced sputum cell counts showed a significantly higher total cell count and neutrophil proportion in cough subjects compared to controls (Table 4.2.1). Neutrophils in induced sputum did not correlate with the duration of cough or age of the patient.

There was no correlation between neurotrophin levels in serum/ sputum with age of the patient, gender, baseline VAS cough severity scale, FEV_1, FEV_1 % predicted, total IgE level, atopy to common allergens, total and differential sputum cell counts, blood cell counts, presence of lower airway hyper-reactivity, previous smoking history or whether the patient was recruited from the community or hospital clinics.

There was no major difference in the neurotrophin levels based on the final diagnosis of the cough (Figure 4.2.1). Individual data for the smaller diagnostic sub-groups are not depicted because the numbers were too small for meaningful analysis. There was a significant inverse correlation between serum BDNF and serum NGF (r = -0.24, p = 0.032), but no correlation between the other neurotrophins. Serum NGF levels were related to the duration of cough (r = 0.34, p = 0.002).

Table 4.2.1: Baseline neurotrophin levels (pg/ml) and induced sputum cell counts in chronic cough subjects compared to controls

	Controls		Persistent cough	
Induced sputum cell counts				
	(n=17)		*(n=46)*	
Total cell count	2.7	(2.0-3.4)	7.3	(4.5-15.9)**
Neutrophil %	32.1	(10.1-53.1)	67.3	(44.0-80.5)**
Eosinophil %	0.0	(0.0-0.2)	0.2	(0.0-1.0)
Lymphocyte %	0.35	(0.0-0.7)	0.0	(0.0-0.2) *
Macrophage %	67.1	(46.0-89.5)	30.8	(18.3-52.3) **
Neurotrophins				
Sputum NGF	516	(296-772)	580	(312-880)
Serum	*(n=30)*		*(n=77)*	
Serum BDNF	1157	(1068-1367)	1064	(838-1290)
Serum NGF	116.1	(0-547.4)	44.3	(1.7-541.2)
Serum NT-3	1464.5	(183-9562)	211	(129-1195)

Data entered as median (IQR) ; $*p = 0.005$, $**p < 0.001$

Table 4.2.2. Neurotrophin levels (pg/ml) in different causes of chronic persistent cough.

	PNDS	GORD	CVA	Bronch	Idio	EB
Serum	*(n=28)*	*(n=16)*	*(n=11)*	*(n=7)*	*(n=8)*	*(n=5)*
BDNF	887	1265	1102	1057*	1074	1072
	(636-1322)	(929-1483)	(996-1290)	(829-1069)	(804-1255)	(1054-1080)
NT3	217	172	278	132	150.5	156*
	(126-12000)	(95-1170)	(211-717)	(124-12000)	(128-6427)	(140-193)
NGF	52	145	15.6	16	118.0	15.6
	(16-827)	(16-535)	(16-146)	(16-590)	(40-873)	(16-351)
Induced sputum	*(n=11)*	*(n=9)*	*(n=8)*	*(n=8)*	*(n=4)*	*(n=4)*
NGF	584	656	444	964	728	444
	(432-880)	(520-736)	(288-900)	(344-1096)	(468-1292)	(348-492)

Data depicted as median (IQR); statistical significance: * = p=0.050; Abbreviations: PNDS=post nasal drip syndrome, GORD=gastro-oesophageal reflux disease, CVA=cough variant asthma, Bronch=bronchiectasis, Idio= idiopathic cough, EB=eosinophilic bronchitis

Figure 4.2.1: Neurotrophins [median (IQR)]in chronic cough compared to healthy controls.

The box represents the inter-quartile range; the line in the box is the median value.
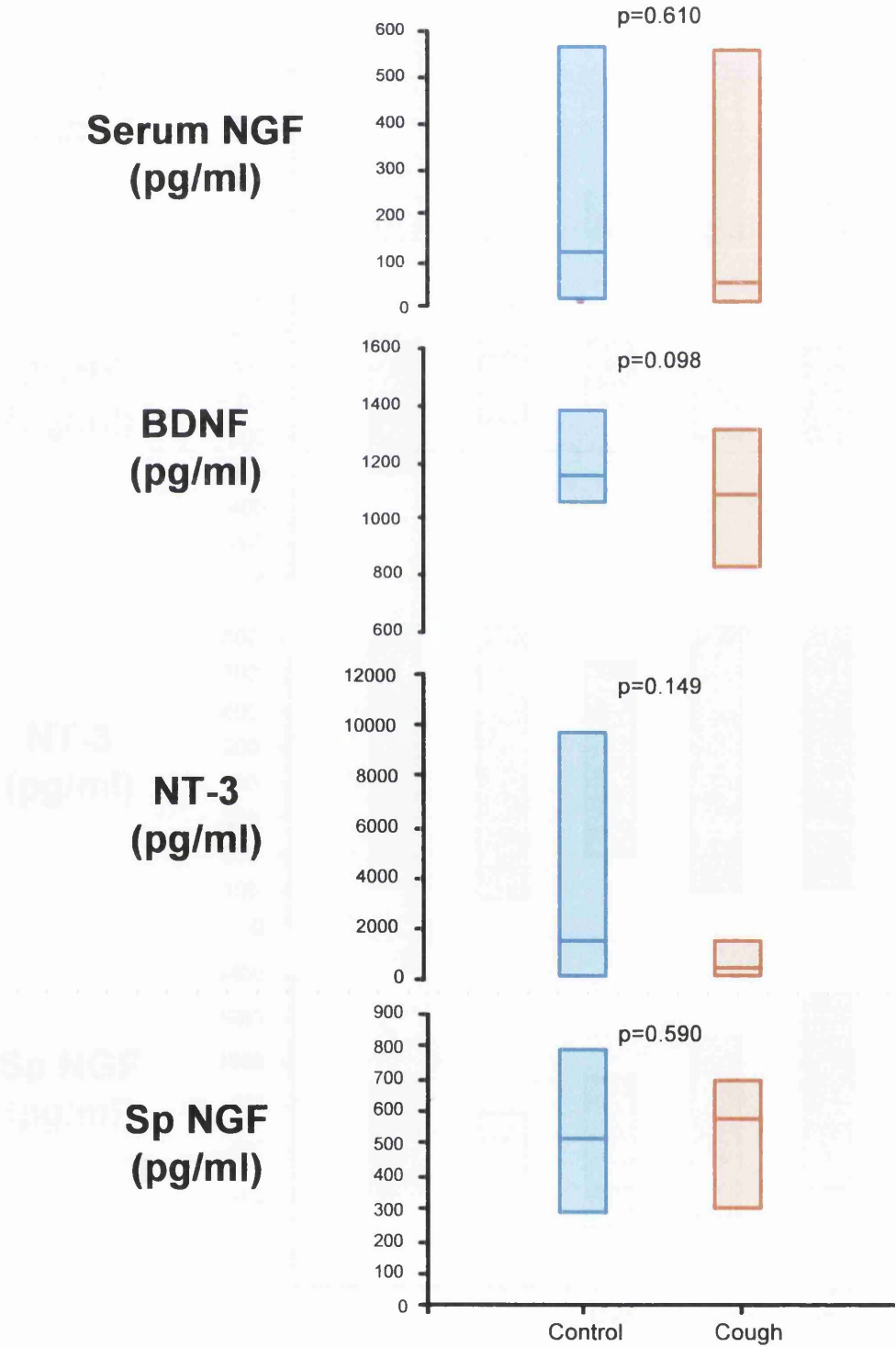
Figure 4.2.2: Neurotrophins [median (IQR)] in different causes of chronic cough

The box represents the inter-quartile range and the line in the box is the median value.
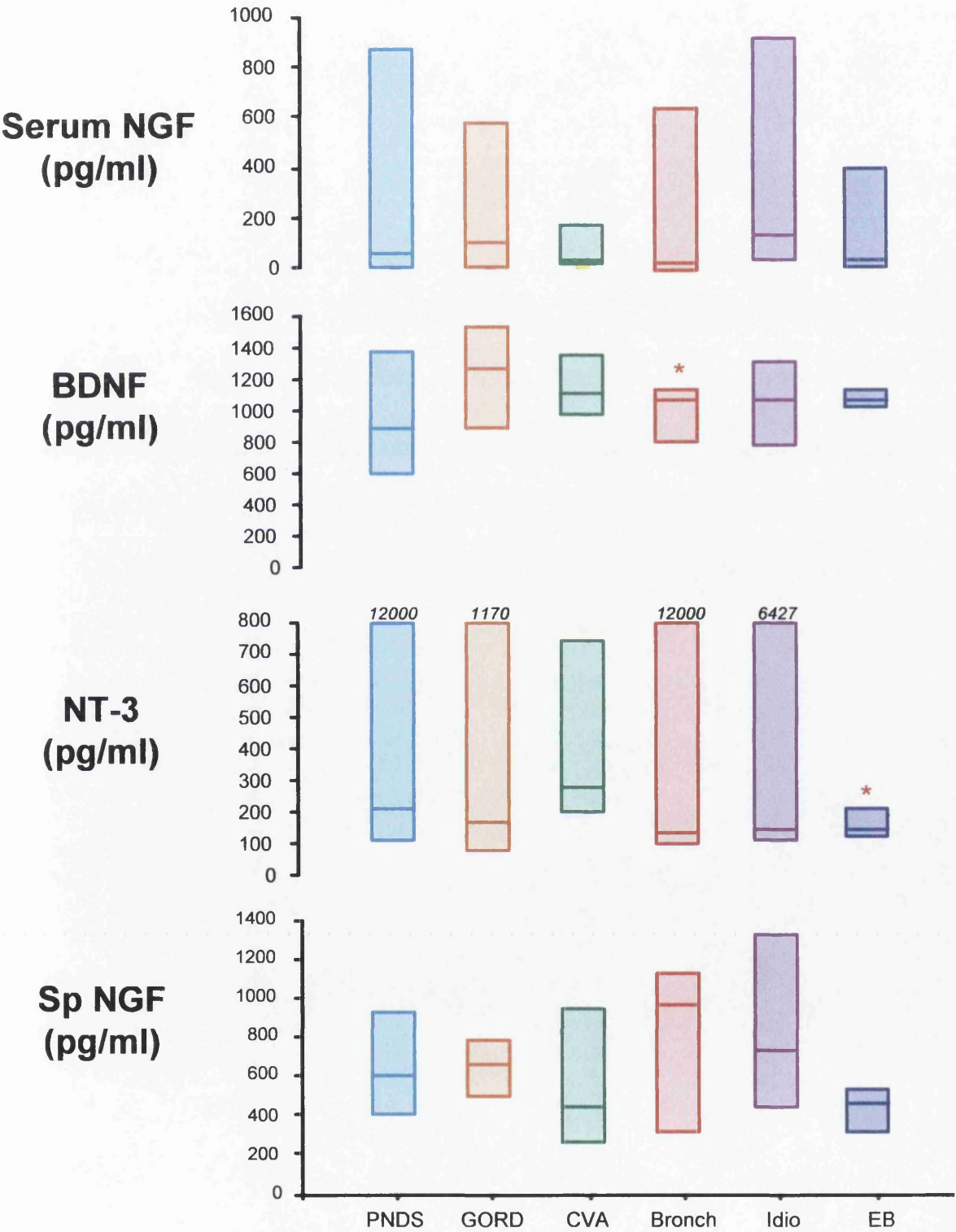
DISCUSSION

Nerve growth factor, brain-derived growth factor and neurotrophin-3 are the common neurotrophins associated with airway inflammation and atopy. This is the first study measuring the levels of these neurotrophins in serum/induced sputum in subjects with chronic cough. We found no increase in neurotrophin levels in cough patients compared to healthy controls, despite the presence of airway inflammation.

There are various factors that could account for the normal neurotrophin levels in our subjects with chronic cough. Firstly, the technique of separation of sputum involves the addition of dithiothreitol (DTT) which has been shown to reduce the levels of certain mediators (241). This is unlikely to account for the normal levels of NGF in our study as the technique was similar in patients and controls and we found full recovery of immunoreactivity of exogenous NGF when added to normal serum. Furthermore, Hope-Gill and colleagues have demonstrated that NGF levels in sputum were unaffected by DTT (175). Secondly, inhaled corticosteroids can suppress levels of NGF and BDNF in serum in subjects with asthma (166). This cannot explain the normal levels we observed, as cough subjects were excluded if they had received inhaled corticosteroids for three weeks or oral corticosteroids for six weeks prior to inclusion. The third consideration was whether the study was adequately powered to demonstrate a difference between groups. We believe it was, as studies in asthma and pulmonary fibrosis have demonstrated differences with smaller group sizes (164, 166, 175). Results could vary in cough of a shorter duration, but we found a positive correlation with serum NGF and the duration of cough. There was no correlation

between age/gender and the levels of any of the neurotrophins therefore it was unlikely that any difference between the study groups were important.

We chose to measure only the NGF in sputum as it has been measurable in previous work (175) whereas NT-3 has not and levels of BDNF would have been affected by DTT used in our method of sputum processing (175). Besides, NGF is the neurotrophin most expressed in pulmonary tissue, followed by BDNF and then NT-3 as revealed by microdensitometric analysis of bands of immunoreactivity (176) and is usually representative of the other neurotrophins.

Nerve growth factor increased eosinophilic airway inflammation and augmented IL-5 production in an animal model of airway inflammation (242). The major inflammatory cell type in our cough subjects was the neutrophil, in keeping with the findings of others (51). A significant increase in neurotrophins has been demonstrated in allergic conditions (164, 243). Only 31% of cough subjects were atopic and there was no difference in neurotrophin levels based on total or allergen-specific IgE levels. It is possible that neurotrophins are less involved in inflammation associated with neutrophils as compared to eosinophilic inflammation in asthma and allergic conditions.

The main causes of cough in our subjects were post nasal drip syndrome, gastro-oesophageal reflux disease, cough variant asthma, bronchiectasis and eosinophilic bronchitis; similar to other studies on chronic cough (8, 32, 34, 45). We found no significant difference in neurotrophins in any of the causes of cough, including cough variant asthma.

It is possible that rather than absolute concentrations of neurotrophins in body fluids, it is their expression at receptor level that is important. It has been speculated that NGF affects airway nerves through the vanilloid receptor (VR1) in allergic asthma (183). This receptor, originally known as the capsaicin receptor, has been proposed as a receptor for cough (182). Further studies are indicated to determine the effect of nerve growth factor at the receptor level in chronic cough.

In conclusion, we have demonstrated that nerve growth factor, brain-derived growth factor and neurotrophin-3 are not elevated in serum/sputum of subjects with chronic cough, regardless of the aetiology of cough. This implies that these neurotrophins do not have a central role in perpetuating airway inflammation in chronic persistent cough.

4.3: QUALITY OF LIFE IN CHRONIC PERSISTENT COUGH

INTRODUCTION

Chronic cough is a common symptom in general medical practice with significant associated morbidity (10, 184, 185). It can persist for several years (33, 35, 110) and patients are often been told by their physician that they have no option but to learn to live with the cough (8).

Quality of life is an outcome which is a fundamental aim of health care (186). Health related quality of life measurement is a means of quantifying, in a standardised and objective manner, the impact of disease on a patient's daily life, health and wellbeing. It is a process that is similar to a highly structured clinical history, leading to an objective measurement that can be used for scientific purposes (187).

In other respiratory conditions such as asthma and COPD, cough is one of the major symptoms affecting patients' quality of life. A study of patient weighting of the importance of different symptoms of asthma found cough to be the most troublesome of all symptoms(244).

Until recently, the quality of life of people with chronic cough had never been studied. French and colleagues used the Sickness Impact Profile (SIP) and Adverse Cough Outcome Survey (ACOS) developed by themselves and demonstrated, in a prospective study, that chronic cough was associated with adverse psychosocial and physical effects on the quality of life (188). They then developed a cough specific

quality of life questionnaire (the CQLQ) in 2002 (189), followed by the Leicester Cough Questionnaire (LCQ) by Birring and colleagues in 2003 (190). Both are recent additions likely to be beneficial as an outcome measure in cough studies, but they do not allow comparison of quality of life across different respiratory conditions.

Successful treatment of cough improved the QOL in a small study including subjects with cough for over 3 weeks (188). Birring and colleagues reported a similar improvement using the LCQ in 9 subjects (190). The change in quality of life following specific treatment of chronic persistent cough has not been assessed in any large study.

Quality of life is widely used in studies in asthma and COPD with respiratory disease specific questionnaires such as the St. George's Respiratory Questionnaire (SGRQ) and in various medical disorders with the Hospital Anxiety and Depression scale. The SGRQ has been a useful strategy for monitoring the outcomes of clinical interventions (245-247). The SGRQ was designed so the scores would have an 'absolute' property and be independent of patient and study (191). Despite the large numbers of sufferers, chronic cough is a relatively neglected condition in the medical world compared to asthma and COPD. A comparison of the effect of cough on quality of life compared with other medical conditions has not been performed.

The aims of this study were to assess the quality of life of patients with cough persistent for over one year, using the St. George's Respiratory Questionnaire and the Hospital Anxiety and Depression Scale. The secondary aims were to study the effect of treatment of cough on these measures and to see if the specific cause of cough affected these quality of life measurements.

METHODS

Subjects:

Adults with a cough persistent for over one year were recruited from hospital respiratory clinics and by advertisement in a local newspaper. No subject had evidence of any other lung disease based on history, clinical examination, chest radiography and spirometry. Details of study design are included in the general methods section, chapter 3.

Quality of Life assessments:

All subjects were requested to complete the St. George's Respiratory Questionnaire and the Hospital Anxiety and Depression Scales.

