COUGH IN HEALTH AND DISEASE

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Abstract

Cough is a common symptom of both acute and chronic respiratory illness. It is poorly understood and treatment options are sparse. One reason is that there are few validated objective measures of cough frequency and severity.

In this thesis I have further validated the Leicester Cough Monitor, an automated cough detection system capable of detecting coughs over 24 hours. I have demonstrated that automated cough numbers were similar to those derived from the gold standard of manual counting in healthy adults and in patients with respiratory disease.

Cough frequency was then measured using the Leicester Cough Monitor and was almost 16 fold higher in patients with respiratory diseases compared to healthy controls. In the population as a whole there was a correlation between cough frequency and the induced sputum neutrophil count.

I then conducted a randomised, placebo controlled, double blind, parallel group trial of low dose erythromycin taken daily for 3 months in 30 patients with unexplained chronic cough. Active treatment was associated with a reduction in the sputum neutrophil count but no difference in cough counts or other measures of cough severity.

Also, there is no information on the natural history of unexplained chronic cough. In a longitudinal study of 42 patients with unexplained chronic cough followed up for at least 7 years, I found that cough, measure by the cough visual analogue score, improved in 25 percent. Unexpectedly, patients had an abnormally rapid fall in FEV1, whether cough improved or not.

Finally, the assessment of health related quality of life is important in people with acute cough in order to evaluate potential therapies. I have shown that that the minimal important clinical difference in the Leicester Cough Questionnaire for those with acute cough is 2.5 and this will aid in the interpretation of treatment trials.

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Publications arising from this thesis

Papers

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Yousaf N, Lee KK, Javaraman B, Pavord ID, Birring SS. The assessment of quality of life in acute cough using the Leicester Cough Questionnaire (LCQ-acute). Cough 2011;18;7(1):4.

Yousaf N, Monterio W, Birring SS, Pavord ID. The long term outcome of patients with unexplained chronic cough. Respiratory Medicine *in press*

Letters

Yousaf N, Montiero W, Matos S, Birring SS, Pavord ID. Cough frequency in health and disease. European Respiratory Journal *in press*

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Chapters

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Yousaf N, Lee KK, Javaraman B, Pavord ID, Birring SS. Acute cough: Measurement of quality of life with the Leicester Cough Questionnaire (LCQ). Thorax 2009; 64 (Suppl 4)

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Yousaf N, Birring SS, Pavord ID. The long-term outcome of patients with unexplained chronic cough. Thorax 2009; 64 (Suppl 4).

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Table of Contents

List of Tables

List of Figures

1. INTRODUCTION

1.1. DEFINING COUGH

Cough is an important defence mechanism which clears the airways of mucus and debris. In order to measure cough one must first state what is being counted. A widely accepted definition favoured by physiologists is that cough is a three phase motor act characterised by:

- an inspiratory effort (inspiratory phase)
- followed by a forced expiration against a closed glottis (compressive phase)
- the glottis then opens accompanied by rapid expiratory flow and a characteristic sound (expulsive phase)[1].

However a cough is not always a singular event. More often patients describe a 'cough attack', also known as a cough epoch. These cough epochs can consist of one single preliminary inspiratory effort followed by a number of characteristic cough sounds. Quantifying is a pragmatic discipline and using the above definition makes counting numbers challenging and error prone. Another definition, intuitively more suited to counting coughs, is that cough is a forced expulsive manoeuvre, usually against a close glottis and which is associated with a characteristic sound[2]. This is the definition of cough used throughout the rest of this work.

1.2. THE COUGH PATHWAY

Cough can be caused by a number of mechanical and chemical stimuli. It can be elicited from the larynx, trachea, large bronchi and possibly also from the smaller airways, oesophagus and auditory canal. The afferent pathway of the cough reflex has been extensively researched, mostly in guinea pigs but also in rodents, dogs and cats. The conclusions are therefore somewhat confusing and occasionally contradictory and the relevance of the findings to humans are sometimes uncertain. Nonetheless it seems that fibres of the afferent vagus nerve are the sole sensory component of the afferent cough pathway[3].

There are at least 4 known vagal fibres which innervate the airways. These can be classified according to their physiological properties (see TABLE 1). The cell bodies of these afferent nerve fibres reside in the nodose and jugular ganglion of the vagal nerve and then feed into the 'cough centre' in the nucleus of the solitary tract in the medulla oblongata. The efferent arm of the cough reflex involves vagal and phrenic innervation of muscles involved in cough.

Rapidly adapting airway stretch receptors (RAR) are myelinated fibres which terminate in the intrapulmonary airways[4,5]. They are called rapidly adapting because of the rapid decrease in action potentials during sustained lung inflation. They are sensitive to mechanical stimuli such as lung deflation, bronchoconstriction and mechanical distortions caused by airway oedema[4-6] and are relatively insensitive to direct chemical stimuli[7]. Yet they can be activated indirectly by neurogenic inflammation which results in airway oedema, mimicking mechanical bronchoconstriction[4]. Cooling the vagus nerve to a level that blocks myelinated fibres, including RAR activity, blocks mechanical and

acid induced cough in cats[8]. Interestingly however, substances that activate RAR through bronchoconstriction such as thromboxane, histamine and methocholine are relatively ineffective at producing cough[9-11]. This lends to the argument that there are other fibres involved in the cough reflex.

Slowly adapting stretch receptors (SARS) are extra-pulmonary and intrapulmonary myelinated fibres[12-14] that are sensitive to mechanical stimuli and relatively insensitive to direct chemical stimuli[15,16]. They are differentiated from RARs as these receptors are not sensitive to lung deflation and do not show a rapid decline in action potentials during sustained lung inflation. They appear to be involved in regulating the pattern of breathing and may have a role in the Hering- Breuer reflex, but do not seem to have a direct role in cough[9,12].

Pulmonary and bronchial C fibres are non myelinated fibres that terminate in the mucosa and submucosa of upper and lower airways and have also been identified in the lower oesophagus. They have a much higher threshold for mechanical stimulation than RARs. However they are very sensitive to chemical stimuli and are activated by substances such as capsaicin, bradykinin and acid, which are potent protussives in conscious humans and animals[15,17,18], but do not elicit cough in the anesthetised animal or human[19,20]. Also prostaglandin E2, adrenaline and adenosine inhibit RAR by causing bronchodilation but sensitise C fibres to capsaicin[19]. However, C fibres alone cannot be responsible for cough as it has been shown that capsaicin desensitisation, the phenomenon by which a capsaicin challenge results in a loss of response of the neurone to other stimuli[21], abolishes cough due to citric acid without having an effect on mechanical cough[19].

TABLE 1 CHARACTERISTICS OF COUGH FIBRES

	RARS	SAR	C fibres	'cough receptor'
Location	Intrapulmonary & extrapulmonary airways	Intrapulmonary & extrapulmonary airways	Intrapulmonary & extrapulmonary airways	Extrapulmonary
Myelinated	Yes	Yes	No	Yes
Sensitive to mechanical changes	Yes	Yes	Relatively no	Yes
Synthesis of neuropeptides	No	No	Yes	No
Adaptation to inflation/deflation	Activated	Only activated during lung inflation	No	No
Chemosensitive	No – only indirectly through neurogenic inflammation mimicking bronchoconstriction	No	Capsaicin, bradykinin, prostaglandin E2, adrenaline. Adenosine leads to sensitisation to capsaicin	Acid and water

RARS = Rapidly adapting airways receptor, SARS = Slowly adapting airways receptor

It has been suggested that perhaps C fibres augment the action of RARs in that they produce tachykinins such as Substance P (SP), neurokinin A and Calcitonin Gene Related Peptide (CGRP) that cause local neurogenic inflammation. Or perhaps C fibres have a role in 'urge-to'cough' [22]or non-reflex cough.

One other myelinated, rapidly adapting fibre involved in the cough reflex has been identified in the guinea pig. This has rather confusingly been named the 'cough receptor'. They are thought to be located primarily in the larynx, trachea and bronchus and appear to be activated by punctate mechanical stimuli, acid and water[23], all of which provoke cough in both conscious and anesthetised humans and animals[23-26]. They are distinct from RARs, SARs and C fibres as they do not responsive to changes in luminal pressure, capsaicin or bradykinin, nor do they synthesis neuropeptides [27-29].

Reflex cough can occur in the unconscious individual as a result of mechanical stimulation or acid and may be mediated by the putative 'cough receptor'. As a primary defence mechanism cough elicited in this way will protect the unconscious animal from aspiration. However, patients who present to clinic with cough describe an irritant cough while they are awake. This non-reflex cough is probably mediated by vagal chemosensitive fibres, although it is likely that RARs and chemosensitive C fibres have a complimentary role in eliciting non-reflex cough.

1.3. AFFERENT NERVE RECEPTORS

At least 2 Transient Receptor Potential (TRP) receptors may have an important role in the cough pathway: TRP vanilliod 1 (TRPV1) and TRP ankyrin 1 (TRPA1).They are expressed in the membrane of C fibres terminals and consist of 6 transmembrane domains that form tetramers allowing cation permeability[30]. Both are voltage gated cation channels. An influx of calcium through these channels into the cell leads to membrane depolarisation and production of an action potential (AP)[31]. The number of APs will determine the strength of the signal transmitted to the 'cough centre'. It is likely that once a certain threshold of APs is exceeded cough will occur.

The role of TRPV1 receptors in cough is well established. TRPV1 can be activated directly by capsaicin, acid or heat or indirectly by inflammatory mediators involved in neurogenic inflammation[32]. They are located within and beneath the epithelium and within the smooth muscle. Neuropeptides are synthesised in the cell bodies of the vagal ganglions and are then stored in the peripheral nerve endings localised alongside TRPV1 receptors[33]. Stimulation of these receptors causes cough by generating action potentials as described above but alongside this stimulation also results in release of tachykinins resulting in neurogenic inflammation, which in turn will further stimulate TRPV1 receptors and will also indirectly stimulate RARs[31]. Capsaicin, a potent agonist of TRPV1, causes cough in a dose dependent fashion in guinea pigs and humans[34-36]. TRPV1 receptors have been implicated in the pathogenesis of chronic cough, as there is an increase in the number of TRPV1 receptors by up to 4.4 fold humans with chronic cough, as well as a moderate significant

correlation between capsaicin cough sensitivity and the number of TRPV1 receptors[37].

TRPA1 receptors are activated directly by chemicals in cigarette smoke, isothiocyanate (a chemical in mustard oil), cinnamaldehyde (a chemical in cinnamon) and some prostaglandins[38,39]. TRPA1 receptors have been identified in the C fibre terminals of mice and guinea pigs and recent studies have shown that TRPA1 is co-expressed in up to thirty-five percent of TRPV1 expressing neurones[38,40]. Inhalation of TRPA1 agonists produce cough in a dose dependent fashion in guinea pigs[41,42] and in humans[42]. It has also been shown that cough produced by TRPA1 agonists can be inhibited by selective TRPA1 antagonists[41] but not by TRPV1 anatgonists, thereby demonstrating that these chemicals induce cough independent of TRPV1. The evidence for the role of TRPA1 receptors in cough is growing but establishing the importance of their role requires further work, particularly in humans.

A number of G protein coupled trans-membrane receptors are involved in sensitising TRPV1 and TRPA1 to tussive chemicals. Activation of prostanoid receptors, by prostaglandin E2 or thromboxane, has been shown to sensitise vagal nerves to capsaicin in rats and in humans[43,44]. Furthermore thromboxane antagonists have been shown to reduce capsaicin cough sensitivity[45,46]. It has been shown that stimulation of the bradykinin 2 (B2) receptor by bradykinin can mediate activation of TRPV1 and that bradykinin induced C fibre activation can be inhibited by TRPV1 antagonists[47]. The exact pathways by which activation of prostanoid and bradykinin receptors leads to enhanced TRPA1 and TRPV1 activity in the airways is not yet clear but it may be related to indirect effects on protein kinase C resulting activation of TRP receptors[48].

1.4. HIGHER CONTROL OF COUGH

Humans can cough voluntarily and this cough is indistinguishable from reflex cough[49,50]. Recently there has been interest in determining the higher centres involved in the control of cough and this has resulted in a number of interesting studies. Hutchings et al demonstrated the voluntary control of cough associated with an upper respiratory tract infection by asking 79 patients to suppress their cough. 34 percent successfully suppressed their cough for the twenty minute duration of the experiment, while only 25 percent were unable to suppress cough for even a few minutes[51]. In a separate study the authors also demonstrated voluntary control of capsaicin induced cough. 25 healthy volunteers underwent 2 capsaicin challenges 5 minutes apart. In the first they were asked to cough if they felt the urge and in the second they were asked to suppress their cough. Interestingly 24 out of 25 people coughed in the first challenge but only 3 out of 25 coughed in the second challenge[22].

Higher cortical control of cough has also been investigated in recent functional magnetic resonance imaging (fMRI) studies. In a study of 21 patients, widespread higher cortical activity was seen in fMRI images during voluntary coughing, capsaicin induced coughing and cough suppression[52]. It could be argued that the higher cortical activity observed on fMRI was perhaps due to the motor action associated with coughing. However a separate study by Mazzone[53] induced, not a cough but, an ‘urge to cough’ using capsaicin in a group of 10 healthy volunteers while collecting fMRI images. Mazzone also found activation of a variety of higher cortical areas. Variable higher cortical modification of the afferent cough pathway may explain the poor correlation between cough frequency and the measurement of capsaicin cough sensitivity,

as capsaicin seems to induce an urge to cough, which is then under varying degrees of higher cortical control.

1.5. COUGHING IN HEALTH

Even healthy humans cough, but just how much is as yet unclear. Loudon in 1967 reported observing 2.5 coughs per minute in a lecture theatre of 100 people[54] which works out to be about 1.5 coughs per person per hour. A few groups have reported cough frequency in small numbers of healthy controls using ambulatory cough monitors. Hsu et al reported between 0 to 16 coughs in 24 hours[55] in 12 healthy controls. Birring reported a mean of 2 coughs per hour, measured in 9 healthy individuals over a period of 6 daytime hours[56]. It is not clear if a 24-hour range for cough in healthy individuals can be extrapolated from this data, as it is not known how cough frequency will vary over a 24-hour time period in healthy individuals. Marsden and colleagues reported a median of 0.4 cough seconds per hour cough frequency in 19 healthy controls [57]. It is unclear how to compare the absolute values of cough seconds per hour with coughs per hour but it has been shown that log transformed values of individuals cough and coughs per second correlate strongly[58].

1.6. EPIDEMIOLOGY OF EXCESSIVE COUGHING

Excessive coughing is a symptom of almost all respiratory disease and is the commonest reason for consulting a primary care physician. Cough is also an important condition seen in secondary care, accounting for approximately 20 percent of outpatient respiratory referrals[2]. In the UK, antitussive medication sales amount to over £100 million per year, again highlighting the burden of this

symptom[59]. In spite of this, there is still no satisfactory treatment for either acute or chronic cough. This is in part due to our poor understanding of the mechanisms of cough and also because until recently, it has not been possible or practical to objectively measure cough in pharmaceutical treatment trials.

The European Taskforce[59] and The American College of Chest Physicians[60] define an acute cough as lasting less than 3 weeks and a chronic cough as lasting greater than 8 weeks.

1.6.1. Acute cough

Acute cough is poorly understood and an estimate of the prevalence is difficult to gauge as it is often innocuous and self-limiting. Despite this, acute cough is the commonest new presentation to primary care and the cost to the UK economy approaches one billion pounds due to loss of productivity and healthcare costs[2]. Acute cough is mainly caused by upper respiratory tract viral infections, although the exact mechanism by which this occurs is not yet known[61]. It has been suggested that upper respiratory tract infections lead to post nasal drip due to excess mucus production and cough may be stimulated in this way[62]. Another suggestion is that viral infections may result in increased amounts of inflammatory mediators that sensitise the afferent cough fibres. There is some evidence for the latter explanation, as capsaicin cough sensitivity is transiently enhanced during upper respiratory tract infection and returns to normal once the infection has resolved[63,64].

Although self-limiting, acute cough is associated with considerable impairment in health related quality of life in men and women[65].

1.6.2. Chronic cough

European and American estimates of the prevalence of chronic cough vary from between 9 to 44 percent of the population. The imprecise estimate is due to heterogeneity in the populations surveyed, the study methodology, the questions asked and the incidence of smoking. Janson et al[66] performed the most comprehensive assessment of the presence of cough in 20 to 48 year olds. This was a large international population survey involving 36 centres in 16 countries across Europe, the United States of America and Australasia. They conducted an interview led questionnaire in 18,277 randomly sampled individuals. The prevalence of current smoking varied by country from 26 to 54 percent. They asked specifically about nocturnal cough and winter cough and the prevalence amongst non-smokers was 14 percent and 27 percent respectively.

There are several postal survey based estimations of the prevalence of chronic cough, with response rates varying from 60 to 91 percent and therefore with the potential of introducing bias into the estimate. Also, different questions have been asked, from have you coughed on most days for more than 3 months of the year[67,68], to asking about a cough on most days for the past half year[69]. These different questions, needless to say, will provoke different answers from the same individual. A summary of the findings from these surveys is shown in TABLE 2. Recollection bias may affect these survey estimates, however based on this self-reported data on the prevalence of cough in the general population, cough is a common symptom and is reported more frequently by current smokers.

TABLE 2. ESTIMATES OF THE PREVALENCE OF CHRONIC COUGH

Number	Population	Age group (years)	Response rate (%)	Non-smokers (%)	Prevalence of cough (%)		
					Non-smokers	X-smokers	Current smokers
18277[66]	Europe, USA, Australasia	20 - 48	N/A	46 – 74	14	13	24-44
1109[67]	USA (Arizona)	Children & adults	N/A	49.5	9	20	33
25969[68]	Northern Italy	20 - 44	72	67	7	13	18 – 35
12073[70]	Sweden	20 - 59	70	50	10	10.9	16.9
10015[69]	South East UK	5 - 54	91	35		14	
6416[71]	North West UK	adults	60	78	11	Unknown	18

1.7. DIAGNOSING THE CAUSE OF A COUGH

Diagnosing the cause of chronic cough requires a thorough and systematic evaluation. Almost all infectious and non-infectious respiratory conditions can present with a cough. The initial evaluation of a patient with chronic cough involves a thorough history, including details of smoking, travel, drugs, pets, family history and a clinical examination. Initial investigations should include a chest radiograph, spirometry and bronchodilatior reversibility if required. A chronic productive cough in a smoker may be a symptom of chronic obstructive airways disease (COPD). A new cough in a smoker associated with an abnormal chest radiograph should be treated with a high index of suspicion for lung cancer. Angiotensin converting enzyme (ACE) inhibitors are a well-recognised cause of a chronic cough[72,73] and it is thought to result from bradykinin induced sensitisation of afferent cough fibres[74]. ACE inhibitor induced cough resolves on withdrawal of the drug[75,76]. TABLE 3 lists possible causes of a chronic cough.

TABLE 3. CAUSES OF A CHRONIC COUGH

Productive cough	Dry cough
Bronchiectasis	Asthma
Tuberculosis	Post nasal drip
Cystic fibrosis	Gastro-oesophageal reflux
Chronic obstructive pulmonary disease	Interstitial pneumonitis
Foreign body inhalation	Sarcoidosis
Heart failure	Lung cancer
	Pulmonary embolism
	Tonsilar enlargement
	Obstructive sleep apnoea
	Angiotensin converting enzyme inhibitors

It has been widely believed that in non-smokers with a normal chest radiograph and normal spirometry, cough is caused by asthma, post nasal drip, gastro-oesophageal reflux disease (GORD) or a combination of all three[77-81]; the cause can be expounded by thorough and systematic investigations and treatment trials[82]. Irwin et al published their seminal and influential review paper in 1977 in which the mechanisms of the cough pathway were described and the causes of cough reported in the literature were reviewed. It was their view that cough receptors were located in nasopharynx, hypopharynx, paranasal sinuses, the larynx, trachea and bronchi as well as in the stomach, pericardium and diaphragm. And that in order to diagnose the cause of a chronic cough it was important to systematically consider each of these anatomic locations[83]. Later Irwin presented a trial of this approach, which was named the anatomical diagnostic protocol, in which forty-nine unselected patients with chronic cough were diagnosed and treated. A success rate of 100 percent in diagnosing the cause of cough and a success rate of up to 98 percent in treating it was reported. It was concluded that the cause of chronic cough could be diagnosed in 86 percent of patients by taking a good history, performing a thorough clinical examination and performing a methocholine challenge test. It was also concluded that the most common causes for cough were post nasal drip and asthma[77]. This approach was quickly adopted by specialist cough clinics and varying success rates from 60 to 100 percent in diagnosing the cause of chronic cough have been reported. TABLE 4 summarises the studies.

TABLE 4. CAUSES OF CHRONIC COUGH IN PATIENTS PRESENTING TO SPECIALIST COUGH CLINICS

Study	Number	Cough duration	Diagnosis (%)				
			GORD	UACS	Asthma/EB	UCC	Other
Irwin[77]	49	>3 weeks	10	47	43	0	7
Poe[79]	100	>8 weeks	5	26	35	12	7
Irwin[78]	102	>3 weeks	21	41	24	1	5
Pratter[81]	45	>3 weeks	11	87	31	10	
O'Connell[84]	87	>8 weeks	22	25	13	31	15
Smyrnios[85]	78	>4 weeks	15	40	24	3	
Mello[86]	88	>4 weeks	36	40	15	2	
McGarvey[87]	43	>3 weeks	19	21	23	19	
Brightling[88]	91	>3 weeks	8	24	31	7	20
Palombini[80]	78	>3weeks	41	58	59	0	32
Birring[89]	236	Not stated	15	12	24	26	23
Niimi[90]	47	>8 weeks	11	13	32	40	6
Kastelik[91]	131	>8 weeks	22	10	24	7	39

GORD = Gastro-oesophageal reflux disease, UACS = Uper airways cough syndrome, EB = Eosinophilic bronchitis, UCC = Unexplained chronic cough

Not all groups have been successful in replicating the high success rates of Irwin's early study. There may be a number of reasons for this, including differences in patient population, differences in diagnostic and treatment protocols, different definitions of chronic cough and its reputed causes and differences in follow-up. Whereas Palombini[80] extensively investigated patients with a mean of 8.5 investigative procedures to diagnose the cause of cough, others recommend a more pragmatic approach involving a combination of investigations for asthma and treatment trials for GORD and PND[91]. This approach did not result in a particularly low diagnostic yield and the authors noted that the cause of chronic cough could be diagnosed in 26 percent of patients with two simple investigations; a chest radiograph and spiromtery. Multiple investigations make more than one concurrent diagnosis more likely[80].

1.7.1. Disease of airway inflammation

Classical asthma is usually diagnosed by demonstration of variable airflow obstruction and is associated with symptoms other than cough such as wheeze and shortness of breath. In patients with normal spirometry it has been shown that methacholine challenge and induced sputum eosinophil count are particularly useful diagnostic tests in patients with asthma, with a positive predictive value of 97 percent and 100 percent respectively[92].

Although widely reported as one of the main causes of chronic cough, there is sparse objective data about the magnitude of the problem although cough is often self-reported in surveys of patients with asthma as a significant problem[66]. Hsu reported 24-hour ambulatory cough frequency in a group of twenty-one patients with stable classical asthma[55]. Patients continued their usual treatment

during the study and were reported to have a median 24-hour cough frequency of 282. A recent study of patients with classical asthma presenting to a tertiary referral centre attempted to objectively quantify the magnitude of the problem[57]. The population of 56 asthmatic patients included 11 current smokers, 32 patients on inhaled corticosteroids and 3 patients on oral corticosteroids. These patients were compared to matched controls. Cough frequency was higher in the asthmatic group, reported as 2.6 cough seconds per hour, but not as high as expected by the authors.

Cough variant asthma was first described in the nineteen-seventies as an isolated chronic cough with no history of wheeze or shortness of breath and normal spirometry but with airway hyper responsiveness as demonstrated by a methocholine challenge test[93,94]. It accounts for 23 to 42 percent of referrals to specialist cough clinics[79,87,95]. It is associated with a heightened cough reflex[96,97], which is not typically associated with classical asthma. Sputum studies show raised eosinophils[98,99] and it can progress to classical asthma in 14 to 55 percent of patients, particularly if left untreated[100,101]. It has been reported that bronchodilator therapy[94] and/or corticosteroids[102] can result in resolution of the cough.

