A description of cough in tuberculosis and other respiratory conditions

A thesis submitted to the School of Medicine and Dentistry, Queen Mary University, London in partial fulfilment of the requirements of the Degree of Doctor of Philosophy (PhD).

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STATEMENT OF ORIGINALITY

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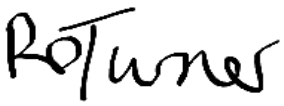

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Turner RD, Bothamley GH. How to count coughs? Counting by ear, the effect of visual data and the evaluation of an automated cough monitor. Respir Med. 2014; 108: 1808-15.

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Correspondence

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Abstract

Cough is common but has been under-researched. In tuberculosis cough is probably of particular relevance in transmitting infection.

This thesis explores several interlinking areas. Regarding how best to measure cough, coughs are shown to be recognizable to the human ear, but automated cough monitors can disagree with auditory cough counting. A novel approach to testing cough reflex sensitivity is described, $E_{62.5}$.

There was no evidence to support the hypothesis that cough has unique characteristics in tuberculosis, in terms of symptoms, frequency and clustering. A significant reduction of cough frequency in tuberculosis is demonstrated overnight. Clinical correlates of 24-hour cough frequency were explored; female sex in unexplained chronic cough, and sputum smear status in tuberculosis were important, and possibly transfer factor in pulmonary fibrosis and duration of symptoms prior to treatment in COPD exacerbations. Cough frequency correlated poorly with symptoms.

There seem to be both generic and disease-specific mechanisms associated with cough. This was further suggested by a faster reduction in cough frequency with treatment in pneumonia than in acute asthma and COPD exacerbations, correlating with C-reactive protein decline only in pneumonia. A serial reduction in 24-hour cough frequency in tuberculosis during the whole course of treatment was demonstrated, a potentially novel approach to measuring treatment response.

The role of genetic polymorphism in the cough receptor gene *TRPV1* was explored, but, at least in chronic cough was not demonstrated to predict coughing.

Regarding the infectiousness of coughs, an airborne particle counter was shown not to be sensitive enough for measuring droplets released during coughing in room air. However, I demonstrate for the first time a significant association between 24-hour cough frequency in TB and household infection.

This work has set a foundation for the further investigation of the mechanisms, processes and patterns of coughing with respect to tuberculosis transmission and other contexts.

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Contents

List of figures

List of tables

Glossary

ANOVA – analysis of variance

BCG - bacillus Calmette-Guérin

Bout - episode of coughing in which the interval between individual coughs is <2 s

c/24h – coughs per 24 hours

c/h – coughs per hour

C2 - concentration of capsaicin solution required to produce two coughs in a cough challenge test

C5 – concentration of capsaicin solution required to produce five coughs in a cough challenge test

CI – confidence interval

COPD – chronic obstructive pulmonary disease

CRP – C-reactive protein

CT - computed tomography

CURB – confusion, urea concentration, respiratory rate, blood pressure

DGH – District General Hospital

$E_{62.5}$ – number of coughs elicited by a 62.5 µM solution of capsaicin in a cough challenge test

E_{max} - maximum number of coughs evoked by any concentration of capsaicin in a cough challenge test

Episode – period of coughing consisting of either a either a lone cough or a bout of cough sounds separated by <2 s

Epoch- episode of coughing in which the interval between individual cough sounds is <2 s

EPTB – extra-pulmonary tuberculosis

FEV_1 – forced exhaled volume in one second

FVC – forced vital capacity

FFP – filtering face piece

HEPA - high-efficiency particulate arrestance

HRCT – high-resolution computed tomography

HUH – Homerton University Hospital (NHS Foundation Trust)

ICC – intraclass correlation coefficient

IGRA – interferon gamma release assay

ILD – interstitial lung disease

IPF – idiopathic pulmonary fibrosis

IQR – interquartile range

KCO - lung transfer factor for carbon monoxide per unit lung volume

LCM – Leicester Cough Monitor

LCQ – Leicester Cough Questionnaire

Lone cough – a cough separated in time from other cough sounds from the same individual by ≥ 2 s

LTBI – latent tuberculosis infection

MIRU-VNTR - mycobacterial interspersed repetitive units - variable number of tandem repeats

Mtb – Mycobacterium tuberculosis

PEFR – peak expiratory flow rate

PTC - phenylthiocarbamide

q – quantum, the lowest dose of *Mycobacterium tuberculosis* required to cause new infection

QFT – QuantiFERON®

RH – relative humidity

SD – standard deviation

SNP – single nucleotide polymorphism

TB – tuberculosis

TLC – total lung capacity

TLCO – total lung transfer factor for carbon monoxide

TRPV1 – transient receptor potential vannilloid 1

UIP – usual interstitial pneumonia

URTI – upper respiratory tract infection

VAS – visual analogue scale

SECTION ONE: INTRODUCTION

1 Background and aims of thesis

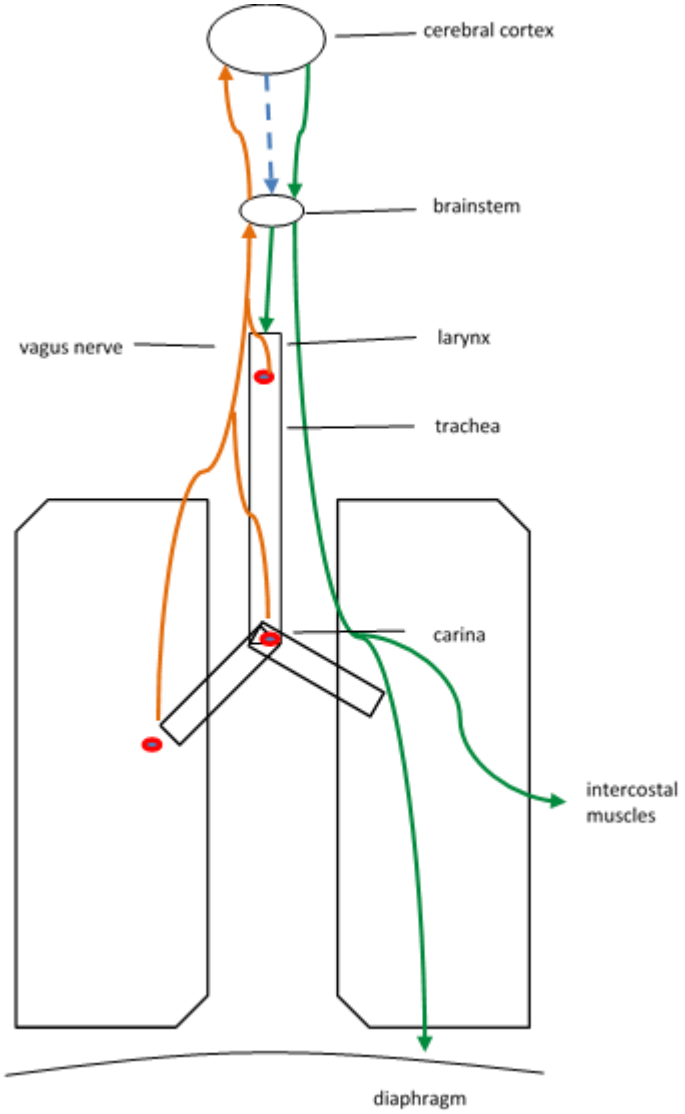
1.1 Definition of cough

Cough is an onomatopoeic word, in English as well as many other languages. The distinctive sound is produced by the rapid release of air through the opening vocal cords which have been held closed against a forced expiratory movement.[1] Correspondingly, the definition of cough proposed by the British Thoracic Society is a 'forced expulsive manoeuvre, usually against a closed glottis and which is associated with a characteristic sound'.[2]

There has been debate about whether a 'true' cough is only that which is preceded by an inspiratory phase in contrast to an expiration reflex which is not.[3] On this basis a burst of cough sounds preceded by a single breath would be classed only as a single cough. However, the majority of doctors with an interest in cough seem to classify each typical sound within a cluster as an individual cough.[4] Presumably the lay person or patient experiencing cough would classify a cough likewise. To avoid ambiguity, particularly when the frequency of coughing is being discussed, individual cough sounds are often distinguished from bouts, peals, series or epochs of coughing.[5,6] A cough epoch has been defined, arbitrarily, as more than one closely-spaced cough sounds, each of which occurs no later than 2 s than the one preceding it.[5] Throughout this thesis a 'cough' has been taken to refer an individual cough sound while a cough bout refers to the equivalent of a cough epoch following the definition above. In turn, a cough 'episode' refers to either a single cough or a coughing bout (see

1.2 The physiological role of cough

The purpose of cough is defensive, for removing particles, mucus and other potentially harmful material from the airways.[1] It is a physiological phenomenon that is shared with other species, but it perhaps more important in humans as our upright posture leads to an increased risk of aspiration.[7] Sensory receptors are located mainly in the proximal airways, the larynx and tracheobronchial tree,[8] and are provoked by various



Sensory afferent cough receptors, predominantly located in the central airways and larynx, input via the vagus nerve (orange) to the brainstem, which in turn triggers coughing via efferent relay (green) to the diaphragm and intercostal muscles. Note the involvement of the cerebral cortex in sensation and the volitional control of cough. Adapted from reference [9].

Figure 1.1. Basic anatomy of the cough reflex.

mechanical and chemical stimuli including smoke, hypotonic and hypertonic saline, acidic and alkaline solutions, bronchoconstriction and heat.[9] The importance of an intact cough reflex is demonstrated by an increased risk of aspiration pneumonia where it is impaired, for example in old age[10,11] and stroke.[12] Also, in some

circumstances, voluntarily suppressing cough is thought to lead to chronic pulmonary infection.[13] Conversely, the use of angiotensin-converting enzyme (ACE) inhibitors, which enhance the cough reflex,[14] has been associated with a reduced risk of pneumonia.[15]

1.3 Mechanisms of coughing

The current paucity of effective anti-tussive treatments reflects the poor state of knowledge of the mechanisms of cough.[16,17] The basic anatomy of the cough reflex has been elucidated mainly from animal studies, predominantly involving the guinea pig and is illustrated in Figure 1.1.[9] The vagus nerve communicates with the nucleus tractus solitarius to trigger the characteristic pattern of muscular activity.[9,18,19] Input from the cerebral cortex is also clearly important, as demonstrated by the ability to cough at will or to voluntarily suppress coughing.[20] Effective placebo treatments for cough probably operate via this route.[21]

Sensory afferents include rapidly adapting Aδ fibres and unmyelinated C-fibres which interact, but broadly speaking are more responsive to mechanical activity and chemical stimuli respectively Figure 1.2.[8,22–24] Although the large majority of the work leading to the characterisation of these different fibre types has been done in animal models of cough (predominantly the guinea pig), a very similar innervation pattern also seems to be present in humans.[25] The exact purpose of different types of afferent nerve continues to be investigated, but is has been suggested that C-fibres could be mainly involved in the regulation of cough, whereas the role of Aδ fibres is in cough initiation.[26]

Important mediators for cough receptors include bradykinin, prostaglandins and adenosine triphosphate.[19]

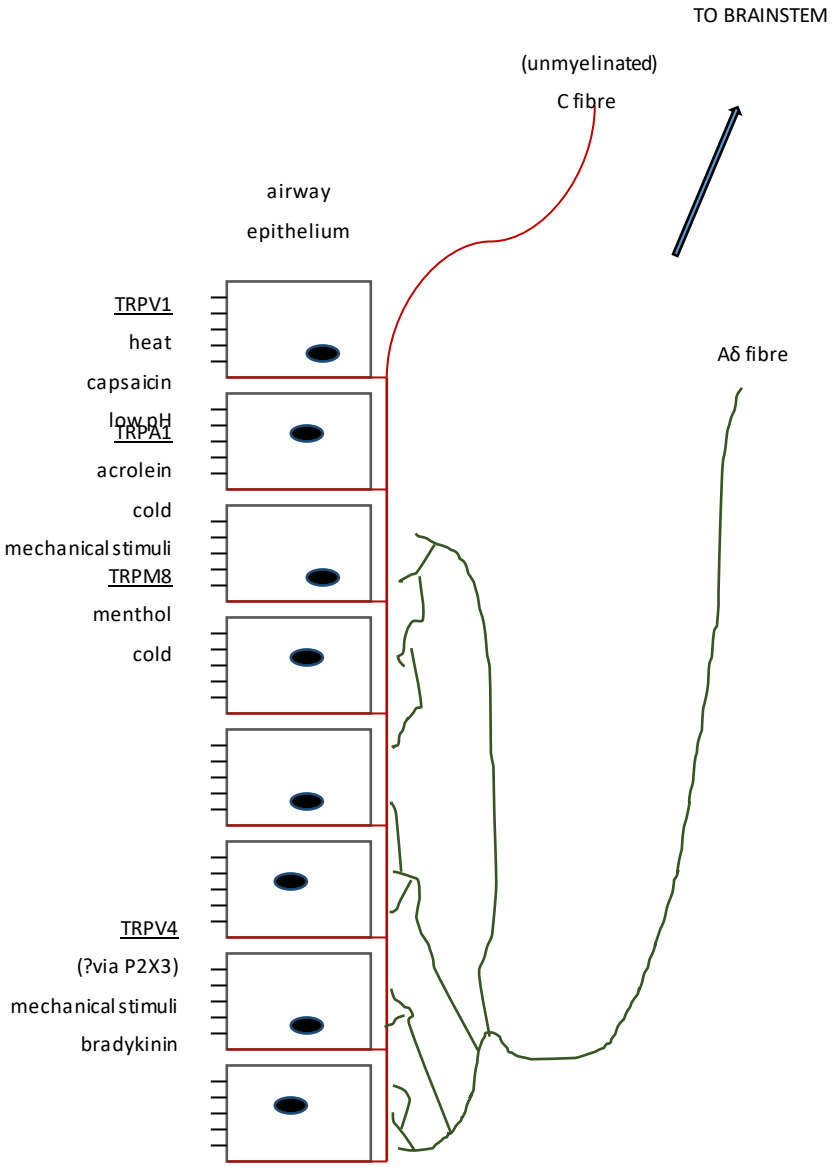


Illustration of the two main afferent nerve types thought to be involved along with their associated TRP receptors and known triggering stimuli. Adapted from references [23,24,27,42].

Figure 1.2. Sensory afferents of the cough reflex.

1.3.1 Cough receptors

1.3.1.1 *C-fibres*

The receptors associated with C-fibres have been better characterized than those triggering the rapidly-adapting Aδ fibres. In particular, the transient receptor potential (TRP) ion channels have attracted attention.[27] TRP channels compose a large group

involved in sensory transduction in response to a number of stimuli in a variety of cell types but in the lung appear to have an important role in cough.[27–31] For example, resiniferatoxin elicits the cough reflex and iodoresiniferatoxin inhibits it by acting on the transient receptor potential vanniloid-1 receptor (TRPV1).[29,32] TRPV1 is also activated by heat, particles and other irritants including capsaicin, the extract of chillies used experimentally for measuring cough reflex sensitivity, as discussed below (Section 1.6.2.1).[31] TRPV1 channels are more abundant in the airways of patients with chronic cough compared to healthy controls[28,33] and have been shown to be upregulated by rhinovirus infection of a human cell culture.[34] TRPA1 also appears to have an important role, by activating the cough reflex directly and indirectly.[35] TRPA1 responds to tussogenic irritants which do not activate TRPV1, such as acrolein which is present in cigarette smoke and industrially-polluted air.[36]

TRPA1 is activated by paracetamol.[37] Epidemiological evidence has linked frequent paracetamol use to asthma,[38] and could perhaps also be associated with cough, potentially via TRPA1, although this has not previously been explored.

TRPM8 responds to menthol and cold temperatures and also seems to be associated with C-fibres in animal models.[39]

Despite the clear roles of TRPV1, TRPA1 and TRPM8 in the cough reflex, inhibitors of these receptors have yet to show clinical utility. For example, in a small study in unexplained chronic cough, the TRPV1 antagonist SB-705498 led to a reduction in sensitivity to capsaicin on cough challenge testing, but no changes in daily symptoms or 24-hour cough frequency.[40]

1.3.1.2 *Aδ fibres*

Another TRP channel, TRPV4, is also widely-expressed in the respiratory tract.[41] Recently-published work has demonstrated that hypo-osmolar solutions and TRPV4 ligands cause firing of Aδ fibres, vagus nerve and cough in a conscious guinea pig model, independent of C-fibre activation, and all blocked by TRPV4 inhibitor.[42] TRPV4-mediated activity in this study was also blocked by an antagonist of P2X3, an ATP-

gated ion channel present in airway nerves.[42] This finding is of particular interest given the substantial reduction in cough frequency observed in a subset of patients with unexplained chronic cough in a recent small phase 2 trial of AF-219, an inhibitor of P2X3.[43]

The mechanisms of cough are clearly complex and probably involve several inter-linking peripheral and central pathways. The lack of efficacy of TRPV1 receptor antagonism on cough frequency compared to the results seen with blocking P2X3 support the idea of a more subtle regulatory role in the cough reflex for C-fibres, in contrast to a role of Aδ fibres in cough initiation. More preclinical and clinical work in this area is required.