The St. George's Respiratory Questionnaire (SGRQ)

The SGRQ is a supervised self-administered measure designed for use in airway disease. [Details are included in the Methods section, Chapter 3]. A difference in the SGRQ total score of four points would indicate clinically significant differences between populations (191, 248) and a large difference would be a change of more than 7 points (191). The SGRQ has been shown to be responsive to therapeutic changes (247, 249).

In a study of 307 normal individuals, the mean (SD) for SGRQ scores were 9.67 (13.24) for symptoms, 13.4 (17.63) for activity, 4.73 (9.92) for impact and total score 8.41 (11.33) (250). In a British study (251), Spencer and colleagues reported similar normal ranges [mean (SD)] for Symptoms 12 (15), Activity 11(13), Impact 3 (5) and total score 7 (7).

The Hospital Anxiety and Depression Scale (HADS)

The HADS was specifically developed for the screening of clinically significant anxiety and depression in patients with somatic conditions as well as for changes in severity of these conditions at subsequent visits (227). Scores of 7 or less represent non-cases, 8-10 indicate possible cases and scores of 11 or more definite cases (227).

Final Diagnosis of Cough

All subjects were offered sequential trials of specific therapy depending on the likely clinical diagnoses for their cough. Final diagnosis or diagnoses of the cause of the cough (described in chapter 3) were based on defined pre-treatment criteria (5) and accepted only when the cough significantly improved or disappeared with specific therapy (8).

Quality of life questionnaires were posted to all participants after a final diagnosis had been achieved along with a stamped self-addressed envelope. If the questionnaire was not returned within ten days, a reminder phone call was made and a second set mailed out if the questionnaires had been misplaced.

In the SGRQ, part 1 refers to symptom assessment over the past year and this had been validated for a symptom recall of one year at the time this study commenced (191, 252). The time of achieving a final diagnosis and repeating the score was expected to vary from two months to over a year, so the symptom domain and hence the total SGRQ score after treatment was not repeated after treatment in this study.

Statistical Analysis

Differences from baseline in the St. George's Respiratory Questionnaire components and the Hospital Anxiety and Depression Scale were analysed by t-tests and the corresponding 95% confidence intervals for the mean changes. Quality of life scores were compared between males and females with two-sample t-tests. Correlations between the quality of life measures were analysed by Pearson correlations. The association between the quality of life measures and other factors was assessed by Spearman rank correlations. The differences from baseline were compared between the diagnostic groups using ANOVA. A p value <0.05 was considered significant. All analyses were done using SAS version 8.02.

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RESULTS

Demography

88 subjects with cough persistent for over one year completed baseline quality of life assessments and were included in this study. One HAD scale was incomplete at baseline and not utilised in the analysis. A final diagnosis for the cough was obtained in 78/88 (89%) of subjects. The pattern of primary diagnoses is as depicted in Experiment 1, figure 4.1.1. Baseline characteristics of the patients are shown in table 4.3.1. Individual data for the smaller diagnostic sub-groups are not depicted because the numbers were too small for meaningful analysis.

74 (84 %) subjects returned the final sets of questionnaires. Fourteen subjects did not return the second set of questionnaires despite reminder telephone calls and letters. Analysis of this sub-group revealed no major differences from the group who returned their questionnaires following treatment. The mean age was 55.04 (10.03) years, 9/14 were female, 10/14 were recruited from the community compared to 4 from hospital clinics, 6 were ex-smokers compared to 8 never smokers and the mean (SD) duration of cough was 14.5 (12.92) years. The primary diagnosis was PNDS (8), CVA (2), GORD (1), EB (1) and Idiopathic (2). There was no significant difference in any domain of the SGRQ score and the HADS in these subjects at baseline compared to those who completed the study.

There were a higher number of females in the study; 56/88 subjects (64%). The differences in quality of life scores based on gender are depicted in Table 4.3.3. Activity and Impact sections of the SGRQ were significantly higher in females compared to males, but there was no difference in the anxiety or depression scores.

The co-existence of another chronic illness was reported by 57/88 subjects. The commonest conditions were hypertension (23), angina (6), previous myocardial infarction (4), diabetes mellitus (4), previous stroke (30) and glaucoma (2). Chronic illnesses were associated with the depression score in the HAD Scale (p=0.022) but not with the other quality of life assessments.

Factors unrelated to the quality of life scores were age, previous smoking history and duration of cough. Baseline cough severity, marked on a 0-10 cm visual analogue scale, correlated with the impact domain of the SGRQ (r=0.22, p=0.042). Quantity of sputum and cough during sleep correlated with the symptoms, impact and total SGRQ. Baseline FEV_1, FVC and FEF_{25-75} significantly correlated with total, activity and impact scores of the SGRQ, but not with the symptom score or the HAD scale (table 4.3.4).

Quality of life scores in cough before and after treatment of cough

The baseline HADS scores of the 74 subjects who completed the study are depicted in Figure 4.3.1. Scores before and after treatment for the individual domains of the St. George's Respiratory Questionnaire are depicted in table 4.3.2 (a) and figure 4.3.2 The changes in the Hospital Depression and Anxiety Scales are depicted in Table 4.3.2b and figure 4.3.3.

The most significant change in the SGRQ in all causes of cough following treatment is in the Impacts Domain (figure 4.3.4) with a mean (SD) change of [-9.82 (15.32), p<0.001]. The symptom score was not repeated following treatment and hence the total score could not be accurately calculated following treatment of the cough.

Keeping the symptom score unchanged, the change in the total score after treatment was a mean (SD) of [-6.17 (11.45), p<0.001]. A cut-off value of 4 points has been considered the minimal clinically significant change by the developers of the SGRQ, and a value of 7 a very significant difference. For the activity section, 27 subjects (36%) had an improvement > 4 points of whom 20 had improved over 7 points. For the impact domain of the SGRQ, a 4 point improvement was seen in 46 (62%) subjects of whom 43 had a change of 7 or greater points.

Although the anxiety and depression scores were not high at baseline, there was a significant reduction in both scores following treatment (Table 4.3.2.b).

Quality of life in different causes of cough

The quality of life scores in the major causes of cough are depicted in table 4.3.5 and changes with treatment in table 4.3.6. There was no statistical difference in the scores at baseline or the change with treatment, based on the final diagnosis of cough.

Correlation between SGRQ and the HAD Scale and demographic features

These 2 scales measure different components of quality of life. The anxiety scale correlated only with the total SGRQ, whereas the depression scale correlated significantly with the total as well as the individual domains of the SGRQ. Correlations of SGRQ domains and HAD Scale before and after treatment of cough are depicted in table 4.3.7.

Table 4.3.1: Baseline characteristics of chronic cough subjects.

Number	All causes 88	PNDS 30	GERD 18	CVA 13	Bronchiec 9	Idio 10
Age, years*	59.0 (12.7)	58.8 (12.4)	67.7 (4.4)	48.5 (17.3)	64.0 (9.6)	57.7 (8.7)
Gender, male/female	32/57	12/18	8/10	2/11	3/6	4/6
Duration of cough, years	16.2 (16.1)	18.6 (17.4)	14.9 (15.8)	13.6 (9)	17.8 (19.4)	13.9 (18.6)
Baseline cough VAS, cm.	4.1 (2.0)	3.9 (2.3)	3.9 (1.9)	4.7 (2.1)	4.3 (1.7)	4.4 (2.2)
Ex-smokers: number	35	13	8	4	4	4
Years quit smoking	19.7 (12.9)	20.7 (13.7)	21.6 (13.9)	7.1 (8.9)	20.3 (6.9)	25.8 (16.4)
Pack years smoked	14.8 (15.8)	10.0 (13.1)	24.7 (19.9)	14.6 (24.4)	20.3 (8.7)	7.4 (7.0)
FEV_1, L	2.6 (0.8)	2.7 (0.8)	2.4 (0.6)	2.4 (1.0)	2.1 (0.8)	2.9 (0.9)
FEV_1% predicted	94 (16)	96 (12)	94 (15)	86 (24)	88 (12)	101 (13)

*Data listed as mean (SD), unless stated otherwise, *ANOVA test $p < 0.001$ between the sub-groups.*

Table 4.3.2a: St. George's Respiratory Questionnaire Scores before and after treatment of chronic persistent cough.

QOL score	Before treatment	After treatment	Difference	Statistical significance
TOTAL SGRQ	34.66 (14.45	-	-	-
SGRQ SYMPTOMS	55.24 (18.74)	-	-	-
SGRQ ACTIVITY	29.33 (22.97)	26.82 (23.31)	-2.51 (17.45)	P=0.220
SGRQ IMPACT	30.35 (14.64)	20.53 (16.35)	-9.82 (15.32)	**P<0.001**

Table 4.3.2b: Hospital Anxiety and Depression Scale before and after treatment of chronic persistent cough.

QOL score	Before treatment	After treatment	Difference	Statistical significance
ANXIETY SCORE	6.41 (3.56)	5.47 (3.28)	-0.96 (2.97)	**P=0.008**
DEPRESSION SCORE	3.72 (2.70)	2.96 (2.47)	-0.75 (2.32)	**P=0.007**
TOTAL HADS	10.12 (5.15)	8.43 (5.08)	-1.68 (4.31)	**P=0.002**

Figure 4.3.1a : Baseline Anxiety score on the Hospital Anxiety and Depression Scale; frequency of cough patients in different severity stages
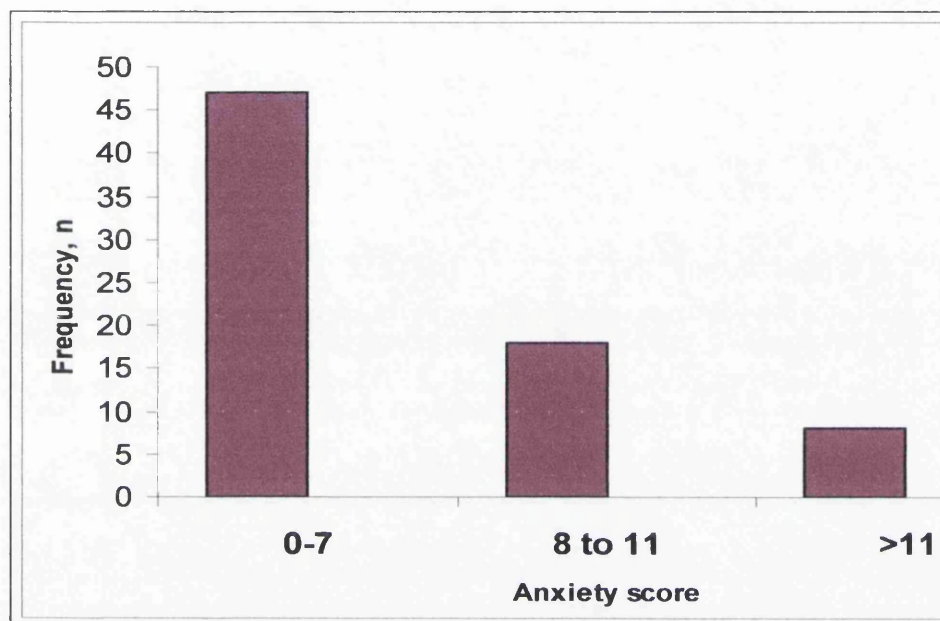
Figure 4.3.1 b : Baseline depression score on the Hospital Anxiety and Depression

Scale; frequency of cough patients in different severity stages
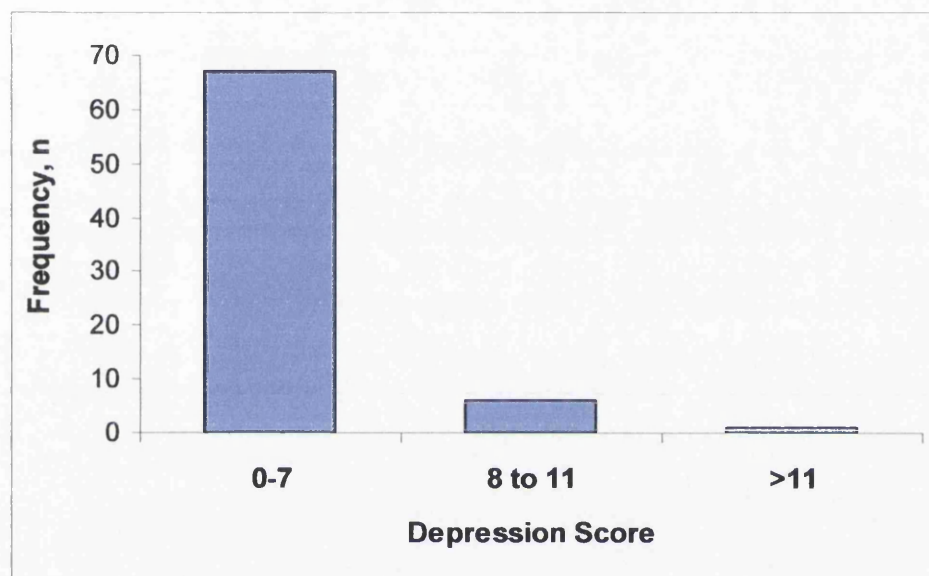
Figure 4.3.2 : SGRQ scores before and after treatment of chronic persistent cough.

a. Change in the Activity Domain of SGRQ
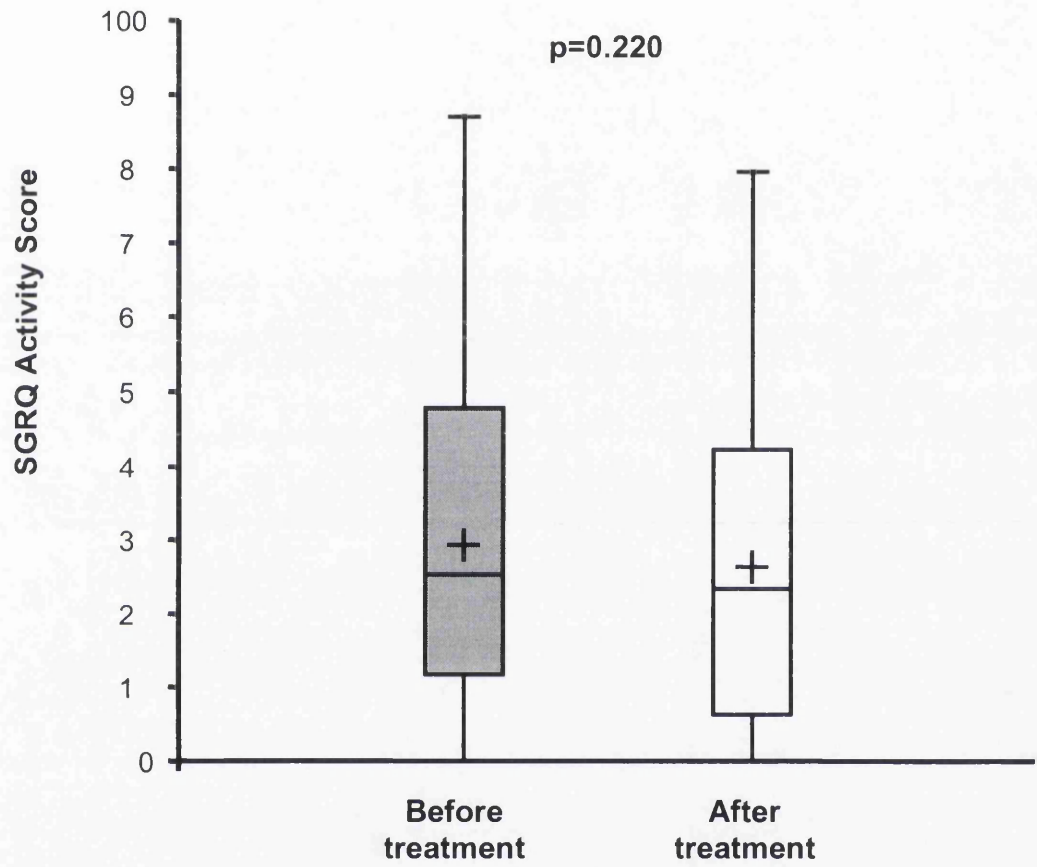
The box represents the interquartile range, the line within the median value, + is the mean value and maximum and minimum values are depicted by the error bars.

Figure 4.3.2 : SGRQ scores before and after treatment of chronic persistent cough.

b. Change in the Impact domain of SGRQ
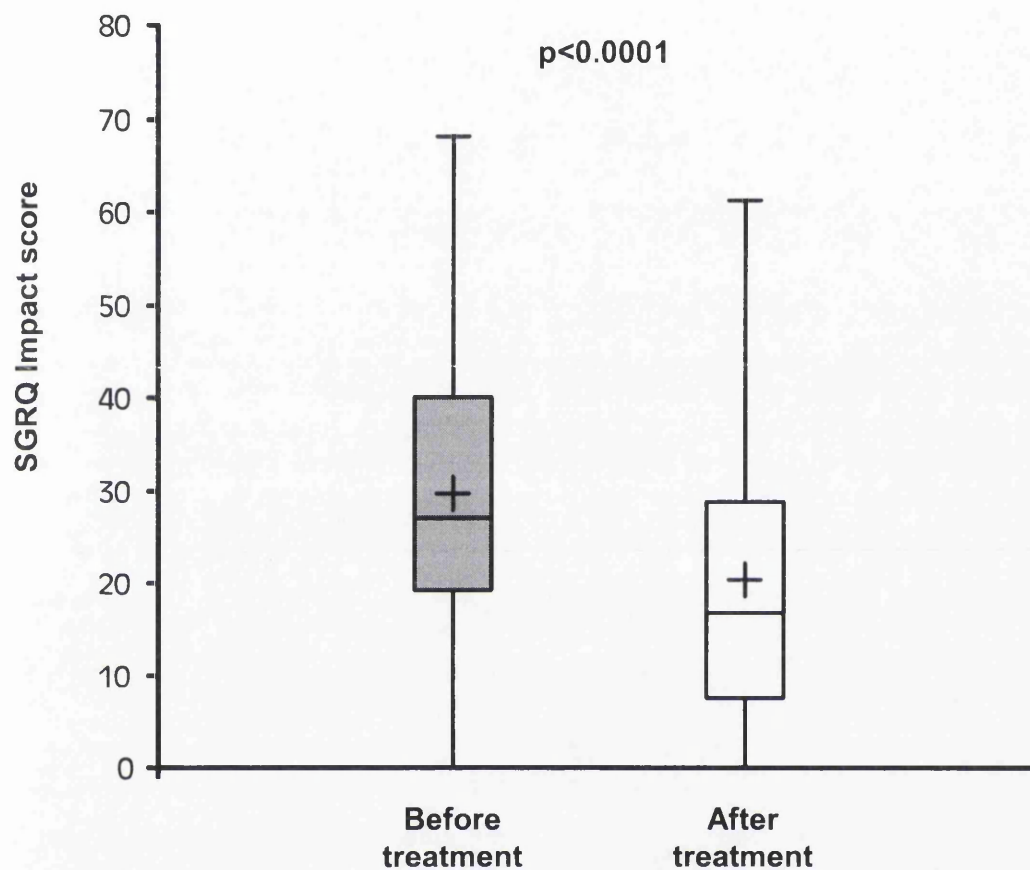
The box represents the interquartile range, the line within the median value, + is the mean value and maximum and minimum values are depicted by the error bars.