Localisation of mast cells within airway smooth muscle in asthma are associated with airways hyperresponsiveness[103]. Mast cells in bronchial biopsies of patients with eosinophilic bronchitis are mainly epithelial and therefore there is an absence of airways hyper-responsiveness. Eosinophilic bronchitis accounts for approximately 10 to 33 percent of patients referred to specialist cough clinics[88,104,105]. It was first described by Gibson in 1989. They

described a chronic cough in non-smokers with raised sputum eosinophils but no evidence of airways hyper-responsiveness, therefore distinguishing this from asthma[103]. In a later study involving 9 patients they also showed that sputum eosinophils fell with steroid therapy and used cough symptoms scores to show that cough also improves[106]. In addition to an improvement in sputum eosinophils, Brightling also reported an improvement in capsaicin cough sensitivity following successful treatment[107], suggesting that a heightened cough reflex may be an important factor in the development of cough in these patients. Limited longer term follow-up has been reported on Gibson's cohort showing that half these patients remained symptom free at 10 years[108]. In contrast to this Berry reported long term follow-up data on 32 patients with eosinophilic bronchitis and showed a resolution of cough and sputum eosinophils in only 1 patient; 3 developed asthma as evidenced by airways hyper-responsiveness and 6 developed fixed airflow obstruction. The remainder had either on-going cough or raised sputum eosinophils[109]. The causes of treatment failure were not addressed and compliance with steroid therapy was not corroborated; but importantly it was demonstrated that eosinophilic bronchitis can be associated with long-term morbidity. Interestingly, raised sputum eosinophils have been reported in 20 to 40 percent of patients with COPD[110-113]. It is not clear whether coughing is a particular feature of COPD in these patients but it is possible that eosinophilic bronchitis may precede the development of COPD in these patients. The features of asthma, cough variant asthma and eosinophilic bronchitis are shown in TABLE 5.

TABLE 5. FEATURES OF ASTHMA, COUGH VARIANT ASTHMA AND EOSINOPHILLIC BRONCHITIS

	Classical asthma	Eosinophilic bronchitis	Cough variant asthma
Symptoms	Cough, wheeze and dyspnoea	Cough	Cough
Variable airflow obstruction	Present	Absent	Usually absent
Bronchial hyperresponsiveness	Present	Absent	Present
Capsaicin cough sensitivity reflex	Can be heightened	Heightened	heightened
Sputum eosinophils	Usually	Always	Usually
Smooth muscle mast cells	Present	Absent	Present
Basement membrane thickness	Increased	Increased	Increased
Steroid responsive	Yes	Yes	Yes

These diseases of airways inflammation associated with cough can be grouped together as steroid responsive causes of chronic cough and may represent a spectrum of disease flanked by classical asthma at one end and eosinophilic bronchitis at the other.

1.7.2. Gastro-oesophageal reflux disease

Gastro-oesophageal reflux disease (GORD) is common; it is thought to be present in up to 28 percent of the UK population[114] and has been reported in 5 to 73 percent of patients with chronic cough presenting to specialised clinics (see TABLE 4) The wide range is due to different study populations and different means of diagnosing GORD; from a characteristic history, 24-hour oesophageal

pH monitoring or response to treatment trials with a proton pump inhibitor. Although ambulatory 24-hour oesophageal pH monitoring is the gold standard diagnostic investigation, it has been noted that symptoms of heartburn or regurgitation, particularly after meals, have a specificity of 97 percent for GORD and treatment trials can be initiated on this basis[115,116].

It is feasible that GORD can cause cough as vagal C fibres have been identified in the lower oesophagus of the mouse[117] and the guinea pig[118] and they have been shown to express TRPV1 receptors[118]. It has also been shown that infusion of acid into the oesophagus of the anesthetised rat causes stimulation of the nucleus of the solitary tract, suggesting activation of the afferent cough pathway[119].

There may be 2 possible mechanisms by which reflux can cause cough.

- Micro-aspiration of gastric contents into the larynx/trachea
- Direct stimulation of the vagal C fibres

The role of micro-aspiration in respiratory disease was studied in 4 patients with asthma and gastro-oesophageal reflux and 3 controls. The presence of tracheal micro-aspiration was demonstrated in the asthmatic group using oesophageal and tracheal pH probes. Some of these episodes of micro-aspiration were accompanied by a fall in peak expiratory flow rate (PEFR) suggestive of bronchoconstriction[120]. Controls did not have any episodes of micro-aspiration or PEFR variability. Although it is possible that bronchoconstriction resulting from micro-aspiration can result in cough, this hypothesis is currently unproven.

Ing and colleagues studied 22 patients with cough and GORD as well as 12 matched controls, by infusing acid and water into the lower oesophagus via a nasogastric tube in a double blind fashion. They found that cough could be consistently elicited in patients with chronic cough with acid infusion but not in control subjects. Cough could also be elicited in some patients with chronic cough with infusion of normal saline, perhaps suggesting hypersensitivity of the oesophageal cough receptors[121]. It is important to note that cough was not elicited in control subjects, implying that direct stimulation of vagal C fibres alone is not sufficient to elicit cough. These findings have not been consistently demonstrated as Irwin failed to reproducibly elicit cough in twelve patients with chronic cough using a similar methodology[121,122].

The association between reflux episodes and cough in patients with chronic cough has been studied by a number of groups using 24-hour pH oesophageal monitoring and (most often) patient completed cough diaries[123-127]. The association between reflux episodes and cough appears variable with between 13 and 48 percent of cough episodes related to reflux episodes. More recent studies have reported symptom associated probability scores (SAP). This validated statistical method in which 2 minute signals from the oesophageal pH monitor preceding a cough are analysed for episodes of reflux. Fishers exact test is then used to evaluate the probability (P) of the reflux and cough event being unrelated. The SAP is then calculated as 1 – P and expressed as a percentage[128]. The most robust of these is a recent study in an unselected group of 71 patients with chronic cough using twenty-four hour oesophageal impedance/pH monitoring and cough assessed using a non-automated

ambulatory cough monitor[129]. The findings are interesting for a number of reasons.

1. The number of coughs recorded was far higher than in previous studies which had used symptom diaries or oesophageal impedance probes, suggesting that cough frequency cannot be reliably measured using these methods.

2. In this unselected group of patients in which 53 percent had evidence of other causes of cough, 48 percent of patients had a positive SAP score and this score was unaffected by the type of reflux (acid or non-acid). Other studies have also described an association between non-acid reflux events and cough[125,126].

3. Patients with a positive SAP had a more sensitive cough reflex.

Others have also demonstrated an increased capsaicin cough sensitivity in patients with GORD[84] and in fact Ferrari demonstrated enhanced cough sensitivity in patients with GORD both with, and without cough[130].

It is likely that patients with a heightened cough reflex cough in response to a variety of stimuli and perhaps it is not surprising that studies investigating improvements in cough with treatment with proton pump inhibitors have yielded poor results[131].

1.7.3. Post nasal drip

The diagnosis of post nasal drip or rhinitis is solely made on the basis of a characteristic history of a 'sensation of something dripping down the back of the throat'. There are no objective tests to demonstrate this and sinus radiographs and computerised tomography scans have a low diagnostic yield. It may cause cough indirectly through inflammation of the upper airways[132] or by mechanical stimulation of cough receptors in the larynx by dripping nasal secretions. An improvement in cough following treatment with first generation antihistamines has been interpreted as confirmation that the cough was caused by post nasal drip[62]. Similar improvements in cough have not been observed with newer antihistamines. First generation antihistamines are not particularly specific antagonists of the H1 receptor and are known to cross the blood brain barrier leading to their most significant side effect of drowsiness.

1.7.4. Unexplained chronic cough

There is an increasing recognition that the cause for a chronic cough cannot always be identified. More recent studies of chronic cough have identified up to 40 percent of patients in whom a cause cannot be found following thorough investigations and treatment trials (see TABLE 4). Unexplained chronic cough is challenging to manage and causes considerable physical and psychological morbidity. Little is understood about the pathogenesis of unexplained chronic cough, the natural history has not yet been described and there is a lack of effective targeted therapeutic options.

It is well documented that the majority of patients with unexplained chronic cough are women with an onset of cough around the menopause[65,87,133,134].

There also appears to be an association with organ specific autoimmune disease, with a higher than expected prevalence, particularly of hypothyroidism in patients with unexplained chronic cough[96,135]. In a retrospective review of 180 well-characterised patients with unexplained chronic cough seen in a specialist cough clinic between 1999 and 2006[136], over 80 percent of patients were female and had a mean age of 51 at the onset of cough. One third had organ specific autoimmune disease compared to a prevalence of 6 to 8 percent in the general population[137].

Sputum studies comparing patients with unexplained chronic cough to normal controls reveal evidence of increased sputum neutrophils and mediators associated with neutrophillic airway inflammation such as interlukin-8, tumour necrosis factor alpha and prostaglandin E2[96,138,139]. It is difficult to establish if these inflammatory changes are responsible for causing cough or are caused as a consequence of coughing. Bronchoscopy studies show a lymphocytosis in bronchial biopsies[140,141] and an increased proportion of lymphocytes in bronchioalveolar lavage[134,142]. It has been postulated that these findings suggest an auto-immune origin for unexplained chronic cough[135]. Subclinical lymphocytic alveolitis has been described in patients with both systemic and organ specific auto-immune disease but the respiratory sequelae of this are not clear from the literature[143-145].

Airway remodelling can occur as a result of airway inflammation and is well described in patients with asthma. Bronchoconstriction from airway remodelling may result in cough as it has been shown that bronchodilators can improve cough in this group of patients. It is unclear if airway remodelling occurs in

patients with unexplained chronic cough but Birring retrospectively reviewed a population of non-smokers with COPD and found a group of predominantly women with normal sputum eosinophils but raised sputum neutrophils[146]. In this group approximately fifty percent had organ specific autoimmune disease. This population appears remarkably similar to those with unexplained chronic cough and the possibility of airway remodelling in these patients requires further examination.

Mundet al observed in a sample of sixteen healthy non-smoking women aged between 26 and 63 that there was an increase in CD4/CD8 lymphocyte ratio in women aged over 43[147]. The same was not true of the sixteen healthy males also studied. This has led to some speculation that the increased prevalence of unexplained chronic cough in women in this age group may be associated with hormonal related lung changes. Sex hormone levels were not measured in this study and the study sample is too small to draw any real conclusions. Furthermore, another study of healthy volunteers aged 25 to 78 showed increasing CD4/CD8 lymphocyte ratio which was age related, although data on males and females was not presented separately[148].

Also studies have reported that healthy women have a heightened cough reflex compared to men[149-151]; perhaps this makes women more susceptible to chronic cough in the context of other generalised inflammatory disorders.

There are no known drug treatments for unexplained chronic cough, but respiratory physiotherapy aimed at cough suppression may be useful in reducing cough frequency in patients with unexplained chronic cough[152]. A randomised control trial of placebo versus speech therapy showed a significant

improvement in cough symptom scores in the active treatment arm[153]. This benefit may be due to management of paradoxical vocal fold movement which is present in a large proportion of patients with unexplained chronic cough[154]. Paradoxical vocal fold movement is characterised by abnormal adduction of the vocal cords during inspiration, causing airflow obstruction at the level of the larynx[155]. This technique requires further evaluation as the study described was not blinded and assessment of the improvement of cough was subjective. Nonetheless it may aid in providing higher cortical control of cough.

1.8. MORBIDITY ASSOCIATED WITH CHRONIC COUGH

Chronic cough is a distressing symptom and has been reported to cause significant impairment in quality of life and social isolation[156]. French studied quality of life in 28 patients attending a cough clinic at baseline and after the cough had improved with treatment. They reported significant health related physical, social and psychological dysfunction in this group of patients due to exhaustion, retching, concern that something serious is wrong and embarrassment. Many of these adverse events improved with successful treatment of the cough. In addition to psychosocial symptoms, Birring also reported a prevalence of a number of secondary physical symptoms such as urinary incontinence, vomiting, sleep disturbance and musculoskeletal chest pains (see TABLE 6)[157]. A study involving 147 patients with a range of respiratory diseases associated with cough such as asthma, COPD, bronchiectasis, unexplained chronic cough, and cough associated with GORD showed that cough specific impairment in quality of life was similar across the range of disease groups[158].

As a result of this a number of cough specific quality of life questionnaires have been developed and will be discussed later (French 2003, Birring 2003).

Given the psychosocial morbidity, it is not unanticipated that patients with chronic cough suffer from significant depressive symptoms. Dicpinigaitis administered the Centre for Epidemiological Studies Depression Scale to a group of 97 patients before and after treatment for chronic cough. This questionnaire has been used in at least twenty-five other patient populations from those with cancer, to those with other chronic conditions including asthma, epilepsy, arthritis and diabetes. The authors reported one of the worst depression scores, exceeded only by inner city patients with asthma and patients with type 2 diabetes mellitus and erectile dysfunction [159]. Importantly the depressive symptoms improved with successful treatment of the cough.

TABLE 6. ADVERSE SYMPTOMS ASSOCIATED WITH COUGH

Physical	Psychological	Social
Syncope	Depression	Relationship tensions
Vomiting	Anxiety	Fear of public places
Chest pains	Embarrassment	Avoidance of social events
Hoarse voice	Fear of illness	Interference with work
Incontinence	Frustration	Interruption of telephone calls
		Interruption of meals

1.9. TOOLS TO ASSESS COUGH

There is a lack of validated tools to objectively measure cough frequency or intensity. Consequently, the factors that influence the severity of cough are poorly understood. The inability to make objective assessments has been a major factor behind the poor quality clinical trials of cough therapy reported in the past. The recent increase in the resources and amount of research in this field has generated a number of novel tools to assess cough severity and provided much needed validation and standardisation of old tools. All the international cough management guidelines endorse the assessment of cough severity, both in clinical practice and research[59,60]. The assessment of cough severity in secondary care outpatient clinics should be performed at first consultation to confirm the presence of cough and its severity, facilitate individually tailored therapy and importantly to objectively assess the response to treatment trials, which are an integral part of the diagnostic algorithm.

1.9.1. Symptom scores

Cough symptom scores are the simplest and most practical of the assessment tools. They are brief and easy to complete whether self-administered, interview based, completed over the telephone or used as part of a diary. Cough scores[160], diaries[161-163], symptom questionnaires[164,165] and Visual Analogue Scores (VAS)[157] are commonly used in clinical studies to evaluate cough severity. All lack thorough validation for this purpose. Cough diaries comprise of questions relating to cough frequency but they correlate poorly with objectively measured cough frequency[55]. The reproducibility and responsiveness of cough diaries has not been reported and the relationship to other parameters of cough severity such as cough reflex sensitivity is not known

for patients with chronic cough. In children, the repeatability of parent-completed cough symptom questionnaires is poor[165]. Interestingly, self-completed cough diaries for children have good responsiveness and correlate better with objective cough frequency[161].

Cough VAS is a 100mm linear scale on which patients indicate the severity of cough. They are the best validated of the symptom scales. Cough VAS is highly repeatable over a two-week period[166] and responsive when used as an outcome measure in clinical studies of patients with a chronic cough[107,157,167]. Cough VAS relates well to cough specific quality of life but not to cough reflex sensitivity[134]. Although cough VAS many be useful as a simple and cheap tool to measure the benefit of a treatment trial it is not useful in comparing cough between people or groups.

1.9.2. Quality of life questionnaires

Chronic cough is often trivialised. For the individual affected, it is a major symptom that should be taken seriously[168]. Anxiety and psychological symptoms are prevalent[159,169]. A recent study from the USA found that fifty-three percent of patients with chronic cough, assessed in a specialist clinic, had significant depressive symptoms. This was comparable to other chronic disorders such as chronic obstructive pulmonary disease and chronic heart failure[159,169]. Physical symptoms associated with cough such as syncope, sleep deprivation, incontinence, chest pains and vomiting are readily apparent during consultation but psychosocial aspects are often neglected. Cough can interfere in relationships, meetings, work and social events. This can be distressing for the patient. There has been a general trend to assess psychosocial aspects of

chronic illness and health related quality of life questionnaires are designed to help capture this. The questionnaires assess the overall impact of the condition on the patient and provide a quantifiable, validated and reliable measure of health status. Generic health status questionnaires have been used to assess patients with cough. They are not specific for cough and hence are less responsive to changes[157]. Three cough-specific, health related, quality of life questionnaires have recently been developed and validated. They can be used to quantify the overall impact of chronic cough and establish the range of problems affecting patients[157,170,171].

The Leicester Cough Questionnaire (LCQ) is a brief, self-administered and well validated, cough specific, health related, quality of life questionnaire[157] (Appendix 1). It comprises of 19 items and takes less than five minutes to complete. Patients respond to questions about their health on a seven-point Likert scale. Early experience with the LCQ suggests that patient comprehension of the questionnaire and data completion is very good. Electronic scoring systems are available to facilitate the calculation of LCQ scores. The LCQ has been translated, validated and used successfully in research and clinical trials in the Netherlands, Turkey and Hong Kong[172-174]. There is no evidence as yet that the LCQ is a valid tool to assess heath status of patients with acute cough. The LCQ has been used in several chronic cough clinical trials[174-177].

The Cough Specific Quality of Life Questionnaire (CQLQ) is a 28-item questionnaire that has been developed and tested in North America[170]. The items are divided into six domains: physical complaints, extreme physical

complaints, psychosocial issues, emotional well-being, personal safety fears and functional abilities. The CQLQ has been well validated and shown to be repeatable and responsive. The CQLQ has been translated into other languages and used in clinical trials[173,178]. Studies to determine the minimal important difference of the CQLQ are underway.

The Chronic Cough Impact Questionnaire (CCIQ) is the most recent of the three to be published. This was tested in a large number of patients and is well validated[171]. It consists of 21 items and 4 domains. The minimal important difference for the CCIQ has not yet been determined. All three cough specific health questionnaires assess the impact of cough on psychosocial health.

The minimal important difference (MID) of a health related quality of life measure (HrQL) has been defined as the smallest change in score that is considered beneficial by patients[179]. It is useful as it ties the magnitude of change in the quality of life to treatment outcomes and can thus help with study design and the choice of sample size. It emphasises the importance of the patients perspective and can link this to clinical decision making, although caution must be employed as the MID is a pooled measurement in a specific population and therefore may not apply directly to individual patients or other patient populations.

There is no consensus as to the best methodology of calculating the MID, however it has been suggested that a triangulation of a number of methods yields the best result[180] and that confidence in a specific MID value grows over time[179]. Distribution based methods such as effect size are widely used and determine the MID as a measure of magnitude of change standardised by a

measure of variance (usually the standard deviation). This method of determining the MID is quick and easy but is greatly affected by the heterogeneity of the study population on whom the MID is based.

Anchor based methodologies to determine MID are also commonly employed. Clinical anchors such results of blood tests or patient reported anchors such as global rating of change (GRCQ)[181] assign subjects into groups of no change, small change and large change. The change in the HrQL corresponding to a small change in the anchor is deemed the MID[181]. The assumptions of this methodology are that the anchor is independent of the HrQL tool being assessed and that there is at least a moderate correlation at baseline[182]. Single anchors or multiple anchors can be used.

The GRCQ is a retrospective anchor and may be subject to recall bias[183]. Critics state that there is evidence to suggest that patients find it difficult to recall their previous state and the GRCQ is correlated more closely with the present state, rather than the change from baseline. These criticisms are less important in studies in which there is a short timeframe between repeat HrQL assessments. However, in response to these criticism a prospective anchor based methodology has been proposed in which multiple anchors based on separate domains assessed by the HrQL ask, not about the change from the previous state but, about the patients current state over the previous 2 weeks[184]. This methodology is not currently in wide use. The MID of the Leicester cough questionnaire as determined by retrospective anchor based methods is 1.3[185] The MID of other cough specific quality of life questionnaires is not yet known.

1.9.3. Cough reflex sensitivity

Patients who cough frequently tend to have heightened cough reflex sensitivity[96]. Cough sensitivity testing is widely used to assess abnormalities of the cough reflex. It is objective and provides important information about the mechanisms of cough. The methodology is well established and shown to be very safe[34,59]. Cough reflex testing is commonly used in clinical trials to assess the effect of antitussive drugs, since cough reflex sensitivity is heightened in most patients with cough and successful treatment results in a measurable reduction[89]. Several tussive agents can be used to stimulate cough. Capsaicin and citric acid are used most frequently; distilled water, ammonia and low chloride solutions are alternatives. There is extensive experience and well-validated standardised methodology available[59]. Single breath inhalation of the tussive agent is more commonly used than tidal breathing methods, but there are no particular advantages of one method over the other. Cough reflex sensitivity testing does not discriminate between healthy subjects and patients with chronic cough, as there is considerable overlap in the results[96]. This limits its clinical usefulness. It is however a responsive tool that can be used to assess the effects of drug therapy and therefore is useful as an objective outcome measure.

1.9.4. Cough monitors

Cough monitoring is considered the gold standard cough assessment tool. Its importance lies in its objectiveness and ability to validate the presence of cough and assess response to treatments. Attempts to develop cough monitors have been hindered by inadequate recording memory, limited battery life and laborious manual analysis. Developing automated systems has been difficult

because of the variability of cough sounds and interference from speech and background noises. Recent advances in digital recording devices and improved battery life have led to a renewed interest in cough monitor development. A variety of methods can be used to detect cough; sound recordings are emerging as the favoured medium rather than abdominal electromyography (EMG) and chest wall movement detectors[55]. Sound recordings are more practical and can be used to derive a number of cough frequency outcome parameters such as coughs per hours (coughs counted individually), epochs (bursts) of cough and cough seconds (number of seconds containing coughs). There are no significant advantages of one parameter over the other but coughs per hour may be easier to interpret[58].

There are at least four automated cough detection systems in advanced stages of development and validation. All generate 24-hour ambulatory recordings. A summary of cough monitors is given in TABLE 7. Sensitivity of cough detection and false positive cough rate are amongst the most important validation parameters. The Leicester Cough Monitor[186] and the Hull Automated Cough Counter [187] use sound only and use cough detection algorithms based on voice recognition techniques and probability neural networks respectively.

TABLE 7. A COMPARISON OF COUGH MONITORING DEVICES

	LCM	HACC	LifeShirt	VitaloJak
Ambulatory	Yes	Yes	Yes	Yes
24-hour recording	Yes	Yes	Yes	Yes
Recording parameter	Sound	Sound	Sound +chest wall	Sound
Outcome measure	Coughs/hr	Coughs	Coughs/hr	Coughs
Calibration necessary	No	No	Yes	Yes
Operator input time (min)	10	45	NK	NK
Sensitivity (%)	91	80	78	NK
Specificity (%)	99	96	99	NK
False positives / hour	2.5	NK	NK	NK

NK: not known; LCM: Leicester cough monitor; HACC: Hull Automated Cough Counter

The Lifeshirt combines a microphone with a vest that contains inductance plethysmograph sensors that detect respiratory flow[188]. The VitaloJak records sound from a coupled microphone taped to the skin and is currently being validated[189].

Both the VitaloJak and Lifeshirt monitors require a calibration procedure for each subject prior to recording sound. For all monitors, studies to investigate cough intensity measurement and its potential clinical relevance would be valuable. It would also be useful to explore whether cough monitors can distinguish cough from other physiological respiratory reflexes[190]. The minimal important clinical difference for cough frequency needs to be studied in order to interpret results appropriately. Cough monitoring is likely to be used initially in specialist cough clinics and clinical trials assessing antitussive therapy.

1.9.5. Measuring acute cough

The measurement of acute cough is largely limited to the research and clinical trial setting. Symptom scores, diaries and cough reflex sensitivity measurement are frequently used. More recently, cough specific quality of life measures have been introduced.[65,157] These look promising as they provide more patient centred data but require further evaluation as they have only been fully validated in chronic cough. Ambulatory 24-hour automated cough monitors have not been evaluated in acute cough.

1.9.6. Measuring cough in clinical trials

Once again, the measurement of cough symptoms in clinical trials has largely been subjective using cough diaries. Several recent studies have used cough specific quality of life questionnaires successfully[59] but cough monitors have not been used.