1.3.1.3 *Taste receptors*

Other sensory receptors in the respiratory tract may also be important in cough. One such group are bitter taste receptors. Their evolutionary role is presumably to detect toxins and initiate their expulsion, and they have been found in human airway smooth muscle,[44,45] where they may have a role in asthma.[46,47] For example, the G-protein coupled T2R bitter taste receptors may promote bronchodilatation.[44,48] This would aid airway clearance of noxious particles, but it is not known if T2R receptors are directly involved in the cough reflex. Conversely sweet tasting substances have been used traditionally as cough remedies. One study showed a reduction in cough reflex sensitivity after rinsing the mouth with sucrose solution, while a bitter substance had no effect.[49]

That cough is linked with taste sensation is also suggested by the gustatory side effects observed alongside cough suppression with P2X3 inhibition,[43] presumably a result of inhibition of P2X receptors which are known to have an important role in taste.[50]

One simple way of gaining further insight in this area could be through correlating variation in taste sensation in the population, such as exists for phenylthiocarbamide (PTC), with variations in coughing patterns.

1.3.2 Genetic influences on coughing

The genetic influence on cough patterns has been investigated rarely. Angiotensin converting enzyme receptor polymorphism may be important for developing ACE inhibitor-induced cough[51] but probably not for chronic cough of other causes,[52] although if may affect cough reflex sensitivity.[53] Polymorphism in the tachykinin gene *NK-2R* has been associated with cough reflex sensitivity to capsaicin but not with symptoms of chronic cough.[54] There appear to be very few other studies comparing objectively-measured cough to genetic variation.

Single nucleotide polymorphisms (SNPs) of the *TRPV1* and *TRPA1* gene have been described.[55] Several SNPs of *TRPV1* have been associated with cough symptoms in a large observational study involving two independent populations whereas *TRPA1* SNPs did not have this association.[55] It is not known whether SNPs of *TRPV1* influence objectively measured coughing patterns.

1.4 Pathological and other causes of excessive coughing

Cough is extremely common, being one of the most common reasons for medical consultation in the UK and other countries.[2] Excessive coughing is associated with a range of pathological states affecting the upper respiratory tract, lower airways, lung parenchyma and pleura.[56] Gastro-oesophageal reflux disease (GORD) is also stated to be a common cause[56] although this is debated,[57] the purported cause being direct vagal activation of the cough reflex via cough receptors in the oesophagus, or laryngeal irritation by gastric contents.[58]

The majority of coughs are short-lived, self-limiting and due to upper respiratory tract infections. Chronic coughs are defined arbitrarily as those persisting longer than eight weeks.[2] It is estimated that approximately 10% of individuals in the community experience chronic cough.[59] Up to 40% of patients referred to respiratory clinics with chronic cough may have symptoms that are unexplained in spite of investigation and trials of treatment,[60] although rates this high have mainly be reported from tertiary referral cough clinics. Unexplained chronic cough may represent hypersensitivity of the

cough reflex, possibly a neuropathic disorder caused by upregulation of cough afferent pathways.[61,62]

Trials of treatment are often used empirically in chronic cough. While often successful, a problem with this approach is that where cough has not been measured objectively, it is difficult to confirm or refute the level of response. Furthermore, chronic cough can improve spontaneously,[63,64] and responds to behavioural training,[65,66] and placebo, as in randomized controlled trials of proton pump inhibitor therapy.[67] The true epidemiology, burden and causes of chronic cough have therefore not been adequately defined in longitudinal cohort studies.

Infectious organisms and the substances they produce may stimulate cough chemo- and mechanoreceptors,[68] as may components of the host immune response including increased mucus production and inflammatory mediators.[19] Infectious diseases and other causes of cough also seem to affect the overall sensitivity of the cough reflex, as seen in causes of acute,[34,68,69] and chronic cough.[34,68–71]

Aside from pathological causes, excessive coughing is associated with smoking,[2,72] and environmental occupational irritants.[73] This probably results from both the direct activation of cough receptors by inhaled particles and chemicals, and via the resultant inflammatory response which follows. ACE inhibitors cause cough through the accumulation of the pro-tussive peptides bradykinin and substance P in the lung.[74]

Female sex influences cough in some respects; women appear to have a more sensitive cough reflex than men,[70] unexplained chronic cough is more common in women than men, and women with this disorder have a higher cough frequency.[75] Sex also seems to affect cough frequency in asthma,[76] but not in idiopathic pulmonary fibrosis[77] or COPD,[78] and data in acute cough due to upper respiratory are conflicting.[79,80] Reasons for an effect of sex are unclear but there is a suggestion of a link between idiopathic chronic cough and predisposition to autoimmune disease, which also shows a female bias.[81] It is not clear exactly how age influences coughing patterns on the whole.[82]

1.4.1 Variation in cough with pathological cause

Variations in cough with pathological cause have been studied rarely. Effect on quality of life by coughs due to stable asthma, COPD, asthma and bronchiectasis and unexplained chronic cough in one study of 147 patients did not significantly vary with the underlying cause.[83] The relative subjective appreciation of coughing between diseases appears to have been little studied elsewhere. This could be relevant for the transmission of infection as will be further discussed.

One method of investigating cough mechanism is measurement of the minimal dose of the tussogenic compounds capsaicin or citric acid required to cause a certain number of coughs, as discussed below (Section 1.6.2.1).[5] However, cough reflex sensitivity measured in this way is variable and is not always distinguishable between health and disease,[69,70,78,84] although a new approach to the capsaicin cough challenge may be more useful in this regard.[85]

As will be discussed later (Section 1.6.2.2), ambulatory cough monitoring is a relatively recent development.[86] Where cough frequency has been objectively measured, variability between individuals within the same disease group is high.[75–78,80,87,88] Although the mechanisms for cough may vary between disease it is therefore not clear if there are significant differences in overall cough frequency between diseases. Cough seems to be less frequent at night than during the day,[89–91] but it is also unknown if there are significant differences in diurnal variation in cough between diseases. It is plausible, for example, that early morning coughing in asthma is more pronounced than in other diseases to coincide with the diurnal variation in lung function, and therefore presumable disease activity, in this disease.[92] Another difference between diseases which needs further investigation is the way in which coughs are clustered within bouts. For example, repeated short bursts of coughing might be more efficient than recurrent single powerful coughs at clearing mucus[93,94] or aerosolizing pathogens for the transmission of disease.

The Manchester cough research group has reported predictors of objectively-measured daily cough frequency in several diseases. In chronic obstructive pulmonary disease (COPD) cough frequency seems to relate to current and previous cigarette smoking and sputum neutrophila.[78] In idiopathic pulmonary fibrosis (IPF) the cause of cough is unclear and cough frequency correlates poorly with lung function.[77] Cough in asthma also does not relate well to pulmonary function but perhaps to measures of airway inflammation.[76,95] In cystic fibrosis cough associated with airway inflammation, sputum production and lung function impairment.[96] Loudon and Brown in the 1960s reported that in pulmonary tuberculosis overnight cough frequency increased with radiological extent of disease.[88]

Further work in this area across disease groups, through clinical observation and ambulatory cough monitoring, should give further insights into the associations between pathological processes and cough.

1.4.1.1 *Cough sounds*

The notion of there being different types of cough in different disease is consistent with the range of terms used to describe coughs by medical textbooks and the layperson alike.[97] The sound of the cough is said to predict pathology, a brassy cough for example equating with malignant tracheal compression and a bovine cough associated with laryngeal palsy.[97] The practical utility of these terms is unclear as there is no evidence that health professionals are able to make diagnoses based on cough sounds, at least when they are heard in isolation for COPD, asthma, pulmonary fibrosis and bronchiectasis in adults.[98] As will be discussed later (Section 1.6.2.4), attempts have been made to compare cough sounds between diseases, both in terms of the duration of component phases of the cough sound and, more promisingly, composite acoustic frequencies. The presence of airway mucus,[99] pulmonary consolidation[100] and bronchoconstriction[101] may be significant.

1.4.2 Respiratory mucus

Dry *versus* productive might be one of the commonest ways of describing coughs by both doctors and the general public, to refer to the absence or presence of associated sputum production respectively, which does seem to be easily recognizable.[98] Although one of the roles of cough is the clearance of respiratory secretions,[102] sputum production correlates more with cough in some diseases, such as cystic fibrosis,[96] and COPD[79] than in others such as asthma.[103] Airway mucus may not only influence the presence of cough but also patterns of cough: sputum expectoration in COPD has been observed to follow bouts of coughing rather than less closely-associated individual cough sounds.[93]

The mucociliary escalator functions continuously to remove respiratory secretions of relatively low volume and viscosity. Any impairment of ciliary function, for example by smoking or infection, will require the airway to be cleared by coughing.[104,105] Both the quantity and characteristics of respiratory secretions are also likely to be important determinants of cough; any significant increase in mucus production or viscosity will also overwhelm ciliary clearance.[104] Mucins are the large glycoproteins responsible for the properties of respiratory mucus.[105] They are stimulated by toxins, inflammatory states, and viruses (including respiratory syncytial virus, influenza and rhinovirus) and bacteria (including *Staphylococcus, Streptococcus, Pseudomonas, Haemophilus* and *Mycoplasma*).[104,106]

MUC5AC and MUC5B are the principal secreted mucin components of respiratory mucus.[104] MUC5B appears to have a particularly important role in host defence through the functioning of the mucociliary escalator. The corresponding *MUC* genes exist as variants in the human population[107] and a large population study has shown single nucleotide polymorphisms (SNPs) of *MUC5AC* to be associated with self-reported respiratory diagnoses including bronchitis and asthma.[108] It is not clear if there is a direct association between SNPs of *MUC5AC* and objectively-measured

cough frequency in health and disease due to effects on mucus quantity and composition but this requires investigation.

1.5 Cough and the transmission of disease

In the public consciousness 'coughs and sneezes spread diseases'.[109,110] There is a natural aversion to the coughs of others and it is considered good etiquette to avoid coughing openly in company, particularly if unwell.[111] Despite this, it is unclear exactly how much coughing contributes to the transmission of infection and which characteristics of cough are likely to be more infectious.

1.5.1 Aerosol transmission of disease

Many infectious diseases are spread by the airborne route, either as the only mode of transmission or one of several modes available to an organism.[112] True airborne transmission is distinguished from droplet spread, the latter involving larger particles which quickly fall to the ground under the influence of gravity.[113] Large droplets have the potential for infection only if a susceptible individual is positioned in close proximity to the source, said to be less than 1 m,[114] unless secondarily doing so via objects in the environment acting as fomites. Airborne microorganisms, by contrast, have the potential to travel a far greater distance.[115] To overcome gravity, particles probably need to measure around 5 µm or less, in order that frictional forces are of sufficient relative magnitude as predicted by Stokes' law.[113] Airborne particles, or droplet nuclei, may have started off as larger droplets on release from the respiratory tract, depending if there has been enough time for evaporation to lead to shrinkage down to an airborne size range before reaching the ground, as discussed further in Chapter 10. Further particle movement and changes in composition and size will be affected by ambient conditions such as air circulation, temperature, humidity, ultraviolet light, the presence of other particulate matter, and duration of transit through the air.[113,115,116] The relative importance of the airborne route of infection varies between diseases; infections may be obligate, preferential or opportunistic airborne spreaders.[112,114]

1.5.1.1 *Tuberculosis*

Tuberculosis (TB) is possibly the only communicable disease which is almost completely transmitted by the airborne route.[112,117] Aerial infection was demonstrated convincingly by Riley in the 1950s when tuberculosis was induced in laboratory-reared guinea pigs by air vented into otherwise sterile incubation chambers from a TB ward below.[118]

Evidence for the dominance of the airborne route in TB transmission comes from epidemiological data, particularly where outbreaks have occurred. For example in the 1960s almost half of the crew of 306 on board the *USS Byrd* became tuberculin skin test positive after sharing the ship with a case of pulmonary TB. The air on this ship was completely recirculated. The chance of contracting the infection was no higher amongst sailors who had had direct contact with the source case than those who had not, suggesting transmission by large droplets or direct contact was relatively insignificant.[117,119] Another TB outbreak was studied in a juvenile correctional facility where the daily movements of the residents were closely monitored. The apparently random pattern of new acquisition of infection amongst the two wings of the building where the boys slept would have been very difficult to explain if direct contact were necessary for TB transmission, but much better favoured airborne spread.[120]

Further support for the aerial infection comes from what is known about the initiating events in new infection. It was shown in the 1940s in rabbits that inhaled particles need to be small enough to reach the alveoli; bacteria deposited more proximally in the airways do not seem to cause tuberculosis.[121] Interaction between *Mycobacterium tuberculosis* and alveolar macrophages seems to be a necessary early step in new infection.[122,123] Respiratory particles containing *M. tuberculosis* (Mtb) are expelled from infected hosts during talking, singing and coughing.[120,124–126] To reach the alveolus they need to measure <10 µm in diameter,[127] ideally probably <2 µm,[128] as larger particles will be deposited in the upper airway or more centrally due to their physical size and inertial mass.[128] Larger droplets would therefore be too large to cause TB,

unless they evaporate quickly to a size small enough to resist gravity and become potentially capable of reaching the alveolus of a new host.

1.5.1.2 *Measles*

Transmission of measles seems to occur via virus-containing particles expelled from the respiratory tract. Droplet spread over short distances is probably the predominant mechanism, as the virus gains entry through the upper respiratory tract.[129] Epidemiological studies have also shown the airborne route to be important. For example in one school outbreak in Illinois, 69 cases occurred in a very low prevalence area several days after exposure to a strongly symptomatic individual. Because only 22-65% of the pupils were likely to have had close contact with the source case whilst changing classrooms, droplet spread was unlikely to have been the only means of transmission.[130] In 1991, 16 cases of measles were directly traced to attendance at an sporting event in which the index case was one of the athletes.[131] 10 of the cases had no known direct contact with the index and two of them were spectators sitting >30 m above the athletes' entrance to the indoor stadium.

Indirect evidence for airborne transmission of measles comes from the pre-vaccine era.[117] The effectiveness of ultraviolet air decontamination at controlling measles epidemics in the 1940s was observed by comparing test schools with controls in Pennsylvania. Over a five year period there were significantly fewer cases in the UV-air disinfected schools. Other evidence from measles outbreaks, and data from air travel-acquired cases,[132] also support the airborne route of measles transmission. The relative contribution of airborne compared to droplet spread however is unclear.

1.5.1.3 *Influenza*

Influenza RNA has been detected in the air of clinical environments.[133] The virus can retain its infectiousness to laboratory animals in airborne particles for periods of at least 24h depending on ambient conditions,[134] and also possibly on disease strain.[135] Mice and ferrets can infect each other when separated by distances up to 2.5 m,[136] too far to be explained by droplet spread of disease. Influenza can be induced in humans

and other animals experimentally by exposure to aerosolized virus[137] and the dose required seems to be lower than that necessary to cause infection if applied directly to the nasal mucosa.[135,138] Although airborne transmission of influenza therefore can and probably does happen,[139] the majority of epidemiological evidence suggests that droplet spread or direct contact is the predominant mode of infection.[135] For example hospital clusters show that nurses having direct contact with an index case have a much higher chance of infection than others in the same vicinity.[135] The comparative effectiveness of the ventilation systems in two aeroplane influenza outbreaks was associated with no significant difference in viral transmission, and contact or droplet control measures (e.g. cohorting, isolation rooms and gloves) seem to be the most effective ways of limiting new infections in healthcare settings.[140] Influenza probably enters the body via the conjunctiva and gastrointestinal tract as well as the upper and lower respiratory tract[141] allowing transmission via larger droplets or fomites.

1.5.1.4 *Other pathogens*

Rhinovirus probably is transmitted predominantly by hand contact and self-inoculation,[142] although droplet spread seems to be important and airborne transmission possible.[143] The same seems to be true for respiratory syncytial virus.[144] Smallpox is an opportunistic airborne transmitter,[117] and so may be *Neisseria meningitides.*[145] Pneumococcus does not appear to be particularly contagious and is probably transmitted by direct contact with respiratory secretions, entering via the upper respiratory tract.[145,146] For pneumococcal pneumonia and bacterial meningitis, acquisition of the responsible organism may not be as important a step in new infection as the transition between asymptomatic carriage in the upper airway and invasive disease.[145] Chickenpox, norovirus, rotavirus and *Aspergillus* may also be opportunistically spread by the airborne route.[115] Pertussis has also been shown convincingly to be capable of airborne transmission in a baboon model, although again direct contact in humans is likely to be the predominant mode of spread.[147]

There therefore remains a significant lack of knowledge about the extent to which true airborne transmission of infection occurs although the evidence for tuberculosis is strongest by far.