Figure 4.3.3. : HADS scores before and after treatment of chronic persistent cough.

a. Change in the Anxiety Score

The box represents the interquartile range, the line within the median value, + is the mean value and maximum and minimum values are depicted by the error bars.
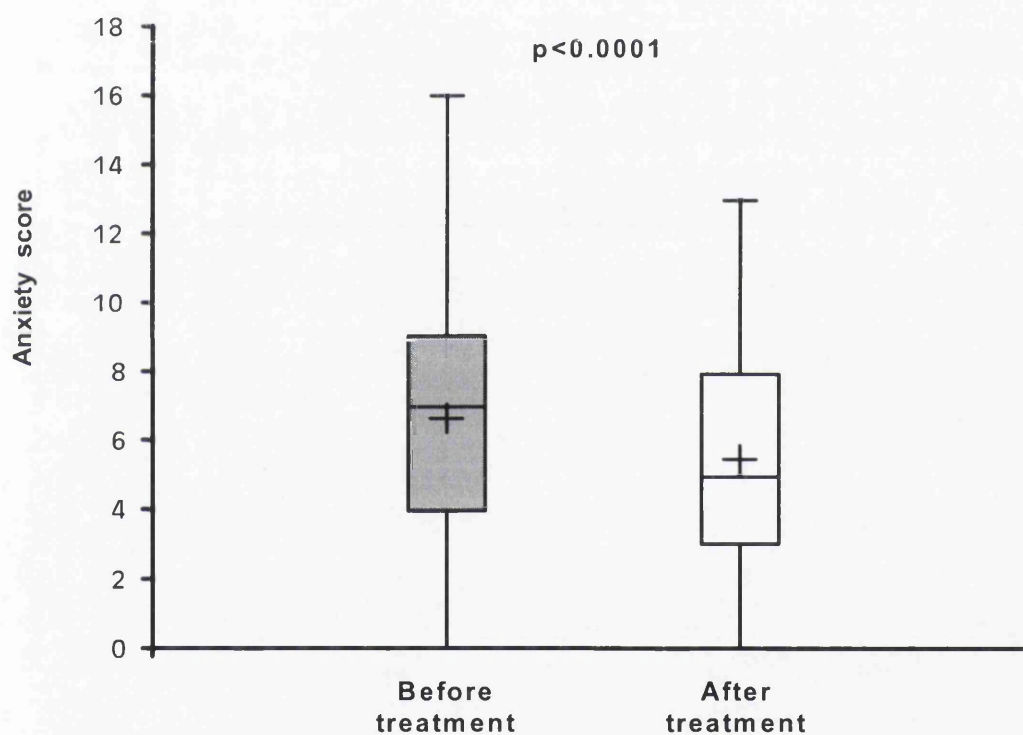
Figure 4.3.3. : HADS scores before and after treatment of chronic persistent cough.
b. Change in the Depression Score
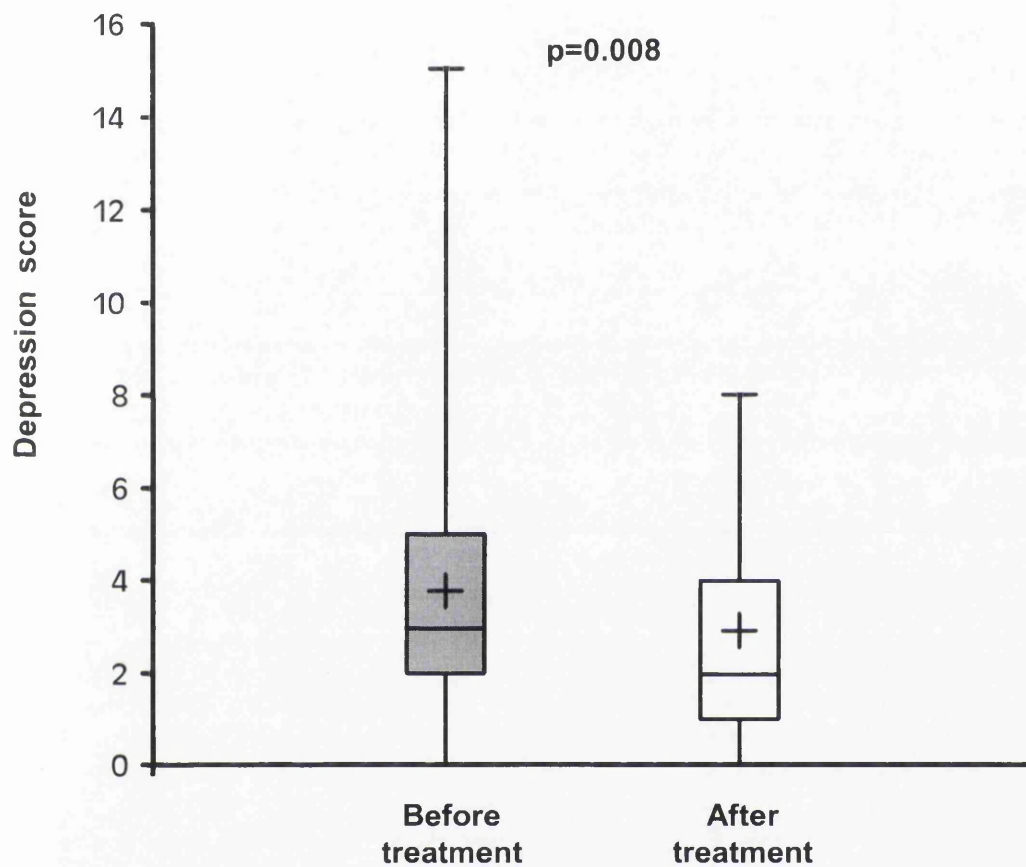
The box represents the interquartile range, the line within the median value, + is the mean value and maximum and minimum values are depicted by the error bars.

Table 4.3.3. Quality of life scores in males versus females with chronic persistent cough.

	Males (n=32)	Females (n=56)	Statistical significance
Total SGRQ	29 (13)	38 (15)	**p=0.011**
Symptom	53 (16)	57 (20)	p=0.415
Activity	22 (20)	34 (23)	**p=0.019**
Impacts	26 (11)	33 (16)	**p=0.027**
Total HADS	9 (5)	11 (5)	p=0.187
Anxiety	6 (3)	7 (4)	p=0.264
Depression	3 (3)	4 (3)	p=0.250

Data depicted as mean (SD) and statistical difference as t tests.

Table 4.3.4 a: Correlation of St. George's respiratory questionnaire with baseline demography in chronic persistent cough

	TOTAL SGRQ	SYMPTOMS	ACTIVITY	IMPACT
Age	r =0.10	r = -0.02	r =0.11	r =0.01
	P=0.349	P=0.888	P=0.290	P=0.908
Duration of cough	r =-0.10	r =-0.08	r =-0.14	r =-0.02
	P=0.348	P=0.449	P=0.189	P=0.882
Baseline cough severity	r =0.12	r =0.06	r =0.03	**r =0.22**
VAS scale, cm	P=0.264	P=0.593	P=0.784	**P=0.042**
Sputum quantity, ml	**r = 0.33**	**r = 0.48**	r = 0.12	**r = 0.35**
	P=0.002	**P<0.0001**	P=0.266	**P=0.0008**
Cough during sleep	**r = 0.26**	**r = 0.26**	r = 0.10	**r = 0.27**
	P=0.017	**P=0.016**	P=0.377	**P=0.012**
FEV$_1$ pre salbutamol	**r = - 0.40**	r = - 0.20	**r = - 0.38**	r = -0.31
	P=0.0001	P=0.064	**P=0.003**	**P=0.004**
FVC pre-salbutamol	**r = - 0.40**	r = - 0.21	**r = - 0.39**	r = - 0.29
	P=0.0001	P=0.054	**P=0.0002**	**P=0.007**
FEF$_{25-75}$ pre-salbutamol	**r = - 0.38**	r = - 0.20	**r = - 30**	r = - 0.32
	P=0.0003	P=0.058	**P=0.005**	**P=0.002**

Table 4.3. 4b : Correlation of the HAD scale with baseline demography in chronic persistent cough (using Spearman Rank Correlations)

	TOTAL HADS	ANXIETY	DEPRESSION
Age	r = -0.15	**r = - 0.25**	r = 0.10
	P=0.177	**P=0.021**	P=0.339
Duration of cough	r =0.01	r =0.03	r =-0.04
	P=0.897	P=0.817	P=0.735
Baseline cough severity	r =0.09	r =- 0.00	r =0.16
VAS scale, cm	P=0.386	P=0.992	P=0.128
Sputum quantity	r = 0.09	r = - 0.02	**r =0.24**
	P=0.371	P=0.843	**P=0.030**
Cough during sleep	r =0.16	r = - 0.14	r =0.04
	P=0.154	P=0.207	P=0.703
FEV$_1$ pre salbutamol	r =0.00	r =0.08	r =- 0.19
	P=0.917	P=0.436	P=0.085
FVC pre-salbutamol	r = - 0.00	r =0.05	r = - 0.19
	P=0.971	P=0.635	P=0.084
FEF$_{25-75}$ pre-salbutamol	r =0.00	r =0.10	r = -0.14
	P=0.999	P=0.338	P=0.196

Table 4.3.5: Quality of life scores in different causes of cough

	Control*	PNDS	GORD	CVA	Bronchiec	Idiopathic
Number	(307)	(30)	(18)	(13)	(9)	(10)
Total SGRQ	8 (11)	29 (13)	33 (9)	34 (12)	39 (21)	43 (18)
SGRQ symptoms	10 (13)	51 (18)	54 (19)	51 (14)	66 (20)	63 (23)
SGRQ activity	13 (18)	21 (20)	28 (18)	33 (19)	27 (29)	46 (26)
SGRQ impact	5 (10)	26 (12)	27 (14)	29 (12)	37 (19)	34 (14)
Total HADS		11 (5)	9 (4)	8 (4)	10 (5)	11 (7)
Anxiety		7 (4)	6 (3)	6 (3)	6 (5)	7 (3)
Depression		4 (2)	3 (2)	2 (2)	4 (2)	5 (4)

* Normal ranges for SGRQ for comparison, obtained from Ferrer et al (250)

Table 4.3.6: Change in St George's Respiratory Questionnaire and Hospital Anxiety and Depression Scales in different causes of cough, following specific treatment for the cough.

	PNDS	GORD	CVA	Bronchiec	Idiopathic
Number	*22*	*17*	*11*	*9*	*8*

Change in SGRQ after treatment of cough

	PNDS	GORD	CVA	Bronchiec	Idiopathic
ΔSGRQ activity	1 (17)	- 3 (19)	- 5 (15)	6 (15)	-14 (20)
ΔSGRQ impact	-6 (15)	-12 (15)	-12 (17)	-10 (15)	-12 (18)

Change in HADS after treatment of cough

	PNDS	GORD	CVA	Bronchiec	Idiopathic
ΔTotal HADS	-3 (5)	-1 (4)	-8 (12)	-1 (4)	-11 (13)
ΔAnxiety	-2 (3)	-1 (3)	0 (4)	0 (2)	0 (3)
ΔDepression	-1 (3)	-1 (2)	0 (1)	-1 (3)	-1 (3)

ΔTotal SGRQ and ΔSGRQ symptoms was not done as the symptom score had not been repeated after treatment (refer methods section)

There were no significant differences between the sub-groups of cough.

Table 4.3.7: Correlation between the St. George's Respiratory Questionnaire and the Hospital Anxiety and Depression Scale before and after treatment of chronic cough

a. Anxiety Scale

	Before treatment	After treatment	difference with treatment
Total SGRQ*	r = 0.17	**r = 0.32**	**r = 0.19**
	P=0.107	**P=0.007**	**P=0.117**
Symptoms	r = 0.18	-	-
	P=0.094	-	-
Activity	r = 0.12	r =0.34	r =0.20
	P=0.249	P=0.003	P=0.094
Impacts	r = 0.20	r = 0.22	r = 0.16
	P=0.067	P=0.058	P=0.175

b. Depression Scale

	Before treatment	After treatment	difference with treatment
Total SGRQ*	r =0.49	r =0.59	r =0.40
	P<0.0001	P<0.0001	P=0.0005
Symptoms	r =0.22	-	-
	P=0.044	-	-
Activity	r =0.49	r =0.65	r =0.31
	P<0.0001	P<0.0001	P=0.008
Impacts	r =0.45	r =0.47	r =0.39
	P<0.0001	P<0.0001	P=0.0007

Total SGRQ calculated keeping symptom domain constant; Pearson correlations used.

Figure 4.3.4: Mean change in SGRQ Impact Score in Different Causes of Cough.

A reduction of 4 points is considered the minimally significant clinical difference.
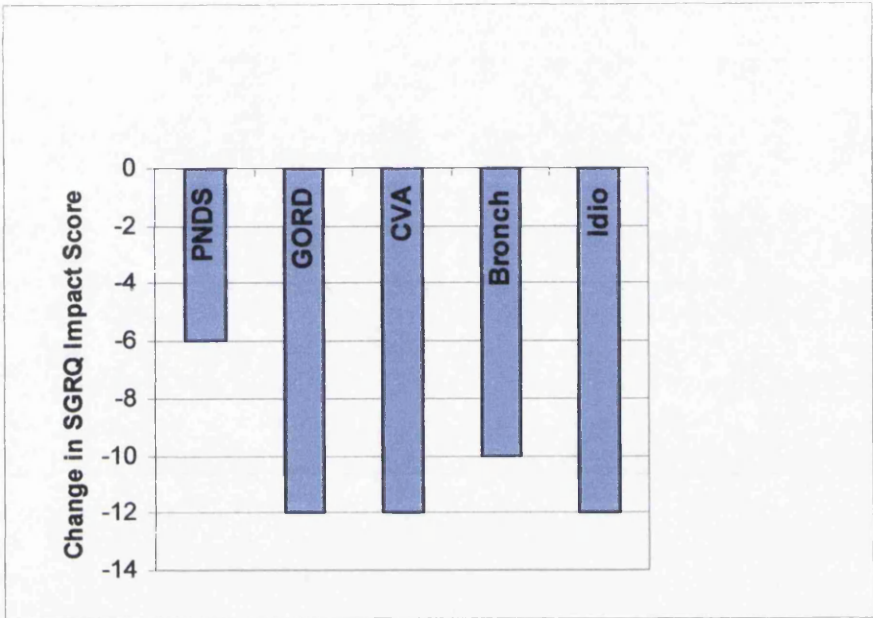
DISCUSSION

Chronic cough is a common condition with a prevalence of 14-23% in the general population (10, 184, 185). Successful treatment of all patients with chronic cough is difficult (32, 33, 35, 110). This is the first study of the quality of life in subjects with a cough persistent for over a year. We demonstrated that the impairment of quality of life parameters improves significantly following specific treatment of cough. Clinically significant levels of anxiety and depression are not present in cough, nevertheless, there is an improvement following treatment of cough.

The SGRQ score has been widely used in respiratory conditions such as asthma and COPD and has been validated in patients with bronchiectasis (253). It correlates moderately (r=0.54) with the Leicester Cough Questionnaire in patients with chronic cough lasting over 3 weeks (190). In our study, the total score and each individual domain were higher than reference values obtained from people with no respiratory symptoms (250, 251).

Following treatment of the cough, the Impact domain mean difference (SD) was - 9.8 (15.3). A difference of 4 points is considered a minimal clinically significant difference in each of the sub-scales of the SGRQ (191, 248) and a large difference would be a change of more than 7 points (191). The symptom domain and hence the total score were not repeated after treatment in this study, as they had only been validated to record a symptom recall of one year at the time the study commenced (191, 252). Barr et al have

now modified the SGRQ into American English and validated it for a one month duration symptom recall (254) and Wilson and colleagues have validated it for a change in bronchiectasis after six months (253).

French and colleagues (188) studied 28 subjects before and after treatment of their cough using the Sickness Impact profile (SIPS) score and the adverse cough-specific outcome survey (ACOS) and found a resolution of the deterioration in quality of life following successful treatment of the cough. They modified the ACOS into the Cough-Specific Quality-of-Life Questionnaire (CQLQ) and found it responsive to changes with treatment in 24 subjects with cough persistent for over 3 weeks. Effect of treatment in modifying a cough persisting for over a year has not been studied to the best of our knowledge.

Our results show that both the anxiety and depression scales of the HADS and the impact domain of the SGRQ are highly responsive to treatment of cough persisting for over one year. The mean duration of cough in our subjects was 16 years but the duration of cough did not correlate with either the SGRQ or the HADS.