1.9.7. The gold standard assessment of cough

The assessment of cough severity is recommended by all international cough guidelines. A wide range of cough assessment tools are available for clinical and research use. They measure differing aspects of cough severity, so should ideally be used in combination. Cough monitors provide an objective quantitative measure of cough which is particularly useful in establishing the success or failure of a treatment trial. Quality of life questionnaires compliment this data by providing a qualitative assessment of the overall impact of cough on health, particularly psychosocial wellbeing.

1.10. TREATING COUGH

As demonstrated earlier, cough is, to some extent, under voluntary control which explains why it is highly suggestive to the placebo effect. Reports suggest that up to eighty-five percent of the efficacy of some antitussives can be explained by the placebo effect[191]. Uncontrolled treatment trials are uninterpretable and I will consequently only discuss placebo controlled trials.

1.10.1. Acute cough

There have been a number of placebo controlled trials in patients with acute cough associated with upper respiratory tract infections. These trials are summarised in TABLE 8 below. They have shown no effect of codeine[192,193]. Dextromethorphan, a synthetic derivative of morphine, has also been tested in a number of randomised placebo controlled trials. Parvez at al conducted 3 separate single dose trials of dextromethorphan over 3 years and reported these as a single trial of 451 patients. The study methodology was similar in each trial, in that baseline ambulatory cough frequency was recorded and manually counted for an hour. Patients were then given a single dose of dextromethorphan or placebo and cough frequency was again measured for 3 hours[194]. One of these studies showed a statistically significant improvement in cough at 120 and 150 minutes in the group of patients treated with dextromethorphan. As there was no power calculation and as the positive study had the smallest number of patients in each arm, the positive result may be due to a type I error.

A meta-analysis of these three trials and three other unpublished trials showed a marginal but statistically significant reduction in cough as measured by 4 hour

cough recordings[195]. It is therefore unlikely that dextromethorphan has a clinically significant impact on acute cough.

Non-sedating antihistamines also have no effect on acute cough[196,197]. Two studies have looked at first generation antihistamines. Gaffey investigated non-sedating antihistamines in combination with a decongestant pseudoephedrine and demonstrated a subjective improvement in cough severity scores[62]. Berkowitz undertook a more robust study using 4 hour cough recordings to measure cough frequency before and after administration of a single dose of a first generation antihistamine. This study did not show an active treatment effect[194].

Studies of the expectorant guaifenesin have generally been poorly conducted and have relied on self-reporting of improvements in cough. Nonetheless, in one well conducted study using cough frequency as the primary endpoint the reported effect was no greater than placebo[198].

TABLE 8. PLACEBO CONTROLLED TRIALS FOR THE TREATMENT OF ACUTE COUGH

Anti-tussives			
Trial intervention	Number of participants	Outcome measures	Efficacy
[192]Codeine 30mg four times a day for 4 days	81	Cough severity scores	No significant differences
[193]Codeine 50mg single dose	82	Measurements taken for 90 minutes before and after administration of drug • Cough scores • Cough sound pressures • Cough frequency	No significant differences
[194]Dextromethorphan 30mg single dose	3 separate trials with 108, 134 and 209 participants	Cough recordings over 4 hours measuring: • Cough bouts • Cough visual analogue scores	1 significant result with a peak difference in cough counts of 36% at 120 minutes, $p < 0.05$
[199]Dextromethorphan 30mg single dose	44	• Cough frequency recordings • Cough sound pressure levels • Cough severity questionnaire	No significant difference
Moguisteine 600mg 3 times a day for 4 days	108	Cough scale	No statistical difference at end of treatment visit

Antihistamines			
Trial intervention	Number of participants	Outcome measures	Efficacy
[196]Terfenadine 60mg twice a day for 4 days	250	Symptoms cards	No significant difference
[197]Terfenadine 120mg twice a day for 4 days	97	• Symptoms cards • Clinical examination	No significant difference
[194]Diphenhydramine 25mg single dose	134 (3 arms)	Cough recordings over 4 hours measuring: • Cough bouts. • Cough Visual Analogue Scores	No significant difference
Antihistamine and decongestant combinations			
[62]Dexbrompheneramine 6mg and pseudoephedrine 120mg twice a day for 1 week	73	Cough diary Cough scores	Mean cough score 1.4 (trial drug) vs 2 (placebo) $p<0.05$
Loratadine 5mg and pseudoephedrine 120mg twice a day for 5 days	283	Cough diary Cough scores	No significant difference

Expectorant			
Trial intervention	Number of participants	Outcome measures	Efficacy
[200]Guaifenesin 200mg four times a day for 3 days	239	Patient questionnaires Cough scores	33 (placebo) compared with 79 (trial drug) found the treatment helpful. P<0.01
[198]Guaifenesin 480mg every 6 hours for 30 hours	65	Cough recordings for cough frequency – 6 hours analysed over successive days Cough symptoms questionnaires	No significant difference

1.10.2. Chronic cough

Most trials of cough suppressants in chronic cough have taken place in populations of patients with COPD. The applicability of these findings in patients with other causes of cough or unexplained chronic cough is uncertain. TABLE 9 summarises these trials.

Codeine has been shown to reduce cough in 3 small placebo controlled trials of patients with chronic bronchitis and COPD conducted over 20 years ago[201-203]. However a more recent, well conducted randomised trial which included both cough symptoms questionnaires and ambulatory cough monitoring did not show a benefit of codeine in this patient group[204].

One recent double blind randomised control trial of morphine in unexplained chronic cough showed an improvement in the LCQ scores and cough diaries in patients taking 5mg of slow release morphine twice a day[205]. A statistically significant difference in the citric acid cough challenge was not observed in this study. Theoretically, morphine may supress cough by acting centrally within the brainstem or it may exert its effect peripherally, as it blocks TRPV1 sensitisation by preventing its phosphorylation by protein kinase A[206]. But due to the discordance between the subjective and objective measures of cough in this study, it is still unclear if morphine is an effective treatment in patients with unexplained chronic cough.

One other study, despite not being placebo controlled, is worth a mention. 28 well characterised patients with unexplained chronic cough, were randomised to receive 10mg of amitriptyline or codeine/guaifenesin for a duration of 10 days.

TABLE 9. PLACEBO CONTROLLED TRIALS FOR TREATING CHRONIC COUGH

Intervention	Number of participants	Patient group	Measurements	Outcome
[201]Codeine 30mg single dose	8	Chronic bronchitis	Cough scores	Reduction in cough by 40%, $p<0.05$
[202]Codeine 30mg single dose	10	Chronic bronchitis	Cough frequency	Reduction in cough by 47%, $p<0.01$
[203]Codeine 30mg single dose	12	COPD	Cough frequency	Reduction in cough by 60%, $p<0.01$
[204]Codeine 60mg twice a day for 7-10 days	21 (power calculation presented)	COPD	• 20 hour cough frequency • Cough symptom questionnaire • Citric acid cough challenge • Cough visual analogue score	No significant difference

Intervention	Number of participants	Patient group	Measurements	Outcome
[207]Moguisteine 1200mg 3 times a day for 4 days	87	COPD, lung cancer, pulmonary fibrosis and unexplained chronic cough	2 hour cough frequency	28% reduction in cough frequency compared with placebo, $p<0.03$
[201]Dextromethorphan 630mg single dose	8	Chronic bronchitis	Cough scores	Reduction in cough by 50%, $p<0.05$
[175]Morphine sustained release 5mg twice a day	27	Unexplained chronic cough	• Leicester cough questionnaire • Cough diary • Citric acid cough challenge	Significant improvement in LCQ score and cough diary

A significant subjective improvement in cough in patients in the amitriptyline arm was reported by a majority of patients[178]. This interesting finding merits further investigation using objective markers of efficacy, as chronic cough may share molecular pathways with neuropathic pain and the mood altering effects of amitriptyline may impact on subjective reporting of cough.

1.10.3. Pitfalls in new drug development

It is clear that the current selection of non-specific antitussive agents are unsatisfactory and that new efficacious drugs for the treatment of cough are needed. Modern drug development begins with the identification of a 'druggable' target. Either, medicinal chemists can engineer a molecule that modulates the target or, more commonly, the target is screened against a large bank of compounds in a process known as high throughput screening. A few lead compounds are identified that are able to modulate the target. These lead compounds require optimisation to make them more specific to the target, to make them more potent and to improve their pharmacokinetic properties. The final compound from this process is a new drug, which is initially tested in-vitro to obtain preliminary efficacy data and pharmacokinetic data, which is used to supplement data from animal studies and to help inform dosing. Although in-vitro studies can inform choices regarding dosing and schedule and to help the researcher understand basic pharmacokinetic properties of a new drug, more detailed studies must be performed in animal models to establish efficacy, a safe dose and a dosing schedule to take forward to first-in-human studies.

This is where the difficulties begin. Although our understanding of the physiological process underpinning cough have advanced significantly in recent

times, and ‘druggable’ targets have been identified, the animal models are not sufficiently robust. Potential antitussive agents are usually tested in capsaicin induced cough in the guinea pig. This is a poor mimic of unexplained chronic cough as we know that pathological cough in humans is a complex physiological and inflammatory process, possibly augmented by coughing itself. Animal models of capsaicin induced cough cannot imitate this and may be misleading in selecting the appropriate drug to take forward to human trials.

1.10.4. Promising targeted agents

The TRPV1 receptor is an obvious target for a drug treatment for chronic cough associated with a heightened cough reflex. TRPV1 receptor antagonists for cough have been evaluated in three published preclinical studies. Two proof of concept studies investigated the ability of a TRPV1 antagonist in inhibiting capsaicin and acid induced cough and showed a reduction in the number of coughs in a dose dependent manner[208,209]. A third study investigated TRPV1 antagonists in allergen induced cough in the guinea pig and have shown promising results with a reduction in cough frequency of up to 60 percent[210]. Similar results must now be replicated in humans but although TRPV1 antagonists are currently undergoing phase II trials for pain. The role of TRPA1 in chronic cough is discussed elsewhere and this may also be a potential target for drug development.

Other molecular pathways which sensitise the TRPV1 receptors may also make good targets for potential drugs. G protein coupled receptors have been discussed in details earlier in this work and may play an important role in augmenting the sensitivity of the cough reflex.

1.11. HYPOTHESIS AND AIMS

1.11.1. Hypothesis

I hypothesise that the Leicester Cough Monitor is a valid ambulatory tool to measure cough frequency.

I hypothesise that low dose long term macrolide antibiotics reduce cough frequency by attenuating neutrophillic airway inflammation.

I hypothesise that unexplained chronic cough is associated with long-term morbidity.

1.11.2. Aims

The aim of the work described in this thesis is:

1. To assess the validity of the Leicester Cough Monitor in assessing the 24-hour cough frequency in healthy and diseased groups of subjects.

2. To assess the range of cough in a group of subjects with respiratory disease and compare this to other markers of disease severity.

3. To estimate a normal range for cough in healthy subjects.

4. To conduct a double blinded randomised drug trial to assess the impact of erythromycin on unexplained chronic cough and to compare this data to other markers of disease severity.

5. To present novel data on the natural history of chronic cough.

6. To assess the minimal important difference of the Leicester Cough Questionnaire in acute cough.

2. METHODS

2.1. RESPIRATORY PHYSIOLOGY

2.1.1. Spirometry

Spirometry was performed with a Vitalograph spirometer (Vitalograph, Buckinghamshire, UK). Participants inhaled 200 micrograms (µg) of salbutamol via a volumatic device. After 15 minutes the FEV1 and FVC was recorded as the best of at least 3 successive readings within 100 millilitres (mL).

2.1.2. Sputum induction and processing

Prior to starting the sputum induction process, the test was explained to the patient and written instructions were given. Patients were asked to spit out saliva generated during the nebulising process into a container. Patients were advised to sit up straight during nebulisation and lean forward during expectoration. They were also asked to blow their nose, rinse their mouth and swallow water prior to expectoration attempts. Patients were asked not to swallow sputum but to cough it into a sterile container.

2.1.2.1. Induction

All participants were pre-treated with 200 µg of salbutamol via a volumatic device 15 minutes prior to sputum induction to minimise bronchoconstriction. A 3 percent saline solution (Nova Laboratories Ltd, Leicester) was breathed in tidally via an ultrasonic nebuliser (DeVilbissUltraneb, Sunrise Medical,Pennsylvania) for 5 minutes. Subjects were then asked to rinse out their mouth and blow their nose to minimise contamination of the sputum. Any sputum produced after the saline inhalation was expectorated into a sterile pot.

FEV1 was then measured. If no sputum was obtained the procedure was repeated with 4 percent saline and again with 5 percent saline. However if there was a drop in FEV1 of more than 10 percent the same concentration of saline was administered again. If FEV fell by more than 20 percent, or if significant symptoms (i.e. wheeze) were encountered during the induction, the investigation was abandoned and the patient was treated with nebulised bronchodilators until their symptoms settled.

If no sputum was expectorated after the administration of 3 doses of saline, sputum was not obtained.

2.1.2.2. Processing

Sputum was processed at 4°C within 2 hours of expectoration and was viewed using an inverted microscope. Sputum plugs were separated from saliva using fine forceps. Plugs were then gathered into one mass using larger blunt-ended forceps. A polypropylene centrifuge tube with the lid on top was weighed and the concentrated sputum transferred into it with the blunt ended forceps. The weight of the sample was calculated by subtracting the weight of the empty centrifuge tube from the weight of the centrifuge tube plus selected sputum to obtain the weight of sputum portion to be processed. A fresh stock of 1 percent dithiothreitol (DTT, Sigma-Aldrich, St Louis) was made every 30 days [200 milligrams (mg) DTT in 20mLs of water at 4ºC]. It was diluted daily to 0.1 percent using Dulbecco's phosphate buffered saline (D-PBS, Sigma-Aldrich, St Louis). 4 mL of 0.1 percent DTT per gram (g) of sputum was added to the centrifuge tube. The sputum was then dispersed by repeated gentle aspiration into a plastic Pasteur pipette, vortexed (Whirlymixer, Fisher Scientific, Loughborough, UK) for 15 seconds and then incubated for 15 minutes while

rocking on a bench (Spiromix 5, .Denley Instruments Ltd, Colchester, UK). An equal volume of D-PBS was added and this was then vortexed for a further 15 seconds, then filtered through a 48 micrometre (µm) nylon gauze into a clean 15 mL centrifuge tube and centrifuged at 2000 revolutions per minute (rpm) for 10 minutes. The sputum supernatant was removed and stored at -80°C for future mediator assays. The cell pellet was re-suspended in a small volume of PBS.

10 microlitres (µL) of cell suspension was mixed with 10 µL of 4 percent Trypan blue solution (Sigma-Aldirch, St Louis) that stained non viable cells blue. The neubauer haemocytometer (Fisher Scientific, Loughborough, UK) was then flooded with this mixture and a cell count was performed within 5 minutes. All cells in 5 fields of the haemocytometer were counted. Cells were classified as viable leukocytes, dead leukocytes, and squamous cells (whether viable or not). The mean number of cells per square were counted and the percentage of viable and squamous cells were calculated.

The cell suspension was then centrifuged at 2000rpm for 10 minutes to produce a cell and debris free supernatant. The supernatant was removed without disturbing the cell pellet and divided equally into labelled cryotubes. These were stored at -70°C.

The cell pellet was re-suspended in 0.5 – 1 mL of D-PBS and aspirated gently to give a single cell suspension. It was then adjusted to a suspension of 0.5 - 0.75 x 10^6 cells per mL with D-PBS. The volume of D-PBS required was calculated by dividing the total number of cells (10^6) by 0.5 x 10^6.

Two slides of 50µL of cell suspension were prepared and labelled a and b. Another 2 slides of 75µL of cell suspension were prepared and labelled c and d. These were then spun at 450 rpm for 6 minutes using a Shandon III cytocentrifuge to produce a monolayer of cells on the slide (Shandon Southern Instruments, Sewickley, PA, USA).

The slides were then air dried for 15 minutes at room temperature and then fixed with methanol for 10 minutes. The cells were stained in neat Romanowski stain for 5 minutes and fixed in dilute stain for 25 minutes. The slides were stored in a clean and dry environment in slide storage boxes at room temperature.

Preparation of the Romanowski stain was as follows. 1.5 g of Azure-B-thiocyanate dye content 98 percent (Sigma-Aldrich, St Louis) was dissolved in dimethyl sulphoxide (DMSO, Sigma-Aldrich, St Louis) at 37^{o}C and 0.5 g Eosin in 300 mL methanol at room temperature. The Azure blue solution was slowly added to the Eosin and stored away from light.

2.1.2.3. Differential counts

More than 400 non-squamous cells on Romanowski stained slides were counted. The percentage of squamous cells, epithelial cells, neutrophils, eosinophils, macrophages and lymphocytes were calculated.

2.1.2.4. IL-8 concentrations

The concentrations for IL-8 in sputum supernatants were determined by a commercial sandwich enzyme-linked immunosorbent assay set (BD Biosciences Pharmingen, San Diego, CA). The set contained pre-titred capture and detection antibodies, pre-titred streptavidin-horseradish peroxide and

lyophilized recombinant human IL-8. The sensitivity levels of the assay were 0.8 X 10^{-3} nanograms (ng) per mL of sputum.

The sputum supernantant was warmed to room temperature. The lyophilized recombinant human IL-8 standard was reconstituted with 1 mL of deionised water to yield a standard stock. The standard was allowed to equilibrate for 15 minutes and then vortexed gently to mix (Whirlymixer, Fisher Scientific, Loughborough, UK).

Anti-human IL-8 monoclonal capture antibody was diluted to 1.4 µg per mL in 0.1 molar (M) ph 9.5 sodium carbonate coating buffer (Sigma-Aldirch, St Louis). 50 µL of diluted antibody was added to each well of a microtitre plate (Sigma-Aldrich, St Louis). The plates were sealed and incubated overnight at 4^{o}C. The next morning the plate was warmed to room temperature and the wells were aspirated and washed 3 times with more than 300 µL of washing buffer, phosphate buffered saline with Teen 20 (PBS-T, Sigma-Aldrich, St Louis) per well. The plate was then inverted and blotted on absorbent paper to remove any residual buffer. The plates were then blocked with more than 200 µL of assay diluents per well and incubated at room temperature for 1 hour. The wells were again washed with washing buffer as above.

A 200 picogram (pg) per mL standard was prepared from the stock standard and vortexed to mix. 300 µL of assay diluent (phosphate-buffered saline (PBS) with 10 percent fetal bovine serum (FBS), PharMingen, San Diego) was added to 6 tubes labelled 100 pg per mL, 50 pg per mL, 25 pg per mL, 12.5 pg per mL, 6.3 pg per mL and 3.1 pg per mL. The assay diluent served as the 0 pg per mL

control. Serial dilutions were then performed by adding 300 µL of each standard to the next tube and vortexing between each transfer.

100 µL of each standard, sputum supernatant and the control was measured into each well using a pipette. This process was duplicated. The plate was then sealed and incubated at room temperature for 2 hours. The plate was then washed 5 times with washing buffer as described above.

Working detector was prepared by adding biotinylated anti-human IL-8 monoclonal detection antibody to assay diluent. Streptavidin-horseradish peroxidise conjugate enzyme reagent was then added and mixed well.

100 µL of working detector was added to each well and the plate was then sealed and incubated for 1 hour at room temperature. The plate was then washed 7 times with washing buffer as described above.

100 µL of Tetramethylbenzidine (TBM) liquid substrate (Sigma-Aldrich, St Louis) was added to each well and incubated in the dark at room temperature for 30 minutes. 50 µL of stop solution (1M phosphoric acid, PharMingen, San Diego) was added to each well to stop the colour reaction.

A Victor2 1420-018 multilabel counter microplate reader (Wallac, Massachusetts, USA) set to 450 nanometres (nm) was used to read the optical density for each well. The mean absorbance was calculated for each set of duplicate sputum supernatants and controls. The mean zero standard absorbance was subtracted from each mean. Standard curves were plotted on a log scale with a curve of best fit though the standard points.

2.1.2.5. Bacterial colony forming units

900 µL of sterile PBS was measured into 5 sterile micro-tubes using a pipette. Each micro-tube was labelled from 1 - 5. In tube 1, 100 µL of sputum filtrate was added and the lid was closed securely. The micro-tube was inverted 5 times. Serial dilutions were made by removing 100 µL of solution from micro-tube 1 transferring it to micro-tube 2. This procedure was repeated for all micro-tubes providing 5 serial dilutions. Quadrants were drawn on chocolate agar plates (Sigma-Aldirch, St Louis). Using sterile P20 tips aliquot 3, 20 µL of each concentration from micro-tubes 2 to 4 were put onto each quadrant of the chocolate agar and allowed to air dry under a class II hood. Once dried the chocolate agar plates were put into an incubator at 37^{o}C for 24 hours. The colonies were then counted from only one concentration using an inverted microscope and a tally counter.

The colony forming units (CFU) per mL were then calculated using the average all 3 aliquots using the formula below.

Mean number counted x 50 x concentration of solution = CFU per mL

2.1.3. The capsaicin cough sensitivity reflex

A stock solution of capsaicin (Sigma-Aldrich; St.Louis, MO. molar mass 305.41 g per M) was made up once a week using 10 mL of IMS and 0.003 g of capsaicin. This was stored in a fridge at 4ºC. 1 mL of this solution was removed and diluted with 1 mL of IMS and 8 mL of normal saline to give a solution with a concentration of 1000 µM per litre of capsaicin. Serial dilutions of this solution were used to produce doubling concentrations from 0.49 µM to 500 µM per litre. The dilutions were discarded after 24 hours.

An air powered dosimeter (KoKodigidoser; Pulmonary Data Services Instrumentation Inc; Louiseville, CO) was calibrated to administer a breath activated dose of 10 µL of solution and the inspiratory flow was standardised to 0.5 litres per second with an inspiratory flow regulator valve.

Subjects inhaled a single vital capacity breath of 10 µL of 0.9 percent saline solution followed by doubling concentrations of capsaicin every 1 minute. Coughs were counted for 30 seconds following dose administration. The counting of coughs was facilitated using a sound recording device. The investigation was stopped when 500 µM per litre solution was inhaled, or when the subject coughed 5 times or more. The concentration of capsaicin required to make the subject cough 2 (C2) and 5 (C5) times was calculated by the linear interpolation of the log-dose response curve.

2.2. MEASURING COUGH

2.2.1. Cough Visual Analogue Score

Subjects with respiratory disease were asked to complete cough symptom scores. These were recorded using a 100 millimetre (mm) visual analogue score. The bottom end (0 mm) represented no cough and the top end (100 mm) represented ‘worst cough ever’. Patients were able to refer to previously completed cough analogue scores.

2.2.2. Leicester Cough Questionnaire

Subjects with a cough were asked to complete the Leicester Cough Questionnaire. This is a 19 item validated quality of life questionnaire for patients with chronic cough. It assesses 3 domains (physical, psychological and

social). The total score range is 3 - 21 and domain scores range from 1 - 7; a higher score indicates a better quality of life. A modified version of the Leicester cough questionnaire was used for subjects with acute cough (Leicester Acute cough Questionnaire). Both versions of the questionnaire can be found in Appendix 1 and Appendix 2 respectively.

2.2.3. Measuring cough frequency using the Leicester cough monitor

2.2.3.1. Recording

Cough frequency was recorded using the Leicester Cough Monitor (LCM) which consists of an mp3 player/recorder (iRiver iFP-799, iRiver Europe GmbH, Eschborn, Germany) and a free-field microphone (Sennheiser MKE 2-5, Sennheiser Electronic GmbH & Co. KG, Wedemark, Germany). The subject wore the microphone around their neck and the mp3 player/recorder was housed in a small bag which the patient carried (either across their shoulder, around their waist, or on their belt). After the LCM was attached, the patient resumed normal activities and returned to the hospital 24 hours later to have the LCM taken off. The mp3 player/recorder samples all sounds at a frequency of 16Hz and with an encoding bit rate of 64kbps.

2.2.3.2. Analysing

The recording obtained was then downloaded onto a computer and analysed by the Leicester cough algorithm software, based on the pattern recognition approach followed in speech recognition software[211]. This process took approximately 60 minutes.

2.2.3.3. Training

Following initial analysis the algorithm identified sounds as cough and non-cough. A selection of these sounds were then presented to the user to be confirmed as either cough events or non-cough events. The algorithm then refined the analysis based on this user input. This process lasted approximately 5 to10 minutes per recording. Following the completion of this process the software generated a PDF report giving the total number of coughs for the recording and a bar chart showing the number and distribution of the coughs per hour

2.3. STATISTICAL ANALYSIS

All statistical analysis was performed using SPSS version 16 and Graphpad Prism version 5.