1.5.2 Particle production during respiratory activities

The relative importance of respiratory activities in the production of infectious particles has been little studied. Relevant factors are likely to include the relative frequency and force of each activity and the size, quantity and composition of particles at the point of expulsion from the body.[127] There are some data in tuberculosis, but most work on particle production has been done in healthy volunteers.

1.5.2.1 *Particle size*

Following on from the discussion above, the ultimate size of particle released during respiratory activities will be an important determinant of its potential mode of transmitting infection, with the cut off often made between predominantly airborne transmission for particles <5 µm in diameter and mainly droplet spread if >5 µm.[114] As mentioned for tuberculosis, a particle's size will also be a major determinant of its potential to reach and interact with particular target sites within the new host, for example a diameter of <10 µm is required for the possibility of reaching the alveolus.[128]

Several studies have attempted to measure the range of sizes of particles produced during normal respiratory activities through a variety of techniques.[127] Older studies predominantly used the method of particle impaction against a surface and light microscopy.[148] This work is now thought to have been insensitive to smaller particles as the average measured diameter of particles produced by coughing and talking was 50-150 µm.[127] More recent studies have used aerodynamic particle sizers, charge separation methods or laser diffraction methods,[127,149,150] and generally agree that the predominant particle size during breathing, coughing and talking is in the range 0.01-5 µm.[127,149] Recent data are limited but coughing seems to produce a larger range of

particle sizes than talking.[150] When healthy individuals cough openly perhaps 97% of particles are smaller than 1 µm and 99% smaller than 10 µm.[149]

To my knowledge only one study has looked at particle production during singing, using an impaction technique, and found the predominant size range to be similar to that for coughing.[124] There appears to be no recent study measuring particles produced by sneezing, but the fact that a large number of emitted droplets are visible by eye with stroboscopic photographic techniques[151] suggests that the mean size is larger than that of those produced by other respiratory manoeuvres, although of course there will presumably also be a large number in the microscopic size range.

1.5.2.2 *Particle number*

Few studies have attempted to compare the number of particles produced by different respiratory manoeuvres and differences in methodology makes comparing studies difficult. Older studies used particle impaction methods and light microscopy, and therefore had a relative high limit of detection. Gerone *et al*. reported a single sneeze from one volunteer produced 1.6 million particles compared to around 200,000 for a single cough in an individual with a viral upper respiratory tract infection.[152] Loudon and Roberts estimated that 3 healthy volunteers produce a mean of approximately 7,000 particles by coughing 15 times and 1,800 when talking loudly (counting from 1 to 100).[148]

More recent studies have used a variety of methods. Papineni and Rosenthal used two methods, an optical particle counter and impaction onto glass with electron microscopy with 5 healthy volunteers. There was wide variation between subjects but the mean numbers of particles per L produced by coughing, mouth breathing, nose breathing and talking were 483, 72, 27 and 113 respectively.[153] Xie *et al*. used a solid impaction method and reported an average of 108 particles were produced by seven healthy individuals both by talking (counting from 1 to 100) and voluntarily coughing 20 times, although the predominant particle size measured was 50-75 µm.[154] Morawska *et al.* used an aerodynamic particle sizer to compare particle production by

15 healthy volunteers. The concentration of particles produced by vocalizing during a 2 minute period (alternating an "aah" sound for 10 s with breathing normally for 10 s) was greater than that by voluntarily producing 'mild throat clearing coughs' for around 30 s (0.75 compared to 0.48 particles/cm^3). Breathing, whispering and talking (voiced counting) produced lower concentrations (*c*. 0.05, 0.08 and 0.28 particles/cm^3 respectively).[150]

It is therefore difficult to make conclusions about absolute numbers of particles from these limited and varied studies, but the relative numbers of particles produced during coughing and talking seems to be of the same order of magnitude, and higher than those produced during normal breathing. Although this applies for healthy individuals, the relative numbers of particles produced by different activities in disease is not known.

1.5.2.3 *Particle composition*

Infectious particles obviously need to contain viable microorganisms. The other components will also presumably have an effect on a particle's potential to cause infection by influencing its size and its movement, both through the air and within the respiratory tract of a potential secondary host, and by promoting the viability of organisms contained within, through effects on protection from desiccation, ultraviolet light and first lines of defence of the secondary host. There have been few studies specifically exploring this. However, Zayas *et al*. recently used a cough simulator machine and a mucus simulant solution to show that increasing the cohesivity of the solution decreased the number of particles expelled and aerosolized during a 'cough'.[155]

The composition of mucus varies with disease,[105] smoking,[156] and possibly genotype.[108] The origin within the respiratory tract of an expelled particle will affect both its content of mucus and microorganisms. Particles originating in the mouth will contain a higher content of saliva whereas those from the airways will contain more mucus. Organisms causing upper respiratory tract symptoms, such as the common cold,

influenza and measles, will presumably occur in higher concentrations in the upper airway whereas those causing lung disease, such as pneumonia and tuberculosis, will be at their highest concentrations in the lower airways. The predominant site of origin of resultant respiratory particles will vary with respiratory activity. Sneezing is initiated by receptors in the nasal mucosa[157] and the majority of particles produced seem to originate in the mouth and nose whereas cough presumably more efficiently expels particles from the lower respiratory tract.[102]

1.5.2.4 *Expiratory flow*

The number and size of particles formed during expiratory manoeuvres will be influenced by the speed with which air moves outwards over respiratory epithelia. In turn, the range and behaviour of aerosolised particles in the environment will be affected by the force at which they are released.

Peak flow rates during expiratory manoeuvres will vary depending on lung size, physical fitness, gender and other factors including disease.[158,159] The maximum velocity of air expelled by coughs from healthy volunteers in one study using shadowgraph imaging was 2.2-5.0 m/s for women and 3.3-14 m/s for men.[160] The same authors report that for these values are similar for sneezing (4.5 m/s) and higher than for breathing, either through the mouth (1.3 m/s) or nasally (1.4 m/s).[161] Using a laser particle detection technique another group measured the initial air velocity in healthy volunteers during coughing to be 15.3 m/s in men and 10.6 m/s in women; during speaking these figures were 4.07 m/s and 2.31 m/s respectively.[162] Blowing on certain musical instruments may produce particularly high expiratory flow velocities.[163]

1.5.2.5 *Activity frequency*

Given that all expiratory manoeuvres have the potential to release infectious particles, the relative importance of each respiratory activity in disease transmission will clearly in part depend on the frequency with which it occurs. In individuals free of disease coughing and sneezing are rare events. Sneezing is a symptom of the common cold and influenza and also measles.[129] Coughing is typical in all these conditions but is also

usual in pneumonia and bronchitis, and is the cardinal symptom of pulmonary tuberculosis. Persistent coughs in particular are typical of TB, so much so that the presence of chronic cough is used as a screening strategy for tuberculosis in endemic parts of the world.[164] Talking, shouting, playing wind instruments and singing will vary with personal and social factors but will presumably be diminished in frequency in those who are unwell. The frequencies of respiratory activities in infectious diseases have been rarely reported.

1.5.3 The importance of cough in the transmission of tuberculosis

Mechanisms of bacterial aerosolization deserve special attention in TB since it is perhaps the only infection to be spread entirely by the airborne route. Coughing was first shown experimentally to be capable of spreading tuberculosis in the 1890's, at least from humans to guinea pigs, by Carl Flügge, a key early proponent the role of respiratory particles in TB transmission.[165–167] Coughing is likely to be of prime importance for TB transmission for the reasons above: cough frequency is increased in TB, coughing clears material from the distal airways and coughs produce large numbers of particles at high velocity, the majority of a size small enough to be carried as true aerosols and reach the alveoli of susceptible hosts.

1.5.3.1 *Determinants of the infectiousness of tuberculosis*

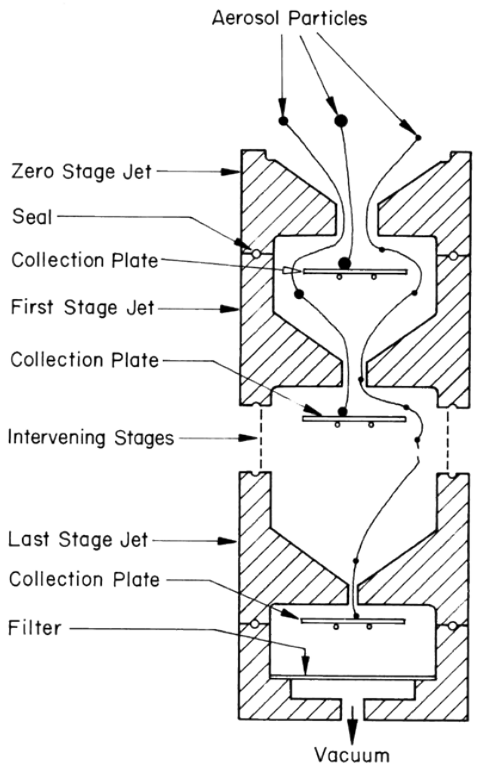
For the transmission of TB there are several broad stages: (1) the clearance and release of bacilli from an index case, (2) the aerial passage of viable bacilli through the environment, (3) inhalation into the alveoli of a new potential host and (4) initiation of new infection. Variability in TB transmission clearly exists. This is demonstrated by the outcomes of routine contact tracing, where overall probably fewer than half of sputum smear-positive cases of pulmonary disease pass infection on to household contacts,[168,169] and is suggested by TB outbreaks, in which unusually large numbers of secondary infection are related to a particular epidemiological setting and individual source case.[170,171] This variability must be explainable by factors affecting one or more of the four stages above. Smoking in the secondary host may increase susceptibility to

the acquisition of infection through effects on the innate immune system.[172] Other personal epidemiological risk factors for tuberculosis, such as very young age,[173] alcohol misuse,[174] malnutrition,[175] diabetes,[176] and HIV infection,[177] probably act mainly on the adaptive immune system, thereby influencing progression of latent Mtb infection rather than its acquisition. Relevant environmental variables for transmission include UV light,[178] and factors leading to the shared air:[179] crowding,[180] and ventilation of indoor space,[181] whilst social factors relate to the number, duration and nature of episodes of contact with others.[119] Features of the organism strain are also of interest, potentially affecting every stage of transmission and disease in ways that are yet to be ellucidated.[182–185]

A large part of the variability in the transmission of tuberculosis is related to features of the source case. This has been shown most clearly by the classic experiments of Riley mentioned above (Section 1.5.1.1), in which the majority of infections in exposed guinea pigs were caused by only a small percentage of patients with untreated smear positive tuberculosis.[118,186] Similar studies have been conducted recently with very similar results.[187]

Expectorated bacillary load, measured as the presence and number of mycobacteria visible on microscopy of sputum smears before culture (sputum smear status) is probably the factor most commonly used to estimate the infectiousness of source cases.[188,189] Various studies have showed that compared to community controls the rates of tuberculin positivity are increased by 30-50% in contacts of smear positive individuals and by around 5% in contacts of smear negative, culture positive cases.[119] For example, in one study of 4,445 TB contacts in Edinburgh, 18% of close contacts of smear positive cases had active TB compared to <2% of contacts of smear negative pulmonary disease.[190] Despite this, only <50% of smear positive source cases seem to transmit infection, indicating that smear positivity alone is insufficient for transmission.[168,169]

The presence of visible lung cavities in the source case is also linked with tuberculosis transmission. This is at least partly because cavities are associated with higher bacterial loads, both in the lung itself[191] and in sputum,[192] presumably leading to an increased chance of releasing infectious particles into the environment. However, cavitary disease may be associated with infectiousness independently of the presence of a high sputum bacillary content.[193–196] This might be because cavities are a marker of more advanced disease and a longer period over which transmission could have occurred. Alternatively, cavities themselves might promote coughing and the aerosolization of Mtb. This has not been directly explored as far as I am aware. Another possibility is that exposure to air in cavities might promote a phenotypic change in TB bacilli which improves their ability to resist exposure to the open environment once released into aerial transit.[197] The type of bacilli which end up in sputum are not necessarily the same as those which are released into the air in droplet nuclei.



Airborne particles separated by inertial mass. Adapted from ref [201].

Figure 1.3. Andersen cascade impactor.

1.5.3.2 *Aerosolization of Mycobacterium tuberculosis*

The ability to produce aerosols of a respirable size which contain viable TB organisms is a prerequisite for transmission.[121,198] This fact received little direct attention for many years until the recent work of Fennelly and colleagues.[126,199,200] The group has developed apparatus involving an Andersen cascade impactor[201] for separating out particles released during voluntary coughs into sizes based on inertial mass (Figure 1.3) which are collected on selective media for mycobacterial culture.[126] The majority of particles from which TB was cultured were in the diameter range 0.65-4.7 µm,[126,199] the optimum size for aerial transmission. Positive cough aerosol cultures of *M. tuberculosis* were achieved in only 28 of 101 patients with culture-positive pulmonary tuberculosis. Individuals on TB treatment and with a longer time to positive sputum culture were less likely to produce aerosol cultures; those with a higher sputum bacillary content and a salivary as opposed to a purulent appearance of the sputum were more likely to be aerosol culture positive.[199] Further work correlated positive cough aerosol cultures with transmission by individuals with smear positive pulmonary tuberculosis.[200] As was the case in the earlier study, <50% of patients with smear positive disease produced cough aerosols, although some had received several days of TB treatment. The only predictor of infectiousness, as measured by conversion to a positive from a negative test for latent TB amongst 110 household contacts of culture-positive tuberculosis, was a higher number of colony forming units in the aerosol culture of the index case, even though other factors including source sputum smear grade and radiographic extent of disease, age and BCG vaccination status of the contact and house size were taken into account.[200] Correlating with epidemiological evidence mentioned above, the presence of bacteria in the sputum did not necessarily equate with the ability to aerosolize and transmit tuberculosis, even though this has been considered the most important determinant of infectiousness.

Even though Fennelly's work is a significant step forward from evaluating sputum smears as the sole method of estimating the infectiousness of individuals with TB it has limitiations.[202] The ability to expectorate airborne particles containing culturable *M.*

tuberculosis does not necessarily equate with whether the composition and other characteristics of the particles are sufficient to resist transit through the air until reaching a new potential host. Also, the production of infectious particles when coughing voluntarily into a collection system on one occasion does not necessarily equate to what individuals with pulmonary tuberculosis do normally over the course of their illness prior to treatment. The frequency of coughing in tuberculosis is presumably important, and certain types of cough might be better at aerosolizing bacilli. These factors have rarely been investigated.

1.5.3.3 *Features of cough in tuberculosis*

The effect of objectively-measured cough frequency on TB transmission has to my knowledge been studied only once. In a Texas hospital in the 1960s, Loudon and Spohn measured coughing frequency between the hours 11 pm and 7 am in patients yet to be started on treatment for pulmonary tuberculosis.[125] This was compared to the proportion of 130 household contacts aged 14 years or less with cutaneous reactions to tuberculin. They demonstrated only a small and statistically insignificant effect of nocturnal cough frequency on the infectiousness of untreated pulmonary TB on univariate analysis ($p < 0.20$). Source case sputum smear status and radiological extent of disease were better predictors of tuberculin skin test result in contacts, and no attempt at multivariate analysis was made to look for independent effects of any of the variables. The study was also limited by the short duration of cough recordings due the technology available at the time. Cough in other diseases is more prevalent during waking hours.[89,91] The relevance of objectively-measured daytime cough frequency in tuberculosis transmission has therefore not been investigated, nor has the significance of patterns of cough in terms of clustering of coughs into epochs.

The force of coughing was assessed subjectively by Fennelly *et al*. and shown to relate to a higher chance of producing culturable cough aerosols in tuberculosis.[199] This has not been addressed elsewhere although is also presumably relevant. The observation of the same authors that salivary rather than mucoid sputum in better at producing aerosol[199] corroborates the experimental work of Zayas with a cough simulator

machine,[155] but other work by Fennelly *et al.* failed to show a direct association between salivary characteristics and TB transmission.[200] Further work is needed here; pharmacologically modulating sputum properties may be a way of reducing TB transmission in cases of drug resistant disease where conventional medication has little effect.

Individuals' awareness and interpretation of their coughs must also be important in the transmission of TB in terms of modifying behaviour such as cough hygiene, reducing social contact, wearing a mask and seeking medical attention for TB treatment. For example, there is evidence that smokers may delay seeing a doctor about symptoms of TB,[203] presumably because coughing is less of an unusual occurrence than in non-smokers.[72] As in other diseases, cough frequency probably correlates poorly with subjective awareness of coughing in tuberculosis,[88] but the strength of correlation has not been compared with that in other diseases as far as I am aware. Other TB symptoms which accompany cough are probably important in reducing social contact and seeking medical advice, as shown in a recent study in Tanzania.[204]

Given the central role that cough has in tuberculosis it has been investigated very infrequently. The characteristics of cough, particular patterns which associate with transmission and direct mechanisms are largely unknown. Much of the variation in transmissibility between individuals, and also perhaps between strains of tuberculosis, may be explained by variation in coughing patterns which promote the efficiency of aerosolization of viable bacilli into the environment. A better understanding of tuberculosis transmission will help global efforts to control the disease as drug resistance becomes an increasing threat.[205] The efficiency of contact tracing would be much improved if it could be estimated with greater accuracy which individuals are likely to have transmitted the most disease. The use of isolation measures in hospitals could also be utilized more efficiently for the same reason, particular in areas of the world with high TB burden and low levels of resources.