In COPD, both anxiety and depression correlated with SGRQ (255). In our cough patients, the depression score, but not the anxiety score of the HADS correlated with the individual domains of the SGRQ, similar to findings in bronchiectasis (253).

Cough reflex sensitivity has consistently been shown to be higher in females compared to males regardless of whether the stimulant was capsaicin (256, 257), inhaled citric acid

(258) or inhaled tartaric acid (259). We found a significant increase in the total SGRQ score as well as the individual Activity and Impact domains in females compared to males but no difference in anxiety or depression scales. In asthma, anxiety but not depression has been shown to be higher in females compared to males using the HADS (260, 261). Using the CQLQ in chronic cough > 3 weeks, French et al have reported that HRQoL is adversely affected in women compared to men (189), with the speculation being that women were more likely to suffer from stress urinary incontinence due to coughing and became embarrassed by it. However, Thompson et al used a 5 point Likert scale to record the effect of cough on psychosocial and physical aspects of health and found an overall moderate affection of these parameters with no difference in male and female patients (262).

In smokers without COPD, abnormal SGRQ scores were due to cough and phlegm (263). The quality of life measured by the SGRQ in our study showed a direct relation with sputum quantity and cough during sleep. Age, duration of cough and baseline cough severity were not factors affecting the quality of life.

The baseline quality of life as well as the improvement in the persistent cough was not related to the final diagnosis of the cough. This was similar to the findings of French and colleagues using the Adverse Cough Outcome Survey in their study of 28 subjects with cough persisting longer than three weeks (188). The fact that there was no difference in improvement in quality of life questionnaires in subjects with idiopathic cough compared to other groups who reported an improvement in their cough following treatment implies

that the entire effort of being treated and investigated for the cause of the cough contributes to reported well being rather than elimination of the cough alone.

A double-blind placebo-controlled study would be an ideal study design to measure the effectiveness of treatment in any condition. Given the numerous different causes of cough and the variety of therapeutic strategies, this would not have been a feasible option for this study.

The SGRQ and HADS are not cough-specific questionnaires, but allow for certain comparisons with other medical conditions. Comparison of scores in this study with random studies in asthma and COPD (appendix 5) show that for subjects with chronic persistent cough, there is an impairment of quality of life similar to that with chronic asthma (table 4.3.8). In comparison with COPD, the Impact domain is similar, but patients with COPD have a higher activity and symptom score, as would be expected due to their persistent breathlessness.

Objective measures of cough such as portable cough counters of good quality along with randomised controlled treatment trials will eventually be the best method to assess the true effect of therapy on cough. Quality of life instruments are designed to quantify the overall benefit of health-care interventions to the patient and currently, these subjective measures are likely to be the ones that best reflect the frequency and intensity of cough as well as its effect on patients' daily lives.

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Table 4.3.8: SGRQ in Chronic Cough compared with Asthma and COPD.

Mean values for normal and cough subjects, weighted means for asthma and COPD

	Normals	Cough	Asthma	COPD
Totals no of studies	1	1	9	10
Total no of subjects	307	88	1962	2234
Total SGRQ	8	34.6	34.23	40.92
Symptoms	10	55.6	48.95	57.08
Activity	13	29.6	37.94	57.74
Impacts	5	30.1	27.51	34.44
Anxiety Scale of HADS		6.6	7.09	4.27
Depression Scale of HADS		3.8	4.74	5.06

To calculate the mean value for scores for SGRQ, a total of 9 asthma studies with 1962 subjects were used. For COPD SGRQ scores, a total of 10 studies with 2234 subjects were used. HADS Asthma and COPD means were calculated from 2 studies each, with data from 474 asthma subjects and 202 COPD subjects (Appendix 6)

For each study a total score was obtained by multiplying the mean score with number of subjects. These totals for each study were added to form a grand total; which was then divided by the total number of subjects in all studies. This was to account for the different weights the varying number of participants would add to the average score.

4.4: BRONCHIAL AND EXTRATHORACIC AIRWAY HYPERREACTIVITY IN CHRONIC PERSISTENT COUGH

INTRODUCTION

Airway hyperresponsiveness or hyperreactivity is an increased ability of the airways to narrow after exposure to constrictor agonists. Airway hyperresponsiveness is believed to be affected by genetic as well as environmental factors (192, 193). Acute allergen exposure causes an increase of airway hyperresponsiveness that is consistently associated with an influx of inflammatory cells in the airways (194), which suggests a possible causal relation between airway inflammation and hyperresponsiveness (84, 195-197). As upper and lower airway disease often co-exist there could be a continuum of inflammation along the airway (210, 264).

Chronic cough is a very common condition, with the main causes identified as post nasal drip syndrome, gastro-oesophageal reflux disease and asthma (265). Since cough can be initiated anatomically by conditions that are outside the lower airways, measuring lower airway hyper-reactivity alone may be inadequate in explaining airway responsiveness in cough.

Bucca and Rolla matched flow-volume loops with fibreoptic laryngoscopy during histamine challenge inhalation and found that the maximal mid-inspiratory flow (MIF_{50})

best reflected changes in mid-inspiratory glottis area (204). They used this index to study patients with upper respiratory disorders and found evidence of hyperresponsiveness of the upper airways or extrathoracic airway hyperresponsiveness (EAHR) in subjects with sinusitis, laryngitis and pharyngitis (204, 205). This was associated with damage to the epithelium and proliferation of submucosal fibres (206).

Asthma like symptoms, especially cough, have been associated with extrathoracic airway dysfunction (208). In 7 patients with gastro-oesophageal reflux and cough, Rolla et al demonstrated a significantly lower $PC_{25}MIF_{50}$, indicative of extrathoracic airway hyper-reactivity compared to GORD without cough (207).

Carney and colleagues considered the possibility that upper airway hyperreactivity could be a unifying mechanism in cough, but found it present in only 12/30, 40% of subjects with chronic cough persistent for longer than 4 weeks (46).

The aim of this study was to record extrathoracic airway hyperreactivity in a large group of subjects with chronic cough persistent for over one year, to see if it was a common occurrence and whether EAHR was associated with increased airway inflammation, measured by induced sputum cell counts, exhaled gases and sputum inflammatory mediators.

METHODS

Subjects:

Adults with a cough persistent for over one year were recruited for this study. No subject had evidence of any other lung disease based on history, clinical examination, chest radiography and spirometry. Details of study design are included in the general methods section, chapter 3.

Procedures:

Methacholine challenge test was performed as described in the methods section, chapter 3. Extra thoracic airway threshold was expressed as the methacholine concentration causing a 25% drop in MIF_{50} ($PC_{25}MIF_{50}$) and the concentration of 8 mg/ml was expressed as the cut-off to define extrathoracic airway hyperresponsiveness (207, 224).

Other tests performed included:

- Baseline flow volume loops with reversibility to nebulised salbutamol.
- Induced sputum with the sample used for cell counts and supernatant for mediator measurements.
- Exhaled nitric oxide (eNO)
- Exhaled carbon monoxide (CO)
- Nasal nitric oxide (Nasal NO)

Inhaled corticosteroid treatment: All subjects received inhaled fluticasone for 2 weeks compared with placebo as described in Chapter 3 and experiment 1.

Statistical Analysis:

The variables were compared by chi-squared tests and Wilcoxon tests. A p value < 0.05 was considered significant. All analyses were performed using a statistical package SAS Version 8.02.

RESULTS

88 patients were included in the study. Demography for the subjects is described in the results section of Experiment 1 and table 4.1.1.

Extrathoracic airway hyperreactivity (EAHR) alone was seen in 4 subjects, bronchial hyperreactivity (BHR) alone in 12, both EAHR and BHR in 8 and no hyperreactivity in 64 subjects with chronic persistent cough. Demography, final diagnosis, exhaled gases, induced sputum cell counts and sputum mediator levels values are described for the four sub-groups individually in tables 4.4.1-4.4.4. The individual group sizes were not large enough for meaningful statistical comparisons. However, certain trends were noted. There were no major differences in demography except in gender where females accounted for 100% with EAHR alone, 92% with BHR alone, but 53% in the group where no hyperreactivity was present. Baseline lung functions, especially MIF_{50}, were lowest in the group with EAHR alone. Duration of cough and quantity of sputum were similar in all groups.

Final diagnosis of cough

EAHR alone was not specific to any single diagnosis of cough (one case each for PNDS, GORD, asthma and bronchiectasis). BHR was commonest in those with asthma.

Inflammatory Markers:

Total cell count and neutrophil % in induced sputum were similar in all groups. Eosinophils were higher in those with BHR alone. Exhaled NO and CO were similar in all groups. Nasal NO values were highest in those with UAHR alone.

Mediators in sputum were similar in all groups. LTB_4 levels were highest in those with EAHR alone. The numbers of subjects for whom sputum mediator measurement was possible in the EAHR group alone was very low [n=1- 4].

Mean (SD) difference of difference in the cough VAS scale (in cm) following inhaled fluticasone compared to placebo was 1.39 (2.85) for those with BHR alone, 0.11 (1.66) for those with EAHR alone and 0.89 (2.71) for subjects with no hyperreactivity.

Comparison of all subjects with EAHR compared to those without:
We analysed all subjects who had evidence of EAHR (n=12) compared to those who had no EAHR (n=76). 11/12 subjects with EAHR were female, compared with 45/76 in those without. Final diagnosis in the 12 subjects with EAHR was asthma in 6, GORD in 3, PNDS in 2 and 1 was termed idiopathic. MIF_{50}, L was significantly lower at baseline in those with EAHR [3.4 (1.2) compared to 4.5 (1.8), p=0.050].

Wilcoxon tests were utilised and showed no significant differences in baseline cough severity, eNO, nasal NO, CO, sputum cell counts and fluticasone responsiveness. Levels of cys-LT, ECP, MPO, IL-8, TNF-α and PGE_2 were similar in both groups. LTB_4 levels

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were higher in those with EAHR [mean (SD), ng/ml 37.1 (11) in the EAHR group, 28.2

(13) in those without, p = 0.051].

Table 4.4.1: Demography according to airway hyperreactivity; mean (SD)

	EAHR alone	BHR alone	Both EAHR and BHR	No hyperreactivity
Number	*4*	*12*	*8*	*64*
Age, years	62.2 (4.6)	56.6 (20.2)	55.8 (14.6)	59.9 (11.1)
Height, cm	163.8 (1.5)	161.3 (9.1)	166.4 (9.7)	168.9 (10.5)
For ex-smokers, number	2	5	4	24
Pack years smoked	15 (1.4)	19.4 (21.5)	17 (19.6)	13.4 (15.2)
Duration of cough, yrs	14.8 (10.7)	12.8 (10.8)	14.4 (12.5)	17.4 (17.7)
Baseline cough severity	4.8 (3.6)	4.6 (1.8)	4.0 (2.0)	4.0 (2.1)
Sputum quantity, ml	16 (23)	13 (14)	6 (10)	10 (13)
Total IgE, IU/ml	34 (19)	31.2 (38.5)	32.5 (19.6)	118.9 (221.6)
FEV_1 pre salbutamol, L	1.8 (0.4)	2.0 (0.8)	2.5 (1.1)	2.7 (0.8)
FVC pre salbutamol, L	2.6 (0.6)	2.7 (0.9)	3.1 (1.1)	3.5 (1.0)
FIF_{50} pre salbutamol, L	3.2 (0.8)	3.4 (1.1)	3.5 (1.5)	4.7 (1.8)

EAHR=extrathoracic airway hyperreactivity, BHR = bronchial hyperreactivity

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Table 4.4.2: Demography (2) according to airway hyperreactivity

	EAHR alone	BHR alone	Both EAHR and BHR	No hyperreactivity
Number	*4 (4.6%)*	*12(13.6%)*	*8 (9%)*	*64 (73%)*
Gender; Female (%)	4 (100)	11 (92%)	7 (88%)	34 (53%)
Atopic, specific IgE positive	3 (75%)	1 (8%)	3 (38%)	21 (33%)
Primary Diagnosis				
PNDS	1	1	1	27
GORD	1	0	2	15
CVA	1	7	5	0
Bronchiectasis	0	3	0	6
Idiopathic	1	1	0	8
Eosinophilic bronch	0	0	0	5
Other	0	0	0	1

EAHR=extrathoracic airway hyperreactivity, BHR = bronchial hyperreactivity

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Table 4.4.3: Non-invasive test of inflammation according to airway hyperreactivity; [mean (SD)]

	EAHR alone	BHR alone	both EAHR and BHR	no hyper reactivity
Number	4	12	8	64
Exhaled NO, ppb	11.1 (4.7)	10.8 (5.3)	12.8 (8.7)	10.0 (5.5)
Exhaled CO, ppm	3.44 (0.67)	3.69 (1.43)	3.45 (0.95)	3.7 (1.1)
Nasal NO, ppb	**1023.8 (218.7)**	796.0 (281.2)	805.9 (251 0)	813 0 (357.7)
Induced sputum				
Total cell count, x10^6	11.8 (11.9)	12.8 (8.0)	8.5 (6.4)	10.1 (9.7)
Neutrophil %	62.5 (24.9)	77.0 (15.4)	63.4 (19.8)	59.2 (23.9)
Eosinophil %	0.3 (0.5)	**2.4 (4.3)**	0.4 (0.5)	0.8 (1.8)
Lymphocyte %	0.0 (0.0)	0.1 (0.2)	0.5 (0.6)	0.2 (0.4)

EAHR=extrathoracic airway hyperreactivity, BHR = bronchial hyperreactivity

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Table 4.4.4: Sputum mediators according to airway hyperreactivity; [mean (SD)]

	EAHR alone	BHR alone	both EAHR and BHR	No hyperreactivity
Number	4	12	8	64
Cys-LT, ng/ml	1.1 (0.4)	1.3 (1.2)	1.0 (0.6)	1.4 (1.1)
LTB_4, ng/ml	**39.9 (9.4)**	28.3 (11.4)	36.1 (12.1)	28.1 (13.0)
IL-8, ng/ml	12.4 (10.5)	51.7 (77.7)	31.8 (66.9)	29.4 (69.7)
PGE_2, ng/ml	10.4 (12.0)	14.7 (8.5)	18.9 (18.0)	16.8 (9.7)
MPO, µg/ml	10.9 (12.4)	26.6 (32.0)	25.0 (29.9)	16.9 (23.9)
ECP, ng/ml	532 (277)	1192 (932)	1012 (1092)	1183 (883)
TNF-alpha, ng/ml	0.6 (n=1)	1.6 (4.3)	1.5 (3.6)	1.2 (4.3)
NGF, ng/ml	736 (n=1)	572.0 (374.6)	393.2 (198.3)	1728 (4198)

EAHR=extrathoracic airway hyperreactivity, BHR = bronchial hyperreactivity

Table 4.4.5: Cough VAS response following inhaled fluticasone compared to placebo according to airway hyperreactivity; mean (SD)

	EAHR alone	BHR alone	Both EAHR and BHR	No hyperreactivity
Number	*4*	*12*	*8*	*64*
VAS change, cm	**0.11 (1.66)**	1.39 (2.85)	1.42 (1.52)	0.89 (2.71)
VAS % difference	-10.9 (41.6)	25.22 (58.1)	62.55 (73.64)	18.79 (137.8)

EAHR=extrathoracic airway hyperreactivity, BHR = bronchial hyperreactivity

DISCUSSION

Hyperresponsiveness of the upper airways or extrathoracic airway hyperreactivity has been demonstrated in asthma and in a small number of subjects with rhinitis and gastro-oesophageal reflux disease (205, 207). In this study, we have demonstrated that in a large group of people with chronic persistent cough, EAHR is present in only 14% of subjects and is not associated with lower airway inflammation or sputum inflammatory mediator levels.

Irwin and colleagues selected 9 patients with cough and post-nasal drip and demonstrated upper airway obstruction which improved following specific treatment of the cough (266). Bucca et al also selected patients with sinusitis and found evidence of EAHR which improved following treatment (205). It is unknown whether the hyperreactivity is a cause or effect of the nasal condition. It has been suggested that extrathoracic airway hyperresponsiveness in the absence of bronchial hyperresponsiveness may indicate upper airway disease as a cause of cough (46). Our findings are not in support of this hypothesis as only 2/30 subjects with PNDS had EAHR.

In our study where all subjects with chronic cough were grouped, EAHR was an uncommon finding. This is in keeping with the study by Carney and colleagues in 30 subjects with cough persisting for over 4 weeks (46).

The difference in our methodology compared to others was the use of methacholine as a broncho-provocative agent rather than histamine. Methacholine, a direct stimulus of bronchoconstriction, is currently the gold standard for the assessment of bronchial hyper-reactivity (201, 202). As results for histamine and methacholine are similar for BHR and we were performing the methacholine challenge test for the lower airway hyperreactivity, we used methacholine for the upper airway test as well. This may account for some differences from other studies and further work on comparison of these agents in the upper airways is indicated.

Bronchial hyperreactivity has been suggested as an indirect method of assessing airway inflammation (201, 202) and airway responsiveness can be associated with the severity of airway inflammation in asthma (267). We found no difference in induced sputum cell counts or exhaled nitric oxide levels in those subjects with EAHR compared to those without. Recently several studies have questioned the association of airway inflammation and hyperresponsiveness (198, 199). In chronic cough, Carney and colleagues found BHR in 23% subjects and sputum eosinophils in 50%, showing the dissociation between lower airway hyperreactivity and eosinophilic inflammation of the airways.