3. STUDIES

3.1. COUGH FREQUENCY IN HEALTH AND DISEASE

3.1.1. Abstract

Little is known about cough frequency in health and disease and factors associated with it.

We assessed the validity of 24-hour cough frequency measurements obtained using the Leicester cough monitor (LCM) against the gold standard of manual counting in 12 patients with respiratory diseases and 8 healthy adults. We assessed cough frequency using the LCM and other measures of cough and airway inflammation in 44 healthy adults and 78 subjects with conditions associated with or defined by chronic cough.

The validity of LCM as measured by the intra-class correlation coefficient (ICC) was 0.98 (95 % CI 0.92 - 0.99) in patients with cough and 0.85 (0.33, 0.97) in healthy individuals. The geometric mean (log SD) number of coughs per 24 hours assessed using the LCM was 18.6 (0.5) in healthy individuals and 275 (0.37) in patients with cough. Women coughed 3.5 fold (p=0.001) and 1.9 fold (p=0.01) more than men in health and disease. Cough frequency correlated modestly with other measures of cough and with the induced sputum differential neutrophil count ($r = 0.36$; $p<0.001$).

Cough frequency can be estimated accurately using the Leicester cough monitor. Cough frequency is higher in men than in women in health and disease.

3.1.2. Introduction

Cough is an important defence mechanism in healthy individuals. However, cough can also be a symptom of almost all respiratory diseases and isolated chronic cough is a common clinical problem. The European Community Respiratory Health Survey reported a prevalence of non-productive cough in a large population of young people as 8 % in non-smokers and 18 % in heavy smokers [66]. Other epidemiological surveys have reported that up to 16 % of a South East English population and 11 % of a Swedish population report a chronic cough[70,212,69].

Isolated chronic cough is a difficult clinical problem and treatment options are limited[213,214]. One difficulty has been the absence of a well validated means to assess cough objectively [2]. Recent advances in digital sound recording, battery life and voice recognition software have made 24-hour ambulatory cough monitoring and automated cough detection a possibility. The LCM uses an MP3 player/recorder, attached to a field microphone to record sound in the patient's own environment over 24 hours. This recording is then analysed using methods based on key word recognition techniques and Hidden Markov Models[186]. We have previously reported preliminary evidence that the LCM detects coughs accurately over 6 hours and that cough frequency is increased in patients with chronic cough compared to controls [215]. However, relatively few normal controls were studied and there remains uncertainty on the performance of automated cough detection systems over a 24-hour period and across the range of expected cough frequency.

We set out to assess the validity of 24-hour ambulatory cough frequency assessed using the automated LCM in two groups of subjects: adult volunteers

with no reported respiratory symptoms and had no objective evidence of respiratory disease and adult volunteers with conditions characterised by or associated with chronic cough. We also investigated factors associated with cough frequency in these groups.

3.1.3. Methods

3.1.3.1. Subjects

44 healthy volunteers were recruited from those responding to a poster advertisement. All healthy volunteers had no past or current respiratory symptoms, were non-smokers with a <5 pack year past smoking history and had normal spirometric values, methacholine airway responsiveness and induced sputum inflammatory cell counts.

78 patients with respiratory disease were recruited from respiratory clinics. Asthma was defined by consistent symptoms and objective evidence of abnormal variable airflow obstruction and/or airway hyper-responsiveness as indicated by one or more of the following: a greater than 15 % increase in FEV1 after 200 µg inhaled salbutamol and/or a provocative concentration of methacholine required to cause a 20 percent fall in FEV1 (PC20) of less than 8 mg per mL. Patients with classical asthma complained of wheeze and breathlessness as the predominant symptoms, whereas those with cough variant asthma complained of cough as the predominant symptom. Eosinophilic bronchitis was diagnosed in subjects with a chronic cough, normal spirometry, a PC20 greater than 16 mg per mL and an induced sputum eosinophil differential count of greater than 3 %. COPD was defined as a post bronchodilator FEV1/FVC of less than 70 % and a less than 15 % or 200mL (whichever greater) increase in FEV1 20 minutes after 200µg inhaled salbutamol.

Bronchiectasis was diagnosed in subjects with a consistent clinical picture and a high resolution computerised tomographic scan (HRCT) showing bronchiectatic changes. Interstitial lung disease was defined as a consistent clinical picture, restrictive spirometry and an HRCT showing abnormal interstitial shadowing. Patients with unexplained chronic cough were recruited from the Leicester Cough Clinic. All these patients had a cough for longer than 8 weeks, normal spirometry, a PC20 of greater than 8 mg per mL and a normal HRCT scan of the thorax; subjects had failed treatment trials with a proton pump inhibitor and/or a nasal steroid spray given as per British Thoracic Society guidelines[2]. Six current smokers and four men taking angiotensin converting enzyme inhibitors (ACEi) were also recruited. Our main motive for including these subjects was to assess the effect of these factors on cough frequency in asymptomatic patients and none reported chronic cough at the time of assessment. With the exception of 2 patients with ILD, 2 patients with asthma and 2 patients with COPD where there was clinical concern about discontinuing therapy, all assessments were done in patients who were taking no prophylactic treatment for at least 2 weeks. The study was approved by the Leicestershire, Northampton and Rutland Research Ethics Committee.

3.1.3.2. Materials

All volunteers underwent spirometry on the first visit. This was performed with a Vitalograph spirometer (Vitalograph, Buckinghamshire, UK) as the best of at least 3 successive readings within 100mL. In patients with an FEV_1/FVC of less than 0.7 spirometry was repeated 15 minutes after inhaling 200µg of salbutamol via a volumatic device; those with normal spirometry had a methacholine inhalation test using the tidal breathing method[216].

The LCM (iRiver iFP-799 mp3 device, iRiver Europe GmbH, Eschborn, Germany and Sennheiser MKE 2-5 field microphone, Sennheiser electronic GmbH & Co. KG, Wedemark, Germany)[211] was then attached. The subject wore the microphone around their neck and the mp3 device was housed in a small bag which the patient carried (either across their shoulder, around their waist or on their belt). After the LCM was attached subjects were encouraged to resumed normal activities. Audio was recorded using a sampling frequency of 16Hz and saved in mp3 format with an encoding bit rate of 64kbps. All volunteers attended the hospital again 24 hours later when the LCM was removed.

The recording obtained was downloaded onto a computer and analysed by the Leicester cough monitor software. The software is based on hidden Markov models which are used in speech recognition software. The basis of this is that cough sounds can be recognised by a distinct spectral pattern, however cough sounds between individual subjects and disease states can have a different spectral pattern. The software algorithm has been trained on over 300 different cough sounds and over 400 non-cough sounds therefore enabling it to analyse 24 hour recordings and identify all sounds as either cough or non-cough[186]. This process takes approximately 60 minutes. A selection of these sounds are then presented to the operator to be confirmed as either cough events or non-cough events. The algorithm then refines the analysis based on this operator input. This process takes approximately 5 to 10 minutes per recording. Following completion of this process the software generates a PDF report giving the total number of coughs for the recording and a bar chart showing the

number and distribution of the coughs per hour. A separate file with the time stamps for cough events is also generated.

The validity of the Leicester cough algorithm was assessed by randomly selecting 20 recordings [8 healthy volunteers, 12 patients with respiratory disease (UCC n=6, asthma n=3, EB/CVA n=2, COPD n=1)] which were analysed manually first by the same blinded observer and then by the Leicester cough algorithm. Manual analysis was done using a method we have previously shown to result in highly reproducible cough counts [215]; it involved uploading the mp3 file onto a computer and converting it into an uncompressed sound format (WAV). The recording was then played at double speed in 10 second segments while viewing the signal waveform on the screen. When a sound thought to be a cough was heard, it was played back at normal speed. Each individual cough sound was counted as one cough. Other non-cough sounds were categorised and counted as described before[217].

A capsaicin cough challenge was performed after removal of the cough monitor. Subjects inhaled a single vital capacity breath of 10µL of 0.9 percent saline solution followed by doubling concentrations of capsaicin from 0.49µM per litre every 1 minute. Coughs were counted for 30 seconds following each inhalation. The counting of coughs was aided by a sound recording device. The investigation was stopped when 500 µM per litre solution was inhaled, or when the subject coughed 5 times or more. The concentration of capsaicin required to make the subject cough 2 (C2) and 5 (C5) times was calculated by the linear interpolation of the log-dose response curve[96].

Sputum was induced and processed using nebulised hypertonic saline and the sputum selection protocol as described before[218]. Patients with respiratory diseases completed the Leicester Cough Questionnaire[157] (LCQ), a 19 item validated quality of life questionnaire for patient with chronic cough. It assesses 3 domains (physical, psychological and social). The total score range is 3-21 and domain scores range from 1-7; a higher score indicates a better quality of life. Patients also completed a 100mm cough Visual Analogue Score set at the bottom end by no cough and the top end represents the worst cough ever.

3.1.3.3. Statistical analysis

Cough numbers per 24 hours, C2 and C5 were log normally distributed and log transformed prior to analysis. An independent-sample t-test was used to compare means between groups. Agreement was assessed by the Intra-class correlation coefficient. Correlations between variables were analysed using the Pearson correlation coefficient for normally distributed data and Spearman's rank correlation coefficient for non-parametric data. The upper limit of normal cough frequency was calculated as antilog [log mean + (1.64 x log SD)]. Statistical analysis was performed using SPSS version 16.

3.1.4. Results

Demographic details of all subjects are shown in TABLE 10. Patients reported that the cough monitor was easy to wear and did not interfere with ambulation, daily tasks or with sleep.

3.1.4.1. Validation

The geometric mean (log SD) manual and Leicester cough algorithm counts in 12 patients with conditions associated with cough and 8 healthy volunteers was

467 (0.32) vs. 446 (0.32) and 16 (0.7) vs. 22 (0.4) respectively (FIGURE 1). The mean (95% CI) fold difference in manual and automated counts was 0.9 (0.8, 1.1; p= 0.4) and 1.4 fold (0.7, 3; p=0.3.) respectively. There was stronger agreement as assessed by the intra-class correlation coefficient (ICC) between log cough frequency assessed manually and using the automated system in patients with cough (0.98, 95 percent CI 0.92 - 0.99) than in healthy volunteers (0.85, 95% CI 0.33, 0.97). The sensitivity and specificity of the automated system in patients was 83.8 percent and 99.9 percent in patients with cough and 82.3 percent and 99.9 percent in healthy volunteers. The geometric mean false positive rate was 0.3 per hour in healthy volunteers and 2 per hour in patients with cough.

In healthy adults the geometric mean (log SD) number of cough sounds in 24 hours was 18.6 (0.5) (FIGURE 2). Women coughed more than men [geometric mean (SD) 29.5 (0.4) vs 8.3 (0.5); mean difference 3.5 fold; 95% CI 1.9, 6.8; $p < 0.001$]. The upper limit of normal 24-hour cough frequency was 55 in men and 133 in women. There was no correlation between 24-hour cough frequency and C2 ($r = -0.08$) or C5 ($r=-0.03$) (FIGURE 3). Day time cough counts (0800hrs to 2200hrs) were higher than night time cough counts and hourly cough frequency tended to be higher in early morning and mid-afternoon (FIGURE 4). Cough frequency was not related to body mass index ($r=0.08$) or age ($r=0.12$).

In adults with conditions associated with or characterised by cough, the geometric mean (log SD) number of cough sounds detected using the automated system in 24 hours was 275 (0.37),15.8 fold (95% CI 9.7, 21.9; $p < 0.001$) greater than healthy controls. Geometric mean (log SD) cough frequency

C2, C5 and the mean (SD) cough VAS and LCQ score by group were as shown in TABLE 10.

Women with conditions associated with or characterised by cough coughed more than men [381 (0.43) vs. 198 (0.4); mean difference 1.9 fold; 95% CI 1.1,3.2; p=0.012]. Cough frequency was not different in the 6 current smokers (TABLE 10, FIGURE 2) but was significantly higher in the four men taking ACEi compared to healthy men [geometric mean (SD) 128 (0.2) vs 8.3 (0.5); mean fold difference 15.7 fold; 95% CI 4.2, 58.7; p<0.001;FIGURE 2]. There was a significant correlation between the 24-hour cough frequency and the cough VAS (r = 0.66; p<0.001), C2 (r=-0.35; p<0.001), C5 (r=-0.5; p<0.001) and the Leicester Cough Questionnaire (r= -0.44; p=0.001; FIGURE 3). In the group with respiratory disease the cough frequency by hour followed a similar pattern to that seen in healthy controls (FIGURE 4).

FIGURE 1. BLAND-ALTMAN PLOT OF AUTOMATED AND MANUAL 24-HOUR COUGH COUNTS IN 8 HEALTHY CONTROLS AND 12 PATIENTS WITH CHRONIC COUGH

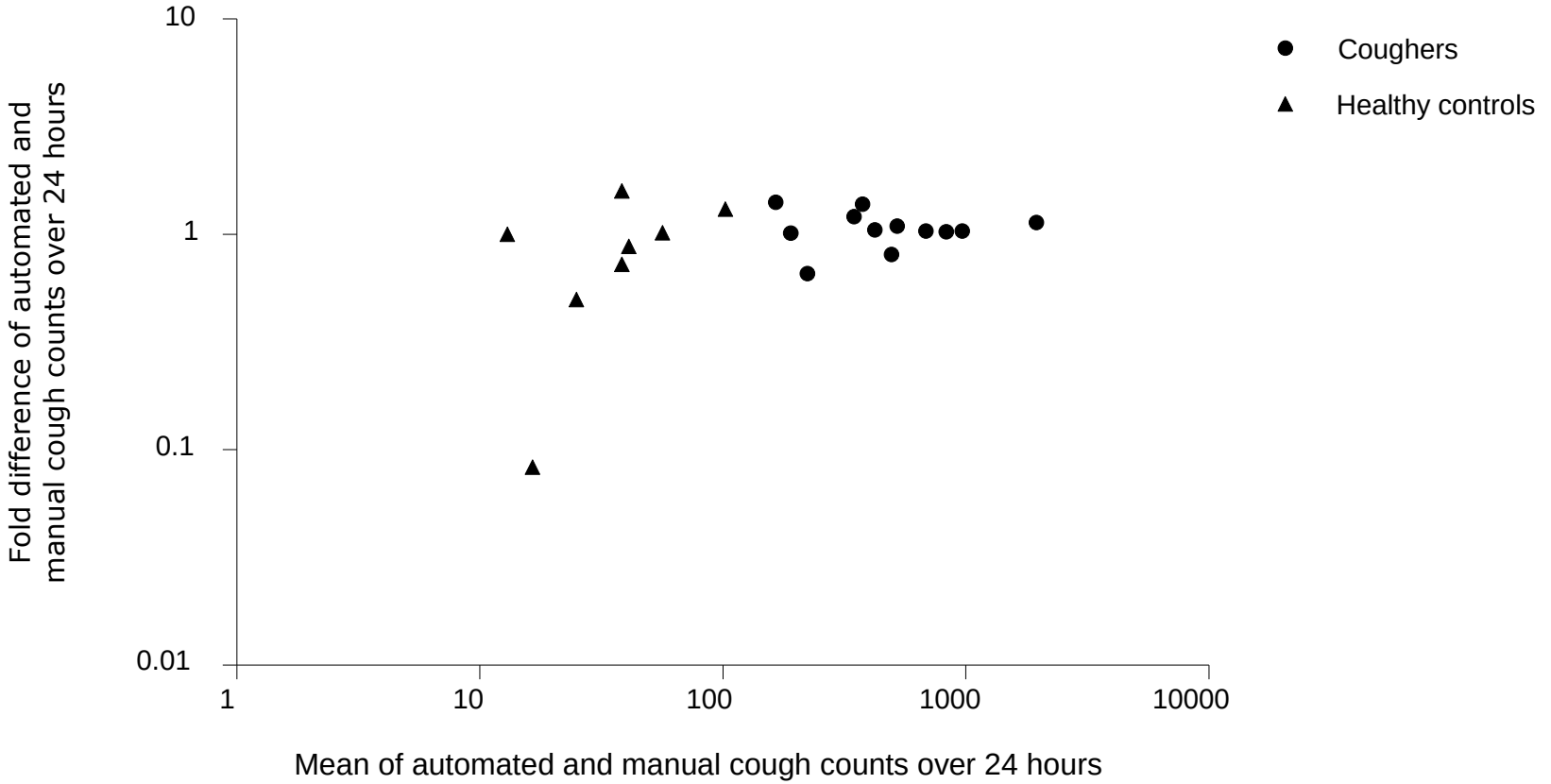


TABLE 10. DEMOGRAPHIC DETAILS OF SUBJECTS

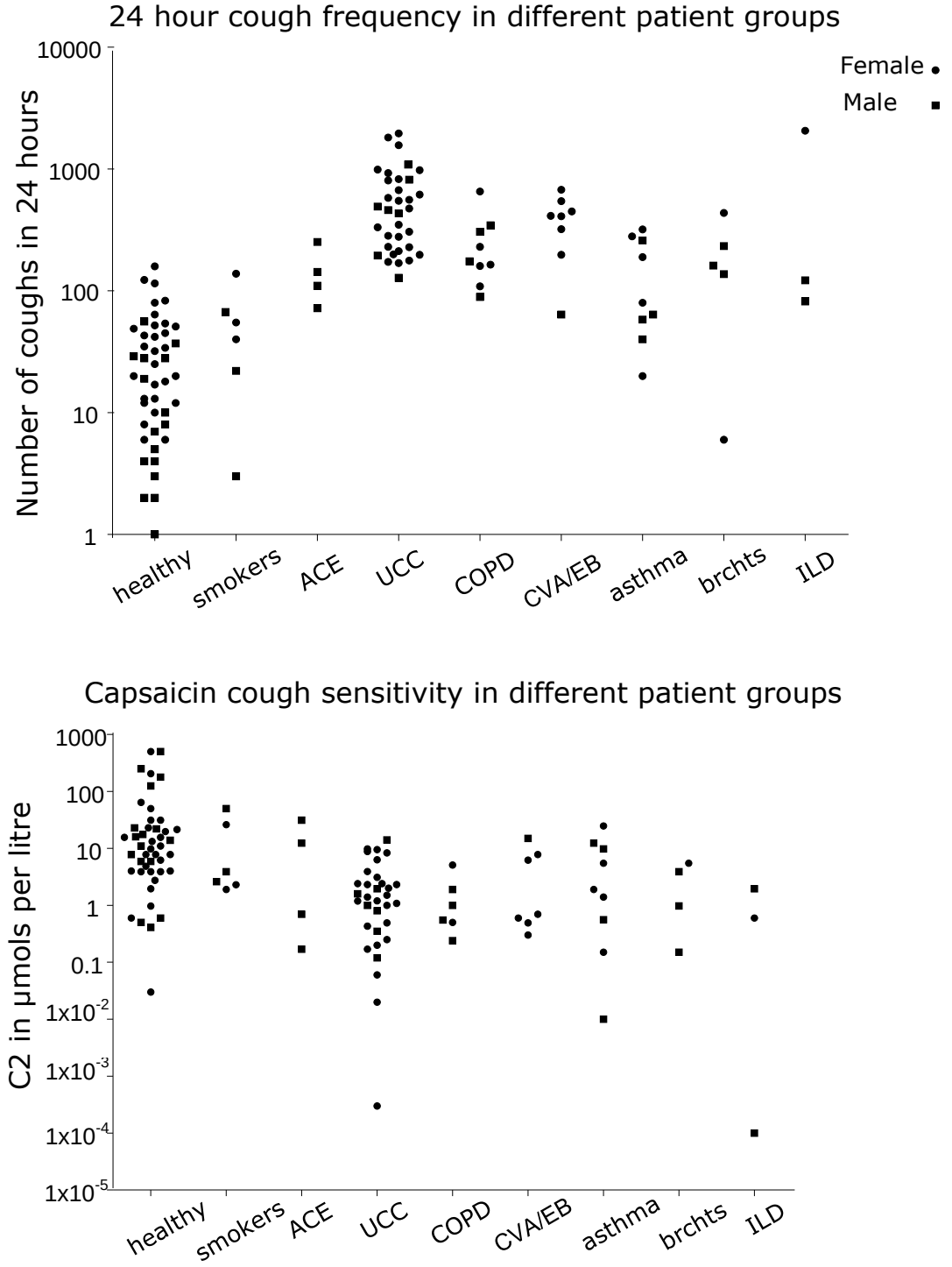
	Healthy	Smokers	On ACEi	UCC	CVA/EB	COPD	Brchts	Asthma	ILD
Number (female)	44 (27)	6 (3)	4 (0)	34 (27)	8 (7)	9 (5)	5 (2)	9 (5)	3 (1)
Median age (range)	55 (20, 80)	40 (23, 55)	64 (37, 80)	60 (46, 80)	64 (43, 68)	68 (59, 80)	65 (59, 69)	52 (22, 77)	54 (35, 67)
BMI*	26 (6)	23 (4)	28 (3)	27 (4)	34 (8)	23 (4)	28 (4)	31 (5)	30 (3)
FEV1 percent predicted*	99 (14)	93 (11)	102 (19)	91 (10)	96 (16)	64 (16)	63 (33)	85 (12)	87 (10)
FEV1/FVC*	80 (5)	81 (5)	80 (5)	76 (6)	80 (6)	56 (13)	68 (21)	74 (6)	87 (10)
Sputum eosinophils^ (%)	0.4 (0.6)	0.4 (0.1)	1.3 (1.2)	0.5 (0.5)	0.5 (0.7)	0.7 (0.5)	0.4 (0.7)	0.2 (0.6)	0.5 (0.7)
Sputum neutrophils (%)	49 (29)	76 (16)	75 (22)	72 (15)	60 (26)	59 (28)	84 (14)	63 (19)	66 (11)
C2 (μmol/L)^	8.8 (0.8)	6.2 (0.6)	2.6 (1.1)	1.3 (0.5)	1.5 (0.8)	0.6 (0.5)	1.3 (0.7)	1.5 (1.1)	0.1 (2.3)
C5 (μmol/L)^	85.7 (1.2)	26.3 (0.6)	61.7 (2.1)	3.6 (0.4)	6.1 (0.9)	2.4 (0.8)	6.4 (0.5)	15.7 (0.8)	1.9 (0.5)
LCQ*				11.4 (3.9)	8.2 (3.7)	12.9 (3.6)	14.1 (5.8)	11.5 (5.3)	14.2 (7.1)

	Healthy	Smokers	On ACEi	UCC	CVA/EB	COPD	Brchts	Asthma	ILD
VAS (mm)*				52 (4)	45 (12)	40 (6)	30 (9)	23 (5)	53 (19)
Total coughs/24 hours^	18.6 (0.5)	33 (0.6)	128 (0.2)	477 (0.3)	321 (0.3)	213 (0.3)	106 (0.7)	107 (0.3)	274 (0.8)

*Mean (SD), ^geometric mean (log SD), UCC = unexplained chronic cough, CVA = cough variant asthma, EB = eosinophillic bronchitis, COPD = chronic obstructive pulmonary disease, Brchts = bronchiectasis, ILD = interstitial lung disease, ACEi = angiotensin converting enzyme inhibitor, VAS = cough visual analogue score, LCQ = Leicester Cough Questionnaire, C2 = concentration of capsaicin required to elicit 2 coughs, C5 = concentration of capsaicin required to elicit 5 coughs.