1.5.4 The aerial movement of particles released by coughing: a theoretical discussion

Human-to-human transmission of infection depends on characteristics of one or more of the disease-causing organism, the infecting source case, the environment, and the secondary host (Section 1.6.2.5). For aerially-transmitted disease such as tuberculosis, infectiousness of a source case relates largely to the nature of infectious particles and the way in which they are released into the air. The number and size of infectious particles are important for infectiousness, but so is the initial horizontal distance travelled on release from the respiratory tract before the particle enters Brownian motion, as I shall now discuss.

1.5.4.1 *Space, time and quantity: relevant variables in the aerial release of Mycobacterium tuberculosis*

Modelling the aerial transmission of TB in confined spaces often assumes complete mixing of the air and ignores the proximity of the source case to other individuals.[206] However, epidemiological studies of TB transmission in aeroplanes and other contexts have shown that the spatial distribution of individuals in an enclosed space is important,[206–208] as infectious particles are at a higher concentration closer to the infector.[209] For example, 31% of 13 passengers seated within two rows of an index case of untreated smear-positive pulmonary tuberculosis during a nine-hour flight developed positive tuberculin skin tests compared to 3.6% of passengers seated elsewhere[208] (although laminar air recirculation patterns in the aircraft may have effectively divided cabins into sections[210]).

It is of interest to consider the size of the zone of highest concentration of airborne bacteria surrounding an infecting individual, and in particular how rapidly and how far infectious particles move at head height before falling to the floor or entering random Brownian motion. Such considerations are perhaps not so important for longer durations of contact with an infectious source case, particularly in small, enclosed and poorly-ventilated locations, but are of most relevance for situations in which brief casual contact leads to infection. Such contacts are probably more common than is

classically assumed, particularly in high-incidence settings, as molecular epidemiological studies suggest.[211–216] For brief contact to result in infection, the rapid direct propulsion of an ejected bacillus in the airstream of a cough towards the nose and mouth of a new potential host will much more likely achieve this than Brownian motion.

The time during which Mtb remains capable of causing infection after release into the open environment is highly relevant. For example, if bacilli were to be rendered non-viable within seconds of exiting the mouth, even prolonged contact between two individuals may not necessarily result in transmission, even if the source case was coughing out large numbers of initially viable bacilli. Over 99% of artificially aerosolized Mtb from broth culture became non-culturable almost immediately after entering the airborne state in one study.[217] It is not clear how closely this represents bacilli released during coughing, and the half-life of *M.tb* in open air is uncertain,[202] although it is variable and affected by ambient conditions, including ultraviolet and ionising radiation,[178,218] and possibly features of the organism itself.[184,219] For example live bacillus Calmette-Guérin (BCG, a strain of *Mycobacterium bovis*) is light sensitive and supplied in dark glass vials for vaccination, and when reconstituted it is recommended that it be used within six hours,[220] and probably 30 min if not kept out of the light. A time limitation on the infectious potential of airborne bacteria nevertheless reduces their effective range, strongly influenced by the speed of release into room air.

A crude indication of the importance of the effects of space and time on the airborne movement *M. tuberculosis* is given by a comparison of the results of studies which have sampled room air from TB wards with those from collecting aerosols at the point of exit from the mouth in pulmonary TB. Experiments with artificially-produced aerosols,[121] and studies exposing guinea pigs to air from TB wards (Section 1.6.2.5.1)[118] have shown that, at least in animal models, a single infectious dose is sufficient to cause infection with a single pulmonary lesion. This dose probably equates to a single viable bacillus, although this has not been proven without doubt.[121,221] Through measurement of airflow over the guinea pigs, observation of the rate of new guinea

pig infections, and calculation of the total air volume breathed by the animals, Riley and Wells, and later others, have estimated the rate of addition of infectious doses (or 'quanta' (q)) to ward air by patients with smear-positive pulmonary TB.[118,187,218,219,222] Recent molecular techniques have linked source cases to the number of guinea pig infections.[219] For one particularly infectious case, the rate of release of aerially-transmittable particles exceeded 200 q/h although the median (IQR) rate in patients causing infection in guinea pigs was about 12 (4.3-39) q/h.[219] In Fennelly *et al.*'s cough aerosol sampling work (Section 1.6.2.5.2), the smear-positive TB cases who released cultivable aerosols into the collection system produced a median of 16 (5-30; maximum 710) Mtb colony forming units over only 10 min when asked to cough repeatedly,[199] although these subjects may have coughed a lot more frequently in the 10 min period than they would have done under normal condtions.[126] However, part of the reason for higher viable Mtb aerosol numbers close to the source case than reach further parts of room air in normal circumstances might be explained by what happens to airborne particles, and the bacilli contained within, as they travel through air.

1.5.4.2 *Droplets and droplet nuclei: modelling the aerial movement of respiratory particles*

The aerial movement of respiratory droplets is complex and has been discussed surprisingly infrequently since the classic (largely theoretical) work of William Wells.[113,223] Important factors are the size, speed, and composition of the droplet, the size of the mouth at the point of exit, and the relative humidity (RH), temperature, pressure, movement and quality of the ambient air, as I shall summarise, largely taken from the recent paper by Xie *et al*.[223]

The size of a newly-formed respiratory droplet will decrease on contact with the external environment due to evaporation of its aqueous content. As originally theorised by Wells,[113] a droplet of pure water released at a height of 2 m above the ground will either fall to the ground or evaporate completely before doing so, depending partly on its starting size. The critical size determining the droplet's fate will vary depending on a number of factors including the ambient temperature and RH,

and the velocity at which it is released from the mouth. For example, evaporation is slower with greater RH, leading to further vertical movement under the influence of gravity. Respiratory droplets clearly do not consist entirely of pure water, but contain glycoproteins, ions and, of interest to this discussion, microbes. Complete evaporation therefore does not occur but results to the formation of 'droplet nuclei' consisting of microbes and solute residue.[113] As previously discussed (Section 1.5.2.1), for *M. tuberculosis* such infectious particles measure approximately 0.5-5 µm, as can be inferred from the dimensions of the bacterium itself and the anatomy of the distal airways which they must reach to cause infection.[121] Direct measurements with an Andersen cascade impactor within a cough aerosol collection system have confirmed this directly (Section 1.5.3.2).[126,202]

For the current discussion of infectiousness within the close proximity of a source case of tuberculosis, droplet movement in the horizontal direction from the mouth is of most interest. The closer the starting diameter of an expiratory droplet is to that of an airborne droplet nucleus, the less vertical movement there will be due to gravity after release from the mouth. Aerially suspended particles resist gravity due to the relatively large frictional force acting on them in movement, as explained by Stokes' law.[113] The temperature of the air within a cough air jet relative to ambient conditions will determine whether the jet is isothermal, with a linear trajectory, or non-isothermal, with a trajectory that is slightly curved, hotter air for example moving upwards. At the same time, droplets with greater starting speeds have greater momentum and take longer to leave the cough air jet to become suspended in air, although they also take longer to shrink by evaporation and escape the pull of gravity, due to larger Reynolds, and therefore Sherwood numbers.

The Reynolds number of the airborne particle, Re_p, is the ratio of inertial forces to viscous forces defined as

$$\mathrm{Re_p} = \frac{\rho_g d_p |\vec{V}_p - \vec{V}_g|}{\mu}$$

where ρ_g is air density, d_p, the particle diameter, $\vec{V}_p$ and $\vec{V}_g$, particle and air velocity, respectively, and μ the dynamic viscosity of air. Where inertial forces dominate, the Reynolds number is high and flow is turbulent, whereas laminar flow occurs at low Reynolds numbers where viscous forces dominate.

The Sherwood number, Sh, is the ratio of the total rate of mass transfer to the rate of purely diffusive mass transport, and is defined as

$$\mathrm{Sh} = 1 + 0.3\mathrm{Re}_p^{1/2}\mathrm{Sc}^{1/3}$$

where Sc is the Schmidt number, the ratio of momentum diffusivity (viscosity) and mass diffusivity, $\mathrm{Sc} = \frac{\mu}{\rho_g D}$

where D is the diffusion coefficient of vapour through air. The size of the mouth opening affects properties of the air jet and therefore of droplet evaporation and movement such that a larger cross-sectional area increases the propulsive distance of a cough.

Together, airborne droplet movement can be estimated from the following simultaneous differential equations for evaporation and deceleration. The rate of evaporation (change in droplet radius, r_p) can be estimated as

$$\frac{dr_p}{dt} = \frac{CM_v D_\infty pSh}{\rho_p r_p RT_\infty} \ln \frac{p - p_{va}}{p - p_{v^\infty}}$$

where C is a correction factor due to temperature dependence of the diffusion coefficient, M_v, the molecular weight of vapour, D_∞, the diffusion coefficient of vapour far from the droplet, ρ_p, the particle density, R, the universal gas constant, T_∞, the ambient temperature, p, total air pressure, p_{va}, vapour pressure at the droplet surface, and p_{v^∞}, ambient vapour pressure. Droplet deceleration can be estimated as

$$\frac{d\vec{V}_p}{dt} = \vec{g}\left(1 - \frac{\rho_p}{\rho_g}\right) - \frac{3C_d\rho_g\left|\vec{V}_p - \vec{V}_g\right|(\vec{V}_p - \vec{V}_g)}{8\rho_p r_p}$$

where $\vec{g}$ is gravitational acceleration, and C_d, the drag coefficient. In turn droplet displacement is given by $d\vec{x}_p = \vec{V}_p dt$.

From this, it is estimated that with an ejection velocity of 20 m/s, in a cough air jet of initial temperature 33 °C, through an oral aperture of diameter 4 cm into a room of ambient temperature 20 °C and RH 50%, the horizontal distance moved before commencing Brownian motion would be greatest (3.5 m) for a droplet of starting diameter *c.* 40 µm. Assuming a constant deceleration, it would take 0.35 s to cover this distance. For a slower cough air jet speed of 10 m/s, the same particle in the same conditions would move about 2.3 m in 0.46 s, or about 1.4 m in 0.28 s if the mouth opening was 2 cm in diameter.

Therefore, according to the modelling of Xie *et al*., the most infectious individuals (who spread infectious particles quickly over a relatively large distance) will be those with higher cough velocities and larger mouth openings during coughing who produce large numbers of respiratory droplets of initial diameter 40-60 µm. For example, men would be expected to be potentially more infectious than women on this basis, due to larger mouths and higher cough velocities (3.3-14 *vs.* 2.2-5.0 m/s, respectively, in one study[160]). For a stationary individual repeatedly coughing openly with velocity varying up to 10 m/s into a still room of RH 50% and temperature 20 °C, it is therefore possible to imagine a zone following the cough air jet extending up to 2.3 m containing rapidly moving shrinking droplets of high concentration, surrounded by zones of ever smaller concentrations of droplet nuclei, some of which will contain viable microbes.

The above estimated distances and speeds of droplets are of course approximate and the modelling of Xie *et al.* ignores many variables of importance. These include droplet composition (assumed above to be 0.9% saline), fluid flow within droplets, physical interactions between multiple droplets in an air jet, electrostatic charges, room air purity and room air currents, and body movements of the source individual, including covering of the mouth while coughing. Airborne droplets in motion are also assumed to be spherical, whereas *Mycobacterium tuberculosis* is a bacillus, i.e. rod-shaped.

Although droplets presumably start off spherical, during evaporation an airborne droplet nucleus containing a single bacillus may approach the same shape, therefore altering the Reynolds number and drag coefficient compared to a sphere of the same volume and increasing the potential range. The difficulties of visualizing moving respiratory droplets clearly makes verification of such theoretical models a problem. This is an important area for further theoretical and experimental research.

Mathematical modelling can therefore predict the movement of respiratory particles in air on release from the mouth and the influence of various variables. It might be possible to test some of these predictions by measuring particles in air.

1.6 Clinical evaluation of coughs

Cough is one of the most common medical presentations to be assessed by clinicians. A simple line of enquiry would include the duration of the cough, any associated symptoms, and perhaps the daily pattern, relieving and exacerbating factors and the presence of mucus along with smoking habit and known respiratory disorders.[2] Where the cough is more prolonged, particularly despite investigation and trials of treatment, a more detailed assessment of the characteristics of the cough is helpful.[5]

1.6.1 Subjective assessment

As well as asking patients to describe the frequency, severity and other characteristics of their cough, tools have been developed to help document subjective cough severity. Asking individuals to score the severity of their cough out of ten or to mark a line on a visual analogue scale (VAS) are the simplest ways.[5]

Cough severity visual analogue scales are widely-used in cough research, although predominantly in chronic cough.[63] In stable COPD the VAS score is generally consistent after an interval of two weeks and, in studies of chronic and acute cough, it is responsive to clinical improvements.[5,79,80,224] The minimum clinical important difference of a 0-100 mm cough severity VAS has not been formally defined but is probably 17 mm.[225]

1.6.1.1 *The Leicester Cough Questionnaire*

There are cough-specific quality of life questionnaires, the most frequently-used of which is the Leicester Cough Questionnaire (LCQ, Appendix).[226,227] It is composed of 19 questions, each with seven possible responses on a Likert scale, concerning the effect of cough on physical, social and psychological domains, and has a total possible quality of life score of 3-21.

The LCQ was originally developed in patients with cough of duration >3 weeks.[226] The process involved the generation of an initial list of items following a literature review, multidisciplinary expert meeting, and discussion with 15 patients with chronic cough, and then reduced down after testing the questions on a further 104 patients. Concurrent validity of the final questionnaire was shown by correlation of responses with those to the St George's Respiratory Questionnaire,[228] the Short Form 36 item (SF36) health status questionnaire,[229] and a cough severity visual analogue scale. Further evaluation demonstrated repeatability of LCQ scores in patients with stable symptoms, and responsiveness to reported improvements in cough.[226]

Although used mainly in the context of chronic cough, where the minimum clinically important difference in the total score has been defined as 1.3,[230] the tool has also been successfully evaluated for validity, repeatability and responsiveness in acute cough due to upper respiratory tract infection (with a minimum important difference in the total score of 2.5),[231] bronchiectasis, [232] and COPD.[233] The LCQ has also been used in asthma,[76] idiopathic pulmonary fibrosis,[77] and, on only one occasion to my knowledge (without validation), in tuberculosis.[234] It has been translated into a number of languages.[235–237]

The LCQ therefore seems to be a useful and versatile tool for assessing cough symptoms. A potential omission from the questionnaire, however, concerns urinary incontinence. This is a frequent and often distressing feature associated with chronic cough[238] and is addressed in another specific cough assessment tool, the Cough Quality of Life Questionnaire (CQLQ).[239] However, this problem is generally more

frequent in women than men, meaning the LCQ would be less gender non-specific were it to specifically ask about urine incontinence. Furthermore, the impact of incontinence should be captured within responses to other questions in the LCQ concerning control, embarrassment, frustration and feeling fed up Appendix

In unexplained chronic cough and asthma the strength of association between both LCQ score and cough VAS score and objectively-measured cough frequency is similar and statistically significant but mild to moderate.[76,84,87,227] Early cough monitoring studies similarly noted the weak correlation between cough rates and patients' appreciation of them.[88,240] Cough frequency therefore only seems to explain part of cough-related quality of life in these conditions. Data in other diseases are lacking.

1.6.2 Objective assessment

The objective evaluation of cough is required for the adequate exploration of mechanisms of cough and the direct assessment of provoking or relieving factors. Different features of cough have the potential for measurement including coughing frequency, the force or intensity of cough, cough reflex sensitivity, characteristics of the resultant cough sound, associated lung or peripheral biomarkers and the potential of the cough to transmit disease in infections by production of particles.

1.6.2.1 *Cough reflex sensitivity*

Cough can be experimentally induced with a variety of agents, including citric acid, acrolein and ultrasonically-nebulized distilled water, but the most commonly-used substance in cough challenge testing is capsaicin.[241] Cough reflex sensitivity to capsaicin has been shown to be repeatable[242] and responsive to improvements in cough symptoms.[243] It is usually defined as the inhaled concentration of capsaicin required to produce 5 coughs (C5) within a specified time (usually 15 s),[241] and there is expert consensus on the method of dose administration.[5] The main role of capsaicin cough challenge testing has been in investigating potential antitussive treatments by measuring the effect on cough reflex sensitivity.[244] However, as it is currently used, a limitation of the test is the relative inability to differentiate between health and

disease due to wide inter-subject variability in capsaicin cough response.[70] There is also poor correlation between C5 and 24-hour cough frequency.[75,245]

A novel test protocol has been proposed which involves gradually escalating the concentration of capsaicin and noting the largest number of coughs produced in 15 s.[85,246] 'E_{max}', the maximum number of coughs evoked by any concentration of capsaicin, seems to be more predictive of 24-hour cough frequency than C5.[85] However, because the test protocol terminates at the maximum-tolerated concentration of capsaicin, it is not clear from the reported data how often E_{max} reaches a plateau for particular patients. Published data so far appear to only include <40 patients.[85] Poor tolerance of higher doses of capsaicin could be a limitation to the wider use of this protocol. Another drawback of this proposed new approach is that it is time-consuming, requiring several repeated inhalations of each concentration of capsaicin solution.