It has been suggested that upper airway hyperresponsiveness is possibly due to release of inflammatory mediators (46). We measured inflammatory mediators in induced sputum and found that levels of cys-LT, PGE_2, MPO, ECP, TNF-α and IL-8 were similar in subjects with and without EAHR. It is possible that measurement of mediators in nasal secretions may yield different results. Leukotriene B_4 was the only mediator marginally

increased in the EAHR group. Nasal provocation in allergic rhinitis can increase levels of LTB_4 and IL-8 in nasal fluid, which respond to an anti-histamine, astemizole (268) Responsiveness to inhaled corticosteroids was unrelated to the presence of upper airway responsiveness.

We measured MIF_{50} and found baseline values lower in the group with EAHR. This must be interpreted with caution, as predicted values for MIF_{50} are not available for women of all ages. Age, gender and height would affect the predicted values as in all lung functions. Although age and height were not very dissimilar, there was a definite female preponderance in the EAHR group.

The female:male ratio was higher in the group with EAHR. This is identical to the findings of Bucca and colleagues (204). Airway hyperresponsiveness as well paroxysmal glottic closure are usually seen more commonly in women compared to men (269, 270) with reasons such as reduced airway calibre and hormonal changes considered.

The mechanism of EAHR is unknown. It could be triggered by stimulation of pharyngo-laryngeal receptors (208), or simply a reflex narrowing of the glottis (209). EAHR may represent an entity similar to vocal cord dysfunction (269, 271), a condition frequently associated with asthma.

In conclusion, we studied extrathoracic hyperreactivity in 88 subjects with chronic cough and found it present in only 14%. There was no association with lower airway inflammation other than an increase in leukotriene B_4 levels in induced sputum of these

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subjects. There was a marked preponderance of women with EAHR. Currently the utility

of EAHR measurements remains a research tool as it has no clinical utility in identifying

the aetiology of chronic cough.

4.3.5: SENSITIVITY AND SPECIFICITY OF SYMPTOMS IN PREDICTING THE CAUSE OF COUGH

INTRODUCTION

Chronic cough is a common condition with prevalence rates between 14-23% (10, 184, 185). There are many known causes of chronic cough (tables 1.1.1 and 1.1.2, Introduction chapter) but several studies have demonstrated that in a non-smoker with normal chest radiography, chronic cough is usually caused by post nasal drip syndrome (PNDS), gastro-oesophageal reflux disease (GORD) or cough variant asthma (CVA), singly or in combination (8, 32, 34). Despite narrowing the commonest causes of cough to three, the actual diagnosis and control of cough remains a challenge. This is because patients can have symptoms suggestive of these conditions and yet treatment for them may not improve the cough. On the other hand, these conditions may be 'silent' with no obvious suggestive symptoms and the cough may improve with treatment for the condition (34, 272). Specialist clinics have a higher success rate in treating cough than general clinics (7), as these issues are better appreciated.

The key to successful management of the cough is to establish a diagnosis and then treat the specific cause of cough (6). The final diagnosis is dependent on response to specific treatment. This is currently the most commonly utilised method of confirming a diagnosis, advocated by Irwin and colleagues (273), although it has been questioned

recently (6, 274). The treatment trials are fairly long, lasting usually up to two months for each possible condition. GORD can take up to six months to resolve (8, 275, 276). When there are multiple causes of cough, time to response can be even longer. Evidence on response to treatment in cough in large, double-blind, placebo-controlled studies is lacking.

Investigations in chronic cough could reduce the overall treatment duration (213) and play a definite, but limited role. The positive predictive value for most tests is not high [88% for histamine challenge, 68% for 24 hours pH monitoring and 67% for CT scan sinuses] (33). Fibre-optic bronchoscopy does not contribute significantly to the diagnosis of cough (212). The tests commonly used in chronic cough subjects are expensive (213) and require special hospital set-ups to perform.

With cough being such a common clinical problem for a general practitioner, if a clinical solution like a 'good history' could predict a final diagnosis, it would be invaluable in saving time, inconvenience to the patient and expense. Mello and colleagues found that general features of character, timing and complications of cough could not predict a diagnosis (211). McGarvey and colleagues concluded that a specific clinical history was not helpful in diagnosing the cause of cough, as it was predictive of the final diagnosis in only 56% cases of asthma, 52% with PNDS and 40% with GOR (33). Simpson suggested that their choice of questions could have been a factor for their low success rate (277).

The aim of our study was to evaluate the value of specifically selected clinical questions

in making a final diagnosis of chronic persistent cough.

METHODS

Subjects

Adults with a cough persisting for longer than one year were recruited from hospital respiratory clinics and by advertisement in a local newspaper. No subject had evidence of any other lung disease based on history, clinical examination, chest radiography and spirometry. Details of study design are included in the general methods section, chapter 3.

Clinical History

All subjects were asked a detailed clinical history at baseline, entered in our study proforma (appendix 6).

General Cough Questions:

These included duration of cough, approximate quantity of sputum production daily, positional and diurnal variation and whether the cough felt like a 'tickle/itch in the throat'. Subjects were all asked if the cough occurred during sleep (either awakening them or being informed by family members).

Triggers for the cough were noted with specific questions about dust, pollen, pet dander, cold air, exercise, sprays and aerosols.

Disease specific questions:

For GORD: History of heartburn, acid regurgitation, waterbrash, dysphagia and odynophagia (278). *For Asthma:* History of associated wheeze or breathlessness

(paroxysmal or exertional). *For PNDS*: Sensation of something dripping down the nose, need to frequently clear the throat and nasal discharge (8).

To assess the value of specific questions for *rhino-sinusitis*, a scoring system was used adapted from Osguthorpe and Hadley, where the presence of 2 major or 1 major and 2 minor criteria represented the presence of rhino-sinusitis. The major criteria were facial pain/pressure, facial congestion/fullness, nasal obstruction/blockage, nasal discharge/purulence by history or examination and hyposmia. The minor criteria were headache, halitosis, fatigue, dental pain, cough, ear pain/pressure/fullness (265). *For allergic rhinitis*, history of frequent sneezing, itchy nose/eyes/ throat and profuse watery nasal discharge was recorded (279).

Final diagnosis of cough

To achieve a final diagnosis for the cause of the cough, all subjects were offered sequential trials of specific therapy depending on the likely clinical diagnoses for their cough. Final diagnosis or diagnoses of the cause of the cough (described in chapter 3) were based on defined pre-treatment criteria (5) and accepted only when the cough significantly improved or disappeared with specific therapy (8).

Pre-treatment diagnostic criteria:

Certain pre-treatment criteria were set to aid the preliminary diagnoses of cough.

Asthma: History of cough with or without paroxysmal dyspnoea and wheeze or evidence of reversible airflow obstruction (15% improvement in FEV_1 following salbutamol) and/or >/= 20% lability on peak flow readings. The PC_{20} on methacholine challenge testing was $\leq$ 8 mg/ml.

Post nasal drip syndrome: Any one of the following on history or examination: sensation of something dripping down the throat, nasal discharge, need to clear the throat frequently, mucoid/mucopurulent nasal secretions on examination or cobblestone appearance of the oro-pharyngeal mucosa.

Gastro-oesophageal reflux disease: History of heartburn and/or acid regurgitation.

Bronchiectasis: History of cough with large volumes of sputum production.

Eosinophilic bronchitis was considered when sputum eosinophilia > 2%, normal PEF variability, spirometry and methacholine challenge test.

Final diagnosis

The final diagnosis was based on the above criteria and a response to appropriate treatment. When the cause of cough was unknown, it was termed '*Idiopathic cough'*.

Treatment plan:

All patients were treated with medication for their cough, starting with the most likely diagnosis based on their history and baseline tests as above. As cough is known to be frequently due to multiple causes, for each patient a plan was drawn up with a list of potential diagnoses and the order in which to treat them.

Treatment trials were done sequentially, for two months each, with the clinical response noted to each treatment. This was based on the patient's view of the effectiveness of the treatment in reducing their cough compared to their baseline level prior to that treatment trial.

The treatment responses were noted as

Very good response: if the improvement was between 75-100%

Good response: if the improvement was between 50-75%

Minimal response: if the improvement was between 25-50%

No response: if the improvement was < 25%.

A final diagnosis was made when there was a 'very good' response to treatment.

If the response was 'good', then patients were asked to continue with further treatment trials, until the cough resolved. If subjects declined further treatment, then the final diagnosis of the cause of cough would be accepted based on a 'good response' to treatment. If a subject had good responses to two treatment lines, then a combined diagnosis was made with the primary diagnosis being the one for which there was a better therapeutic response.

If there was 'minimal' or 'no response' to treatment, patients were requested to continue with further treatment trials using alternative medication. If they declined further treatment at any stage, the cause of cough was considered idiopathic.

A high resolution CT scan was performed prior to confirming a diagnosis of bronchiectasis.

Therapy used for the common causes of cough:

For each of the common conditions, a plan of treatment was made. First line therapy was used initially, if possible, in all subjects. Second line therapy was used when the first line treatment did not work or was not suitable for an individual patient based on adverse events or contraindications due to other medical conditions. Third line therapy was very occasionally used to tailor to the requirements of an individual patient and consisted of a combination of first and second line treatment.

a. Gastro-oesophageal reflux disease:

First line therapy for GORD (GORD$_1$):Specific first line therapy used for GORD was oral omeprazole 20 mg bid for a month, followed by 20 mg od for a second month along with advice on life-style alterations to reduce acidity, including elevation of the head end of the bed and alterations in diet.

Second line therapy for GORD (GORD$_2$):

This was a combination of metoclopramide and ranitidine for two months along with advice on life-style alterations to reduce acidity.

b. Cough variant asthma and eosinophilic bronchitis

First line therapy for CVA and EB (CVA$_1$ and EB$_1$):

Inhaled corticosteroid (budesonide turbohaler) was prescribed at a dose of 400 mcg bid for two months along with a short acting beta$_2$ agonist as required for asthma.

Second line therapy for CVA (CVA$_2$):

Inhaled corticosteroid dose was 800 mcg bid for two months along with a short acting

beta$_2$ agonist as required for asthma.

c. Post nasal drip syndrome

First line therapy for PNDS (PNDS$_1$):

A combination of a local nasal decongestant (pseudo-ephedrine drops, 2-3 drops to each

nostril, tid) for a week, along with two months of intra-nasal corticosteroid (mometasone,

50 mcg/day, 2 sprays od) and an oral non-sedating anti-histamine (loratadine 10 mg od).

Second line therapy for PNDS (PNDS$_2$):

This comprised of three weeks of an oral decongestant (pseudo-ephedrine, 60 mg tid) and

a first generation antihistamine (brompheniramine maleate, 4 mg tid).

Subjects were treated with first and second line treatment for each of the common causes

of cough. A high resolution CT scan of the chest, CT scan of the sinuses and an

oesophageal pH monitoring test were performed if the patient was agreeable, to look for a

specific diagnosis for the cough.

Statistical Analysis

The association between each test and the diagnosis was tested by a Mantel-Haenszel

Chi-squared test. The sensitivity, specificity, positive and negative predictive values were

also calculated.

A p value < 0.05 was considered significant. SAS version 8.2 was used for all analyses.

RESULTS

Patient demography

88 subjects with chronic persistent cough were recruited. Of these, 52 were recruited from the community (GP referrals and advertisement in a newspaper) and 36 were referred from hospital clinics.

The flow of patients through the different treatment trials is described in Figure 4.5.1. A final diagnosis for the cough was obtained in 78/88 (89%) of subjects; 74% had a single diagnosis, 24% had 2 diagnoses and 2% had 3 diagnoses. The pattern of primary diagnoses [figure 4.5.2] was PNDS (30), GORD (18), CVA (13), bronchiectasis (9), eosinophilic bronchitis (5), habitual cough (2) and bronchitis (1). In 10 subjects, the cough was termed idiopathic. Baseline characteristics of the patients are shown in experiment 1, table 4.1.1. Individual data for the smaller diagnostic sub-groups are not depicted because the numbers were too small for meaningful analysis.

Value of general cough questions

The general cough questions were compared with each of the major final diagnoses (as primary or secondary cause of cough) and there was no significant association for cough during sleep, tickly cough, positional or diurnal variation in any condition other than bronchiectasis where cough during sleep had a 97% NPV, 23% PPV, p=0.039 (Tables 4.5.1-4.5.4)

Presence of sputum with the cough was strongly associated with bronchiectasis and had a 100% negative predictive value for this condition although a low PPV of 37% (Table 4.5.4).

Value of symptoms in predicting final diagnosis of cough

For GORD, clinical history had a 100% NPV if the 5 questions were considered together. The PPV was low (35%). Heartburn alone had the highest sensitivity (87%, p=0.001). Odynophagia, dysphagia and waterbrash had a high specificity (80%-97%) but low sensitivity (Table 4.5.1).

For asthma, wheeze and dyspnoea had a high sensitivity but low specificity leading to a PPV of 20% and NPV of 86%; (table 4.5.3). Triggers for cough as a group were not specific for asthma, but cough triggered by cat/dog dander or grass pollen had a high specificity for a diagnosis of asthma (90-99%).

Questions for PNDS had the lowest sensitivity and specificity for a definitive diagnosis, individually and combined. The rhino-sinusitis specific questions or allergic rhinitis specific questions did not increase this significantly (table 4.5.2).

A summary of the results of the grouped questions for each diagnosis is depicted in table 4.5.5 and figure 4.5.3.

Figure 4.5.1: Flow of patients through different treatment trials for a final diagnosis of cough

TREATMENT 1   (n=88)

$PNDS_1$ n=32 $GORD_1$ n=16
CVA_1 n=32   EB   n=5
$GORD_3$ n=1 $PNDS_3$ n=1
Bronch n=1

Very good response   n=25   (28%)
$PNDS_1$ n=7, $GORD_1$ n=6, CVA_1 n=8, EB n=2, $PNDS_3$
n=1, $GORD_3$ n=1

Declined further treatment trials   n=6
Unknown cause n=3, spontaneous improvement
1 avian antibody positive, termed pigeon fancier's
bronchitis, 2 good responses to $PNDS_1$.

TREATMENT 2   (n=57)

$PNDS_1$ n=18 $GORD_1$ n=23
CVA_1 n=6   Bronch n=3
$PNDS_2$ n=3 CVA_2 n=1
$GORD_2$ n=2

Very good response   n= 17   (30%)
$PNDS_1$ n=6, CVA_1 n=3, $GORD_1$ n=4, CVA_2 n=1,
$PNDS_2$ n=1, $GORD_2$ n=2.

Declined further treatment trials   n=8
(2 good responses to $PNDS_1$, 3 good response to EB
during treatment 1, 1 bronchiectasis + $GORD_1$, 1
bronchiectasis and 1 unknown cause of cough)

TREATMENT 3   (n=32)

$PNDS_1$ n=5 $GORD_1$ n=10
CVA_1 n=7   Bronch n=2
CVA_2 n=2 $PNDS_2$ n=6

Very good response   n= 7   (22%)
$PNDS_1$ n=3, $GORD_1$ n=2, $PNDS_2$ n=1, CVA_2 n=1.

Declined further treatment trials   n=5
2 good responses to $GORD_1$, 1 bronchiectasis, 2
unknown causes of cough

TREATMENT 4   (n=20)

$GORD_2$ n=3 CVA_1 n=4
$PNDS_2$ n=7   Bronch n=6

Very good response   n= 4   (20%)
$PNDS_2$ n=4

Declined further treatment   n=11
2 good responses to combined $PNDS_1$, 1 habit cough,
5 bronchiectasis and 3 unknown causes of cough

TREATMENT 5   (n=5)

$GORD_2$ n=2 CVA_2 n=1
Bronch n=2

Very good response   n= 1   (20%)
$GORD_2$ n=1

Declined further treatment trials   n=3
1 good response to P_2 during Rx 4, 1 bronchiectasis, 1
unknown cause of cough

TREATMENT 6   (n=1)

$GORD_2$ n=1

Very good response   n=0

Habit cough   n=1
Continued to further treatment n=0

Figure 4.5.2: Pattern of primary diagnoses in chronic cough
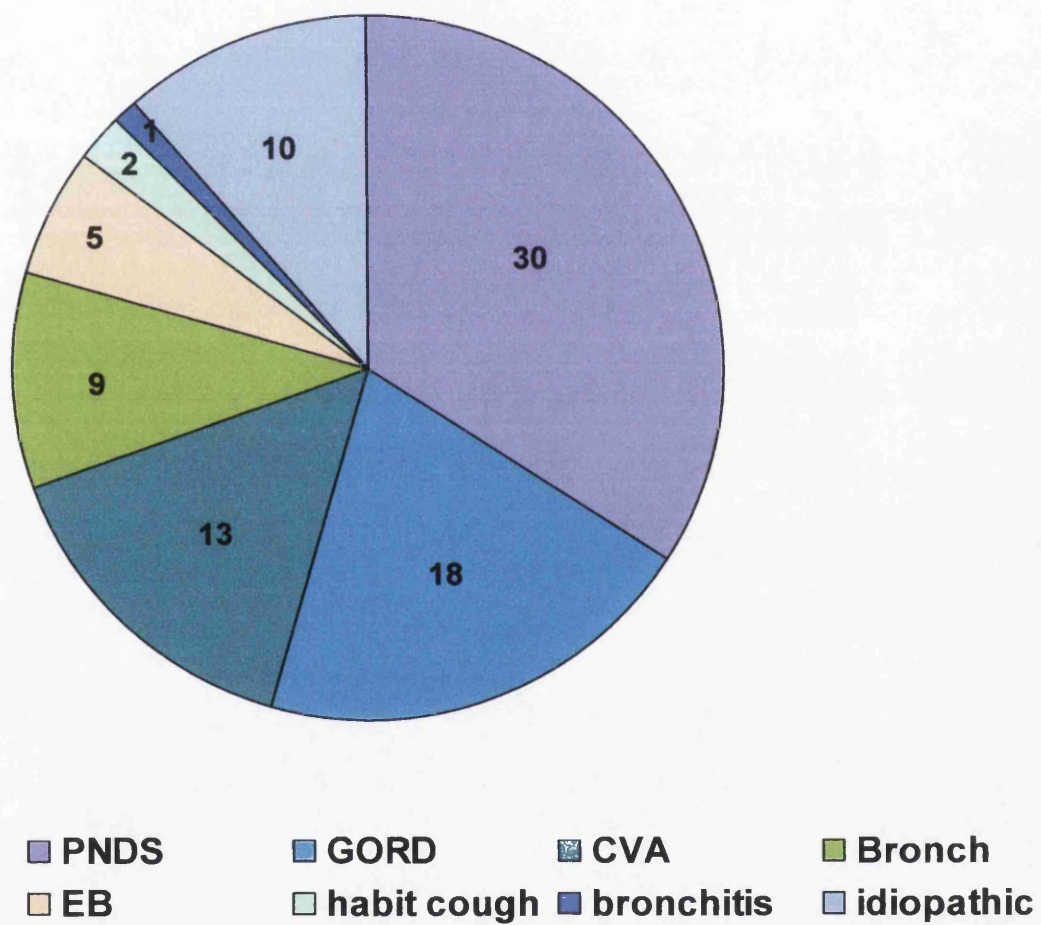
Table 4.5.1. Sensitivity and specificity of history in diagnosing GORD