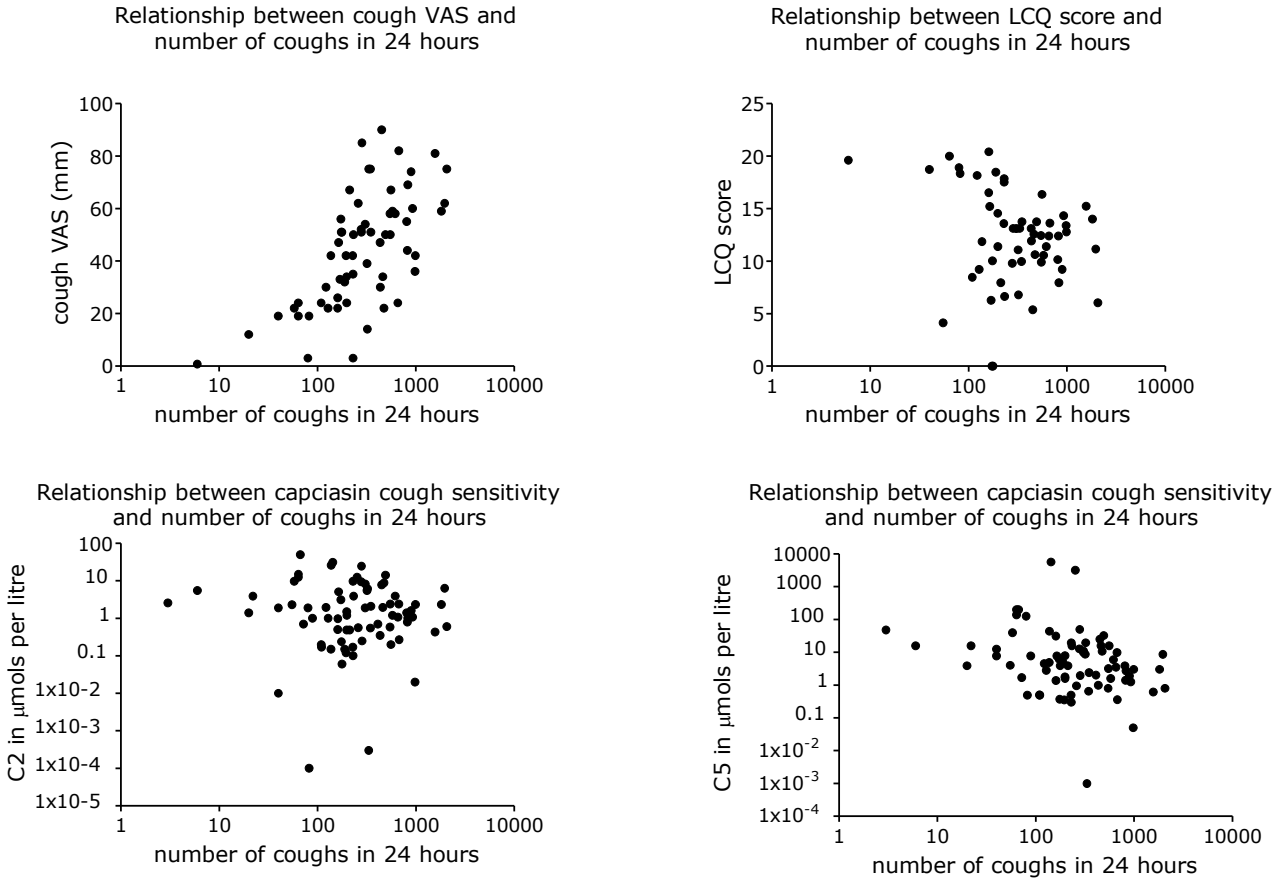
The LCQ is a 19 item validated quality of life questionnaire for patients with chronic cough. It assesses three domains (physical, psychological and social). The total score range is 3-21; a higher score indicates a better quality of life. The cough VAS is a 100mm analogue score set at 0 and 100 by ‘no cough’ and ‘worst cough ever’ respectively.

FIGURE 2. 24-HOUR COUGH FREQUENCY AND CAPSAICIN COUGH SENSITIVITY



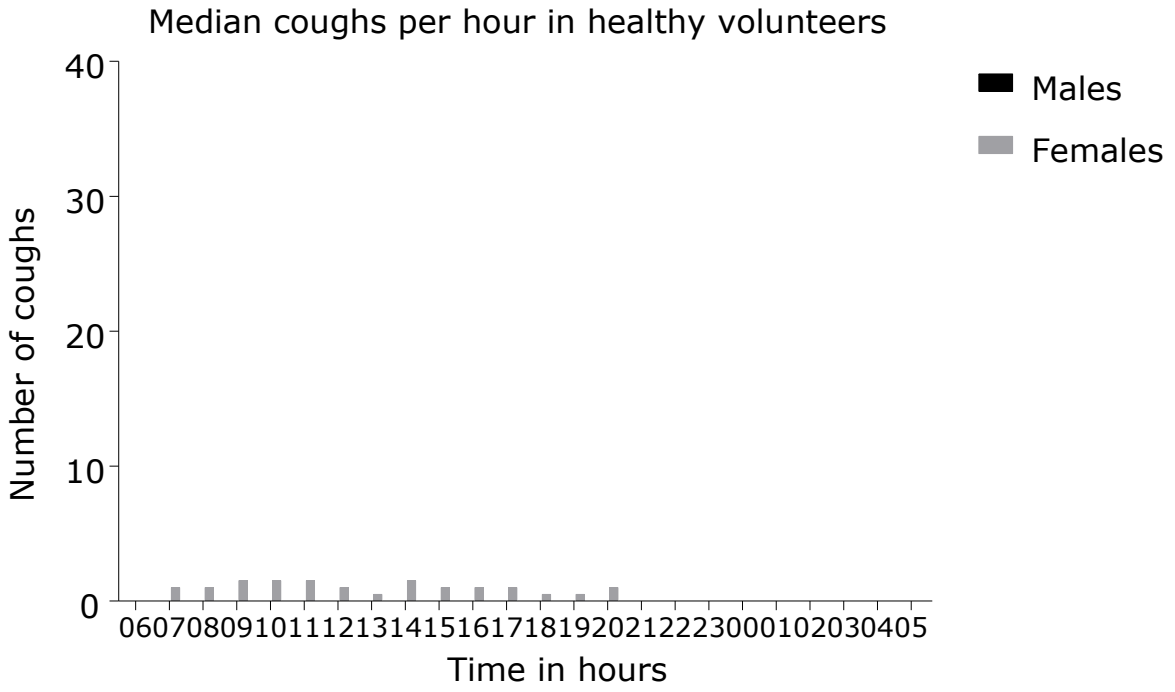
ACE = angiotensin converting enzyme inhibitor, UCC = unexplained chronic cough, COPD = chronic obstructive pulmonary disease, CVA = cough variant asthma, EB = eosinophillic bronchitis, brchts = bronchiectasis, ILD = interstitial lung disease,

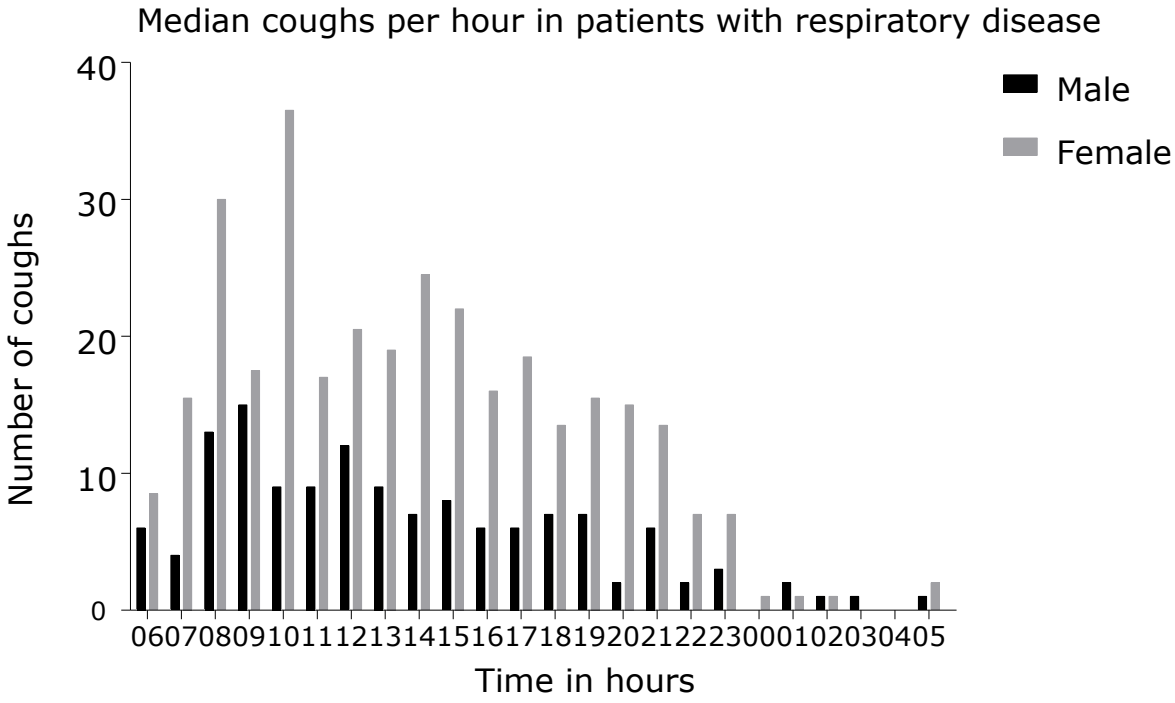
FIGURE 3. RELATIONSHIP BETWEEN 24-HOUR COUGH FREQUENCY AND COUGH VISUAL ANALOGE SCORE (VAS), LECIESTER COUGH QUESTIONAAIRE (LCQ), THE CONCENTRATION OF CAPSIACIN CAUSING 2 COUGHS (C2) AND 5 COUGHS (C5)



C2 = concentrations of capsaicin require to produce 2 coughs, C5 = concentration of capsaicin required to produce 5 coughs, VAS = visual analogue score, LCQ = Leicester Cough Questionnaire. 24-hour cough count, C2 and C5 expressed as geometric mean. Neutrophils, cough VAS and LCQ expressed as mean

FIGURE 4. MEDIAN NUMBER OF COUGHS PER HOUR





There was no difference in the diurnal pattern of coughs by disease (data not shown). In all subjects log cough frequency was significantly associated with sputum neutrophil count [r = 0.36; p<0.0001] and there was an inverse association between log C2 and sputum neutrophil count [r = -0.215; p 0.047].

3.1.5. Discussion

The results of our more extensive validation of the Leicester cough algorithm for ambulatory 24-hour recordings are consistent with our previously reported validation of 6 hour daytime cough numbers. The overall sensitivity of the automated system is 82 to 84 % and the specificity over 99 %. The large difference in cough frequency in health and disease, the ability of the system to detect expected differences in cough frequency and the correlation seen with other measures of cough severity suggest that the automated system produces valid and potentially clinically useful data. One important caveat is that automated cough analysis inevitably results in false positive detection of cough like sounds. This may lead to important bias and over estimation of cough frequency, particularly when the cough frequency is low. This potential over estimation of cough frequency may be a bigger issue in healthy men than women due to the very low 24 hour cough frequency demonstrated in healthy men in this study. Consistent with this, we found less close agreement between automated and manual counts in the healthy volunteers compared to patients with chronic cough and a tendency for cough counts to be higher when assessed using the automated system. In contrast, in patients with chronic cough the automated detection system had good measurement characteristics. This was probably because the false positive cough rates were low in relation to the total number of coughs. Further work is required to determine whether it is

possible to increase the sensitivity of the system without impacting on the false positive rates. Until then, some caution is required when using the Leicester Cough Monitor when the anticipated cough frequency is low.

We have made the novel observation that healthy adults cough on average 18.6 times in 24 hours, mostly during the day and that the upper limit of normal cough frequency is 55 in men and 133 in women Patients with conditions associated or characterised by cough have a 15.8 fold increase in 24-hour cough frequency but the diurnal pattern of coughing was similar and we found no evidence of a disease specific alteration in this pattern. Conditions characterised by chronic cough (i.e. cough variant asthma, eosinophilic bronchitis and unexplained chronic cough) were associated with a higher cough frequency than those associated with chronic cough.

Our estimation of normal cough frequency is similar to that made by Loudon in 1967. He counted 2.5 coughs per minute in a lecture theatre containing 100 people[54]. The findings of more limited ambulatory cough monitor studies are also consistent with ours: Hsu et al[55] found that cough frequency was between 0 and 16 per 24 hours in a group of 12 healthy individuals; Marsden et al[57] reported 0.4 cough seconds per hour in 19 healthy controls with a median age of 43; and we[219] reported 2 coughs per hour over a six hour daytime recording in 9 healthy patients who had 6 hour daytime cough recordings using the LCM.

This is the largest series of healthy control subjects tested, providing us with the opportunity to begin assessing the effect of factors potentially related to cough frequency. Age and body mass index were not associated with cough frequency but women coughed more than men. Our findings on gender

influence on cough frequency are consistent with findings of a study of manually counted ambulatory 24-hour cough frequency in 86 subjects with chronic cough[220] and with compelling evidence from a number of studies showing that women have a more sensitive cough reflex than men[150, 149, 221, 96]. Collectively, these findings provide a potential explanation for the over-representation of women in cough clinics[222]. Chronic cough is particularly prevalent in menopausal women[213], but we found no evidence of age related change in 24-hour cough frequency or in cough reflex sensitivity in women.

We did not show a significant increase in cough frequency in healthy smokers. Our power to show such an effect was limited and larger studies are required to address this interesting question. These studies need to be appropriately designed to detect the healthy smoker effect. There was a significant increase in cough frequency in men taking ACE inhibitors, a finding that is consistent with considerable evidence that ACE inhibitors cause cough and a increase sensitivity of the cough reflex[73]. This is unlikely to be a selection bias due to concerned individuals being more likely to volunteer to participate in a cough study as none of these volunteers reported cough. Our finding raise the possibility that ACE inhibitors cause an increase in cough frequency in all healthy subjects.

Our estimation of cough frequency in respiratory diseases is broadly similar to findings of cough frequency counted manually in asthma[57], chronic cough[223] and COPD[224], although comparison across studies is not straightforward because of differences in methods used to quantify chronic cough. Notable findings include the similarity in diurnal pattern of coughing between health and disease and the exceptionally high cough frequency in patients with

unexplained chronic cough. Our primary motive for studying patients with respiratory disease was to further validate our cough detection system, not to explore differences in cough frequency or the pattern of cough frequency between disease categories. The numbers studied were small and larger studies are required to address these questions. Cough frequency peaked in the early morning and there was a second smaller peak in the late afternoon. We have not investigated the factors responsible for this pattern of cough frequency during the day; one possibility is that coughing is stimulated by eating.

We found moderate correlations between 24-hour cough frequency and other objective and subjective measures of cough severity, consistent with earlier work with manual cough counts[223, 225, 224, 57] and with more limited day time automated counts[219]. Interestingly, 24-hour cough frequency was clearly different in healthy controls and patients with respiratory disease, whereas there was considerable overlap between the groups in capsaicin cough reflex sensitivity. This implies that cough in disease is a function of an increased cough reflex and other potentially disease-specific factors. We found a correlation between sputum neutrophil count and cough frequency across the whole population, suggesting that neutrophilic airway inflammation may be one factor. We acknowledge that we have performed multiple correlations and this finding could have arisen by chance. However, similar correlations between cough frequency and sputum neutrophil counts have been described in children with mild asthma[226] and primary ciliary dysfunction[227] and there is evidence of an increase in sputum neutrophil numbers in patients with chronic cough compared with normal healthy controls[138, 228]. Finally, pro-tussive mediators

associated with neutrophilic airway inflammation have been shown to be present in increased concentration in the airway of patients with chronic cough[229]. Our findings and these earlier demonstrations of a correlation between cough frequency and the sputum neutrophil count do not prove a causal link and the possibility that neutrophilic airway inflammation is a function of rather than a cause of excess coughing cannot be discounted[138].

In conclusion, we have shown that the Leicester Cough Monitor detects cough automatically with good accuracy in patients with high cough frequency but it may over count low cough frequency. Healthy volunteers cough less than once an hour during the day and very rarely at night. Cough frequency is higher in women than men but is not influenced greatly by age or BMI. Conditions associated with and characterised by cough are associated with a marked increase in cough frequency but no change in the diurnal pattern of coughing. There is a much bigger difference between health and disease in cough frequency than cough reflex sensitivity, implying that other factors contribute to excess coughing.

3.2. LONG TERM LOW DOSE ERYTHROMYCIN IN UNEXPLAINED CHRONIC COUGH: A RANDOMISED DOUBLE BLIND PLACEBO CONTROLLED TRIAL

3.2.1. Abstract

Unexplained chronic cough is a common condition with no satisfactory treatments. Previous work has suggested that cough may be linked to neutrophilic airway inflammation. We have tested the hypothesis that long term low dose erythromycin reduces the induced sputum neutrophil count and 24-hour cough frequency in patients with unexplained chronic cough.

30 patients with an unexplained chronic cough lasting more than 8 weeks were randomised to take 250mg of erythromycin once daily (n=15) or placebo (n=15) for 12 weeks in a double blind parallel group study. Cough frequency, cough reflex sensitivity and cough severity were assessed at baseline, 6, 12 and 24 weeks. The primary outcome measure was change in 24-hour cough frequency at 12 weeks.

There was no statistically significant difference in the change in cough frequency between the erythromycin and placebo groups at 12 weeks [mean difference in fold change 1.1; 95% CI 0.7 to 1.5; p=0.585] or at other times. There was a statistically significant between treatment difference in the change in sputum neutrophils at 12 weeks (-10.2 vs +6.6 percent with erythromycin and placebo; mean difference 16.8 percent; 95% C I 1.6 to 32.1; p=0.03) but not at other times. There was no difference in the change in other measures of cough between treatments.

Treatment with low dose erythromycin for 12 weeks reduces the induced sputum neutrophil count but not cough frequency or severity in patients with unexplained chronic cough.

3.2.2. Introduction

No cause is found for chronic cough in up to 40 percent of patients presenting to specialist cough centres[222,230,231]. Patients with unexplained chronic cough remain a challenge to manage, as treatment options are limited. Patients are mainly women and they have a higher than expected prevalence of organ specific autoimmune disease; many report the onset of cough around the menopause[134,167,222]. Quality of life studies have shown that in some domains of a generic quality of life score, patients with unexplained chronic cough suffer from impairment equivalent to that seen in patients with severe COPD[168].

Cross sectional studies have shown that patients with unexplained chronic cough, cough on average 477 times per 24 hours, 13 times more frequently that controls[215]. They have an increased cough reflex to inhaled capsaicin, an induced sputum neutrophilia and raised concentration of mediators associated with neutrophilic airway inflammation, including IL-8, TNFα and PGE_2[96,138,139]. We have previously noted a significant, independent association between the induced sputum neutrophil count and 24-hour cough frequency[232], suggesting that there may be a causal link between neutrophilic airway inflammation and cough.

Long term low dose macrolides have been used successfully to treat respiratory conditions associated with neutrophilic inflammation of the airways and have been shown to reduce the induced sputum neutrophil count[233-235]. We

conducted a randomised double blind placebo control study to test the hypothesis that 250mg of erythromycin given for 3 months reduces neutrophilic airway inflammation and 24-hour cough frequency in patients with unexplained chronic cough.

3.2.3. Methods

3.2.3.1. Subjects

Patients with a chronic cough lasting greater than 8 weeks were recruited from consecutive consenting patients attending the Leicester cough clinic from May to November 2008. They had normal spirometry, a provocative concentration of methocholine required to cause a 20 percent fall in forced expiratory volume in one second (FEV_1) of greater than 8 mg per mL, a normal induced sputum eosinophil count and a normal high resolution CT scan of the thorax. All subjects had failed treatment trials with a proton pump inhibitor and a nasal steroid spray of 2 months or more as per British Thoracic Society guidelines[2].

Exclusion criteria were current smokers or past smokers with a greater than10 pack year history, those with a history of intolerance to macrolide antibiotics and pregnant or breastfeeding women. No patients took any specific therapy for cough during the study. Written consent was obtained from all patients and the study was approved by the Leicestershire, Northamptonshire and Rutland research ethics committee and the Medicines and Healthcare Regulatory Authority. The international standard randomised control trial number (ISRCTN) for this study is ISRCTN75393495.

3.2.3.2. Study Design

Patients attended for two initial baseline visits. Spirometry was performed on the first visit. The Leicester Cough Monitor was then attached and the patients were asked to return 24 hours later. On the second visit the Leicester Cough Monitor was removed after which the patients underwent a capsaicin cough challenge, sputum induction and completed the Leicester Cough Questionnaire and a cough visual analogue score. They were then randomised to receive 250mg of erythromycin stearate or placebo (lactulose) as identical capsules once daily for 12 weeks in a double blind parallel group study. Baseline measurements were repeated at the same time of day, 2 hours after ingestion of the study drug at 6, 12 and 24 weeks. The study measurements are outlined in Chapter 2 (Methods). .

Trial medications were concealed in identical capsules. Randomisation was carried out prior to commencement of the study by pharmacy independent of blinded trial staff. Randomisation was in blocks of 4 (2 erythromycin/2 placebo) and numbers from 1 to 6 from a geigy random number generating table. Patients were automatically dispensed the next allocated treatment.

3.2.3.3. Analysis

Cough frequency, C2, C5, sputum eosinophil differential count and sputum supernatant IL-8 concentrations were log transformed to assume normality prior to analysis. Our primary endpoint was a change in log 24-hour cough frequency from baseline to 12 weeks as we anticipated that any drug effect would be greatest at this time. Secondary endpoints were changes in induced sputum neutrophils differential cell count, sputum bacterial colonies, C2, C5, cough VAS and LCQ. Mixed design ANCOVA with sidak's correction[236] was

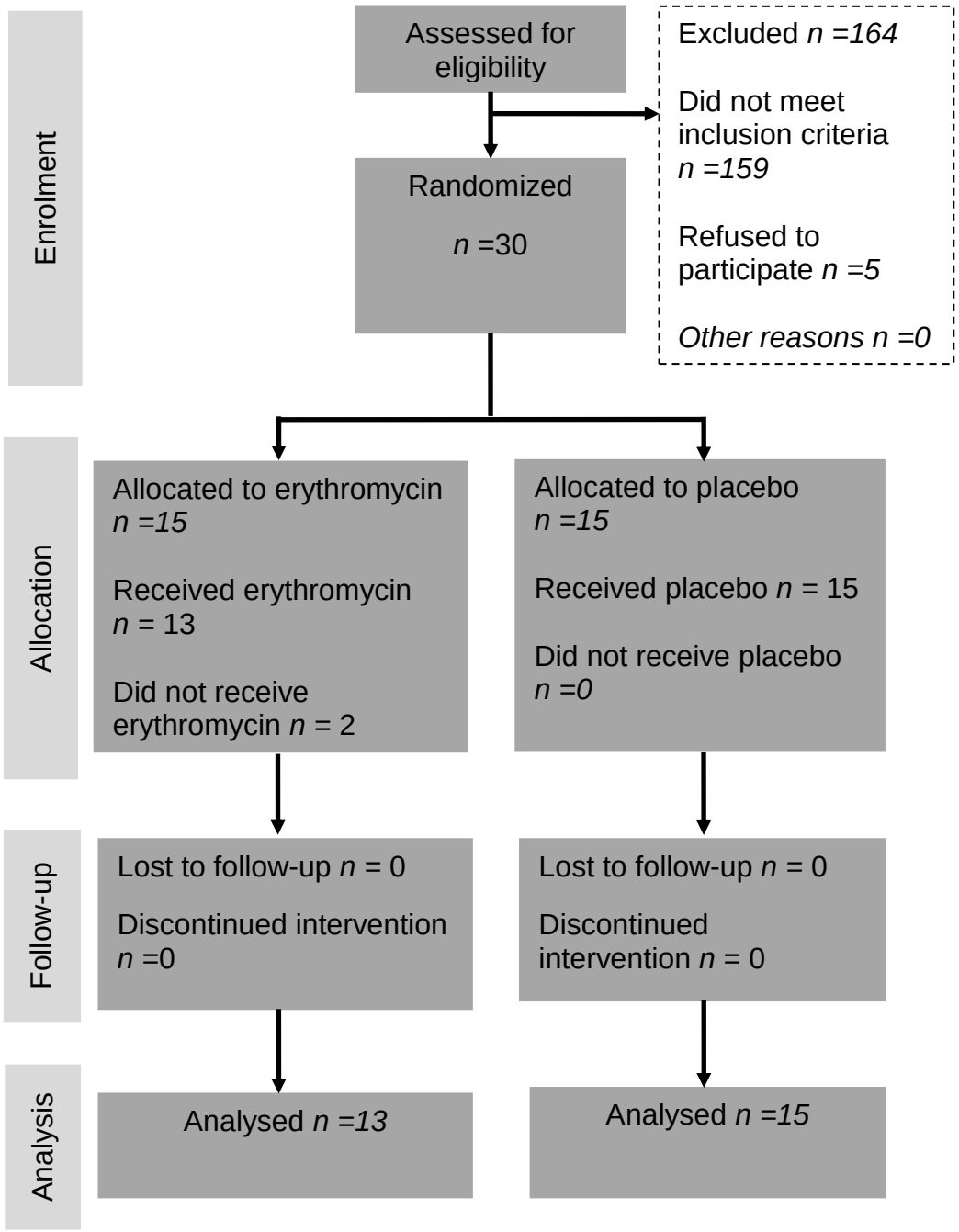
used to compare results with the pre-treatment result used as the covariate to account for baseline differences. We performed an intention to treat analysis; subjects withdrawing after randomisation were assigned post-treatment values using the last value carried forward. Our original target recruitment population was 20 patients per treatment arm. This gave us 80 percent power to detect a 50 percent reduction in 24-hour cough frequency ($\alpha = 0.05$) with active treatment compared to placebo based on our previous data showing a mean log 24-hour cough frequency of 2.68 and a within subject log standard deviation of 0.3. Statistical analysis was performed using SPSS version 16.