An alternative approach might be to compare the number of coughs produced after administration of a one-off relatively high concentration of capsaicin, or to measure cough latency, the time between dose inhalation and the first cough. This latter measure might reflect central processing and the influence of (semi-) conscious cough suppression.

1.6.2.2 *Cough frequency*

The number of coughs in a particular time period can only be assessed objectively. As cough is an episodic symptom with diurnal variation an estimation of cough frequency in health and disease requires measurement over hours or days. Direct observation over this time frame is clearly impractical hence methods for recording and playback of coughs have been developed.

1.6.2.2.1 Recording coughs

Different methods for recording cough activity have been tried, including video information to visualize body movements, electromyogram signals from chest wall

movement, and sound and vibration data from the chest wall or close to the patient.[86,247–251] Video data probably do not add much to sound information: in one study almost exactly the same number of cough sounds were counted in each of eight 8-hour recordings whether or not video information was shown.[252] The cough monitors about which most has been published are based on prolonged sound recordings with either a single free-field microphone,[253] or a combination of one free-field microphone and another in contact with the chest wall.[86] The potential advantage of the contact microphone is it picks up little background noise,[254] making it easier to distinguish coughs of the individual being monitored from background noises, including other coughs. However, sound characteristics are altered when recording through the chest wall, which seems to alter the recognition of cough sounds.[251] The problem of background coughs during cough monitoring will obviously vary with the environment in which recordings are performed. Cough monitoring a patient using only a free-field microphone on open respiratory ward, for example, may lead to over-estimation of cough frequency if coughs of others are misinterpreted as belonging to the patient under observation. This potential problem does not seem to have been examined in detail but it could perhaps be minimized by placing the microphone close to the mouth of the subject and using a device with the capability of filtering out background noise. On listening to the recordings some background coughs may be obvious due to differences in sound quality (for example lower volume and clarity) and characteristics, particularly if the sex of the person coughing is recognizable and different to that of the subject being monitored.

Whereas early attempts at prolonged monitoring of cough required bulky equipment and confinement of the subject to a single room,[240,255] digital technology and advances in sound recording have allowed devices to become small and portable enough to be worn during normal activities[86] for continuous monitoring for 48 h or longer.[256]

Monitoring over several days in the stable state of respiratory disease, or in health, is probably necessary to establish normal intra-individual variability in cough frequency, but does not appear to have been done. Another benefit of doing this might be to

minimize any effect on cough frequency of the actual process of being monitored. It is possible that cough monitoring alters the subject's awareness of his or her cough, leading to fewer or more voluntary coughs, or avoiding activities which produce more coughing such as smoking.[72,240] Wearing a cough monitor over longer periods than 24 h might allow the subject to become accustomed to the device and not adapt behaviour as a result.

1.6.2.2.2 Non-automated cough counting

'Manually' counting coughs in patient audio recordings is the accepted standard for objectively determining cough frequency.[252,253] Different basic units of cough for counting have been proposed[87] but individual cough sounds seem to be preferred,[6,78,253] in keeping with part of the definition of cough by the British Thoracic Society as a "characteristic sound".[2] However, it is assumed there is universal agreement on what a cough sound is, and while this may seems a safe assumption, cough sounds vary between patients and between diseases[257] and this assumption has been little-tested. Studies measuring observer consistency in cough counting have shown good agreement but only on comparing two different observers or the same observer on different occasions, and generally with individuals in research groups who have perhaps learned a particular method of classifying coughs from other sounds such as throat-clearing and other non-verbal vocalisations.[96,247,248,253,258] Inter-observer agreement in cough is in need of further investigation. It would be a reflection of the ability both to discriminate individual coughs in bouts of repeated closely-spaced coughing and to differentiate between coughs and other sounds.

A further matter in non-automated cough counting is the use of audio editing software to identify coughs by the visual appearance of their typical amplitude waveforms coincident with the cough sounds.[87,259] While this method presumably simplifies the recognition and counting of coughs, it is not clear if the approach is valid if the definition of cough is based on its sound alone. No study appears to have evaluated

the influence of the visual inspection of sound activity on cough counting, and the interaction of auditory and visual sensory inputs is complex.[260]

A basic method for measuring cough frequency has therefore not been fully established and evaluated. This is important for the study of cough on the whole and to create a reference standard for the testing of automated coughing counting devices.

1.6.2.2.3 Automated monitors

Counting cough by ear is highly labour-intensive. The automated detection of coughs has been attempted several times and has been reviewed elsewhere.[86,251] Challenges have been the development of software which will correctly recognize coughs across a variety of disease types, patients and environmental conditions and reject other sounds or respiratory events. Three recently-developed automated or semi-automated systems are described below.

1.6.2.2.3.1 PulmoTrack™

To my knowledge only one automated cough monitor, PulmoTrack™ (KarmelSonix, now iSonea, Haifa, Israel) has been made commercially available.[261] It records input from chest wall movement, a free-field microphone and two microphones in contact with the trachea and chest wall to give completely automated counts. The device has been tested in only one study which reported good agreement between its algorithm and non-automated cough counting.[262] However, the evaluation was only carried out on 12 healthy individuals who were asked to voluntarily cough and make other noises during a 25-minute monitoring period. No published research into cough frequency in disease has been published using this device. Further confirmation of its accuracy is required.

1.6.2.2.3.2 VitaloJAK™

VitaloJAK™ (Vitalograph, Buckinghamshire, England) has been developed by the cough group in Manchester, currently only for their research purposes.[263] It is a semi-automated device which uses a combination of a free-field and chest wall contact

microphone to produce ambulatory recordings of up to 24h in length. The software algorithm compresses 24-hour recordings down to approximately one hour or less by removing silences and all non-cough audio data but retains information about the time of occurrence of the preserved sounds.[264] The resultant digital sound file then requires human analysis for counting coughs. There have so far been two published reports on the evaluation of this algorithm in a total of 30 individuals comprising 24 patients (with chronic cough, asthma or COPD) and 6 healthy controls.[263,264] The developers report an almost zero error rate of the algorithm in transferring the original cough sounds to the condensed recording apart from in one patient with asthma and apparently muffled cough sounds.[263] The overall cough rates in the individuals tested did not seem particularly high and the background conditions of the recordings were not described. Although the algorithm has been used in clinical studies of cough frequency[78,79] more published evidence on its performance in a greater range of patients is required.

1.6.2.2.3.3 The Leicester Cough Monitor

The cough monitoring system for which there are the most published data is the Leicester Cough Monitor (LCM).[253] The device consists of a portable digital voice recorder and lapel microphone which can record continuously for over 48h.[256] Recordings are analyzed by a software algorithm using techniques similar to those for speech recognition.[265] It requires human calibration by classifying a selection of candidate noises as coughs or non-coughs.

Initial evaluation of the system by the developers compared its performance to cough counting by ear in 6-hour sequences from 15 patients with chronic coughs due to asthma, gastroesophageal reflux, eosinophilic bronchitis, viral infection or an idiopathic cause. The sensitivity of detecting cough sounds was reported as 86% and sensitivity 99% with a median false-positive rate of 1.0 sound/hr.[253] Further evaluation by an independent researcher from another institution compared non-automated cough counts in 24h recordings from 7 patients with idiopathic chronic cough to analysis by the machine. Automated and non-automated cough counts were very

similar (mean ± SE: 23±7, compared to 24 ± 6 coughs/patient/h respectively; intra-class correlation coefficient 0.98).[266]

More recent testing of the Leicester Cough Monitor in 24-hour recordings from 20 individuals (8 healthy volunteers and 12 with chronic cough) showed a sensitivity of the system of 83.8% in patients and 82.3% in healthy volunteers with a specificity of 99.9% in comparison to counting by ear.[89] Indirect evidence of the accuracy of the LCM is the strong similarity between its reports of 24-hour cough counts in patients with chronic cough compared to those calculated by ear by the Manchester group in recordings from similar patients.[75,89] The two research groups with different methods of counting cough also report similar cough counts in patients with acute cough.[79,80] Reports of coughs frequency using the LCM are consistent with subjective cough severity in chronic cough as measured by questionnaire[89] and improvement in LCM-calculated cough frequency correlated with the primary endpoint of improvement in cough-specific quality of life score in a recent randomised controlled trial of gabapentin in chronic cough.[267]

Data supporting the accuracy of the LCM are therefore accumulating and it would seem to be a useful tool for the automated measurement of cough frequency. However, the total number of patients in whom the system has been rigorously evaluated remains low. There are no reported data for use of the device in lung cancer, bronchiectasis, pneumonia, acute exacerbation of COPD, acute asthma or tuberculosis and possible differences in cough sounds between diseases might be relevant. Because the Leicester Cough Monitor relies on one single lapel microphone for audio input, background coughs may be represented, but this has not been adequately tested. This is less likely to be important for VitaloJAK because of the simultaneous input from a chest wall contact microphone.

Automated cough detection systems therefore remain in development but are much needed for acquiring data about cough in respiratory disease and to enable the use of cough frequency as an outcome measure in clinical research.

1.6.2.3 *Cough intensity*

The intensity of coughing, in terms of the force of chest wall movement, intrathoracic pressure changes, and resultant expiratory flows, are probably important in terms of symptom severity and also the transmission of infection. This does not seem to have been addressed very often. Using measurements of oesophageal and gastric pressure, and peak cough flow rate, one study of 49 subjects showed that cough intensity was higher in individuals with chronic cough than healthy volunteers.[268] The expiratory flow rate of a cough is related to the volume of air within the lungs prior to its onset, such that repeated coughs within a single breath become sequentially weaker and therefore probably less effective at clearing debris from the airways.[94,269] The force of a spontaneous or involuntary cough may be different to when coughing involuntarily.[268,270] No study appears to have yet attempted to measure the intensity of force of 'normal' coughs over a 24-hour period in an ambulatory setting. Using cough peak flow may not be practical over 24h, but electromyography or the measurement of oesophageal pressures[94] might be. Alternatively, the force of a cough could be inferred from characteristics of its sound. This has been considered but requires further investigation.[271]

1.6.2.4 *Cough sound analysis*

Coughs have been analyzed acoustically by either measuring the duration of the component phases of coughs or the frequency composition of their sounds. There have been few developments in this area since the literature was reviewed several years ago.[272] The three phases of a cough sound have been described as (1) the 'initial burst', explosive phase or first phase, corresponding to the opening of the vocal cords, (2) the middle, intermediate or second phase, corresponding to the flow of air through the open larynx, and (3) the 'final burst' or third voiced phase as the vocal cords re-oppose at the end of the cough sound (Figure 1.4).[5,257,273–275] This third phase is only present some of the time, for example in only 53% of 234 voluntary coughs from 24 healthy non-smokers in one study.[274] Data are few but in children it is perhaps more likely to be absent in asthma than in health; Thorpe *et al*. observed a third phase more

often in 33 voluntary coughs from 5 children without asthma than in 81 coughs from 12 children with the disease.[273]

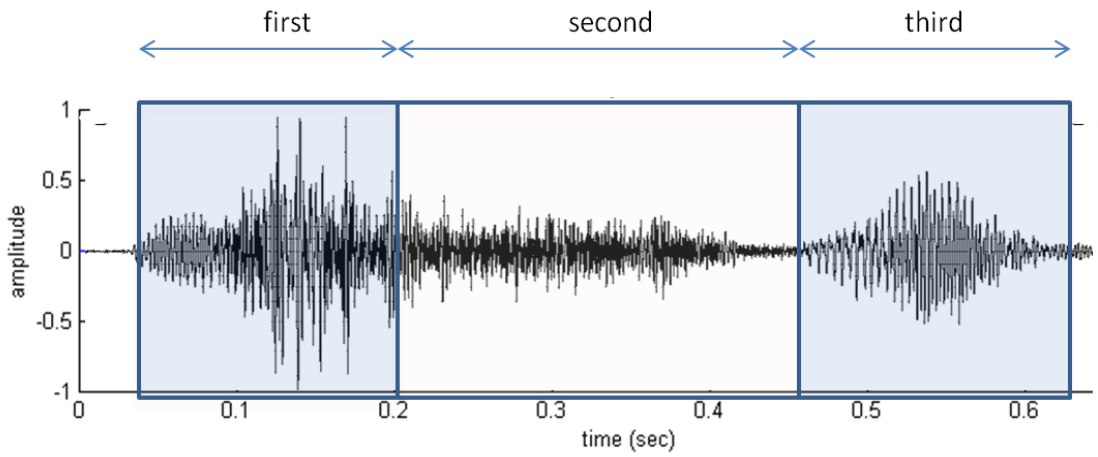


Figure 1.4. Phases of the cough sound, corresponding to, (1) opening of the vocal cords, (2) air flow through the open larynx, and (3) re-opposition of the cords.

This same study found the first phase in children to be shorter in asthma than in health.[273] In contrast Piirilä and Sovijärvi found the first phase cough in adults to be longer in asthma than bronchitis, fibrotic pulmonary disease or tracheobronchial collapse syndrome. An average of 12 spontaneous coughs from each of 31 adults with respiratory disease were looked at here, although the definition of 'first cough sound' was not given.[101] Hashimoto *et al.* observed that in voluntary coughs from 15 patients with chronic productive cough the first phases were relatively shorter and second phases relatively longer then in 9 individuals with dry cough.[275]

The same group observed a significant lengthening of the second phase in 10 voluntary coughs productive of sputum compared to 10 coughs which were non-productive in each of 5 patients with chronic bronchitis. The length of the second phase was also greater than for 5 healthy controls in each case.[99] They also correlated sputum physical characteristics with the duration of the intermediate cough phase which was longer when the sputum was more plastic and transportable by cilia.[275] Others have noted the second phase to be longer in the presence of mucus.[98]

The most extensive measurements of phases of the cough sound in respiratory diseases have probably been carried out by Smith *et al*. In a median of 46 spontaneous coughs from each of 35 patients with COPD, asthma, IPF or cystic fibrosis there was no evidence of difference in phase lengths between diseases, variation being higher within diagnostic groups.[254,276] However, the authors did not appear to control for whether coughs occurred individually or as part of a coughing bout. Coughs occurring later during an expiratory breath occur at smaller lung volumes and with lower flow rates,[94,269] and might therefore have a different distribution of phase lengths.

In terms of analysis of the acoustic frequencies of cough sounds there seem to be differences between individuals based on anatomy, gender and voice.[274,277,278] In 31 patients with respiratory diseases Piirilä and Sovijärvi looked at the frequency at which the maximum intensity component of the cough sound occurred.[101] They found there to be a wide variation, particularly for chronic bronchitis and no significant difference between diseases. They also looked at the upper frequency limit of the cough sound: it was lower for asthma than chronic bronchitis and tracheobronchial collapse syndrome. Knocikova *et al.*, carried out a more detailed analysis of one voluntary cough from each of 26 healthy controls, 17 people with stable asthma and 22 with COPD.[279] They observed that the highest amplitude component of the cough sound in asthma was of a higher frequency than that in healthy controls. COPD coughs had greater amplitude in the low frequency range and asthma more components of high frequency. By including other parameters the authors report an ability to discriminate 85-90% of the cough sounds in asthma, COPD and controls according to disease.[279]

In probably the largest and most clinically relevant study of its kind Abeyratne *et al.* recently report a method for discriminating childhood pneumonia from other acute respiratory illness based only on acoustic analysis of cough sounds.[100] From bedside recordings in an Indonesian hospital an average of 6.7 spontaneous coughs from each of 66 children with pneumonia or another diagnosis including asthma, bronchitis and rhino-pharyngitis were analysed and used to develop the algorithm. This was tested

prospectively on a further 25 coughs from each of another 17 patients with pneumonia and 8 children with other acute respiratory illnesses by a researcher blinded to the physician-made diagnosis. In comparison to this reference standard the reported sensitivity of the algorithm for diagnosing pneumonia was 94% and sensitivity 75%.

The term tussiphonography has been coined to refer to the study of cough sounds.[257] There is need for more work in this area. Just as auscultation of the chest reveals information about underlying pathology,[280] analysis of cough sounds, in terms of both cough phase length and acoustic frequency profiles, might do so likewise. For example, cough sounds in tuberculosis may relate to infectiousness, due perhaps to properties of mucus, vibration frequencies associated with particle generation, or force of the release of air, but this has not been explored.