Symptom	Sensitivity %	Specificity %	p value
GORD SPECIFIC QUESTIONS			
Heartburn	**87**	54	**<0.001**
Acid regurgitation	70	42	0.350
Waterbrash	09	**82**	0.274
Dysphagia	22	**80**	0.860
Odynophagia	13	**97**	0.078
Any one of the above	**100%**	**22%**	**0.001**
	PPV 35 %	**NPV100%**	
GENERAL COUGH QUESTIONS			
Cough during sleep	67	34	0.050
Tickly cough	65	40	0.661
Sputum with cough	83	35	0.119
Positional variation:			0.540
[Worst position (n=17): upright 5, stooping 4, supine 8]			
Diurnal variation			0.353
[Worst time of day (n=15): morning 11, afternoon/evening 0, night 4]			

Table 4.5.2. Sensitivity and specificity of history in diagnosing PNDS

Symptom	Sensitivity %	Specificity %	p value
PNDS SPECIFIC			
Sensation of drip down the back of the throat	66	36	0.863
Frequent throat clearing	74	26	0.974
Nasal discharge	76	32	0.657
Any one of the above	**92**	**10**	**0.735**
	PPV 48%	**NPV 63%**	
ENT GROUPED QUESTIONS			
Chronic rhinosinusitis	87	20	0.401
	PPV 45%	**NPV 67%**	
Allergic rhinitis	**28**	**19**	0.251
	PPV 48%	**NPV 66%**	
GENERAL COUGH QUESTIONS			
Cough during sleep	68	26	0.515
Tickly cough	66	42	0.460
Sputum with cough	65	27	0.392
Positional variation			0.038

[Worst position (n=26) : upright 10, stooping 2, supine 14]

Diurnal variation			0.819

[Worst time of day (n=21) : morning 13, afternoon/evening 2, night 6]

Table 4.5.3. Sensitivity and specificity of history in diagnosing CVA

Symptom	Sensitivity %	Specificity %	p value
ASTHMA RELATED QUESTIONS			
Wheeze	56	56	0.395
Exertional dyspnoea	62.5	56	0.193
Either of the above	**75**	**24**	**0.520**
	PPV 20%	**NPV 86%**	
Triggered by dust	38	63	0.917
Triggered by pollen	25	**90**	0.097
Triggered by cat dander	0	**99**	0.637
Triggered by dog dander	13	**96**	0.195
Triggered by exercise	50	56	0.688
Triggered by cold air	63	47	0.482
Triggered by aerosols	56	47	0.802
Triggered by any one	**81**	**21**	**0.853**
	PPV 19%	**NPV 83%**	
GENERAL COUGH QUESTIONS			
Cough during sleep	76	30	0.716
Tickly cough	69	40	0.505
Sputum with cough	63	29	0.486
Positional variation			0.665
[Worst position (n=10): upright 2, stooping 1, supine 7]			
Diurnal variation			0.050
[Worst time of day (n=13): morning 7, afternoon/evening 2, night 4]			

Table 4.5.4. Sensitivity and specificity of history in diagnosing Bronchiectasis

Symptom	Sensitivity %	Specificity %	p value
Sputum with cough	100	37	0.005
	PPV 25%	NPV 100%	
Quantity of sputum			<0.001
0 ml	0% had bronchiectasis		
1-29 ml	20% had bronchiectasis		
≥ 30 mls	56% had bronchiectasis		

GENERAL COUGH QUESTIONS

Cough during sleep	93	33	0.039
	PPV 23%	NPV 96%	
Tickly cough	50	36	0.305
Positional variation			0.571

[Worst position (n=9): supine 6, stooping 2, upright 1]

Diurnal variation			0.873

[Worst time of day (n=11): morning 8, afternoon/evening 1, night 2]

Table 4.5.5: Summary of value of specific question clusters for diagnosis of the cause of cough

	Sensitivity%	Specificity%	PPV%	NPV%	p value
GORD (heartburn, acid regurgitation, waterbrash, dysphagia, odynophagia)	100	22	35	100	0.001
ASTHMA (wheeze, dyspnoea)	75	24	20	86	0.520
PNDS (sensation of drip at the back of throat, throat clearing, nasal discharge)	92	10	48	63	0.735
BRONCHIECTASIS (presence of sputum with cough)	100	37	25	100	0.005

Figure 4.5.3: Positive and negative predictive value of symptoms in diagnosing he

cause of chronic cough

PPV=positive predictive value, NPV= negative predictive value
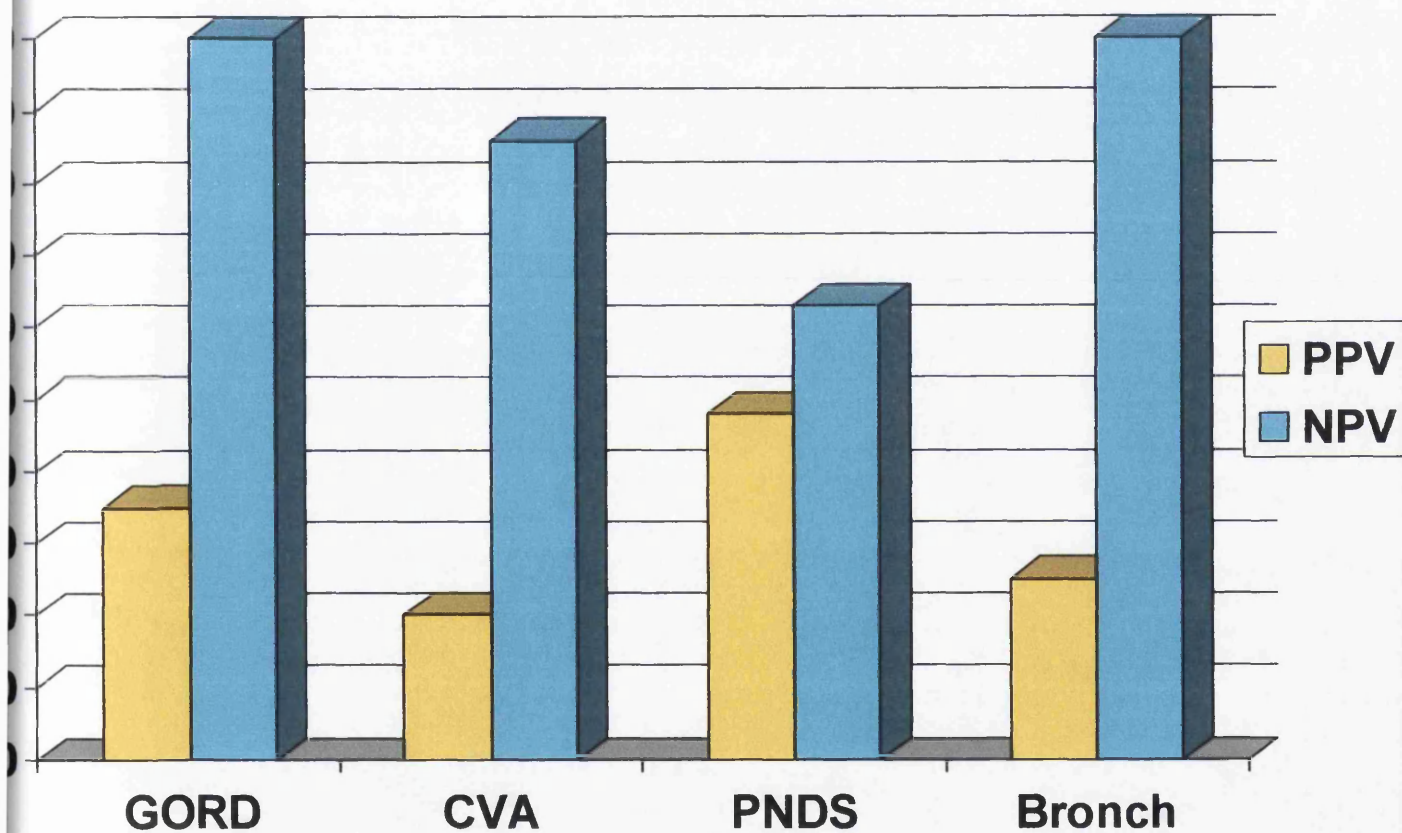
DISCUSSION

Cough is one of the commonest symptoms presenting to a general practitioner. When the cough has persisted for several months it is quite difficult to achieve a clinical diagnosis of the cause of the cough. Even when all investigations are utilised, the success rate can be low (110). As there are a variety of causes of cough, the ability to reduce the differential diagnosis to a minimum would help to achieve a quicker therapeutic success. Various studies have shown that in a non-smoker with a normal chest X-ray, the three commonest causes of cough are PNDS, asthma and GORD (8, 32, 34). Bronchiectasis is another common cause of cough (14, 211). In this study we have shown that certain specific questions have a high negative predictive value for these common causes and could help to guide the choice of specific therapeutic trials.

The pattern of final diagnoses in our study was similar to others, with the commonest diagnoses being PNDS, GORD, CVA and bronchiectasis. 24% subjects had 2 diagnoses for the cough and 2% had 3 diagnoses; which is fairly typical of this condition (8, 14, 34). When assessing the value of symptoms in achieving a diagnosis, both the primary and secondary diagnoses were considered, as successful treatment would require resolution of both. As a tertiary diagnosis was uncommon in our study, this was not incorporated into the analysis.

Of the three commonest conditions for cough, our study showed that GORD can be eliminated in 100% cases and asthma in 86% if there is no suggestive history. However

the positive predictive value for all questions was low, implying a significant overlap of symptoms. Irwin and colleagues studied the key components of diagnostic evaluation in cough and the outcomes of specific therapy (8). For subjects with a diagnosis of GORD based on response to specific therapy, in 43% they found no suggestive symptoms and called them 'silent reflux'. 24 hour oesophageal pH monitoring was positive in all. The two symptoms they utilised were 'heartburn and sour taste in the mouth'. McGarvey and colleagues only comment on PPV of symptoms and found that when subjects were asked about 'dyspepsia and cough worse after meals' they could identify 8/20 subjects correctly with a PPV of 40% (33). It is possible that the choice of questions utilised may account for the difference.

Oesophageal pH monitoring has been shown to have a PPV of 84% an NPV of 100% (14), but is uncomfortable as well as expensive (213). We found that 5 symptoms related to GORD had an identical NPV, but lower PPV. Hence they would be very useful in eliminating GORD from the list of conditions to treat as a cause of cough.

For patients with a dry cough, bronchiectasis was eliminated in 100% cases. Sputum production is typical of bronchiectasis, although present in all common causes of cough (31). Bronchiectasis sicca which is a 'dry bronchiectasis' is seen less commonly in the western world as it is bronchiectatic changes in the upper lobes following tuberculosis. In subjects with a dry cough and a normal chest X-ray, our data suggests that bronchiectasis as a potential cause can be eliminated.

A cough at night was significantly associated with bronchiectasis, but the PPV for a night cough was low as it was present in all causes of cough. Previous studies suggest that in GORD cough diminishes during sleep as the lower oesophageal sphincter tone is maximal (6). In our study, night cough was similar in all sub-groups and did not help to differentiate the cause of cough.

It was difficult to utilise the history to make a definitive diagnosis of PNDS. In our study, although the sensitivity was 92%, the specificity for this diagnosis was only 10%. The PPV and NPV are similar to that noted by McGarvey et al (33).

The sensitivity for wheeze and dyspnoea for diagnosing asthma was 75%, but surprisingly the specificity was 24%, implying that these symptoms were present even when the cough did not respond to anti-asthma treatment.

Positional and diurnal variation of cough did not help in achieving any particular diagnosis, as noted by Mello and colleagues (211). Description of the cough as a 'tickle or itch was common to all diagnoses of cough.

Triggers for cough were common and did not have a high sensitivity for diagnosing cough due to asthma. McGarvey and colleagues used a history of nocturnal cough and cold air, exercise and aerosols as precipitating factors and found a PPV of 56% for asthma (33). We demonstrated that if the trigger factor for cough was cat dander, dog

dander or grass pollen, then the specificity for asthma was 99%, 96% and 90% respectively.

Other studies have examined the value of investigations (14, 34, 273) as well as their cost effectiveness (213) in the management of chronic cough. We did not perform routine 24 hour oesophageal monitoring, CT scan of the sinuses, HRCT chest in this study as the purpose was not a comparison of investigations versus clinical history, but an evaluation of symptoms with response to therapeutic treatment trials.

The value of the choice of symptoms used in this study needs to be assessed in prospective studies on chronic cough.

CHAPTER 5

CONCLUSIONS

5.1 PRINCIPAL FINDINGS

In this study, we included 88 subjects with chronic cough persistent for over one year, with normal chest radiographs and spirometry. The principal findings in the different experiments were:

a. Evidence of airway inflammation in chronic persistent cough, with increased induced sputum cell counts, exhaled nitric oxide and inflammatory mediators (LTB_4, cys-LT, PGE_2, ECP, IL-8 and TNF-α).

The predominant cellular pattern in induced sputum was neutrophilic, which differed from studies by Carney et al (46) and Birring et al (45) but was in agreement with the work from Jatakanon and colleagues (51). A difference in our study population was that we included subjects with cough persistent for over a year, unlike the 3-4 week cut-off in other studies as we felt this group represented a bigger therapeutic challenge in the community.

An interesting observation was the marked increase in inflammatory mediators, especially PGE_2, LTB_4 and cys-LT in all causes of cough. This suggests a certain homology in all causes of cough and presents a therapeutic option that may benefit cough caused by different aetiologies. IL-8, MPO and TNF-α levels were elevated in bronchiectasis alone.

A two-week trial of high dose inhaled fluticasone compared to placebo, lead to a statistically significant improvement in cough as recorded on a 10 cm visual analogue scale. In addition, this treatment was accompanied by a reduction in exhaled nitric oxide, carbon monoxide and sputum ECP levels.

The overall mean% improvement in cough was 22.3 [95%CI, -3.5 to 48.2] and sputum mediator levels, other than ECP, remained elevated, implying that two weeks of inhaled corticosteroids have only a partial effect on the inflammation in cough.

Non-invasive tests as predictors of corticosteroid responsiveness were evaluated. Exhaled nitric oxide levels correlated best with inhaled fluticasone response in cough. This was followed by sputum eosinophil%, total IgE levels, sputum ECP and sputum cys-leukotriene levels.

b. The second experiment measured neurotrophins in serum/ induced sputum of patients with chronic cough. Despite several theoretical reasons for these mediators to be involved in chronic cough, measured levels of neurotrophins in serum and NGF in induced sputum were not elevated compared to controls. This implies that either neurotrophins are not responsible for the neurogenic inflammation in cough or that different methods of evaluating them, possibly at receptor level are required.

c. The third experiment studied the effect of cough on quality of life utilising general questionnaires like the St. George's Respiratory Questionnaire and the Hospital Anxiety

and Depression Scale. We found a marked impairment in all SGRQ domains and a significant improvement following specific treatment of cough. These findings were independent of the cause of cough. Clinical levels of anxiety and depression were not a feature of chronic cough, but there was a significant improvement in the scores after treatment.

d. In the fourth experiment we studied extrathoracic airway hyperreactivity in chronic cough and found that EAHR was present in only 14% of subjects and was unrelated to any particular aetiology of cough. Airway inflammatory cells in induced sputum and mediator levels were similar in the group with EAHR compared to the group without, other than an increase in sputum LTB_4 levels. There was a marked predominance of females noted in the group with EAHR (11/12). This test does not seem to provide any clinically useful addition to the management of chronic cough.

e. In the fifth experiment, the value of symptoms in making a final diagnosis was assessed. The diagnostic pattern was similar to other cough studies with PNDS [n=30], GORD [18], asthma [13], bronchiectasis [9] and eosinophilic bronchitis [5] being the commonest causes. In 10 subjects the cough was termed idiopathic. General cough questions regarding the positional or diurnal variation in cough did not lead to any specific diagnosis, confirming the findings of Mello and colleagues (211). Positive predictive value of symptoms in diagnosing a specific cause of cough was low, similar to the values described by McGarvey et al (33). However, we found that specific history for GORD had a 100% negative predictive value, which could prove useful in a clinical

situation, if evaluation in prospective studies confirms this. For bronchiectasis, history of a dry cough had a 100% NPV. For asthma, the NPV was 86% for history. Certain specific triggers like cat/dog dander and grass pollen had > 90 % specificity for asthma. Questions for PNDS had the lowest positive or negative predictive value and improving the specificity of questions with scoring systems for rhino-sinusitis did not improve this outcome.