3.2.4. Results

35 consecutive patients diagnosed with unexplained chronic cough attending the Leicester cough clinic were approached between May and November 2008. Thereafter recruitment had to be closed because the supplier of erythromycin and placebo ceased production of matched placebo. 30 patients consented to take part in the study and were randomised (erythromycin n=15, placebo n=15). 2 patients withdrew from the study due to personal reasons. 28 patients completed the study (erythromycin n=13, placebo n=15; FIGURE 5).

Patients' baseline demographic information is shown in TABLE 11. Coughs per 24 hours were statistically significantly higher at baseline in the placebo group compared to the erythromycin group. All other baseline characteristics were comparable in both groups. Induced sputum samples were obtained in 76 out of 90 samples (84 %).

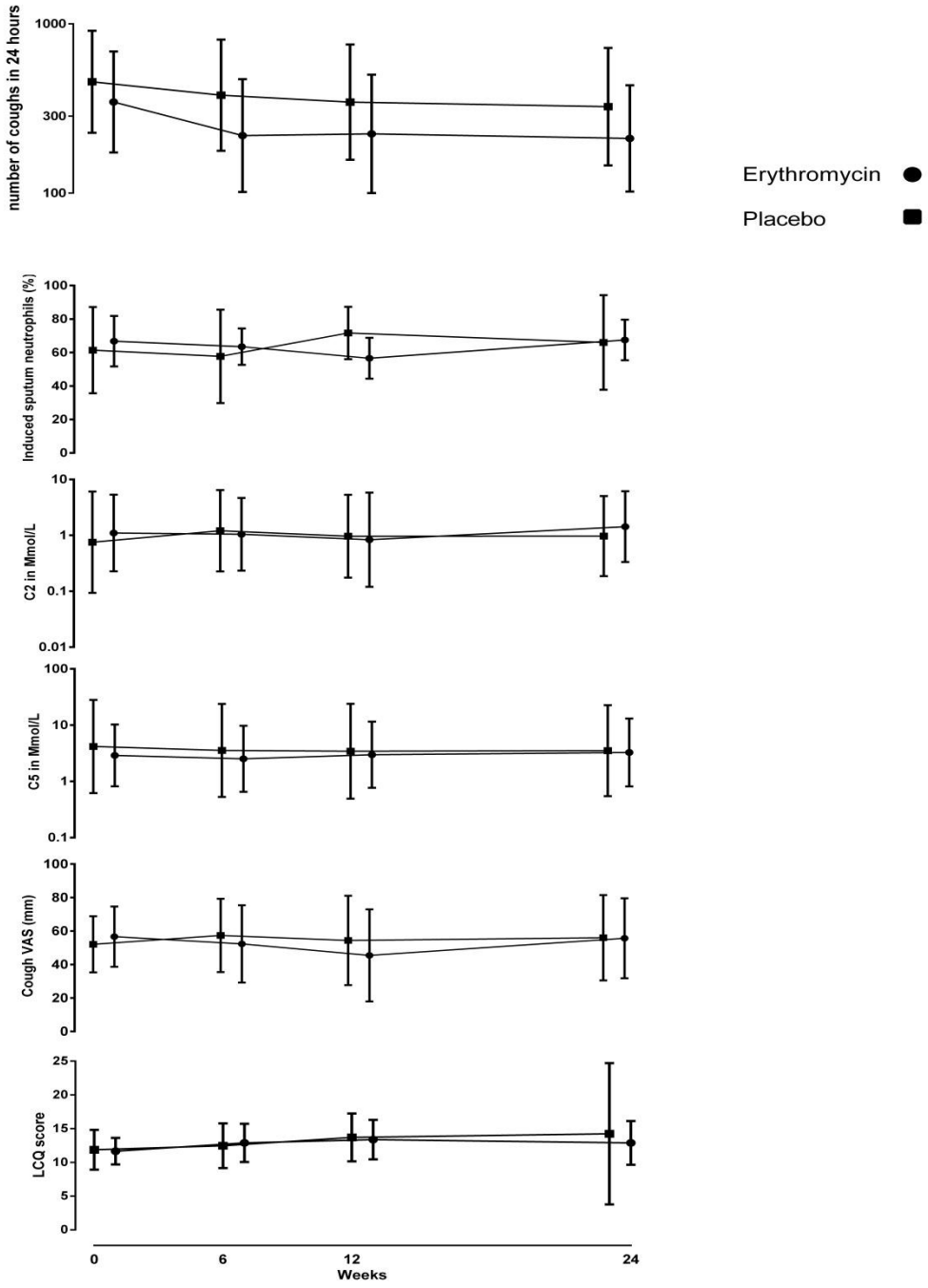
FIGURE 5 PATIENT FLOW WITH ERYTHROMYCIN OR PLACEBO TREATMENT



All patients who completed the study showed greater than 95 percent compliance with the treatment prescribed. 2 patients in the placebo group reported abdominal discomfort at the 6 week visit which resolved by the 3 month visit. 1 patient in the erythromycin group reported dizziness at the second visit that resolved within a week.

Results are shown in TABLE 12. Geometric mean 24-hour cough frequency at baseline and 12 weeks was 536 and 390 (mean fold difference 0.7; 95% CI 0.6 to 0.9) with placebo and 353 and 243 (mean fold difference 0.7; 95% CI 0.5 to 1.0) with erythromycin. After adjusting for baseline differences there was no statistically significant difference in the change in cough frequency between placebo and erythromycin groups at 12 weeks [mean difference in fold change 1.1; 95% CI 0.7 to 1.5; p=0.585] and no difference at other times (FIGURE 6). The induced sputum neutrophil count reduced from 67.8 to 56.6 percent after 12 weeks treatment with erythromycin. There was a statistically significant between treatment difference in the change in sputum neutrophils at 12 weeks (-10.2 vs +6.6 percent with erythromycin and placebo respectively; mean difference 16.8 percent; 95% C I 1.6 to 32.1; p=0.03) but no difference at other times (TABLE 12, FIGURE 6).

FIGURE 6. CHANGE IN NUMBER OF COUGHS IN 24 HOURS, INDUCED SPUTUM NEUTROPHILS, CAPSIAICIN COUGH SENSITIVITY, COUGH VISUAL ANALOGUE SCORE AND LECIESTER COUGH QUESTIONNAIRE OVER 24 WEEKS.



C2 = concentrations of capsaicin require to produce 2 coughs, C5 = concentration of capsaicin required to produce 5 coughs, VAS = visual analogue score, LCQ = Leicester Cough Questionnaire. 24-hour cough count, C2 and C5 expressed as geometric mean. Neutrophils, cough VAS and LCQ expressed as mean.

TABLE 11. MEAN (SD) BASELINE CHARACTERISTICS

	Erythromycin (n=15)	Placebo (n=15)
Number of females	13	11
Age (years)	63 (9)	61 (9)
Duration of cough (years)	12 (4.2)	11.1 (4.8)
Body mass index (kg/m^2)	27.5 (4.6)	26.1 (4.9)
Percentage predicted FEV1	95 (10)	96 (10)
FEV1/FVC	0.77 (0.07)	0.76 (0.04)
^Number of coughs in 24 hours	389 (0.33)	525 (0.33)
Total cells /g of selected sputum	$3.3x10^6$ ($1.9x10^6$)	$3.1x10^6$ ($2.5x10^6$)
Percentage sputum neutrophils	68 (15)	65 (26)
^Percentage sputum eosinophils	0.6 (0.4)	0.54 (0.37)
^Sputum IL-8 (ng/mL)	9.15 (0.27)	12.11 (0.58)
^Bacterial colony forming units (CFU/mL)	47 (0.25)	29 (0.28)
^C2 (mmol/L)	1.1 (0.69)	0.62 (0.91)
^C5 (mmol/L)	2.88 (0.55)	4.17 (0.83)
LCQ score	11.65 (1.97)	11.86 (2.95)
Cough VAS (mm)	57 (18)	52 (17)

^Geometric mean (log standard deviation). FEV_1 = forced expiratory volume in 1 second, FVC= forced vital capacity, IL-8 = interleukin 8, C2 and C5=concentration of capsaicin required to cause 2 and 5 coughs, LCQ = Leicester cough questionnaire, VAS = visual analogue score

TABLE 12. MEAN (SD) AND *GEOMETRIC MEAN (LOG SD) FOLD CHANGE IN PARAMETERS FROM BASELINE TO IMMEDIATELY POST-TREATMENT AT 12 WEEKS

	Change from baseline to 3 months		Difference (95% CI)
	Placebo	Erythromycin	
*Coughs/24 hours	0.7 (0.7)	0.7 (0.3)	1.1 (0.7 to 1.5)
Sputum neutrophils ()	+6.6 (23)	-10.2 (33)	16.8 (1.6 to 32.1)
*C2 (mmol/L)	1.1 (0.4)	1.6 (0.06)	0.7 (0.4 to 1.3)
*C5 (mmol/L)	1.2 (0.1)	0.9 (0.3)	1.3 (0.9 to 2.0)
VAS (mm)	+2 (29)	-12 (33)	10 (-11 to 33)
LCQ	+1.8 (3.8)	+1.8 (3.8)	0 (-2 to 2)
*CFU per mL	1.2 (0.6)	0.7 (0.7)	1.8 (0.6 to 5.8)
*Sputum IL-8 (ng per mL)	2.1 (0.7)	0.8 (0.2)	2.9 (0.8 to 9.5)

C2= concentration of capsaicin required to induce 2 coughs, C5= concentration of capsaicin required to induce 5 coughs, VAS= cough visual analogue score, LCQ= Leicester cough questionnaire, CFU = bacterial colony forming units, IL-8 = interleukin-8

There was no difference in the change in LCQ, cough VAS, log C2 or log C5 between treatments (FIGURE 6). Haemophilus Influenzae, Streptococcal Pneumoniae and Moraxella Catteralis were not isolated from sputum samples at any time point and the log bacterial colony forming units was unchanged with active and placebo treatment (TABLE 12). Sufficient paired sputum supernatant was available to measure IL-8 in 6 patients randomised to erythromycin and 10 to placebo. The geometric mean sputum supernatant IL-8 concentration was 9.15 and 8.09 ng per mL before and 12 weeks after erythromycin treatment and 12.11 and 26.10 ng per mL before and 12 weeks after placebo treatment (mean difference in fold change 2.9; 95% CI 0.87, 9.5; p=0.08;TABLE 12).

3.2.5. Discussion

Unexplained chronic cough is a common condition responsible for considerable morbidity. There are currently no satisfactory treatments. Our study is one of the first to evaluate a potential new treatment. We used a wide range of objective and subjective measures of cough severity, as have been recommended[231]. We found no difference between the placebo and erythromycin group in any measure of cough severity, although there was a reduction in sputum neutrophil percentage with erythromycin at the end of the treatment period. Although we were not able to reach our target recruitment population, our study was sufficiently powered to exclude a halving in 24-hour cough frequency and a clinically important reduction in LCQ[185], making it unlikely that we missed a clinically important effect. Our findings imply that cough in patients with unexplained chronic cough is caused by a mechanism independent of neutrophilic airway inflammation.

Our rationale for investigating the effect of erythromycin was based on evidence from cross sectional data indicating that neutrophilic airway inflammation is present in patients with unexplained cough[138] and is independently associated with 24-hour cough frequency in a mixed population[232]. Moreover, mediators associated with neutrophilic airway inflammation, notably PGE_2, have been implicated in causing a heightened cough reflex[229,237]. Our finding that long-term macrolide therapy reduced the sputum neutrophil count is consistent with findings in other inflammatory airway diseases[234,235]. However, the anti-inflammatory effect of erythromycin was not associated with clinical benefit. Treatment reduced the sputum neutrophil count into the normal range[238] making it unlikely that the absence of clinical effect was due to insufficient dose, a suboptimum macrolide regime, or insufficient suppression of inflammation, although these possibilities cannot be excluded with complete confidence. Further work evaluating a wider dose range, potentially more efficacious macrolide regimes and more efficacious inhibitors of neutrophilic airway inflammation will be required to do this.

The mechanism of the anti-inflammatory effect of long-term low dose macrolide antibiotics in inflammatory airway diseases is incompletely understood. We did not detect clinically significant pathogens on sputum culture and bacterial colony forming units were low and unaffected by treatment suggesting that the effect was not due to the effect of macrolide antibiotics on detectable pathogens. An inhibitory effect on intracellular organisms such as Mycoplasma Pneumoniae or Chlamydia Pneumonia is possible. We found a trend to reduced IL-8 concentration with erythromycin treatment compared to placebo raising the possibility that inhibition of this cytokine might be important. Studies of human

airway epithelium in-vitro show that erythromycin inhibits expression of IL-8[239], and there is evidence that macrolide antibiotics reduce sputum IL-8 concentrations in asthma[234], supporting such an effect. The sputum supernatant data should be interpreted cautiously as only half of the patient population had sufficient paired sputum supernatant to do mediator assays and we cannot exclude the possibility that this represented a biased population. The lack of available sputum supernatant also means that we were unable to measure other mediators potentially more relevant to the genesis of cough such as PGE_2. This means we cannot exclude the possibility that erythromycin had a differential inhibitory effect on neutrophilic airway inflammation and associated tussive mediators.

Our study is notable in that it used an automated measurement of cough frequency as an outcome measure. We have previously shown that the Leicester Cough Monitor is a quick and valid method of measuring cough frequency[215]. Detailed evaluation of day-time cough recordings has shown a sensitivity of 80 to 90 percent and a specificity of 99.8 percent and automated 24-hour cough counts have been shown to correlate closely with blinded manual counts[215]. An unexpected finding of the current study was the large reduction in 24-hour cough frequency and improvement in LCQ seen with placebo treatment. Regression to the mean is an unlikely explanation for this reduction as our patients reported chronic cough for many years before study entry. The existence of such an effect supports suggestions that the traditional uncontrolled ‘treatment trial’ approach to evaluation of patients with chronic cough is flawed, if 24-hour cough frequency is used to assess treatment response[231].

The cause of unexplained chronic cough remains unclear and therefore there is uncertainty as to where drug treatment should be targeted. Opioid drugs have been used as an anti-tussive agent but results are mixed[175,204] and there is uncertainty about how acceptable more potent opiates would be to patients. Nebulised local anaesthetic agents[240] and speech therapy intervention[153] with an emphasis on voluntary cough suppression have been shown to be effective in preliminary studies although these studies were unblinded and did not evaluate cough in sufficient detail to be sure about the benefits or the mechanism of the effect. Thus there remains an important unmet need for effective treatments for an important group of patients. Our study should help researchers design and power future clinical trials; it should also direct researchers away from treatments targeting neutrophilic airway inflammation.

3.3. THE NATURAL HISTORY OF UNEXPLAINED CHRONIC COUGH

3.3.1. Abstract

Up to 40 percent of patients seen in a cough clinic have unexplained chronic cough. The long term outcome of these patients is uncertain. We aim to determine the long-term outcome in patients diagnosed with unexplained chronic cough.

We have performed a longitudinal study of symptoms, airway inflammation and spirometry in a cohort of patients with unexplained chronic cough diagnosed over 7 years ago. Cough was assessed using a 100 mm visual analogue score (VAS). At the first and final visit cough reflex sensitivity was assessed as the concentration of inhaled capsaicin at which the volunteer coughed 2 (C2) and 5 times (C5).

We identified 42 patients (32 females) with unexplained chronic cough who had been assessed at least 7 years previously and agreed to a further assessment. The mean (SD) duration of cough was 11.5 (4.5) years at the time of their final assessment. Nine patients (21 %) had organ specific autoimmune disease and twenty (48 %) had a peripheral blood lymphopaenia. Six (14 %) patients had complete resolution of symptoms and 11 (26 %) had a significant greater than 15 mm improvement in their cough VAS during follow up. Longitudinal spirometry data was available in 30 patients. The median rate of FEV1 decline was 44 mL per year and four (13 %) patients developed a post-bronchodilator forced expiratory volume in 1 second (FEV1)/forced vital capacity (FVC) of less than 0.7. FEV1 decline was similar in patients with persistent cough and those

whose cough improved. No other independent predictors of FEV1 decline were identified. There were no independent predictors of improvement in cough.

Cough persists over time in the majority of patients with unexplained chronic cough. Patients have an increased rate of decline in FEV1 and a significant minority develop fixed airflow obstruction.

3.3.2. Introduction

Cough is an important symptom, responsible for considerable impairment of quality of life[156] and accounting for a large proportion of both primary and secondary care referrals[213,231]. Chronic cough, defined as a cough lasting longer than 8 weeks, can be ascribed to a specific cause in 60 to 80 percent of patients[213]. The remainder have unexplained chronic cough and suffer impairment of quality of life, equivalent in some domains to that seen in severe asthma and COPD[168]. Unexplained chronic cough is a challenge for the respiratory physician as there are no satisfactory treatments and almost nothing is known about the long-term prognosis. More definitive information on the natural history of unexplained chronic cough is needed both to guide the management of patients in clinic and also to direct further research.

We conducted a longitudinal observational study to investigate the natural history of unexplained chronic cough diagnosed at our institution over 7 years ago.

3.3.3. Methods

We identified all patients attending a specialist cough clinic and diagnosed with unexplained chronic cough at Glenfield Hospital between January 1998 and December 2001. Unexplained chronic cough was defined as a cough lasting

greater than 8 weeks, with normal spirometry [forced expiratory volume in 1 second (FEV1) greater than 80 percent of predicted and FEV1/forced vital capacity (FVC) ratio of greater than 70 percent], a provocative concentration of methocholine required to cause a 20 percent fall in FEV1 (PC20) of greater than 8 mg per mL, a normal induced sputum eosinophil count (less than 3 percent) and a normal high resolution computed tomography (HRCT) scan of the thorax. Subjects had failed treatment trials with asthma treatment, a proton pump inhibitor and a nasal steroid spray as per British Thoracic Society guidelines[2]. The study was approved by Leicestershire, Northamptonshire and Rutland Ethics committee and all patients provided written informed consent before inclusion.

3.3.3.1. Study measurements

Spirometry was performed with a Vitalograph spirometer (Vitalograph, Buckinghamshire, UK) as the best of at least 3 successive readings within 100 mL. A capsaicin cough challenge was then performed. Subjects inhaled a single vital capacity breath of 10µL of 0.9 percent saline solution followed by doubling concentrations of capsaicin from 0.49µM/L every 1 minute. Coughs were counted for 30 seconds following each inhalation. The counting of coughs was aided by a sound recording device. The investigation was stopped when 500 µM/L solution was inhaled or when the subject coughed 5 times or more. The concentration of capsaicin required to make the subject cough 2 (C2) and 5 (C5) times was calculated by the linear interpolation of the log-dose response curve[96]. Sputum was induced using nebulised hypertonic saline and processed for cell differential count using the sputum selection protocol as described before[218]. At the first visit patients had a full blood count and a full assessment

for the presence of organ-specific autoantibodies was done as patients were participants in an earlier case-control study[134]. The following autoantibodies were measured: antinuclear (in house indirect immunofluorescence); rheumatoid factor (nephelometry, Dade Behring BNII protein analyser; UK); islet cell (in house indirect immunofluorescence); adrenal (indirect immunofluorescence; Biodiagnostics Limited, Worcestershire, UK); parietal (in house indirect immunofluorescence); endomysial (indirect immunofluorescence, Binding Site Limited, Birmingham, UK); and thyroid peroxidase (fluorescent enzyme linked immunosorbent assay system, Pharmacia Diagnositics, Milton Keans, UK).

Subjects completed a 100mm cough Visual Analogue Score (VAS) set at the bottom end by no cough and the top end the worst cough ever. Patients were asked to mark a cross at a point indicative of the severity of their cough over the past 24 hours. A change of > 15 mm was regarded as significant.[166]

3.3.3.2. Study design

Subjects had spirometry, cough VAS, sputum differential cell count and a capsaicin cough challenge test at baseline when they first presented to clinic. These tests were repeated 7 to 10 years from diagnosis.

3.3.3.3. Analysis

C2 and C5 were log transformed prior to analysis to assume a normal distribution. Correlations between variables were analysed using Spearmans rank correlation coefficient for non-parametric data. Statistical analysis was performed using SPSS 18. The rate of FEV1 decline was calculated as the total decline in FEV1 (calculated from a baseline FEV1 measurement and

repeat FEV1 measurement 7-10 years later) divided by the number of years between the measurements.

3.3.4. Results

We identified 45 patients who were diagnosed with unexplained chronic cough out of a total of 286 patients with cough seen between January 1998 and December 2001. Of these patients 3 declined to take part in the study. Of the remaining 42 patients (32 female), 12 declined to have repeat airways assessment but agreed to complete a cough VAS.

Patient characteristics are given in TABLE 13. The mean (SD) duration of cough was 11.5 (4.5) years at the time of their final assessment. Nine patients (21 %) had organ specific autoimmune disease (3 with hypothyroidism, 1 with ulcerative colitis, 1 with coeliac disease, 1 with type 1 diabetes mellitus, 1 with vitiligo, 1 with pernicious anaemia and 1 patient with hypothyroidism, vitiligo and pernicious anaemia) and twenty (48 %) had a peripheral blood lymphopaenia. Six (14 %) patients had complete resolution of symptoms; 10 (24 %) patients showed no change in their symptoms (change in VAS of less than 15mm); 11 patients (26 %) had a significant (greater than15 mm) improvement in their cough with a mean (95% CI) decrease in VAS of 31mm (20, 42); and 14 patients (36 %) had significant (greater than 15 mm) worsening of their symptoms (FIGURE 7) with a mean (95% CI) increase in the VAS score 35 mm (20, 50 mm). Repeat C2 and C5 were available in 10 patients. There was no significant difference in the change in these measures and no evidence that the change differed in patients whose cough improved or not (TABLE 13,FIGURE 9).

Longitudinal spirometry data was available in 30 patients. The median (IQR) rate of FEV1 decline was 44 (59) mL per year (FIGURE 8). Four (13 %) patients had an FEV1 rate of decline of greater than 100 mL per year, seventeen (57 %) patients had an FEV1 decline of 30 to 100 mL per year, four (13 %) patients had and FEV1 change ranging from 1 to 29 mL per year and 4 patients had an improvement in FEV1 of up to 30 mL per year . Four (13 percent) patients developed a post-bronchodilator FEV_1/FEC of less than 0.7. FEV1 decline was similar in patients with persistent cough and those whose cough improved. No other independent predictors of FEV1 decline were identified. There were no independent predictors of improvement in cough.