1.6.2.5 *Measurement of the infectiousness of cough in tuberculosis*

Coughing is probably more important for spreading infection in tuberculosis than in other disease (Section 1.5.3). This is because of the need to expel aerosols of a particular size and composition to transit through the air and reach the alveolus of a new host. Where droplet spread and entry to the new host via the upper respiratory tract or conjunctiva are significant, as in influenza, direct contact and sneezing are more significant as previously discussed (Section 1.5).

Not all people with pulmonary tuberculosis and cough transmit disease. Studying the characteristics of cough that associate with infectiousness requires a method of measuring the spread of infection. Unfortunately, attempts to directly capture and culture airborne *M. tuberculosis* from a room in the vicinity of a coughing individual have been unsuccessful,[202] and the perfect method for assessing TB transmission does not exist. All current techniques provide only indirect evidence for the infection of one person by another.

1.6.2.5.1 Guinea pig air sampling

The guinea pig air sampling model is probably the most direct way of observing the transmission of tuberculosis as it occurs. As previously described, laboratory-reared animals free of disease inhale air that is vented from the vicinity of patients with TB. The guinea pigs then require serial tuberculin skin tests to detect infection and autopsy for confirmation of disease.[118,187,222] The technique has been extremely valuable is providing the first clear evidence of aerial transmission in tuberculosis,[118] and has given the strongest indication of the variability of TB infectiousness between patients.[186,219] It has recently also been used to evaluate the effectiveness of interventions to limit TB transmission, such as the use of surgical masks,[222] and UV irradiation or ionization of indoor air.[178] Problems of this method are the expense and time involved, hence one of the reasons it took so long from the 1950s to be reemployed.[168] Also, as this is an animal model the differences in the process of inhaling and becoming infected with *M. tuberculosis* between humans and guinea pigs may be a source of error.[281] The method is also only practical in a health care setting, and in particular types of patients who require hospitalization for prolonged periods of time. The majority of transmission of TB clearly occurs outside of this context hence the model is removed from real world situations.

1.6.2.5.2 Cough aerosol culture

Another technique is the measurement of expectorated airborne bacilli, as carried out recently by Fennelly *et al.* as described above (Section 1.5.3.2).[126,199,200] Only the minority of individuals with TB produced cough aerosols in the sampling system from which positive cultures were obtained.[199] This remained the case even if studying only sputum smear-positive disease,[200] and the technique appears to produce better predictions of infectiousness than considering only sputum smear status.[200] However, the types of cough in these studies may not be typical. Coughing into a mouthpiece on demand might alter usual airflow patterns and therefore the aerosolization of bacteria. Other groups have recently developed larger closed systems for detecting aerosolized

bacteria which do not involve a mouthpiece.[282,283] Although resultant coughing might be more representative of usual behaviour, only limited pilot data in TB have been reported so far.[282,283] Again though, coughing on demand may not be typical of how people with TB cough normally. It would be more preferable to attempt to identify *M. tuberculosis* from a space which had been occupied by an individual coughing normally. Unfortunately it is very difficult to culture TB from an open area due to overgrowth with fungi and environmental bacteria.[202]

1.6.2.5.3 Detection of aerosolized DNA of *Mycobacterium tuberculosis*

One way around this problem is to measure TB DNA in the vicinity of a patient by polymerase chain reaction-based techniques (PCR). This appears to have been done rarely.[284–286] Limitations of these studies for estimating aerial transmission are several. Firstly, the presence of TB DNA does not prove the presence of living organisms capable of causing infection. The source bacilli may have died some time previously or may lack the appropriate phenotype necessary to cause new infection. Secondly, the proximity of the collection system in two studies did not differentiate droplet-sized particles from those capable of aerial spread. Thirdly, qualitative techniques about the presence or absence of DNA gives no indication about the potential inhaled concentration of TB.[287] PCR techniques therefore may have high sensitivity but will have lower specificity for estimating infectiousness, although more recent approaches are quantitative in nature,[286,288,289] and may be complementary to other approaches for estimating infectiousness.

1.6.2.5.4 Quantifying aerosolized particles

Collecting aerosol for TB culture has the disadvantage of requiring a closed system. A technique for quantifying the aerosolized particles in an individual's vicinity during routine activity might indicate infectiousness, even if it is difficult to prove that the particles contain viable tuberculosis. Most of the work on particle counts during expiratory manoeuvres involves closed collection apparatus of various types as reviewed above (Section 1.5.2) and elsewhere.[282] Zayas *et al.* used equipment capable

of measuring particles from 'open bench coughs', perhaps more generalizable to normal situations than coughing into a mouthpiece, but still produced on demand so probably not reflective of normal patterns.[149] However, the advantage of this system is the ability to study the effectiveness of masks and other cough etiquette techniques in impeding the release of particles into the environment.[290]

Airborne particle counters have been developed for measuring air quality with high sensitivity in industrial settings, particularly where cleanrooms are required. This technology has yet to be established as a method for monitoring aerosolized particle production by individuals with tuberculosis or other respiratory infections, although very recent preliminary work has tested its use in pulmonary TB.[283,291] Depending on the background air quality it may be a useful method for predicting infectiousness.

1.6.2.5.5 Evidence of new human infection

A rise in the local rate of TB infection coincident with a new diagnosis of active tuberculosis is the most commonly-used method for estimating transmission, particularly if a close genetic relationship between infecting organisms can be proven.[214,292] The main limitation of this approach is that coincident cases of active disease are generally rare with households, at least in the UK, and contact tracing reveals many more cases of latent TB infection than active disease.[188,293,294] By definition, it is not possible to culture or detect TB DNA in latent disease from clinical samples, hence the origin of infection is impossible to determine with certainty. The local incidence of tuberculosis and an individual's other risk factors for TB will affect the likelihood of latent infection having come from a particular source case. New infection in the very young gives clear indication of recent infection, and conversion in the immune response from negative to positive on repeat testing with tuberculin or an interferon gamma release assay is suggestive.[295] Another limitation of detecting latent infection is the accuracy of tests for diagnosis; where the presence of bacteria can be confirmed (i.e. in active TB), the sensitivity of interferon gamma release assays is about 84%,[296] and that of tuberculin skin tests is lower.[297]

Due to the limitations of any one approach, studies using TB infectiousness as an endpoint either need to involve a large number of patients or should involve more than one approach.

1.6.3 The diagnostic utility of cough

Differences in coughs between respiratory conditions have been little studied (Section 1.4.1). Certain clinical features such as duration of cough, production of mucus, associated symptoms, timing of day and exacerbating and relieving factors are used to help diagnose the underlying cause.[2,56] Further study of cough will reveal if there are specific characteristics of coughs that are unique to particular clinical situations. The frequency of coughing, diurnal variation, amount of clustering of coughs into epochs and features of the cough sound might all be relevant.

Cough can be due to serious disease but because it is commonplace its potential importance may be ignored.[298] For this reason the UK Government recently launched a publicity campaign to encourage people with a persistent cough to seek medical attention.[299] A way of analyzing coughs in more detail may improve diagnosis, particularly in resource-poor areas if the technology were simple. Cough sound analysis may improve the diagnostic rate of childhood pneumonia in developing regions.[100] Combining very basic clinical information (the presence or absence of fever) in the study of Abeyratne *et al*. improved the diagnostic algorithm based on analysis of the cough sound (Section 1.6.2.4).[100]

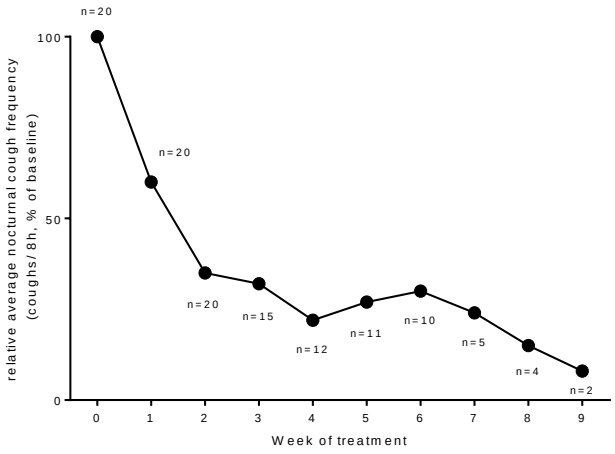
Tuberculosis is also prevalent in low income countries and diagnosis is often delayed.[205] This is due to the reliance on sputum microscopy as the main diagnostic test which is often unavailable. Even when accessible it has sensitivity of <60%, or less in individuals with HIV.[300] New relatively low-cost microbiological and molecular techniques are promising,[301] but simpler diagnostic methods could have a large impact on TB control.

Cough is the most common symptom of pulmonary tuberculosis.[302] A recent large community survey in Kenya suggested that investigating all patients with chronic

cough would detect 60% of cases of active TB but with a specificity of only 2%.[303] It is not known if certain features of cough are more likely to be present in tuberculosis but if this were the case the potential diagnostic usefulness of cough would clearly be enhanced.

1.6.4 The prognostic utility of cough

Serial cough monitoring may be a novel objective laboratory-free method of following the course of respiratory disease. In tuberculosis current methods for assessing the response to treatment are inadequate due to a lack of sensitivity and specificity.[304] They include clinical parameters (improvement of symptoms, control of fever and weight gain), radiographic monitoring (resolution of chest X-ray changes) and microbiological criteria (conversion of sputum smear and cultures). Other tools are needed to guide clinicians through the treatment of individual cases, particularly those with drug-resistant disease.



From data of Loudon and Spohn (reference 115).

Figure 1.5. Reduction in nocturnal cough frequency during treatment of pulmonary tuberculosis.

Cough clearly improves as TB infections resolve; the resolution of the symptom correlates with treatment success.[305] This has only been shown objectively in only one

study to my knowledge,[125] although another describes the early development of a cough detection algorithm for this purpose.[259] In the 1969 study by Loudon and Spohn nocturnal cough counts were repeated on a weekly basis during the treatment of 20 individuals with pulmonary tuberculosis.[125] There was a drop in 65% in the first two weeks, followed by a continued slower decline in cough frequency from then on (Figure 1.5). It is not known how improvements in cough frequency correlate with other indicators of response to TB treatment and whether monitoring coughs over shorter periods than eight hours and more frequently than once a week would be predictive of response to treatment.

Serial cough monitoring has been carried out in acute upper respiratory tract infection to describe the normal range of resolution of cough frequency in this context, useful for the future evaluation of potential anti-tussive medications.[79,80] In chronic conditions such as COPD[78,306] and idiopathic pulmonary fibrosis[77] serial cough monitoring may reveal information about the trajectory of the disease, perhaps with greater sensitivity although probably less specificity than changes in lung function.[307] This also needs further exploration.

1.7 Study aims

This thesis aims to describe the patterns and significance of cough in tuberculosis with reference to other respiratory conditions. The more specific objectives were as follows.

1. To assess and compare the subjective appreciation of cough in respiratory diseases.
2. To evaluate methods of objectively measuring cough frequency.
3. To assess the temporal patterns of cough frequency in respiratory disease, observe the response of cough frequency in tuberculosis and other conditions to treatment, and correlate objectively-measured cough with subjective cough scores

4. To investigate the influence of single nucleotide polymorphisms of *TRPV1* on objective measures of cough.

5. To investigate the infectiousness of coughs by measuring airborne droplets and the association of cough frequency with household infection in tuberculosis.

The thesis addresses some of the areas in which there is a significant lack of knowledge regarding variability, mechanisms and implications of cough in TB and other respiratory disease.

2 Comments on methodology and analysis of data

2.1 Introduction

A variety of methodological approaches were employed to fulfil the aims of this work. Detailed descriptions of the methods follow in the corresponding chapters but common approaches are set out below. This was an observational clinical study, predominantly of a cohort design, with cross-sectional elements.

2.2 Setting

The principal recruitment site was Homerton University Hospital NHS Foundation Trust (HUH), a district general hospital (DGH), in Hackney, North-East London, UK, where individuals were sought with all diagnoses. Patients with pulmonary tuberculosis were also recruited from Whipps Cross University Hospital, Shrewsbury Road Chest Clinic, and Mile End Hospital, East London. The work on capsaicin cough reflex endpoints (Chapter 6) and *TRPV1* genotyping in chronic cough (Chapter 9) took place at King's College Hospital, Denmark Hill, South London. Recruitment took place between January 2013 and June 2015.

2.3 Ethics

Ethical approval was granted for the majority of this study by the London Riverside Research Ethics Committee as part of the project "Cough in respiratory disease" (reference 12/LO/1923). In Chapters 6 and 9 capsaicin challenge testing was carried out (at King's College Hospital) with pre-existing ethical approval given by East London and The City Research Ethics Committee as part of another study, "The assessment of cough intensity with a novel cough monitor" (10/H0703/6). Approval to test for genetic polymorphism related to cough (Chapter 9) was given separately by the London Stanmore Research Ethics Committee ("Genetic variation and cough: the influence of *TRPV1* and *MUC5AC* single nucleotide polymorphisms on cough patterns",

14/LO/0164). The Research and Development departments of the participating hospital trusts granted approval for the conduct of the research under their jurisdiction. All study subjects provided written consent to participate.

2.4 Data cleaning

Data entry was checked primarily by verifying the values of outliers and unexpected results, range checking for continuous data, and verification that all values for categorical data were one of the pre-specified alternatives.[308]

2.5 Statistical analysis

Data were analyzed using Prism version 6.04 (GraphPad Software Inc., San Diego, California) and Stata 13.0 (Stata Corp, College Station, Texas).

Parametric tests were used with all continuous data, unless otherwise stated, after testing that distributions were approximately normal with either the Shapiro-Wilk *W* test (Stata) or D'Agostino-Pearson omnibus normality test (Prism). Log-transformation of continuous data to achieve normality was used where appropriate. Comparisons of means were made with Student's *t*-tests, paired or unpaired as appropriate, or one-way analysis of variance (ANOVA) for two or more groups of data, respectively. Non-parametric data are expressed as medians and inter-quartile range (IQR). Differences in non-normally distributed untransformed groups of data were tested with Wilcoxon rank-sum (Mann-Whitney) or Kruskal-Wallis tests for two or more groups of data, respectively. Dunn's tests were applied for multiple pairwise comparisons.

Categorical data were compared between groups with Fisher's exact tests, with odds ratios calculated with the Mantel-Haenszel method. Associations between two continuous variables were tested with Pearson's coefficient (*r*) or Spearman's rank correlation coefficient (ρ [rho]), for parametric and non-parametric data, respectively.

Multivariate analysis were performed using multiple regression. Candidate explanatory variables were selected on the basis of associated *p* values < 0.15 from univariate analyses. A forward stepwise approach was used to sequentially add variables in order

of magnitude of associated *p* values from univariate analyses (smallest to largest) until the addition of an extra variable was not statistically significant.[308]

All statistical tests were two-sided and statistical significance (α) was taken as $p \leq 0.05$. Power calculations were performed with the aid of an online tool.[309]

2.6 Acknowledged contributions

Necessary applications to ethics committees and research and development departments were made by the author with support from Prof Graham Bothamley. The majority of patients (>97%) involved in this research were recruited by the author. The remainder, all inpatients at HUH with acute respiratory disease, were recruited by Dr Metin Yalcin. All other aspects detailed in the methods in the following chapters were carried out by the author with the exception of DNA extraction and genotyping from saliva samples (Chapter 9) which was carried out by the Barts and the London Genome Centre. Data entry and statistical analysis was also performed by the author, with advice from Dr Richard Hooper. Further details of individuals contributing to this work are given on page 5.

3 Study participants

3.1 Introduction

The majority of the data in this thesis were obtained from the voluntary participation of human subjects, the large majority of whom were patients with respiratory disease. This chapter will describe the criteria for study participation, case definitions and subject characteristics.

The main interest was cough in tuberculosis, with the hypothesis that there may be special features in TB associated with the role of cough in the transmission of infection. Other disease groups were included partly as controls against which to compare cough patterns in TB, but also in attempt to observe particular patterns associated with different types of pathology. Aspects of the pathology of other respiratory conditions are shared to varying degrees with tuberculosis. For example, as well as occurring in TB, chronic sputum production is characteristic of bronchiectasis and chronic bronchitis; fibrosis is a common feature of interstitial lung disease, granulomatous inflammation the mechanism of sarcoidosis, and bacterial infection with consolidation is the hallmark of community acquired pneumonia. Conversely, the airway disease of COPD and asthma is not a feature of tuberculosis, malignant parenchymal and airway invasion by tumour is unique to lung cancer, and individuals with latent TB infection and no respiratory condition, by definition, should cough only as much as anyone else without lung disease. Isolated chronic cough probably represents a group of individuals who cough excessively, or are more aware of their cough, in response to stimuli which to others would be relatively innocuous.[62]

3.2 Subject selection and enrolment

Individuals were invited to participate in the study if they fitted into one of the following categories: isolated chronic cough, stable COPD, stable asthma, bronchiectasis, pulmonary fibrosis, lung cancer, COPD exacerbation, acute asthma, community-acquired pneumonia, pulmonary tuberculosis (TB), or latent TB infection.