5.2 STRENGTHS AND LIMITATIONS OF THE STUDY

With this project, we aimed to study five different aspects about subjects with chronic cough. There are certain strengths in the study design and in retrospect, several limitations as well. Identifying these might help in the design of future projects in chronic cough.

a. Crossover trial with inhaled corticosteroids.

To study the effect of inhaled fluticasone in cough, we utilised a randomised, double-blind, placebo-controlled crossover design. There are relatively few randomised, placebo-controlled studies including large numbers of subjects in treatment of chronic cough. The cross-over design, however, could lead to a carry over effect of the first treatment. In our study, this did not happen and we were able to demonstrate that results were similar whether subjects received fluticasone or placebo first. However, in other cough studies, the cross-over design has been suggested to account for poor results and possibly parallel group studies are better suited for the treatment evaluation in chronic cough (280-282).

We used a high dose (500 mcg bid) of inhaled fluticasone and a placebo inhaler for two weeks each with a two week washout phase in between. The duration of treatment could be considered too short to achieve any significant effect on cough. In a therapeutic trial, when attempting to resolve cough with inhaled corticosteroids, we would use two months as routine treatment, although we would expect a clinical

improvement in a steroid responsive cough within that period. In some studies response to inhaled corticosteroids was seen within two weeks (60, 75). Since we were performing a cross-over trial and aiming for an improvement and not necessarily a resolution of cough we opted for a two-week trial. However, in a study attempting to show a resolution of cough, longer treatment trials should be incorporated.

b. Use of a visual analogue scale as an outcome measure:

For monitoring the effect of treatment we relied on a visual analogue scale where patients recorded their perceived cough severity on a 10 cm scale in a daily diary card. They have been used in several other studies and appear to be responsive to therapeutic interventions (283, 284). Some studies have compared visual analogue scales with capsaicin cough sensitivity (35, 285, 286). However, it requires further comparisons with other measures of cough (287). Of late, portable cough monitors are getting more robust and easier to use, although their value in cough studies with a long duration of treatment is to be developed. Future studies should incorporate an additional objective measure of cough improvement such as this along with daily visual scales.

c. Measurement of inflammatory mediators in induced sputum:

In this study we measured a wide variety of mediators allowing an evaluation of possible activation of different inflammatory cells. This opened up a wider picture than studies demonstrating selective mediator elevation in specific sub-groups of cough (44, 51). A technical problem with measuring induced sputum mediators is that

adequate samples were not available in all subjects at each visit. We were able to measure cell counts in 80% of samples at baseline, similar to results in other studies (72, 288). Higher success rates have been reported using increasing concentrations of hypertonic saline (284) but this runs the potential risk of increased side effects.

d. Quality of life measurements:

In this study we used the St. George's Respiratory Questionnaire and the Hospital Anxiety and Depression Scale to assess the effect of cough on quality of life. These are not cough specific scales, which has the advantage of allowing for comparison with other medical conditions. As they are not disease specific, they are probably less sensitive at identifying all the issues associated with cough as a specific symptom. Birring and colleagues found a good correlation of their cough specific questionnaire and the SGRQ (190). We started our study when no cough specific questionnaires were available, but in future studies, we would like to combine a general with a cough specific questionnaire to allow for a more specific assessment of the effect of treatment in patients with cough.

When we repeated the quality of life questionnaires after specific treatment of cough, we noted a significant improvement in the scores, implying that the questionnaires were sensitive enough to record a response. However, this improvement in quality of life scores occurred in all groups of cough and the effect of participating in a trial with intensive treatment attempts to improve the cough account for some of this improved sense of well being. Ideally, a quality of life questionnaire should be

applied to groups of people treated with medication versus placebo in a double-blinded fashion to truly judge the effect of the medication itself. In this study, by the nature of treatment trials for a variety of causes of cough that would not have been an option.

e. Comparison of lower airway inflammation and upper airway hyperreactivity

Lower airway inflammation could lead to hyperresponsiveness all along the airway and cough. When we compared inflammatory markers of the lower airway in a group of subjects with chronic cough and extrathoracic airway hyperreactivity, we found no correlation other than elevated LTB_4 levels. It is possible that upper airway inflammation may occur independently and future studies of upper airway sensitivity should incorporate nasal fluid inflammatory cell count and mediator levels.

f. Use of non-invasive tests to identify corticosteroid responsiveness:

We identified exhaled nitric oxide and induced sputum eosinophils as the most useful non-invasive tests to identify corticosteroid responders in chronic cough. The practical utility of this is currently limited as these are tests that can only be performed at specialised centres. Portable exhaled nitric oxide machines are now available, but are expensive. A study from Finland (289) looking into the possibility of performing induced sputum in primary health care centres in cough subjects found a high success rate, but as smears of samples were made in the clinic itself, this would be too time consuming out-with a research study.

g. Final diagnosis of cough

Most of our subjects had received some form of treatment in the past from their general practitioners, but it was probably inadequate in dose or duration and no systematic approach to treating all potential causes of cough had been made. This study confirms the finding of other studies in chronic cough subjects, that cough can be significantly improved in the majority of subjects with persistence and accurate utilisation of medication.

5.3: UNANSWERED QUESTIONS AND IMPLICATIONS FOR FUTURE RESEARCH

"Every important scientific advance that has come in looking like an answer has turned, sooner or later, into a question – and the game is just beginning..."

Lewis Thomas
In: Late Night Thoughts on Listening to Mahler's Ninth Symphony

Use of specific treatment trials to make a final diagnosis in cough

The results of our study show that inhaled corticosteroids can be beneficial in a mixed group of subjects with chronic persistent cough. Although the clinical effect was not large, the fact that there was some response in subjects with causes other than asthma and eosinophilic bronchitis suggests that there could be some benefit with an agent powerful enough to suppress inflammation in all subjects with cough. It also highlights the need for large randomised, double-blind, placebo controlled studies to study the effectiveness and specificity of all medication used in cough.

Neurogenic inflammation in cough

Studies to find a common pathway in all causes of cough would be very crucial in improving understanding of the pathophysiology as well as finding appropriate new medications. Interesting work on a possible cough receptor has begun (182) and the role of neurotrophins as possible signalling molecules remains an unanswered question.

Quality of life in chronic cough

We have shown that quality of life in chronic cough can improve with treatment. Further studies utilising double-blind, placebo controlled treatment would answer the question of whether people improve because of the care given to them during intensive treatment trials in a research project or whether the actual change in their cough is the major factor in improving their quality of life. Comparisons with other medical conditions in large meta-analyses will allow the distress faced by cough sufferers to be viewed in comparison with other medical conditions and possibly serve to focus medical attention and research towards this difficult problem.

Inflammation in chronic cough and potential new medications:

Novel therapies can be developed through better understanding of the disease process. Several potential targets for antitussive drugs have been identified recently (6). In this study, we found airway inflammation is an important feature in all causes of cough. This can open up further new avenues for potential suppressants of chronic cough. As the highest levels of mediators in all causes of cough were noted for LTB_4, PGE_2 and cys-LT, these would be the first targets to suppress. It would still be important to pursue efforts to find the cause of the cough and use specific therapy, but suppressing inflammation may provide some respite for patients during this process.

Leukotriene inhibition:

Anti-leukotrienes include leukotriene receptor antagonists and 5-lipoxygenase inhibitors. The currently available receptor anatagonists (montelukast, zafirlukast and pranlukast) are widely used in asthma and are effective in reducing cys-LT levels. Montelukast reduced cough in infants with reactive airway disease following respiratory syncytial virus (RSV) bronchiolitis (126). In patients with cough due to chronic bronchitis, pranlukast, had no significant effect (125). Recently, a randomised controlled trial in 8 patients with CVA demonstrated that zafirlukast 20mg bid for 14 days, reduced cough scores and capsaicin-induced cough compared to placebo (290). Their role in chronic cough in general requires evaluation.

5-lipoxygenase (5-LOX) inhibitors like zileuton may be more effective as they block the effect of LTB_4 (291), which we found at higher levels in all causes of cough compared to cys-LT. Other LTB_4 receptor antagonists being studied for COPD, such as LY293111, may be useful in cough as they reduce neutrophil influx (292).

PGE₂ inhibition:

In asthmatics, indomethacin increased the cough threshold compared to placebo (293) but celecoxib had no effect on cough challenge testing (294).

A combined cycloxygenase and lipoxygenase inhibitor, BW755C, was studied in dogs several years ago, and prevented the development of airway resistance following ozone exposure (295). Liclofelone, a new inhibitor of 5-LOX and COX pathways (296) may have a potential benefit in chronic cough

Neutrophil suppression:

The efficacy of selective phosphodiesterase 4 inhibitors such as cilomilast and roflumilast in the treatment of COPD (297) and their inhibitory effect on neutrophil function suggests that these compounds might be effective in chronic cough. In addition, cilomilast has been shown to reduce levels of TNF-α and IL-8 in patients with COPD (298).

Other mediator suppression:

- *IL-8* production can be downregulated by macrolide antibiotics (299).
- *TNF-α* can be inhibited by etanercept [a TNF-α receptor fusion protein](300)and infliximab [a monoclonal anti-TNF-α antibody] (301). Their role in cough has not been evaluated.

Since the inflammation in cough is complex, with involvement of several different mediators, an ideal anti-inflammatory would be one with the potential to suppress several different pathways simultaneously.

CHAPTER 6

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CHAPTER 7

APPENDICES

ASTHMA RESEARCH

PEAK FLOW CARD

Name: _____

Study number: _____

CONTACT ADDRESS

Dr. Rekha Chaudhuri or Lorna Thomson
Asthma Research Unit
Level 6, Room 18
Gartnavel General Hospital
1053 Great Western Road
Glasgow G12 0YN.

✆ 0141 211 3498
✆ 0141 211 3484 (answer phone)

If yes, the dose is ——— puffs in the morning and ——— puffs at night.
Please enter the number of puffs you take each day.

EXAMPLE

ate		
0		10
	No cough	Maximal cough

0 _____ 10

Inhaler dose ——— puffs (am) ——— puffs (pm)

0 _____ 10

Inhaler dose ——— puffs (am) ——— puffs (pm)

0 _____ 10

Inhaler dose ——— puffs (am) ——— puffs (pm)

0 _____ 10

Inhaler dose ——— puffs (am) ——— puffs (pm)

0 _____ 10

Inhaler dose ——— puffs (am) ——— puffs (pm)

0 _____ 10

Inhaler dose ——— puffs (am) ——— puffs (pm)

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0 _____ 10

Inhaler dose ——— puffs (am) ——— puffs (pm)

0 _____ 10

Inhaler dose ——— puffs (am) ——— puffs (pm)

Date

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0 _____ 1(

Inhaler dose ——— puffs (am) ——— puffs (pm)

St George's Hospital Respiratory Questionnaire

...uestionnaire is designed to help us learn much more about how your breathing is troubling you and how it affects ...fe. We are using it to find out which aspects of your illness cause you most problem, rather than what the doctors ...urses think your problems are.

...e read the instructions carefully and ask if you do not understand anything. Do not spend too long deciding about ...answers.

...STIONS ABOUT HOW MUCH CHEST TROUBLE YOU HAVE HAD OVER THE LAST YEAR.
...SE TICK IN ONE BOX FOR EACH QUESTION.

	Most days a week	Several days a week	A few days a month	Only with chest infections	Not at all
...er the last year, I have coughed:	☐	☐	☐	☐	☐ 08
...er the last year, I have brought ...phlegm (sputum):	☐	☐	☐	☐	☐ 09
...er the last year, I have had ...ortness of breath:	☐	☐	☐	☐	☐ 10
...er the last year, I have had ...tacks of wheezing:	☐	☐	☐	☐	☐ 11

...uring the last year, how many severe or very unpleasant
...tacks of chest trouble have you had:

more than 3 attacks	☐
3 attacks	☐
2 attacks	☐
1 attack	☐
no attacks	☐ 12

...ow long did the worst attack of chest trouble last:
...o to Question 7 if you had no severe attacks)

a week or more	☐
3 or more days	☐
1 or 2 days	☐
less than a day	☐ 13

...er the last year, in an average week, how many good
...ys (with little chest trouble) have you had:

no good days	☐
1 or 2 good days	☐
3 or 4 good days	☐
nearly every day is good	☐
every day is good	☐ 14

...you have a wheeze, is it worse in the morning:

No	☐
Yes	☐ 15

The St George's Hospital Respiratory Questionnaire

Random No	Initials	Date of Visit
☐☐☐	☐☐☐☐	☐☐ / ☐☐ / ☐☐
01	02	D D M M Y Y⊙

SECTION 1
HOW WOULD YOU DESCRIBE YOUR CHEST CONDITION?
(PLEASE TICK IN ONE BOX ONLY)

The most important problem I have

Causes me quite a lot of problems

Causes me a few problems

Causes no problem

IF YOU HAVE EVER HAD PAID EMPLOYMENT, PLEASE TICK ONE OF THESE:

My chest trouble made me stop work

My chest trouble interferes with my work or made me change my work

My chest trouble does not affect my work

SECTION 2
QUESTIONS ABOUT WHAT ACTIVITIES USUALLY MAKE YOU FEEL BREATHLESS THESE DAYS.
FOR EACH ITEM, PLEASE TICK EITHER TRUE OR FALSE AS IT APPLIES TO YOU.

	TRUE	FALSE
Sitting or lying still	☐	
Getting washed or dressed	☐	
Walking around the home	☐	
Walking outside on the level	☐	
Walking up a flight of stairs	☐	
Walking hills	☐	
Playing sports or games	☐	

SECTION 3
SOME MORE QUESTIONS ABOUT YOUR COUGH AND BREATHLESSNESS THESE DAYS.
FOR EACH ITEM, PLEASE TICK EITHER TRUE OR FALSE AS IT APPLIES TO YOU.

	TRUE	FALSE
My cough hurts	☐	
My cough makes me tired	☐	
I am breathless when I talk	☐	
I am breathless when I bend over	☐	
My cough or breathing disturbs my sleep	☐	
I get exhausted easily	☐	

St George's Hospital Respiratory Questionnaire

Random No	Initials	Date of Visit
☐☐☐ 01	☐☐☐☐ 02	☐☐/☐☐/☐☐ D D M M Y Y 03

ON 4

TIONS ABOUT OTHER EFFECTS THAT YOUR CHEST TROUBLE MAY HAVE ON YOU THESE DAYS.
ACH ITEM, PLEASE TICK EITHER TRUE OR FALSE AS IT APPLIES TO YOU.

	TRUE	FALSE
My cough or breathing is embarrassing in public	☐	☐ 06
My chest trouble is a nuisance to my family, friends or neighbours	☐	☐ 07
I get afraid or panic when I cannot get my breath	☐	☐ 08
I feel that I am not in control of my chest problem	☐	☐ 09
I do not expect my chest to get any better	☐	☐ 10
I have become frail or an invalid because of my chest	☐	☐ 11
Exercise is not safe for me	☐	☐ 12
Everything seems too much of an effort	☐	☐ 13

ON 5

TIONS ABOUT YOUR MEDICATION. IF YOU ARE RECEIVING NO MEDICATION GO STRAIGHT TO
TION 6.
MPLETE THIS SECTION PLEASE TICK EITHER TRUE OR FALSE AS IT APPLIES TO YOU.

	TRUE	FALSE
My medication does not help me very much	☐	☐ 14
I get embarrassed using my medication in public	☐	☐ 15
I have unpleasant side effects from my medication	☐	☐ 16
My medication interferes with my life a lot	☐	☐ 17

ON 6

E ARE QUESTIONS ABOUT HOW YOUR ACTIVITIES MIGHT BE AFFECTED BY YOUR BREATHING.
EACH QUESTION, PLEASE TICK TRUE IF ONE OR MORE PARTS APPLIES TO YOU BECAUSE OF YOUR
THING. OTHERWISE TICK FALSE

	TRUE	FALSE
I take a long time to get washed or dressed	☐	☐ 18
I cannot take a bath or shower, or I take a long time	☐	☐ 19
I walk slower than other people, or I stop for rests	☐	☐ 20
Jobs such as housework take a long time, or I have to stop for rests	☐	☐ 21
If I walk up one flight of stairs, I have to go slowly or stop	☐	☐ 22
If I hurry or walk fast, I have to stop or slow down	☐	☐ 23
My breathing makes it difficult to do things such as walk up hills, carrying things up stairs, light gardening such as weeding, dance, play bowls or play golf	☐	☐ 24
My breathing makes it difficult to do things such as carry heavy loads, dig the garden or shovel snow, jog or walk at 5 miles per hour, play tennis or swim	☐	☐ 25
My breathing makes it difficult to do things such as very heavy manual work, run, cycle, swim fast or play competitive sports	☐	☐ 26

The St George's Hospital Respiratory Questionnaire

Draft 03

Random No	Initials	Date of Visit
☐☐☐ 01	☐☐☐☐ 02	☐☐/☐☐/☐☐ D D / M M / Y Y 03

SECTION 7

WE WOULD LIKE TO KNOW HOW YOUR CHEST TROUBLE <u>USUALLY</u> AFFECTS YOUR DAILY LIFE.
PLEASE TICK EITHER TRUE OR FALSE AS IT APPLIES TO YOU <u>BECAUSE OF YOUR CHEST TROUBLE</u>
(REMEMBER THAT TRUE ONLY APPLIES TO YOU IF YOU CAN NOT DO SOMETHING BECAUSE OF YOU
BREATHING).