FIGURE 7. CHANGE IN COUGH AS MEASURED BY COUGH VAS OVER A 7 TO 10 YEAR PERIOD

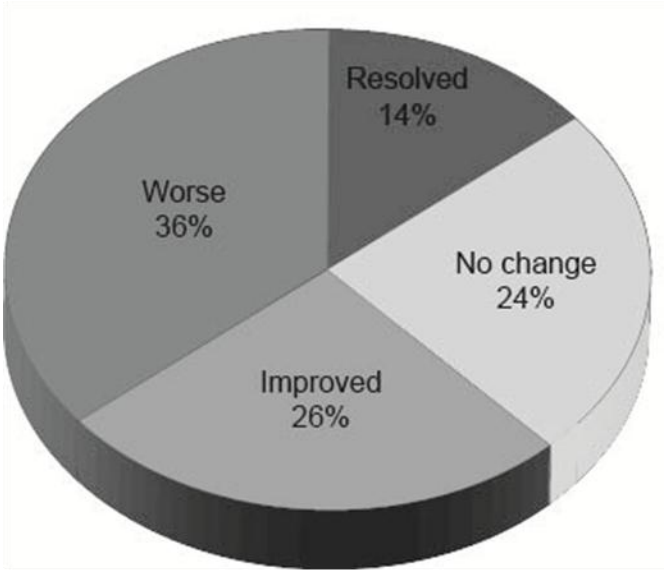
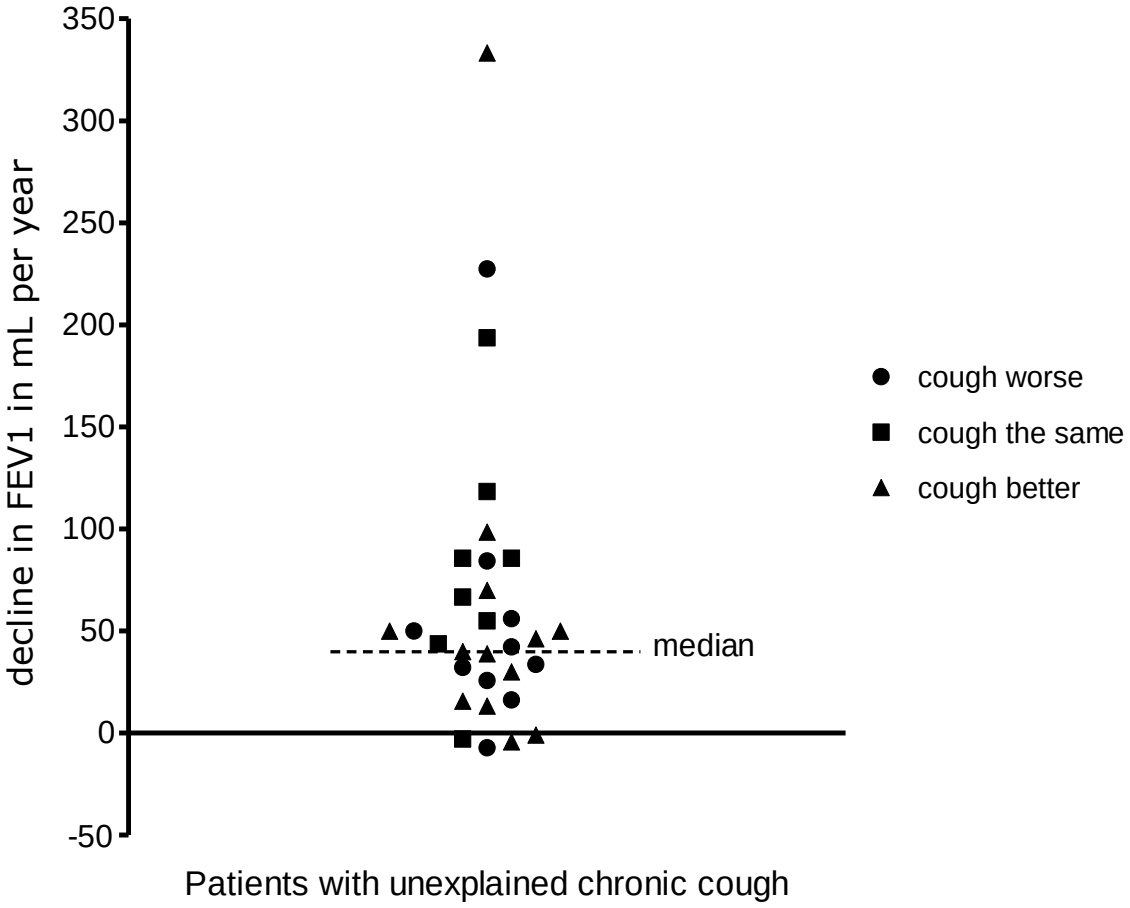


FIGURE 8. ANNUAL RATE OF FEV1 DECLINE



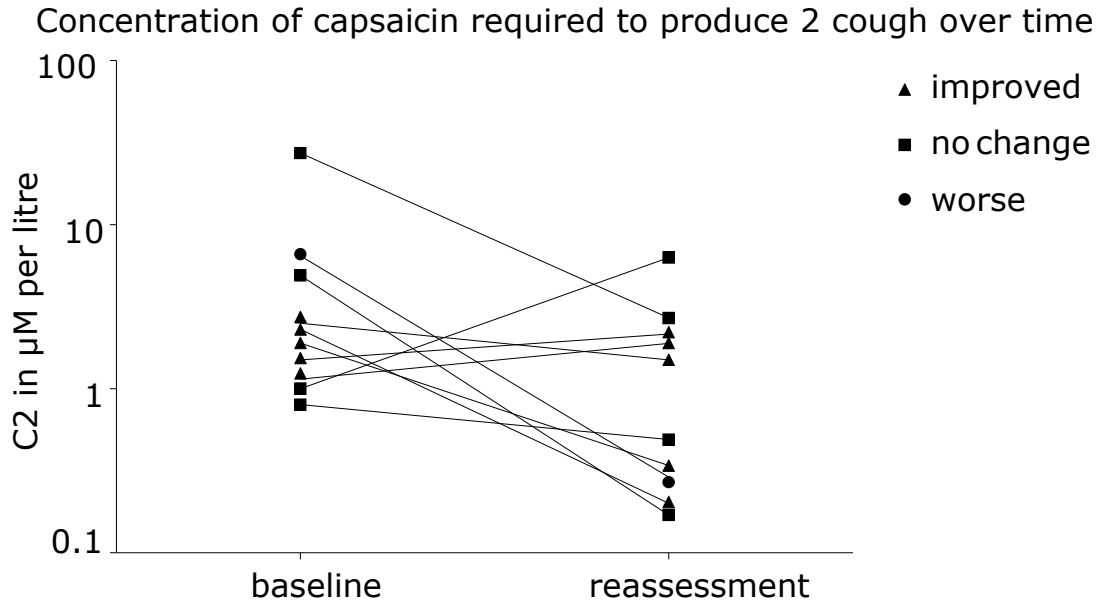
FEV1 = Forced expiratory volume in 1 second. Dotted line represents median FEV1 decline

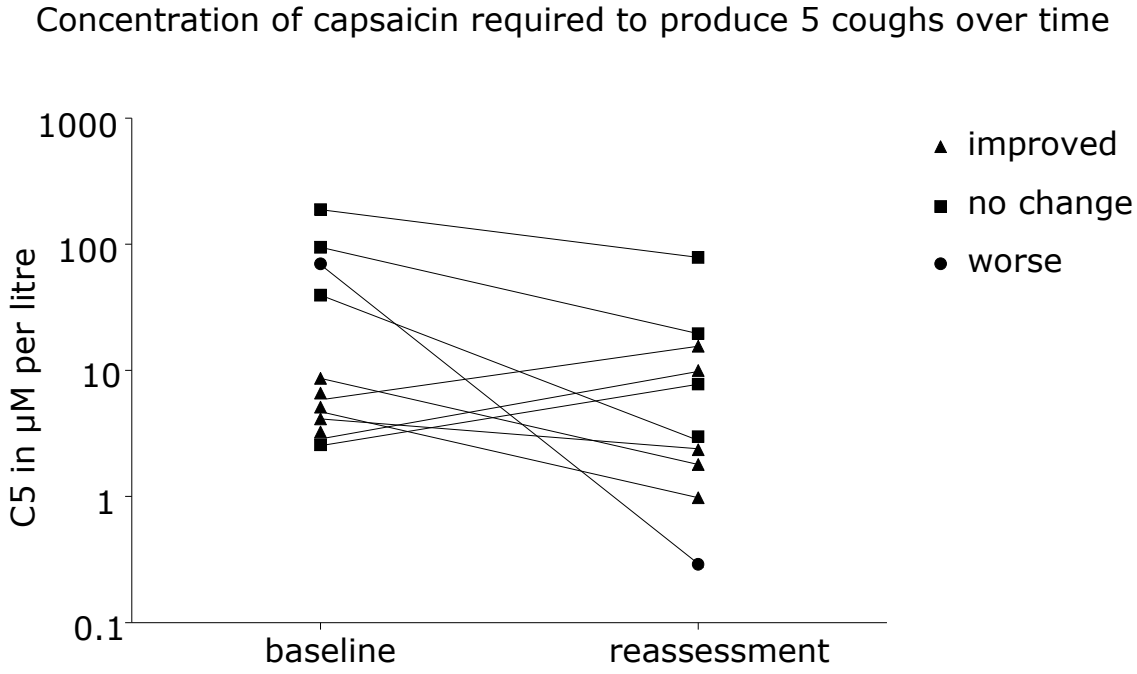
TABLE 13. DEMOGRAPHIC DATA AND STUDY MEASUREMENTS AT DIAGNOSIS AND RE-ASSESSMENT

	Baseline	Re-assessment
Number (females)	42 (32)	
*Age at onset of cough	51(10)	
*Duration of cough at final assessment (years)		11.5 (4.5)
*Percentage predicted FEV1	105 (15)	93.8 (15)
*FEV1/FVC	77 (5)	77 (11)
Sputum neutrophils (%)	49 (24)	61 (23)
^Sputum eosinophils (%)	0.6 (0.7)	0.6 (0.7)
^C2 (μM per litre)	2.4 (0.6)	1.3 (0.9)
^C5 (μM per litre)	10.1 (0.9)	2.7 (0.8)
*Cough visual analogue score (mm)	47 (24)	44 (29)
Percentage of patients with organ specific autoimmune disease (%)	10 (23)	
Number of patients with a peripheral blood lymphocyte count of <1.5 (%)	20 (48)	

* Expressed as mean (standard deviation) or ^ as geometric mean (log standard deviation). FEV1 = forced expiratory volume in one second. FEV1/FVC = forced expiratory volume over one second divided by forced vital capacity. C2= concentration of capsaicin required to induce 2 coughs, C5= concentration of capsaicin required to induce 5 coughs.

FIGURE 9. CAPSAICIN COUGH SENSITIVITY OVER TIME





C2= concentration of capsaicin required to induce 2 coughs, C5= concentration of capsaicin required to induce 5 coughs.

3.3.5. Discussion

This is the first longitudinal study of outcome in patients with unexplained chronic cough. Our patient group was similar to those described previously with a predominance of middle aged women and a high incidence of organ specific autoimmune disease and peripheral blood lymphopenia[167]. Just over half of the patient in this series had either no change or worsening of cough after more than a decade, emphasising the potential long-term morbidly associated with unexplained chronic cough. We were unable to identify predictors of persistence or improvement in cough. Unexpectedly, patients with chronic cough had a decline in FEV1 well above what would be expected in a population of non-smoking patients of this age and a significant minority developed COPD. Decline in FEV1 occurred independently of changes in cough severity.

Our findings should be regarded as preliminary and hypothesis generating rather than definitive as the population was small, not all participants consented to a full follow up assessment, the findings may have been influenced by responder bias and regression to the mean and we used published data regarding FEV1 decline in the normal population rather than a control group. Further work in larger and better characterised populations is clearly necessary. However, the demonstration of a rapid decline in FEV1 is consistent with earlier work by Birring et al[146]. They described a small cohort of older female non-smokers with cough and unexplained fixed airflow obstruction in whom there was a high incidence of organ specific autoimmune disease and peripheral blood lymphopaenia. Collectively, these studies suggest that rather than being a benign condition, non-smokers with chronic cough may develop significant airflow obstruction linked to chronic cough and autoimmune disease.

The magnitude of the decline in FEV1 is striking and also argues against a chance finding. The mean annual rate of decline of FEV1 in non-smoking women and men of this age is 25 mL per year and 29 mL per year [241]. In patients with COPD the rate of decline is 33 mL per year[242]. This compares with a median FEV1 decline of 44 mL per year in our population. The over representation of autoimmune diseases in patients with chronic cough is suggestive of common aetiological factors. Polymorphisms of the gene encoding cytotoxic T-lymphocyte associated antigen 4 (CTLA4), an inhibitor of regulatory T cell activity, are associated with a number of autoimmune diseases including autoimmune thyroid disease and type 1 diabetes mellitus[243,244]. Recently Zhu et al have shown an association between several CTLA4 polymorphisms and the chronic bronchitis phenotype in patients with COPD[245]. Further studies involving genotyping and perhaps involving direct small airway sampling are needed in this patient group to further explore the association with autoimmunity and to evaluate the pathological changes in the airways and the mechanism of decline in lung function. These studies may also help in identifying a potential therapeutic target.

Birring described a cohort of patients with unexplained chronic cough and a bronchoalveolar lavage (BAL) lymphocytosis[134]. Potentially the peripheral blood lymphopaenia seen in patients with cough might be linked to the homing of activated lymphocytes to the lung, as is the case in sarcoidosis. We have previously suggested that aberrant homing of activated lymphocytes from the primary site of autoimmune or chronic infection related inflammation might lead to airway inflammation and damage[16]. This might be expected to be most likely to occur when the chronic inflammatory conditions involve organs that are

embryologically related to the lungs and it is notable that conditions such as inflammatory bowel disease[17], chronic hepatitis C infection[18], autoimmune thyroid disease[8,15], and Helicobacter pylori-induced gastritis[19] have all been linked to airway disease. Alternatively, it is well recognised that many patients with chronic unexplained cough describe a preceding viral infection and following this develop a chronic cough. It may be that the lymphopenia seen in this group of patients is as a result of a prior viral infection. Furthermore, there is a strong association with lymphopenia and both systemic and organ specific autoimmunity[246-248]. Lymphopenia results in homeostatic peripheral T cell expansion which is distinct from normal T cell responses, and can result in T cell proliferation in response to self-antigens, leading to the development of organ specific auto-immune disease[249]. It is possible that the peripheral blood lymphopenia and the BAL lymphocytosis are indicative of an airway specific autoimmue process. The physiological, radiological and pathological features of the airway disease seen in association with chronic inflammatory disorders have not been extensively investigated. It has been suggested that they are due to a low grade obliterative bronchiolitis analogous to that seen in chronic rejection in lung transplant recipients or chronic graft vs. host disease in bone marrow transplant recipients[16]. Unexplained chronic cough is particularly prevalent in menopausal women perhaps because CD-4 positive T-cell numbers increase in the lung at this time[20].

In conclusion, we have shown that a small majority of patients with unexplained chronic cough who consented to a further assessment at least 7 years after the first have persistent morbidity due to cough. Patients with unexplained chronic cough also had an abnormally rapid decline in FEV1 and around 10 percent

developed spirometric features of COPD. Our findings raise the possibility that unexplained chronic cough is due to a persistent damaging airway process and suggest that this condition could be regarded as a risk factor for developing COPD.

3.4. THE ASSESSMENT OF QUALITY OF LIFE IN ACUTE COUGH USING THE LEICESTER COUGH QUESTIONNAIRE (LCQ-ACUTE)

3.4.1. Abstract

Acute cough has a significant impact on physical and psychosocial health and is associated with an impaired quality of life (QOL). The Leicester Cough Questionnaire (LCQ) is a validated cough-related health status questionnaire designed for patients with chronic cough. The purpose of this study was to validate the LCQ for the assessment of health related QOL in patients with acute cough and determine the clinical minimal important difference (MID).

10 subjects with cough due to acute upper respiratory tract infection underwent focused interviews to investigate the face validity of the LCQ. The LCQ was also evaluated by a multidisciplinary team. 30 subjects completed the revised LCQ-acute and a cough visual analogue score (VAS: 0 – 100 mm) within one week of onset of cough and again less than 2 weeks later and at resolution of cough. The concurrent validity, internal reliability, repeatability and responsiveness of the LCQ-acute were also assessed. Patients also completed a Global Rating of Change Questionnaire (GRCQ) that assessed the change in cough severity between visits. The MID was calculated as the change in LCQ-acute score for patients responding to GRCQ category representing the smallest change in health status that patients found worthwhile.

Health status was severely impaired at baseline affecting all domains; median (interquartile range) total LCQ-acute score 13.0 (3.4). All subjects found the LCQ-acute questionnaire acceptable for assessing their cough. Internal

reliability of the LCQ-acute was good for all domains and total score, Cronbach's α coefficients >0.9. There was a significant correlation between LCQ-acute and VAS (r = -0.48, p=0.007). The LCQ-acute and its domains were highly responsive to change; effect sizes 1.7-2.3. The MID for total LCQ and VAS were 2.5 and 13mm respectively.

The LCQ-acute is a brief, simple and valid instrument to assess cough specific health related QOL in patients with acute cough. It is a highly responsive tool suggesting that it will be particularly useful to assess the effect of antitussive therapy.

3.4.2. Introduction

Acute cough impacts significantly on physical and psychosocial health, leading to impairment in quality of life (QOL)[170]. Chest pain, nausea and sleep disturbance are particularly common[168]. 20 million work days are lost each year in the USA due to acute cough according to the National Centre for Health Statistics[250]. The assessment of cough severity in acute cough is limited to self-reported symptom scales, scores or diaries. There is increasing recognition that health related quality of life assessment is important, particularly in the evaluation of therapy. We have previously reported the development and validation of the Leicester Cough Questionnaire (LCQ) which is a brief, self-completed, widely used, health related QOL questionnaire for chronic cough[157]. It is not known if the LCQ can be used to assess QOL in acute cough. The aim of this study was to adapt, validate and assess the LCQ for patients with acute cough and to determine the minimal important difference (MID).

3.4.3. Methods

3.4.3.1. Subjects

30 subjects (10 men) with cough due to acute upper respiratory tract infection were recruited within one week of onset of symptoms. Patients were recruited during the peak cough/cold season October to April. An upper respiratory tract infection was considered a cause of acute cough if subjects had 2 or more symptoms at least 1 day prior to the study of: rhinorrhoea, sneezing, fever, myalgia, malaise, headache and sore throat[251]. Subjects with a history of respiratory disease, chronic cough or those taking antitussive or upper respiratory tract infection drugs or angiotensin converting enzyme inhibitors were excluded. 1 patient had a history of seasonal allergic rhinitis. Informed consent was obtained from all patients and the study was approved by the local research ethics committee.

3.4.3.2. Questionnaires

- Leicester Acute Cough Questionnaire (LCQ-acute)

The LCQ is a 19 item questionnaire that assesses cough-related QOL.[157] It has 3 domains (physical, psychological and social). The total score range is 3-21 and domain scores range from 1-7; a higher score indicates a better quality of life. The questionnaire was revised so that each item related to the patient's experience within a 24-hour time frame and is called the Leicester Acute Cough Questionnaire (see Appendix 2).

- Cough Visual Analogue Score (VAS)

The cough VAS is a 100mm scale on which patients indicate the severity of cough[134].

- Global Rating of Change Questionnaire (GRCQ) (see Appendix 3)

The GRCQ is a 15 point scale widely used to determine the MID of health related QOL questionnaires[181]. Patients were asked to rate global changes in health and sub-domains using 4 GRCQs. The GRCQ response ranged from -7 (a great deal worse) to +7 (a great deal better) and was classified as unchanged (-1,0,+1), small change (-3,-2,+3,+2), moderate change (-5, -4, +5, +4) and large change (-7, -6, +7, +6). MID was defined as the change in LCQ score corresponding to a small change in GRCQ score.

3.4.3.3. Protocol

The LCQ and VAS were completed on three occasions. Patients completed the LCQ-1, VAS-1 and a structured questionnaire designed to record demographics and symptoms associated with acute cough within one week of onset. Patients were asked to complete a GRCQ and a repeat LCQ-2 and VAS-2 within 2 weeks of LCQ-1 and again when the cough resolved (LCQ-3 and VAS-3.)

- Face validity: The suitability of the wording and content of the LCQ for detecting health related QOL in patients with acute cough was assessed by:

    1. A literature review of QOL assessment in acute cough.

    2. Review of the LCQ by a multidisciplinary team (doctor, nurse, physiotherapist, pharmacist)

    3. Focussed interviews with 10 patients with acute cough to assess its impact on QOL and to ascertain their views on the suitability of the LCQ to assess QOL.

- Concurrent validity is the assessment of an instrument against other standards; it was assessed by correlating LCQ-1 scores with cough VAS-1.

- Internal reliability of each domain was assessed by determining Cronbach's alpha coefficients which indicate the extent to which items are interrelated. Internal reliability is generally acceptable if Cronbach's alpha coefficient is greater than 0.7.

- The repeatability of the LCQ was assessed in those patients indicating no change in health status on the GRCQ over 2 weeks.

- The responsiveness of the LCQ and VAS was determined by calculating the effect size of change between baseline and resolution of the cough.

- Minimal important difference (MID) of the LCQ and VAS were determined using anchor based methods using the GRCQ as described by Juniper [181].

3.4.3.4. Statistical analysis

SPSS version 16 was used for data analysis. Data are presented as mean (standard deviation) or median (inter-quartile range) according to its distribution. In accordance with previous studies we expressed global rating scores as absolute numbers i.e. when the change was negative, the sign was reversed as was the sign of change in LCQ score[185]. Spearman's correlation coefficient was used to determine concurrent validity. Mann Whitney tests were used to compare groups. Internal reliability was tested by determining Cronbach's alpha coefficient. Repeatability was assessed by determining the intra class correlation coefficients. Effect size was measured as the change in LCQ score

from baseline to resolution of cough divided by the standard deviation of baseline measurements.

3.4.4. Results

All patients that were interviewed found the LCQ suitable for use in acute cough. The only modification to the LCQ after review by the multidisciplinary meeting was alteration of the time frame for each item from 2 weeks to the past 24 hours. See Appendix 2 for the final version of LCQ-acute. 2 patients did not complete the GRCQ and their data was excluded from the validation of the MID. Subject characteristics are given in

TABLE 14. Health related QOL was impaired at baseline; median (IQR) total LCQ score 12.8 (3.4), physical 4.5 (1.1), psychological 4.9 (1.1) and social 4 (1.4). There were no significant gender differences in VAS, LCQ or GRCQ scores.

There was a significant correlation between the cough VAS and the LCQ total score at baseline (r = -0.48, p=0.007;FIGURE 10). Internal consistency was high for all domains and total LCQ score (

TABLE 15). Only 4 patients indicated a GRCQ score of 0, 2 patients indicated a GRCQ score of 1; this sample size was considered too small to determine intraclass coefficient of repeatability.

QOL improved between visits 1 and 2; median LCQ score 12.8 vs 16.7; p <0.001. QOL improved in all but one patient between visits 1 and 2. The median change in LCQ score for each GRCQ category is given in TABLE 16.