Exclusion criteria were age <16 years, an inability to consent to the study, very severe acute illness (leading to artificial ventilation or palliation), or more than one of the following respiratory diagnoses: isolated chronic cough, COPD, asthma, bronchiectasis, pulmonary fibrosis, community-acquired pneumonia. Patients with lung cancer were not excluded on the basis of co-existent COPD due the common co-occurrence of these two diagnoses.[310,311] Individuals with latent TB infection were excluded if they had any one of the other respiratory diagnoses investigated in this study. Smoking and the use of medication with a possible influence on the cough reflex (in particular ACE inhibitors and opiates or opioids), were not exclusion criteria. Other than for individuals with isolated chronic cough, subjects were invited to participate regardless of whether or not cough was reported.

Patients were recruited both as hospital inpatients and outpatients, from respiratory wards, an acute medical admissions unit, and specialist respiratory clinics. These included a cough clinic, for patients referred from primary care with isolated chronic cough, and TB clinics. For stable COPD, recruitment also took place at pulmonary rehabilitation sessions.

3.2.1 Isolated chronic cough

Participants with isolated chronic cough had cough as the only or predominant symptom for ≥8 weeks.[312] At the time of enrolment they were visiting the cough clinic for the first time after referral from general practice. Their symptoms at that point had not been attributed to a particular diagnosis but the referrer had deemed there to be a low probability of thoracic malignancy.

3.2.2 Stable COPD

The diagnosis of COPD (chronic obstructive pulmonary disease) was confirmed on the basis of chronic respiratory symptoms (including cough and dyspnoea) and a post-bronchodilator ratio of forced expiratory volume in one second (FEV_1) to forced vital capacity (FVC) of <0.70.[313,314] Subjects were considered to have stable disease if they

had not experienced one or more exacerbations requiring medical treatment within the previous four weeks (section 3.2.7).[315]

3.2.3 Stable asthma

Participants with asthma had had their diagnosis confirmed by a consultant respiratory physician. In keeping with national guidelines, in all cases there had been a 'characteristic pattern of symptoms and signs and the absence of an alternative explanation for them' and demonstration of variable airflow obstruction.[316] Patients were considered to have stable disease if, within the previous 10 days neither of the following had occurred: use of a short-acting bronchodilator inhaler ≥4 times/day on two consecutive days, or waking due to symptoms of asthma on two consecutive nights.[317]

3.2.4 Bronchiectasis

Bronchiectasis had been diagnosed on the basis of current or previous symptoms of persistent cough and sputum production in conjunction with a confirmatory high resolution computed tomography (HRCT) which had been interpreted by a thoracic radiologist.[318] Patients with cystic fibrosis were not included, mainly because they did not attend clinics at Homerton University Hospital (HUH).

3.2.5 Pulmonary fibrosis

This category included patients with several underlying interstitial lung diseases (ILDs), including idiopathic pulmonary fibrosis (IPF), sarcoidosis, and connective tissue disease-related ILD.[319,320] All had evidence of pulmonary interstitial fibrosis on HRCT, including architectural distortion, honeycombing, and reticulation.[321]

3.2.6 Lung cancer

Patients in this category had either a tissue or clinico-radiological diagnosis of lung cancer[322] and had not received treatment with curative intent at the time of recruitment.

3.2.7 COPD exacerbations

This group comprised patients with COPD, as defined above (section 3.2.2), but were recruited within 48 h of hospital admission for symptoms of increased breathlessness, possibly accompanied by worsening cough and increased sputum volume and purulence, not explained by pneumonia or another cause.[323]

3.2.8 Acute asthma

Participants with acute asthma had a known previous diagnosis of asthma (section 3.2.3) but were recruited within 48 h of hospital admission for symptoms of increased breathlessness, possibly accompanied by worsening cough and wheeze not explained by pneumonia or another cause, but accompanied by a decline in the baseline or predicted best peak expiratory flow rate (PEFR).[316]

3.2.9 Community-acquired pneumonia

Community-acquired pneumonia (henceforth referred to simply as pneumonia) was defined as an acute illness (of duration ≤21 days) with symptoms consistent with a lower respiratory tract infection (including cough, fever, sputum production, breathlessness, wheeze, chest pain) accompanied by new shadowing on a chest x-ray not attributed to another cause (e.g. pulmonary oedema or infarction) in patients admitted to hospital from the community.[324,325]

3.2.10 Pulmonary tuberculosis

The diagnosis of pulmonary TB was ultimately clinical, made by an experienced physician, based on risk factors, symptoms, physical examination, radiology and sputum microbiology.[326] Sputum culture-negative cases were included. Patients were often invited to participate before the diagnosis was confirmed, and at times were retrospectively excluded if they had not officially been notified, or were later de-notified, with the public health authority as a case of pulmonary tuberculosis. Patients were recruited before they started, or were within 24 h of starting, anti-TB medication.

3.2.11 Latent tuberculosis infection (LTBI)

Subjects with LTBI had immunological evidence of exposure to *Mycobacterium tuberculosis* (Mtb) on the basis of a positive interferon-γ release assay (IGRA) and/or Mantoux test, but a normal chest x-ray and no clinical evidence of active tuberculosis.[327] They had been screened for tuberculosis either as close contacts of cases of active TB, because of recent arrival to the UK from a high TB-incidence country, routine occupational screening prior to working in a health or social care setting, or prior to starting on biological therapy for systemic rheumatic disease or other autoimmune disorder.

The IGRA used in most cases was the QuantiFERON® test (Cellestis, Australia), following manufacturer's instructions, with a positive test equating to a interferon-γ response to TB antigen ≥0.35 IU/ml above the negative control.[328] The Mantoux test was performed in the standard way, by the intradermal injection of 2 TU/0.1 ml tuberculin (PPD RT23, Statens Serum Institut, Copenhagen), and was considered positive if the skin induration at 48-72 h measured ≥6 mm, or ≥15 mm in the context of prior BCG vaccination.[329] Discordance between IGRA and Mantoux results was interpreted by an experienced clinician in light of epidemiological and clinical variables and the greater specificity of IGRAs compared to the Mantoux test.[330]

Individuals with LTBI were included specifically as a negative comparator group to those with respiratory disease. It was assumed that coughing in individuals with LTBI should be relatively infrequent, but individuals with acute coughs (and a normal chest x-ray) and smokers were not excluded.

3.3 Demographic and routine clinical data collection

From clinical records, both electronic and paper, it was verified that subjects fulfilled the diagnostic inclusion criteria. Age, gender, co-morbidities, routine medication use, relevant investigation results and details of routine clinical management were noted.

For patients with tuberculosis, sputum microbiology results were recorded, including acid-fast bacilli counts on smear microscopy, graded as +/-, 1, 2 or 3+.[331] X-ray appearances in TB were noted, including the presence of visible cavities, as reported by a radiologist, and the extent of disease, measured as the number of chest radiograph zones affected, from 0 to 6. *M. tuberculosis* complex lineage was also documented, as inferred from routine molecular typing (mycobacterial interspersed repetitive units, variable number of tandem repeats; MIRU-VNTR), carried out by the local mycobacterial reference laboratory.[332,333] From published literature on associations with virulence particular interest was in the Beijing strain,[182,184] from local (unpublished) cluster data, the Cameroon strain, and from a possible negative association with cough in one study, the Haarlem strain.[234]

For patients with COPD, asthma, bronchiectasis, pulmonary fibrosis and lung cancer, the most recent lung function report from the preceding 12 months was used if available. For pneumonia the CURB-65 score was calculated as an indicator of disease severity from the component variables on hospital presentation of confusion, serum urea concentration, respiratory rate, blood pressure and age.[325]

3.4 Routine management of respiratory disease

What follows is a description of the routine management of conditions relevant to the patients in whom repeated measurements were made.

3.4.1 Acute respiratory disease

Patients admitted to hospital with pneumonia, acute asthma and COPD exacerbations were usually commenced on treatment in the emergency department, transferred to the acute general medical admissions ward, and then a respiratory or other medical ward for continued care. Because an exclusion criterion to the study was very severe acute illness, patients admitted to the intensive care unit were not included. Supportive care included intravenous fluids and oxygen therapy as indicated.[334] Disease-directed treatment followed national guidelines. For pneumonia this included antibiotics, the initial route of administration and choice of drugs depending, at least in

part, on clinical severity of disease.[325] For acute asthma and COPD, treatment included inhaled bronchodilators, usually delivered initially by nebulizer, and systemic corticosteroids.[313,316]

3.4.2 Pulmonary tuberculosis

Treatment for TB was started by an experienced respiratory physician on suspicion or microbiological confirmation of the diagnosis in line with national guidance.[327] The standard regimen of four drugs (rifampicin, isoniazid, pyrazinamide and ethambutol) was given, unless there was confirmation or suspicion of drug resistance (e.g. previous TB treatment or a history of contact with drug-resistant pulmonary tuberculosis), in which case an alternative regimen was used.[327] Treatment of drug-sensitive TB was for six months, the final four months with only rifampicin and isoniazid, unless there was drug intolerance, poor medication adherence or a failure of adequate clinical response, in which case treatment was prolonged, possibly with alteration of the regimen. Common alternative regimens included streptomycin and/ or moxifloxacin as component drugs. Routine clinic appointments with the respiratory physician were scheduled at 0, 2, 8 and 26 weeks during treatment of non-complicated cases. Patients undergoing directly-observed therapy at the HUH TB clinic (usually due to previous TB treatment, previous poor adherence, or social risk factors for reduced treatment adherence) attended daily or on alternate week days. Chest x-rays were performed at baseline and repeated at 8 and 26 weeks in uncomplicated cases. Sputum was sampled at baseline, two and five months during standard treatment, produced either spontaneously or induced by inhaling hypertonic (3%) saline, and sent for mycobacterial microscopy and culture.

3.5 Participant characteristics

A summary of the baseline characteristics of the study participants is shown in Table 3.1. Ethnicity varied between diseases: compared to the whole study population, patients with TB were less likely to be white, as were participants with LTBI. In particular, a higher proportion of patients with stable COPD, acute COPD, and

community-acquired pneumonia was white than in the whole study population and in those with TB. A similar pattern was seen for country of birth, although there were more significant differences on comparing TB with other groups

Table 3.1. Summary of baseline characteristics of study participants

Diagnosis	n	Sex n (%) female	Ethnicity n (%) white	W. Europe-born	Age (y)	Current smokers	ACEi	Opiate use
Chronic cough	143	98 (68.5)$^{\S a}$	54 (37.7)	64 (44.8)†	51 (39-65)‡	5 (3.5)$^{\S d}$	13 (9.1)	4 (2.8)*
Stable COPD	18	9 (50.0)	15 (83.3)$^{\S c}$	16 (88.9)$^{\S c}$	67 (62-73)$^{\S b}$	6 (33.3)	6 (33.3)$^{\ddagger a}$	2 (11.1)
Stable asthma	5	4 (80.0)	2 (40.0)	3 (60.0)	64 (58-73)	0	0	0
Bronchiectasis	9	6 (66.7)	2 (22.2)	3 (33.3)	71 (60-74)‡	0†	0	2 (22.2)
Fibrosis	15	6 (40.0)	5 (33.3)	5 (33.3)	72 (63-74)§	2 (13.3)*	5 (33.3)$^{\ddagger a}$	1 (7.7)
Lung cancer	9	2 (22.2)^{a}	5 (55.6)	6 (66.7)*	64 (58-73)‡	4 (44.4)	0	2 (22.2)
COPD exacerbation	25	13 (52.0)	19 (76.0)$^{\S b}$	19 (76.0)$^{\S b}$	71 (60-77)§	12 (48.0)^{a}	7 (28.0)‡	5 (20.0)^{a}
Acute asthma	18	13 (72.2)†	5 (27.8)	14 (77.8)§	35 (23-44)^{a}	6 (33.3)	1 (5.6)	0
Pneumonia	17	8 (47.1)	12 (70.6)$^{\ddagger a}$	14 (82.4)$^{\S a}$	54 (43-65)*	10 (58.8) ^{b}	5 (29.4)†	0
TB	44	13 (29.5)^{b}	10 (22.7)^{a}	9 (20.5)^{a}	40 (30-43)^{d}	24 (54.5)^{d}	0	4 (9.1)
LTBI	17	7 (41.2)	2 (11.8)	4 (23.5)	31 (29-34)^{d}	2 (11.8)†	1 (5.8)	0
ALL	302	172 (57.0)	129 (42.7)	153 (50.7)	53 (40-67)	69 (22.8)	40 (13.2)	21 (7.0)

TB – pulmonary tuberculosis; fibrosis – pulmonary fibrosis due to any interstitial lung disease; AECOPD – acute exacerbation of chronic obstructive pulmonary disease; LTBI – latent tuberculosis. ACEi – current ACE inhibitor use.
Values are n (%) or median (IQR).
* $p \leq 0.05$, † $p \leq 0.01$, ‡ $p \leq 0.001$, § $p \leq 0.0001$, for differences compared to TB (Fisher's exact tests, except Dunn's multiple comparisons test for age). Differences otherwise not statistically significant.
^{a} $p \leq 0.05$, ^{b} $p \leq 0.01$, ^{c} $p \leq 0.001$, ^{d} $p \leq 0.0001$, for difference in proportions compared to the whole group (Fisher's exact tests, except Dunn's multiple comparisons test for age). Differences otherwise not statistically significant.
* $p \leq 0.05$, † $p \leq 0.01$, ‡ $p \leq 0.001$, § $p \leq 0.0001$, for difference in proportions compared to whole group (Fisher's exact tests).

Patients with TB and lung cancer were more likely to be male, and participants with isolated chronic cough more likely to be female. Age was higher in stable COPD and COPD exacerbations, and lower in TB, LTBI and acute asthma than in the whole group. Current smoking was more prevalent amongst patients with TB than with isolated chronic cough, bronchiectasis, fibrosis and LTBI, but not significantly different from subjects with COPD exacerbations, pneumonia and acute asthma. ACE inhibitors were more likely to be taken in stable COPD and pulmonary fibrosis, and less likely to be

used in TB than in the whole study population. Opiate medication was used significantly less frequently in patients with chronic cough than TB.

Apart from the 302 patients who consented to participate in the study, 32 declined. Their diagnoses were TB (n = 11), isolated chronic cough (n = 6), LTBI (n = 6), pneumonia (n = 3), pulmonary fibrosis (n = 2), asthma (n = 2), COPD (n = 1) and lung cancer (n = 1).

3.5.1 Isolated chronic cough

143 patients were recruited with isolated chronic cough. Final underlying diagnoses were as in Table 3.2, with asthma (30.8%), gastro-oesophageal reflux disease (16.1%) and unexplained chronic cough (16.1%) the most common. Four participants had >1 diagnosis and 18 participants had no diagnosis, due to loss to follow up (n = 8), a decision to be discharged before finding a cause for the cough (n = 6) and ongoing clinic management at the time of the end of the study (n = 4). The median duration of cough symptoms at entry to the study was 6 (3-12) months.

Table 3.2. Final diagnoses of participants with isolated chronic cough.

Diagnosis	n (%)
Asthma	44 (30.8)
Gastro-oesophageal reflux	23 (16.1)
ACE inhibitor-associated	12 (8.4)
Upper airway pathology	10 (7.0)
Post-infective	8 (5.6)
Smoking	4 (2.8)
Lower respiratory tract infection	2 (1.4)
Voluntary coughing / throat clearing	1 (0.7)
Bronchiectasis	1 (0.7)
Sarcoidosis	1 (0.7)
Unexplained chronic cough	23 (16.1)
No diagnosis	18 (12.6)

Values are n (%) or median (IQR). Note 4 patients (2.8%) had 2 diagnoses. n = 143.

The general characteristics of patients referred to the HUH cough clinic and the outcome of the diagnostic approach at the time of the current study have been described elsewhere.[335]

3.5.2 Stable COPD

Eighteen patients had stable COPD. All were recruited from pulmonary rehabilitation sessions. Lung function, exacerbation frequency and regular medication are summarized in Table 3.3.

Table 3.3. Characteristics of participants with stable COPD.

Characteristic	
Post-bronchodilator FEV_1 (% predicted)	59.9 (± 19.0)
Exacerbation frequency (yr^{-1})	6 (3-6)
Regular COPD medication	
long-acting β-agonist	12 (67)
long-acting antimuscarinic	15 (83)
inhaled corticosteroid	14 (78)

Values are mean (± SD), median (IQR) or n (%). Exacerbation defined as acute worsening of respiratory symptoms requiring antibiotics and/or systemic corticosteroids. n = 18.

3.5.3 Stable asthma

Only five patients had stable asthma, all recruited from a secondary care asthma clinic. They all had severe asthma as demonstrated by fixed airflow obstruction, high frequency of exacerbations of symptoms requiring oral corticosteroids and high level of background medication use (Table 3.4). Only one patient had a smoking history, having stopped >20 years previously.