TRUE F

I cannot play sports or games ☐

I cannot go out for entertainment or recreation ☐

I cannot go out of the house to do the shopping ☐

I cannot do housework ☐

I cannot move far from my bed or chair ☐

HERE IS A LIST OF OTHER ACTIVITIES THAT YOUR CHEST TROUBLE MAY PREVENT YOU DOING. (YOU
HAVE TO TICK THESE, THEY ARE JUST TO REMIND YOU OF WAYS IN WHICH YOUR BREATHLESSNESS
AFFECT YOU):

GOING FOR WALKS OR WALKING THE DOG

DOING THINGS AT HOME OR IN THE GARDEN

SEXUAL INTERCOURSE

GOING OUT TO CHURCH, OR PLACE OF ENTERTAINMENT

GOING OUT IN BAD WEATHER OR INTO SMOKY ROOMS

VISITING FAMILY OR FRIENDS OR PLAYING WITH CHILDREN

PLEASE WRITE IN ANY OTHER IMPORTANT ACTIVITIES THAT YOUR CHEST TROUBLE MAY STOP YOU

_____

_____

_____

NOW, WOULD YOU TICK IN THE BOX (ONE ONLY) WHICH YOU THINK BEST DESCRIBES HOW YOUR CH
AFFECTS YOU:

It does not stop me doing anything I would like to do

It stops me doing one or two things I would like to do

It stops me doing most of the things I would like to do

It stops me doing everything I would like to do

THANK YOU FOR FILLING IN THIS QUESTIONNAIRE. BEFORE YOU FINISH WOULD YOU CHECK TO SEE
YOU HAVE ANSWERED ALL THE QUESTIONS.

Random No	Initials	Date of Visit
☐☐☐ 01	☐☐☐☐ 02	☐☐/☐☐/☐☐ D D M M Y Y 03

ors are aware that emotions play an important part in most illnesses. If your doctor knows about these feelings he w
ble to help you more.

questionnaire is designed to help your doctor to know how you feel. Read each item and place a firm tick in the box
osite the reply which comes closest to how you have been feeling in the past week.

t take too long over your replies: your immediate reaction to each item will probably be more accurate than a long
ght-out response.

Tick only one box in each section.

I tense or 'wound up':
- Most of the time ☐
- A lot of the time ☐
- Time to time, occasionally ☐
- Not at all ☐ 04

I enjoy the things I used to enjoy:
- Definitely as much ☐
- Not quite so much ☐
- Only a little ☐
- Hardly at all ☐ 05

t a sort of frightened feeling as if something ul is about to happen:
- Very definitely and quite badly ☐
- Yes, but not too badly ☐
- A little, but it doesn't worry me ☐
- Not at all ☐ 06

n laugh and see the funny side of things:
- As much as I always could ☐
- Not quite so much now ☐
- Definitely not so much now ☐
- Not at all ☐ 07

rrying thoughts go through my mind:
- A great deal of the time ☐
- A lot of the time ☐
- From time to time but not too often ☐
- Only occasionally ☐ 08

l cheerful:
- Not at all ☐
- Not often ☐
- Sometimes ☐
- Most of the time ☐ 09

n sit at ease and feel relaxed:
- Definitely ☐
- Usually ☐
- Not often ☐
- Not at all ☐

I feel as if I am slowed down:
- Nearly all the time ☐
- Very often ☐
- Sometimes ☐
- Not at all ☐ 11

I get a sort of frightened feeling like 'butterflies' in the stomach:
- Not at all ☐
- Occasionally ☐
- Quite often ☐
- Very often ☐ 12

I have lost interest in my appearance:
- Definitely ☐
- I don't take so much care as I should ☐
- I may not take quite as much care ☐
- I take just as much care as ever ☐ 13

I feel restless as if I have to be on the move:
- Very much indeed ☐
- Quite a lot ☐
- Not very much ☐
- Not at all ☐ 14

I look forward with enjoyment to things:
- As much as I ever did ☐
- Rather less than I used to ☐
- Definitely less than I used to ☐
- Hardly at all ☐ 15

I get sudden feelings of panic:
- Very often indeed ☐
- Quite often ☐
- Not very often ☐
- Not at all ☐ 16

I can enjoy a good book or radio or TV programme:
- Often ☐
- Sometimes ☐
- Not often ☐
- Very seldom ☐

Appendix 5: Quality of life in asthma and COPD using the SGRQ and HADS.

a. Asthma studies using SGRQ

No	Reference	No of subjects	Total SGRQ Mean (SD)	Symptom score Mean (SD)	Activity Mean (SD)	Impacts score Mean (SD)
1	Sanjuas (1)	116	39.5 (18.7)	46.2 (20.9	49.2 (24.7)	31.7 (19.9)
2	Bumbacea (2)	29	51.7 (SEM 3.39)	75.65 (SEM 3.22)	60.63 (SEM 5.31)	40.07 (SEM 3.28)
3		37 (severe)	59.57 (SEM 3.2)	79.03 (SEM 2.26)	67.66 (SEM 4.3)	49.37 (SEM 3.55)
4a	Vollmer (3)	347 (controlled)	30	42	41	19
4b		175 (mild)	40	51	53	29
4c		109 (less well controlled)	46	59	57	36
5	Osman (4) Scottish study	396 stable asthmatics	21.5 (12.9)	40.3 (20.9)	15 (11.3)	22.7 (17.2)
6	Jones (5)	368	39	55.2	34.1	39
7	Nishiyama (6)	68	28.6 (18.4)	43.7 (23.4)	32.1 (25.4)	21.8 (17.4)
8	Incalzi (7)	198	36.1 (20.53)	40.4 (20.79)	50.3 (25.11)	14.1
9a	Meszaros (8)	64 education given	35.97	39.44	41.82	31.87
9b		55 control	39.88	49.4	38.87	37.48

b. COPD studies using SGRQ

No	Reference	No of subjects	Total SGRQ Mean (SD)	Symptom Mean (SD)	Activity Mean (SD)	Impacts Mean (SD)
1	Doll H (Quality of Life Research, 2003	230	41.6 (20.4)	61.8 (19.4)	48.8 (27.4)	30.7 (20.9)
2	Vincken W, Eur Resp J 2002	356	40.9 (SEM 0.69)	47.32 (SEM1.07)	55.07 (SEM0.84)	30.95 (SEM0.81)
3	Spencer S etal (9)	>350	48 (18)	66 (21)	61 (22)	35 (19)
4	Miravitles M etal. (10)	336	47.6 (17.2	48.3 (20.2)	62.8 (20.9)	38.7 (17.8)
5	Okubadejo et al. (11)	41 Severe	55.3 (18.2)	64 (21.9)	65.2 (19.8)	46.5 (23.5)
6	Tinkelman D etal (12)	349	53.4 (19.1)	62.2 (17.4)	70.1 (24.4)	40.8 (21.3)
7	Hajiro T etal (13)	161 stable		52.5 (19.9)	47.5 (21.9)	25.7 (16.9)
8	Alemayehu etal (14)	181 stable	58.3 (21.5)	63.6 (28.2)	42.9 (26.6)	50.7 (23.1)
10	Incalzi (7)	230	43.8 (20.4)	53.78 (23.38)	57.2 (24.13)	17.6 (11.52)

c. HAD scale in other studies

No	Reference	No of subjects	Condition	Anxiety mean (SD)	Depression mean (SD)
1	Jones PW (5)	360	Asthma stable	6.7 (3.9)	4.6 (3.3)
2	Rimington et al (15)	40 suburban 74 inner city	Asthma stable	6.2 (3.9) 9.5 (4.1)	3.7 (2.7) 6 (4.3)
3	Okubadejo AA etal. (11)	41	Severe COPD with hypoxemia	5.7 (4.3)	5.3 (3.4)
4	Hajiro T etal (13)	161	stable COPD	3.9 (3.3)	5.0 (3.7)
5	McGarvey LPA (16)	33	Cough > 3 weeks	6.5 (3.75)	3.84 (3.2)

Random No

Initials

| | | | | | | | |

01   02

JGH HISTORY

ation of cough [ ][ ] years [ ][ ] months [ ][ ] weeks

03   04   05

tum production [ ][ ] ml/day

06

vhich <u>position</u> is the cough worst?

Upright [1]

Stooping [2]

Supine [3]

07

what <u>time</u> is the cough worst?

Morning [1]

Afternoon/Evening [2]

Night [3]

08

ich of the following are
ger factors?

a) Dust   Yes [1] No [2]
09

b) Pollen   Yes [1] No [2]
10

c) Cat dander   Yes [1] No [2]
11

d) Dog dander   Yes [1] No [2]
12

e) Exercise   Yes [1] No [2]
13

f) Cold air   Yes [1] No [2]
14

g) Aerosols/sprays   Yes [1] No [2]
15

h) Other   Yes [1] No [2]
16

If Yes, specify _____

_____
17

the cough described as a
kle/itch in the throat?   Yes [1] No [2]
18

the cough originate after an
per respiratory tract infection?   Yes [1] No [2]
19

es the cough occur during
ep?   Yes [1] No [2]
20

sociated <u>wheeze</u>?   Yes [1] No [2]
21

sociated exertional
athlessness?   Yes [1] No [2]
22

C Grade   [1]   [2]   [3]

B. ATOPY/ALLERGIES

1. **Does the patient have**

   (a) **Any known allergies**   Yes [1] No [2]
   24

   If Yes, specify _____

   _____
   25

   (b) **Hay fever**   Yes [1] No [2]
   26

   (c) **Eczema**   Yes [1] No [2]
   27

   (d) **Family history of atopy**   Yes [1] No [2]
   28

C. PATIENT'S VIEW ON THE CAUSE OF COUGH

1. **" I think my cough is caused by ...."**

   _____

   _____
   29

2. **Physician's translation of the patient term above:**

   (a) Rhinosinusitis [1]

   (b) Allergic rhinitis [2]

   (c) Gastro-oesophageal reflux disease [3]

   (d) Cough variant asthma [4]

   (e) Eosinophilic bronchitis [5]

   (f) Atopic cough [6]

   (g) Post-infective cough [7]

   (h) Chronic bronchitis [8]

   (i) Occupational cough [9]

   (j) Habitual cough [10]

   (k) Other cause [11]

   If Yes, specify _____

   _____
   30

   (l) Unknown [12]
   31

Visual Analogue Scale

Version 1.0

A. COUGH SEVERITY: VISUAL ANALOGUE SCALE

1. Ask the patient to complete the Visual Analogue Scale below.
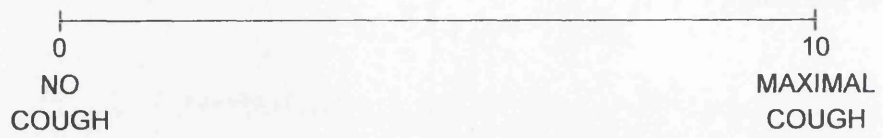
```
   ├─────────────────────────────────────────┤
   0                                          10
   NO                                      MAXIMAL
  COUGH                                     COUGH
```

2. Result: ☐☐.☐ cm
03

Random No

□□□
  01

Initials

□□□□
   02

PER RESPIRATORY TRACT

st Nasal Drip

**sation of something dripping
wn the throat** Yes [1] No [2]
03

ed to frequently clear the throat Yes [1] No [2]
04

sal discharge:

None [1]

Mucoid [2]

Mucopurulent [3]
05

ronic Rhinosinusitis

Major Factors

(i) Facial pain/pressure Yes [1] No [2]
06

(ii) Facial congestion/fullness Yes [1] No [2]
07

(iii) Nasal obstruction/blockage Yes [1] No [2]
08

(iv) Nasal discharge/purulence Yes [1] No [2]
(by history or examination) 09

(v) Hyposmia/Anosmia Yes [1] No [2]
10

Minor Factors

(i) Headaches Yes [1] No [2]
11

(ii) Dental pain Yes [1] No [2]
12

(iii) Ear pain/pressure/fullness Yes [1] No [2]
13

(iv) Fever Yes [1] No [2]
14

(v) Cough Yes [1] No [2]
15

(vi) Halitosis Yes [1] No [2]
16

(vii) Fatigue Yes [1] No [2]
17

lergic Rhinitis

(i) Frequent sneezing Yes [1] No [2]
18

(ii) Itchy nose Yes [1] No [2]
19

(iii) Itchy eyes Yes [1] No [2]
20

(iv) Itchy throat Yes [1] No [2]
21

(v) Profuse watery discharge Yes [1] No [2]
22

B. GASTRO-OESOPHAGEAL REFLUX

1. **Heartburn** Yes [1] No [2]
23

2. **Acid regurgitation** Yes [1] No [2]
24

3. **Waterbrash** Yes [1] No [2]
25

4. **Dysphagia** Yes [1] No [2]
26

5. **Odynophagia** Yes [1] No [2]
27

C. OTHER RELEVANT HISTORY

1. **Any other relevant history?** Yes [1] No [2]
28

   If Yes, specify _____

   _____

   _____

   _____

   _____
   29

...t Interview: Severity of Associated Symptoms

...sion 1.0

Random No						Initials				
				01						02

...PTOM SEVERITY: VISUAL ANALOGUE SCALE

...the patient to complete the Visual Analogue Scales below to indicate the severity of associated symptoms.

...t Nasal Drip?   Yes ☐1 No ☐2
                                                              03

```
 |------------------------+------------------------|
 0                        5                       10
 NO                    MODERATE                MAXIMAL
 SYMPTOMS              SYMPTOMS                SYMPTOMS
```
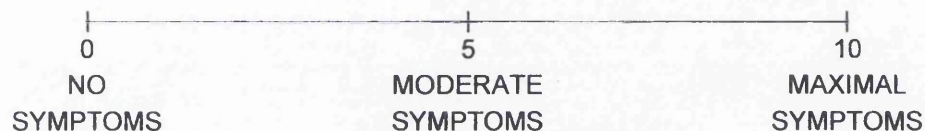
...Result: ☐☐.☐ cm
          04

...tro-Oesophageal Reflux Disease?   Yes ☐1 No ☐2
                                                       05

```
 |------------------------+------------------------|
 0                        5                       10
 NO                    MODERATE                MAXIMAL
 SYMPTOMS              SYMPTOMS                SYMPTOMS
```
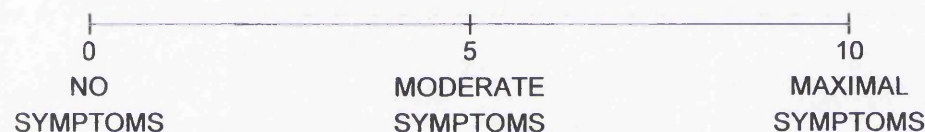
...Result: ☐☐.☐ cm
          06

...gh Variant Asthma?   Yes ☐1 No ☐2
                                                       07

```
 |------------------------+------------------------|
 0                        5                       10
 NO                    MODERATE                MAXIMAL
 SYMPTOMS              SYMPTOMS                SYMPTOMS
```

...Result: ☐☐.☐ cm
          08

...er?   Yes ☐1 No ☐2
                                                       09

...f Yes, specify_____
                                                              10

```
 |------------------------+------------------------|
 0                        5                       10
 NO                    MODERATE                MAXIMAL
 SYMPTOMS              SYMPTOMS                SYMPTOMS
```

...Result: ☐☐.☐ cm
          11

Patient Interview: Medication Record

Version 1.0

Random No		Initials
☐☐☐ 01		☐☐☐☐ 02

A. MEDICATION FOR CURRENT COUGH ILLNESS

		Date Started	Date stopped	Total Daily Dose	Effect on cough (Improved / Same / Worsened)

1. Inhaled Steroids   Yes ☐1 No ☐2 03

   specify _____ 04

   M M Y Y05   M M Y Y06   ☐☐☐☐ µg 07   1 2 3 08

2. Inhaled β₂ Agonist   Yes ☐1 No ☐2 09

   M M Y Y10   M M Y Y11   ☐☐☐☐ µg 12   1 2 3 13

3. Other inhaler   Yes ☐1 No ☐2 14

   specify _____ 15

   M M Y Y16   M M Y Y17   ☐☐☐☐ µg 18   1 2 3 19

4. H₂ Antagonist   Yes ☐1 No ☐2 20

   specify _____ 21

   M M Y Y22   M M Y Y23   ☐☐☐ mg 24   1 2 3 25

5. Proton pump inhibitor   Yes ☐1 No ☐2 26

   specify _____ 27

   M M Y Y28   M M Y Y29   ☐☐☐ mg 30   1 2 3 31

6. Rhinitis treatment   Yes ☐1 No ☐2 32
   If Yes,
   a) Nasal Steroids   Yes ☐1 No ☐2 33

   specify _____ 34

   M M Y Y35   M M Y Y36   ☐☐☐ µg 37   1 2 3 38

   b) Antihistamine   Yes ☐1 No ☐2 39

   specify _____ 40

   M M Y Y41   M M Y Y42   ☐☐☐ mg 43   1 2 3 44

   c) Decongestant (units)   Yes ☐1 No ☐2 45
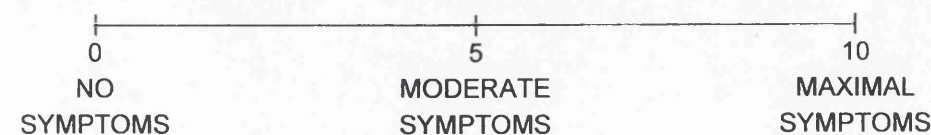
   specify _____ ( ) 46 47

   M M Y Y48   M M Y Y49   ☐☐☐ 50   1 2 3 51

   d) Other (units)   Yes ☐1 No ☐2 52

   specify _____ ( ) 53 54

   55   56   57   1 2 3 58

7. Non-specific cough suppressant (units)   Yes ☐1 No ☐2 59
   If Yes,

   a) _____ ( ) 60 61

   M M Y Y62   M M Y Y63   ☐☐☐ 64   1 2 3 65

   b) _____ ( ) 66 67

   M M Y Y68   M M Y Y69   ☐☐☐ 70   1 2 3 71

   c) _____ ( ) 72 73

   M M Y Y74   M M Y Y75   ☐☐☐ 76   1 2 3 77