TABLE 14. SUBJECT CHARACTERISTICS

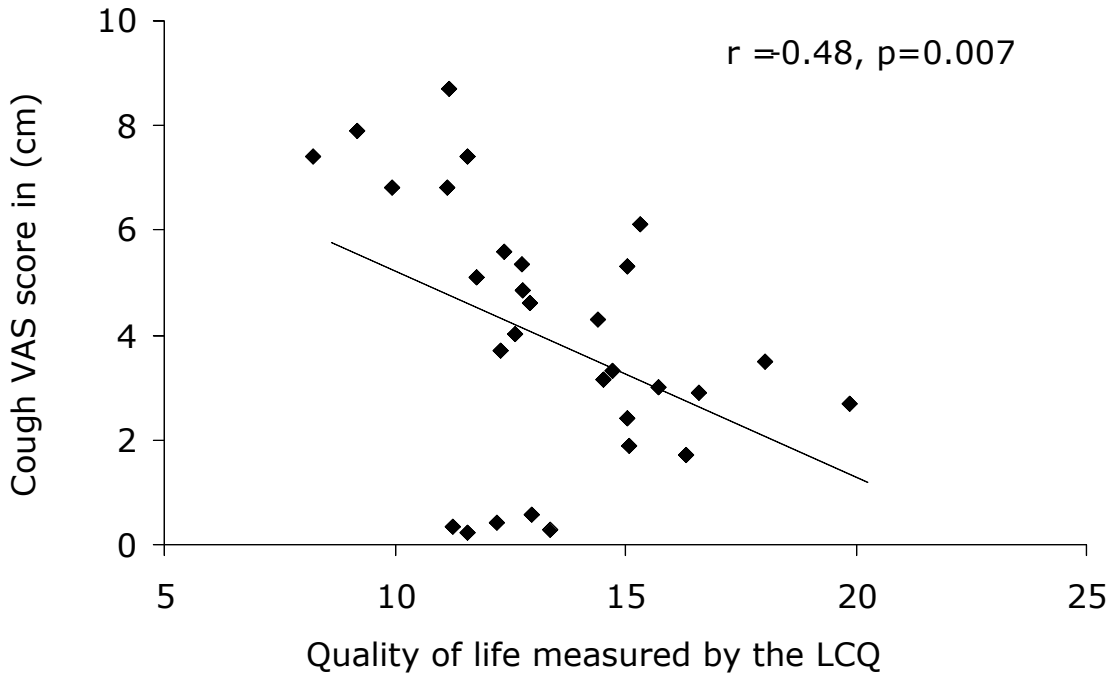
Characteristics (n=30)	
Age mean (SD)	32 (10)
Smokers n(%)	2 (7)
Non-smokers n(%)	28(93)
Duration of cough in days (SD)	12 (9)
LCQ score baseline median(IQR)	12.8 (14.9; 11.5)
VAS score baseline mean(SD)mm	39 (25)
Tiredness n(%)	24 (80%)
Sore throat	18 (60)
Runny nose	17 (57)
Sneezing	16 (53)
Headache	16 (53)
Clear sputum	14 (47)
Coloured sputum	14 (47)
Aches/pains	12 (40)
Fever	10 (33)
Facial pain	9 (30)

The LCQ MID corresponding to a small change in the GRCQ was 2.5 (TABLE 16). The correlation between GRCQ score and change in LCQ total was r = 0.6 (p=0.001) and for domains: physical r = 0.51 (p=0.05), psychological r = 0.46 (p=0.02) and social r = 0.47 (p=0.01). The LCQ and VAS were responsive to reductions in cough severity (TABLE 17). There was a weak relationship between change in VAS score and change in LCQ score (r=0.37, p=0.05). The MID for VAS was 13 mm. There was no correlation between change in VAS and GRCQ score (r = 0.02, p= 0.78).

TABLE 15. INTERNAL CONSISTENCY (CRONBACH'S ALPHA)

LCQ-acutedomain	Cronbach's Alpha Coefficient
Total	0.94
Social	0.90
Psychological	0.90
Physical	0.95

FIGURE 10. CONCURRENT VALIDITY: RELATIONSHIP BETWEEN QUALITY OF LIFE AND COUGH VAS



VAS = Visual analogue score, LCQ = Leicester cough questionnaire. The LCQ is a 19 item validated quality of life questionnaire for patients with chronic cough. It assesses three domains (physical, psychological and social). The total score range is 3-21; a higher score indicates a better quality of life. The cough VAS is a 100mm analogue score set at 0 and 100 by 'no cough' and 'worst cough ever' respectively.

TABLE 16. MEDIAN CHANGE (INTERQUARTILE RANGE) IN LEICESTER COUGH QUESTIONNAIRE SCORE AND VISUAL ANALOGUE SCORE PER GLOBAL RATING OF CHANGE CATEGORY

	Global rating of change questionnaire categories			
	Unchanged (-1/0/1)	Small (-3/-2/2/3)	Moderate (-5/-4/4/5)	Large (-7/-6/6/7)
Change in LCQ-acute total score	n=6 1.2 (0.9)	n=12 2.5 (3.1)	n=6 4.6 (2.9)	n=4 6.8 (3.5)
Change in LCQ-acute physical score	n=1 0.6	n=14 0.6 (0.8)	n=8 1.0 (0.8)	n=5 1.9 (1.5)
Change in LCQ-acute psychological score	n=9 0.1 (1.0)	n=8 0.7 (1.2)	n=7 1.4 (0.9)	n=4 2.2 (1.5)
Change in LCQ-acute social score	n=6 0.6 (0.4)	n=14 0.9(1.4)	n=5 2.3 (0.3)	n=3 2.5 (0.6)
*Change in cough VAS score (mm)	n=6 7.0 (0.6)	n=12 13.0 (0.6)	n=6 13.0 (0.6)	n=4 33.0 (2.3)

n = number of cases. *mean (standard deviation). LCQ = Leicester cough questionnaire, VAS = visual analogue score

TABLE 17. RESPONSIVENESS OF LEICESTER COUGH QUESTIONNAIRE-ACUTE: EFFECT SIZES

	Effect size
LCQ-acute Total	2.3
LCQ-acute Social	1.7
LCQ-acute Psychological	1.8
LCQ-acute Physical	2.3
Cough VAS	1.4

3.4.5. Discussion

The LCQ-acute is a valid health status measure for patients with acute cough. It is easy to use, self-administered and takes less than 5 minutes to complete. The LCQ-acute was highly responsive to change, suggesting it might be particularly useful in assessing the response to treatment both in clinic and in clinical trials. The minimal important difference, the smallest change in health status patients find worthwhile was a change in LCQ-acute score of 2.5.

We validated the LCQ-acute for acute cough using a well-accepted QOL instrument development methodology[252]. The only alteration to the original LCQ was a reduction in the assessment period from 2 weeks to 24 hours to reflect the rapid change in symptoms associated with acute cough. The validity of the LCQ-acute was comparable to the original LCQ used by patients with chronic cough; face and concurrent validity, internal reliability and responsiveness were within acceptable standards for quality of life questionnaires[252]. We were unable to determine the repeatability of the LCQ-

acute since most patients reported improvement in cough severity within the time frame of this study. A shorter time interval between test and retest questionnaires or a much larger study may allow the determination of repeatability coefficients in future. It is possible that symptoms of upper respiratory tract infection other than cough may have influenced quality of life. The LCQ-acute questionnaire items were however individually phrased to be relevant to cough.

The MID for LCQ-acute was 2.5. This should facilitate the interpretation of health status data from clinical studies and calculate sample sizes for future studies. The MID was greater than that for patients with chronic cough (1.3) [185]. This may be because small changes in quality of life have a larger impact in chronic conditions due to the cumulative effect of living with the symptom for many years. We chose anchor based methodology to determine the MID rather than distribution methods based on standard deviations since the latter depend on the heterogeneity of the population under study and utilises arbitrary units of measure[179,253,254]. There are limitations with the anchor based methodology used to determine MID. We included patients with GRCQ scores +/- 1 in the "unchanged" category and it is therefore possible that some patients may have experienced a significant change in cough. We chose this method to be consistent with those described by Juniper[181]; moreover, they have previously reported that a GRCQ score of +/- 1 does not represent clinically significant change. The GRCQ is a subjective instrument and subject to recall bias. Our findings need confirmation with objective assessment of cough severity such as cough reflex sensitivity measurement and cough monitoring. The time-frame for GRCQ was relatively short and this may have minimised the effect of recall

bias. The determination of the MID by prospective methodology avoids some of the limitations of the anchor based methods; this deserves consideration in future studies (Irwin RS, personal communication and data in press). We found a significant correlation between GRCQ and the change in LCQ-acute scores supporting the use of the GRCQ. There was a step-wise increase in change in LCQ-acute scores across GRCQ categories, which suggests that LCQ-acute can discriminate patients with small and large changes in health status. Our study demonstrates that health status improves in the vast majority of patients with acute cough. Further studies will be needed to determine if a MID of 2.5 is applicable for patients whose health status deteriorates.

We were unable to perform a sub-analysis to determine whether the MID varied according to age, gender or strain of virus; this will require further investigation. We determined the LCQ-acute MID in a natural recovery study design. It may be difficult to establish the MID in patients taking currently available antitussive drugs since any improvement in cough severity is more likely to be due to natural recovery or placebo effects rather than a therapeutic effect of the antitussive drug. We suggest that anti-tussive drugs should aim to achieve a clinical benefit that is greater than an increase of LCQ-acute score of at least 2.5 units. This should ideally be achieved at an earlier phase of the illness.

The impairment in quality of life suffered by our cohort of subjects with acute cough was comparable to that of chronic cough[255]. The impairment in QOL was moderate to severe but transient compared to chronic cough. All health domains were affected. A significant impairment in the health status of patients with acute cough was also found in a study using the CQLQ, another validated cough specific health status questionnaire for patients with acute and chronic

cough[170]. Although this seems surprising for such a common and benign condition, it reflects the fact that the LCQ-acute and CQLQ are cough specific health measures. It is likely that general health related QOL determined by generic tools such as the SF36 will demonstrate a lesser impact on QOL in acute compared with chronic cough.

This is the first study to validate the cough VAS in subjects with acute cough and determine its MID. The VAS is easier to use and widely recognised compared to QOL tools. QOL tools however have the advantage that they quantify overall health status and identify the subdomains of health affected. The relationship between VAS and QOL was less strong than that for patients with chronic cough and there was no relationship between the global health assessment tools (GRCQ) and VAS in contrast to the LCQ-acute. This suggests that VAS cannot be used as a substitute for health related QOL tools. Furthermore, we have demonstrated that the LCQ-acute is more responsive to changes in cough severity than the VAS.

In conclusion, there are a range of options available to assess cough severity in acute cough. The LCQ-acute should be used to complement other subjective tools and objective tools such as cough reflex sensitivity and ambulatory cough frequency monitoring. The LCQ-acute represents an advance in the assessment of cough severity and should aid clinicians and researchers in making meaningful interpretations of health related QOL outcomes.

4. CONCLUSIONS

4.1. SUMMARY OF FINDINGS

Cough is a defence mechanism but is also a common symptom of both acute and chronic disease. When pathological, it is associated with substantial morbidity and economic cost. Decades have been spent debating the aetiology of chronic cough with some progress, however with recent advances in molecular medicine, new insights into the pathological mechanisms of cough have been discovered and potential treatment targets identified. Pharmaceutical companies are currently developing a number of potential drugs which show promise for treating chronic cough, albeit the commercial phase II trials planned are for chronic pain. Historically trials of cough treatment have been poorly conducted with a lack of objective outcome measures. It is vital that validated objective outcome measures are used to evaluate the efficacy of potential treatments for cough.

In this thesis I have advanced the validation of the Leicester cough monitor, an objective and practical system capable of detecting coughs over 24 hours in subjects with respiratory diseases associated with cough and in healthy individuals with low cough frequencies. I showed that automated cough numbers were similar to those derived from the gold standard of manual counting in healthy controls and in patients with respiratory disease.

24-hour cough frequency measured using the Leicester cough monitor was almost sixteen times higher in patients with respiratory diseases compared to healthy controls. I also found that healthy women cough more than healthy

men, a finding in keeping with existing knowledge that women in health have a more sensitive cough reflex than men.

In the population as a whole I also found a correlation between cough frequency and the induced sputum neutrophil count. Others have shown pro-tussive mediators associated with neutrophilic airway inflammation to be present in increased concentration in the airway of patients with chronic cough. Also, low dose long term macrolide antibiotics are used in patients with bronchiectasis and COPD and are known to modulate neutrophils. Consequently, I conducted a randomised, placebo controlled, double blind, parallel group trial of low dose erythromycin given once daily for 3 months in 30 patients with unexplained chronic cough with 24-hour cough frequency as the primary endpoint. Active treatment was associated with a significant reduction in the sputum neutrophil count but there was no difference in cough counts or other measures of cough severity. Modulation of sputum neutrophils did not affect the capsaicin cough sensitivity or cough frequency and is therefore not an effective therapeutic approach to the management of unexplained chronic cough. Importantly, I have also demonstrated that it is practical and feasible to incorporate automated measurements of 24-hour cough frequency into a clinical trial.

Quality of life questionnaires are also important measurements in a trial of new treatments as these patient reported outcomes offer a unique insight into the impact of the condition on the patients. The LCQ is a well validated tool in chronic cough and has also been used in trials in patients with acute cough, but interpreting these results is difficult because a statistically significant result is not always a clinically significant result. Understanding the minimal important clinical difference is essential in both the interpretation and design of trials

where the LCQ is used as a treatment outcome measure. Treatment related improvements in acute cough are only relevant if they are deemed significant by the patients. I have demonstrated that the minimal important difference in the LCQ score in subjects with acute cough is 2.5 (out of a possible maximum of 7).

Finally the long term outcome of patients with unexplained chronic cough has not been previously described. I found that cough persists in the majority of patients over at least a seven year period. This finding highlights the urgent need for effective treatments in this patient group. Unexpectedly I also found that patients had an abnormally rapid fall in FEV1, whether cough improved or not.

4.2. PROBLEMS ENCOUNTERED AND CRITICISMS

Some of these problems have been individually addressed in the discussions in each chapter and I will not repeat these discussions, however a number other issues require consideration.

The primary aim of ‘Cough frequency in health and disease’, was to validate the Leicester cough monitor over a range of cough frequencies. A secondary aim was to estimate cough frequency in health and in patients with respiratory disease. Recruiting normal healthy volunteers was a challenge. Our healthy population was self-selected in response to poster adverts and some media publicity about the cough monitor; however the majority of individuals volunteering to take part were ineligible as they reported a chronic cough. This highlighted a significant unmet need in the population but did not assist in recruiting normal healthy volunteers. Unexpectedly, only a small proportion of

volunteers were current smokers. Although the healthy subjects included in the study had normal measurable parameters of lung health, it is possible that I have overestimated cough frequency in the healthy population as people concerned about a cough may be more likely to respond to such poster adverts. Despite this, I was able to demonstrate a clear difference in cough frequency in patients with disease and healthy individuals.

The main weakness of the natural history of cough study was a lack of tissue to explore the long-term inflammatory sequelae of chronic cough. Trans-bronchial biopsies would have helped to explore the reasons behind the persistence of cough and the accelerated decline in lung function. We obtained ethical approval for this as a sub-study, but our population of patients with unexplained chronic cough have been imposed upon frequently to partake in research and did not consent to undergo further invasive tests that were not likely to result in a resolution of their symptoms.

I used anchor based methodology using the global rating of change scores to evaluate the MID. This score requires patients to rate the degree of change in cough related quality of life from the previous questionnaire. There may be some criticism of this retrospective approach as it can be affected by recall bias, although the time between one questionnaire and the next was no longer than a few weeks in this study. To mitigate the effect of recall bias, participants had access to their previous answers and scores. However, one way to overcome the effect of recall bias would have been to use a prospective approach such as the Punum ladder which patients are asked to assess their quality of life over the past week[184].

Also the cough VAS assessed cough severity which, interestingly did not correlate with cough related quality of life. For the purposes of this study a VAS assessing cough related quality of life would have been more pertinent. To further improve the robustness of the study multiple VAS related to LCQ sub-domains could have been employed. Also I was not able to assess the repeatability of the LCQ-acute in patients with acute cough as only one volunteer evidenced no change as per global rating of change questionnaire.

4.3. FUTURE STUDIES

Further quantification of the potential accelerated decline of FEV1 in patients with chronic cough requires additional work and may offer an opportunity to understand the mechanisms behind chronic cough. Non-invasive assessments of airway wall thickness can be performed using limited CTs. This may be one way of investigating pathophysiology, but ultimately tissue biopsies are essential in understanding the mechanisms behind chronic cough.

Tissue biopsies both before and after successful treatment of cough are also crucial in understanding the pathophysiology of chronic cough in humans, giving vital insight into the cellular changes associated with an improvement in cough. Arguably, this will be time consuming, expensive and difficult but we are at an impasse and markers of airway inflammation alone cannot take us any further.

A number of studies have reported bronchioalveoar lavage lymphocytosis in patients with unexplained chronic cough. Additional studies involving flo

cytometry are necessary to further detail this immune response involving by investigating activation of T cells and cytokine production.

Cytotoxic-T-Lymphocyte Anitgen 4 (CTLA4) is a negative regulator of immune responses and polymorphisms of this antigen are associated with the development of autoimmune disease. CTLA4 antigens polymorphism have also been reported in asthmatic and those with COPD. Give the previously association of unexplained chronic cough and autoimmunity, there is value in exploring the possible role of CTLA4 polymorphisms in the susceptibility to unexplained chronic cough.

Despite being a common symptom and despite an increasing understanding of the physiological processes underpinning cough, little progress has been made in terms of treatments. This thesis should highlight that robust treatment trials in patients with cough are feasible and practical and that new treatments must be tested in a robust and clinically relevant way.

APPENDIX 1

LEICESTER COUGH QUESTIONNAIRE

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

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

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

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

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

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

1 All of the time

2 Most of the time

3 A good bit of the time

4 Some of the time

5 A little of the time

6 Hardly any of the time

7 None of the time

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

1 None of the time

2 Hardly any of the time

3 A little of the time

4 Some of the time

5 A good bit of the time

6 Most of the time

7 All of the time

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

1 All of the time

2 Most of the time

3 A good bit of the time

4 Some of the time

5 A little of the time

6 Hardly any of the time

7 None of the time

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

1 All of the time

2 Most of the time

3 A good bit of the time

4 Some of the time

5 A little of the time

6 Hardly any of the time

7 None of the time

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

1 All of the time

2 Most of the time

3 A good bit of the time

4 Some of the time

5 A little of the time

6 Hardly any of the time

7 None of the time

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

1 All of the time

2 Most of the time

3 A good bit of the time

4 Some of the time

5 A little of the time

6 Hardly any of the time

7 None of the time

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

1 All of the time

2 Most of the time

3 A good bit of the time

4 Some of the time

5 A little of the time

6 Hardly any of the time

7 None of the time

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

1 All of the time

2 Most of the time

3 A good bit of the time

4 Some of the time

5 A little of the time

6 Hardly any of the time

7 None of the time

11. In the last 2 weeks, how many times have you had coughing bouts?

1 All of the time (continuously)

2 Most times during the day

3 Several times during the day

4 Sometimes during the day

5 Occasionally through the day

6 Rarely

7 None

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

1 All of the time

2 Most of the time

3 A good bit of the time

4 Some of the time

5 A little of the time

6 Hardly any of the time

7 None of the time

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

1 All of the time

2 Most of the time

3 A good bit of the time

4 Some of the time

5 A little of the time

6 Hardly any of the time

7 None of the time

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

1 All of the time

2 Most of the time

3 A good bit of the time

4 Some of the time

5 A little of the time

6 Hardly any of the time

7 None of the time

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

1 None of the time

2 Hardly any of the time

3 A little of the time

4 Some of the time

5 A good bit of the time

6 Most of the time

7 All of the time

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

1 All of the time

2 Most of the time

3 A good bit of the time

4 Some of the time

5 A little of the time

6 Hardly any of the time

7 None of the time

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

1 All of the time

2 Most of the time

3 A good bit of the time

4 Some of the time

5 A little of the time

6 Hardly any of the time

7 None of the time

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

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

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

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

Thank you for completing this questionnaire.

LCQ Scoring

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

APPENDIX 2

LEICESTER ACUTE COUGH QUESTIONNAIRE

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

1. In the last 24 hours, have you had chest or stomach pains as a result of your cough?

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

2. In the last 24 hours, have you been bothered by sputum (phlegm) production when you cough?

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

3. In the last 24 hours, have you been tired because of your cough?

1 All of the time

2 Most of the time

3 A good bit of the time

4 Some of the time

5 A little of the time

6 Hardly any of the time

7 None of the time

4. In the 24 hours, have you felt in control of your cough?

1 None of the time

2 Hardly any of the time

3 A little of the time

4 Some of the time

5 A good bit of the time

6 Most of the time

7 All of the time

5. How often during the last 24 hours have you felt embarrassed by your coughing?

1 All of the time

2 Most of the time

3 A good bit of the time

4 Some of the time

5 A little of the time

6 Hardly any of the time

7 None of the time

6. In the last 24 hours, my cough has made me feel anxious

1 All of the time

2 Most of the time

3 A good bit of the time

4 Some of the time

5 A little of the time

6 Hardly any of the time

7 None of the time

7. In the last 24 hours, my cough has interfered with my job, or other daily tasks

1 All of the time

2 Most of the time

3 A good bit of the time

4 Some of the time

5 A little of the time

6 Hardly any of the time

7 None of the time

8. In the last 24 hours, I felt that my cough interfered with the overall enjoyment of my life

1 All of the time

2 Most of the time

3 A good bit of the time

4 Some of the time

5 A little of the time

6 Hardly any of the time

7 None of the time

9. In the last 24 hours, exposure to paints or fumes has made me cough

1 All of the time

2 Most of the time

3 A good bit of the time

4 Some of the time

5 A little of the time

6 Hardly any of the time

7 None of the time

10. In the last 24 hours, has your cough disturbed your sleep?

1 All of the time

2 Most of the time

3 A good bit of the time

4 Some of the time

5 A little of the time

6 Hardly any of the time

7 None of the time

11. In the last 24 hours, how many times have you had coughing bouts?

1 All of the time (continuously)

2 Most times during the day

3 Several times during the day

4 Sometimes during the day

5 Occasionally through the day

6 Rarely

7 None

12. In the last 24 hours, my cough has made me feel frustrated

1 All of the time

2 Most of the time

3 A good bit of the time

4 Some of the time

5 A little of the time

6 Hardly any of the time

7 None of the time

13. In the last 24 hours, my cough has made me feel fed up

1 All of the time

2 Most of the time

3 A good bit of the time

4 Some of the time

5 A little of the time

6 Hardly any of the time

7 None of the time

14. In the last 24 hours, have you suffered from a hoarse voice as a result of your cough?

1 All of the time

2 Most of the time

3 A good bit of the time

4 Some of the time

5 A little of the time

6 Hardly any of the time

7 None of the time

15. In the last 24 hours, have you had a lot of energy?

1 None of the time

2 Hardly any of the time

3 A little of the time

4 Some of the time

5 A good bit of the time

6 Most of the time

7 All of the time

16. In the last 24 hours, have you worried that your cough may indicate a serious illness?

1 All of the time

2 Most of the time

3 A good bit of the time

4 Some of the time

5 A little of the time

6 Hardly any of the time

7 None of the time

17. In the last 24 hours, have you been concerned that other people think something is wrong with you, because of your cough?

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7 None of the time

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19. In the last 24 hours, I feel that my cough has annoyed my partner, family or friends

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	Social:	7,8,18,19
2. Domain Scores:	total score from items in domain / number of items in domain (range 1-7)	
3. Total Scores:	Addition of domain scores (range 3-21)	

APPENDIX 3

GLOBAL RATING OF CHANGE QUESTIONNAIRE

Question 1 assesses the change in physical health.

Q1. Since your first cough questionnaire, has there been any change in the impact of your cough related symptoms?

-7 A very great deal worse
-6 A great deal worse
-5 A good deal worse
-4 Moderately worse
-3 Somewhat worse
-2 A little worse
-1 Almost the same, hardly any worse at all
0 No change
1 Almost the same, hardly any better
2 A little better
3 Somewhat better
4 Moderately better
5 A good deal better
6 A great deal better
7 A very great deal better

Question 2 assesses change in psychological health.

Q2. Since your first cough questionnaire, has there been any change in your feelings (e.g. embarrassment, anxiety, frustration) as a consequence of your cough?

-7 A very great deal worse
-6 A great deal worse
-5 A good deal worse
-4 Moderately worse
-3 Somewhat worse
-2 A little worse
-1 Almost the same, hardly any worse at all
0 No change
1 Almost the same, hardly any better
2 A little better
3 Somewhat better
4 Moderately better
5 A good deal better
6 A great deal better
7 A very great deal better

Question 3 assesses the change in social life.

Q3. Since your first cough questionnaire, has there been any change in the impact of your cough on your work or social life?

-7 A very great deal worse
-6 A great deal worse
-5 A good deal worse
-4 Moderately worse
-3 Somewhat worse
-2 A little worse
-1 Almost the same, hardly any worse at all
0 No change
1 Almost the same, hardly any better
2 A little better
3 Somewhat better
4 Moderately better
5 A good deal better
6 A great deal better
7 A very great deal better

Question 4 assesses change in overall health related quality of life.

Q4. Since your first cough questionnaire, has there been any change in the impact of your cough on your health and quality of life?

-7 A very great deal worse
-6 A great deal worse
-5 A good deal worse
-4 Moderately worse
-3 Somewhat worse
-2 A little worse
-1 Almost the same, hardly any worse at all
0 No change
1 Almost the same, hardly any better
2 A little better
3 Somewhat better
4 Moderately better
5 A good deal better
6 A great deal better
7 A very great deal better

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