Table 3.4. Characteristics of participants with stable asthma.

Characteristic	
Post-bronchodilator FEV_1 (% predicted)	66.8 (± 35.0)
Exacerbation frequency (yr^{-1})	6 (3-6)
Regular asthma medication	
inhaled corticosteroid	5 (100)
long-acting β-agonist	5 (100)
montelukast	5 (100)
theophylline	2 (40)
oral corticosteroid	2 (40)

Values are mean (± SD), median (range) or n (%).
Exacerbation defined as acute worsening of respiratory symptoms requiring systemic corticosteroids.
n = 5.

3.5.4 Bronchiectasis

Table 3.5 shows the characteristics of the nine patients with bronchiectasis.

Table 3.5. Characteristics of study participants with bronchiectasis.

Characteristic	
Cause of bronchiectasis	
idiopathic/ post-infective	4 (44.4)
connective tissue disease	3 (33.3)
previous tuberculosis	2 (22.2)
other	1 (11.1)
Number of affected lobes on CT	2 (2-3)
Lung function	
FEV_1 (% predicted)	72.0 (±30.3)
FVC (% predicted)	75.3 (±37.2)
FEV_1/FVC ratio	77.1 (±7.3)
Bacterial colonisation	
Pseudomonas aeruginosa	1 (11.1)
Other	2 (22.2)
Exacerbations/ yr*	4 (2-7)

Values are n (%), mean (± SD) or median (IQR).
*Exacerbation defined as acute worsening of respiratory symptoms requiring specific antibiotic treatment.
n = 9.

3.5.5 Pulmonary fibrosis

The characteristics of the 15 study participants with established pulmonary fibrosis due to ILD are shown in Table 3.6. All the cases of IPF had radiological diagnoses, one of which had been confirmed with histology.

Table 3.6. Characteristics of study participants with pulmonary fibrosis.

Characteristic	
Underlying diagnosis	
idiopathic pulmonary fibrosis	9 (60.0)
connective tissue disease	3 (20.0)
sarcoidosis	3 (20.0)
CT appearance	
UIP	10 (66.7)
Other	5 (33.3)
Lung function*	
FVC	77.9 (±31.7)
FEV_1/FVC ratio	84.0 (±9.9)
TLC	70.9 (±13.2)
DLCO	52.5 (±25.6)
KCO	81.6 (±29.1)

Values are n (%), mean (± SD) or median (IQR).
*all given as % predicted, other than FEV1/FVC ratio (actual values).
TLC – total lung capacity; DLCO – total diffusing capacity of the lung for carbon monoxide; KCO – diffusing capacity of the lung for carbon monoxide per unit alveolar volume
n = 15.

3.5.6 Lung cancer

Only one of the nine patients with lung cancer recruited to this study had received anti-cancer treatment at the time of recruitment, palliative radiotherapy >12 months earlier. There was no evidence of associated pulmonary fibrosis in this, or any other patient. None had undergone surgery. Lung function was only available for four participants: FEV_1 range 46-95% predicted, FEV_1/FVC ratio range 41-74%. One patient did not have any CT imaging due to residence abroad. The characteristics of the group are shown in Table 3.7.

Table 3.7. Characteristics of patients with lung cancer included in the study.

Characteristic	
Location of primary lesion	
RUL	7 (77.8)
RML	1 (11.1)
RLL	1 (11.1)
CT staging	
3b	3 (33.3)
4	5 (38.5)
unknown	1 (11.1)
Histological diagnosis	
no	2 (22.2)
yes	7 (77.8)
adenocarcinoma	4
squamous cell	2
bronchoalveolar cell	1
Co-existent COPD	3 (33.3)

Values are n (%).
RUL, RML, RLL: right upper, middle, and lower lobe, respectively.
n = 9.

3.5.7 COPD exacerbations

The characteristics of study participants with COPD exacerbations are shown in Table 3.8. All were commenced on treatment with antibiotics, oral corticosteroids and nebulised bronchodilators on the day of hospital admission.

Table 3.8. Characteristics of study participants with COPD exacerbations at the time of recruitment.

Characteristic	
Baseline FEV_1 (% predicted)*	44.5 (±16.8)
Exacerbations frequency (yr^{-1})	3 (1-4)
Days from onset of symptom worsening	6 (2-14)
C-reactive protein (mg/mL)	83 (15-119)

Values are mean (±SD) or median (IQR). *data only available for 24 patients. n = 25.

3.5.8 Acute asthma

Table 3.9 shows the characteristics of patients with acute asthma.

Table 3.9. Characteristics of patients with acute asthma at the time of study recruitment.

Characteristic	
Usual asthma medication	
inhaled corticosteroid	14 (78.0)
long-acting β-agonist	9 (50.0)
montelukast	4 (22.2)
theophylline	1 (5.6)
oral corticosteroid	0
Exacerbation frequency (yr^{-1})	0.5 (0-3.3)
Days from onset of symptom worsening	3 (2-5)
PEFR on admission (% best or predicted)	42.4 (±15.1)

Values are n (%), median (IQR) or mean (±SD). n = 18.

3.5.9 Community-acquired pneumonia

Characteristics of the 17 patients recruited with community-acquired pneumonia are shown in Table 3.10.

Table 3.10. Characteristics at time of recruitment of study participants with community-acquired pneumonia.

Characteristic	
Day from onset of symptoms	6 (5-14)
Clinical severity on admission	
bilateral chest x-ray involvement	4 (23.5)
C-reactive protein (mg/L)	288 (123-338)
Neutrophils (x 10^9/L)	13.7 (10.3-14.8)
CURB-65 score	1.5 (1-2)

Values are median (IQR) or n (%). n = 17.

3.5.10 Tuberculosis

Forty-four patients were recruited with pulmonary tuberculosis prior to starting anti-TB treatment (Table 3.11). Two patients also had COPD; no other participant had other chronic respiratory diagnoses. Of the four patients taking regular opiates, the medication used was methadone. Forty patients were recruited at Homerton University Hospital, and four from the other hospitals in East London.

13 of the 44 patients were female (Table 3.1), 10 of whom were sputum smear positive (77%), compared to 18 of 31 males (58%), a non-significant difference (Fisher's exact test, $p = 0.314$). 17 males had disease involving <2 x-ray zones (55%) compared to 7 females (54%; $p = 1.000$). Three women had co-incident extra-pulmonary disease (EPTB; 23%) compared to 4 men (13%; $p = 0.404$).

3.5.11 LTBI

Of the 17 participants with LTBI, reasons for TB screening had been contact with a case of active TB (n = 12), recent arrival to the UK from a high TB-incidence country (n = 3), pre-employment as a healthcare worker (n = 1), and consideration of biological treatment for rheumatoid arthritis (n = 1). One participant had an acute cough of three days' duration at the time of recruitment, presumably due to an upper respiratory tract infection.

Table 3.11. Characteristics of patients with pulmonary tuberculosis recruited to the study.

Ethnicity	
White UK/ Ireland	4 (9)
White other	9 (21)
Black African	10 (23)
Bangladeshi	6 (14)
Pakistani	5 (12)
Indian	2 (5)
Chinese	2 (5)
Other	5 (12)
Region of birth	
UK/ Ireland	8 (18)
Eastern Europe	5 (11)
Sub-Saharan Africa	11 (25)
Pakistan	5 (11)
Bangladesh	5 (11)
Other	10 (23)
Previous pulmonary TB	4 (9)
HIV status	
uninfected	35 (80)
infected	2 (5)
unknown	6 (14)
Coincident extra-pulmonary TB	7 (16)
Referral from screening	2 (5)
Chest x-ray appearances	
cavities present	17 (39)
zones affected	1 (1-2)
Acid-fast bacilli sputum smear	
smear-negative	16 (36)
1+	8 (18)
2+	6 (14)
3+	14 (32)
Sputum culture	
negative	7 (16)
positive	37 (84)
time to positivity (days)	6 (4-9)
organism drug sensitivity	
fully sensitive	30
isoniazid monoresistant	4
unknown	2
organism strain	
Haarlem	7
Cameroon	5
Latin American-Mediterranean	4
Delhi	4
S	3
Beijing	2
other	7
data unavailable	4
Cough symptoms at baseline	
no	7 (16)
yes	37 (84)
duration (weeks)	7 (3-13)

Values are n (%) or median (IQR).
n = 44.

3.6 Discussion

The ethnic origin (43% white) and region of birth (51% western Europe) of the participants in the study overall was similar to those of the wider population of Hackney (52% white, 61% UK-born).[336] Patients with TB were particularly likely to be born overseas and be non-white, in keeping with national data indicating the importance of imported infections.[337] Patients with COPD, both stable disease and with exacerbations, were more likely to be white than the general study population, but were also older. The prevalence of COPD increases with age generally;[338] the ethnic difference was probably a reflection of the fact that white people make up a relatively larger proportion of the local older population compared to younger age groups.[336] In keeping with global figures, individuals with tuberculosis were generally of relatively young age. The relative youth of subjects with LTBI perhaps reflected UK practices of offering preventative anti-TB treatment primarily to those under the age of 35,[327] leading to over-representation of this age group with LTBI in TB clinics.

The male predominance amongst the recruited patients with tuberculosis is representative of wider trends in TB which have a variety of explanations, biological and social.[339] A female over-representation amongst patients with isolated chronic cough has been often reported, and may be representative of a mechanism of the cough reflex (Section 1.4)[340] although women are more likely than men to seek advice about many symptoms other than cough.[341] Smoking is associated with TB,[172] COPD,[310] and pneumonia,[342] and this was reflected in the relatively high proportion of current smokers amongst study participants with these conditions. Smokers were under-represented amongst patients with isolated chronic cough, perhaps because smokers are less likely to seek medical attention for coughs,[343] or they are less likely to be referred by general practitioners (GPs) to secondary care for isolated cough, as symptoms are attributed solely to tobacco use.

ACE inhibitor use was relatively higher in those with COPD and pulmonary fibrosis and lower in TB, presumably reflecting differences in age and the likelihood of the comorbities (hypertension, cardiovascular disease) for which the medication had been

prescribed. The baseline differences in general characteristics between disease groups were therefore predictable and the study participants were representative of the local population. Detailed analysis of differences in baseline characteristic between groups is however limited by sample sizes, particularly for stable asthma, bronchiectasis and lung cancer, in which fewer than 10 participants were recruited in each case.

Disease-specific baseline characteristics within groups also varied. This is most clearly seen for tuberculosis and isolated chronic cough, the largest groups, where baseline variation should make the findings this research more widely applicable to other settings. Studying TB in London is probably unique for the large diversity of host and bacterial strain genotypes.[337]

The underlying attributed diagnoses in isolated chronic cough were similar to those seen in other settings,[9] although comparison with the literature is difficult as many published series contain large proportions of patients referred from primary, as well as secondary care.[224,243,344] It is also a problem for objectivity that diagnoses in isolated chronic cough are to a large extent made on the response to trials of treatment,[2] when cough can also resolve spontaneously,[64] with behavioural training,[65] and in response to placebo.[67]

Most other disease groups were small, which may limit the generalizability of specific findings in these conditions. The patients with stable asthma had severe disease, as is not uncommon in those attending a secondary care asthma clinic, whereas the recruitment of individuals with stable COPD from pulmonary rehabilitation sessions had more moderate pathology. The interstitial lung diseases have varied in their classification with time, and subdivisions in theory and practice are often difficult.[319] This was one of the reasons for grouping together all patients with established lung fibrosis regardless of the attributed underlying cause, in order to observe the effect of chronic lung scarring on cough. Umbrella terms are more commonly used in other areas of respiratory medicine to lump together patients with differing pathology and aetiology of disease. COPD, is the prime example of this, where fixed airway

obstruction might be the only unifying criterion that associates different diseases resulting from, for example, cigarette smoking, longstanding uncontrolled asthma, or the effects premature birth.[345]

3.7 Conclusion

This Chapter has set out the study recruitment criteria and characteristics of the included participants. It is in this context that cough in tuberculosis and other respiratory diseases was explored further.

SECTION TWO:
COUGH AS A SYMPTOM

4 Subjectively-described cough across respiratory conditions

4.1 Introduction and objectives

Cough is a common symptom of many respiratory disorders, either on its own, or in association with other clinical features. There have been few attempts to compare cough in different conditions in the same study.[83,88,89,91,101,276] This chapter will focus on cough as reported by the patient, i.e. the subjective report of cough symptoms, rather than an attempt at objective description of measurable physiological and pathological characteristics of cough, which will be discussed in Section 3.

The hypothesis was that subjectively-described cough varies between respiratory conditions. This might be because of different 'types' of cough, relating to differences in underlying pathology (Section 1.4.1), or differences in the noticeability of cough, either due to its relative novelty across diseases of varying chronicity, relative severity in comparison to other co-existing symptoms, or other factors. As suggested earlier, it would be to the evolutionary advantage of Mtb if the subjective effect of cough in TB were relatively mild so as not to impact significantly on social mixing and reduce opportunities for transmission (Section 1.5.3.3). Patient-reported cough in TB might therefore be particularly distinct.

4.2 Clinical procedures

4.2.1 Study participants

Two cohorts of patients were included. A prospective cohort with various diagnoses, and a retrospective cohort with pulmonary tuberculosis. The recruitment criteria, collection of routine clinical data, participant characteristics, and routine clinical procedures for the prospective cohort are described in Chapter 3. The retrospective cohort comprised all patients who had been treated for pulmonary tuberculosis at Homerton University Hospital (HUH) between 2010 and 2013 inclusive.

4.2.2 Measurement of cough symptoms

4.2.2.1 *Prospective data across respiratory diseases*

At recruitment, if participants reported cough they were asked to rate the severity of their cough over the previous two weeks with a 0-100 visual analogue scale (VAS),[63] and to describe the impact of the cough on quality of life over the same time period with the Leicester Cough Questionnaire (LCQ; Section 1.6.1, Appendix).[226] The possible range of total scores for the LCQ is 3-21. If participants reported an absence of cough, the VAS score was taken to be zero and the LCQ score to be 21, the maximum possible for cough-related quality of life (QOL).

Both the total LCQ scores and the component scores for each of the physical, social and psychological domains were used for analysis. For isolated chronic cough the minimum important difference over time for the same patient for the total LCQ score has been shown to be 1.3,[230] and for the cough VAS is probably 17 mm.[225] For the acute respiratory conditions and TB, these values were taken to be 2.5 and 13 mm, as established for acute cough.[231]

Self-reported sputum production was documented by taking the response from Question 2 of the Leicester Cough Questionnaire, 'In the last two weeks have you been bothered by sputum (phlegm) production when you cough?'.[226,346] Possible responses ranged from 1 to 7 on a Likert scale of frequency from 'every time' to 'never', respectively.

4.2.2.2 *The Leicester Cough Questionnaire in tuberculosis*

As no method appears to have been validated to quantify cough symptoms in TB, the use of the LCQ in participants with this diagnosis was evaluated. The face validity of the LCQ was evaluated by structured interviews with five patients, selected by convenience, and a multi-disciplinary team of a general respiratory physician, two respiratory physicians with specialist interest in tuberculosis, three TB specialist nurses and a TB case worker. Internal reliability of the questionnaire was tested by measuring inter-relatedness between items, concurrent validity by association of cough severity

VAS score with LCQ score, and responsiveness by improvements in LCQ score during treatment of TB.

4.2.2.3 *Retrospective data on cough in TB*

Because of a particular interest in cough in tuberculosis, and due to the limited literature in this area, further data were gathered retrospectively about the local prevalence of cough symptoms in this disease. From clinical records, the absence or presence and, if present, the duration of a cough at initial presentation were noted amongst the retrospective cohort of patients with TB. Gathering this information was made easier by the routine use of a standardized clinical data sheet in the local TB clinic for all patients which included information about cough.

4.3 Analysis of data

Non-parametric data are expressed as medians and inter-quartile range (IQR). Baseline patient characteristics were compared between groups with Fisher's exact tests and odds ratios. Baseline cough scores were compared across diagnostic groups with Kruskal-Wallis tests, and Dunn's tests for multiple pairwise comparisons (or one-way ANOVA and Tukey's tests for parametric data). Univariate analysis were performed on VAS scores, component and total LCQ scores across disease groups with Wilcoxon rank-sum (Mann-Whitney) tests for binary predictor variables and Spearman rank correlation for age. If appropriate, multi-variate analysis was performed of VAS and LCQ scores with linear regression models after the appropriate transformation of non-normally distributed data. The strength of correlation between total LCQ and VAS scores was also tested. Inter-relatedness between items of the LCQ in TB was assessed with Cronbach's α.

For the retrospective data, multivariate analysis was performed of possible predictors of the reported presence of cough in pulmonary TB using logistic regression with a forward stepwise approach (Section 2.5).

4.4 Results

4.4.1 The subjective impact of cough in respiratory disease

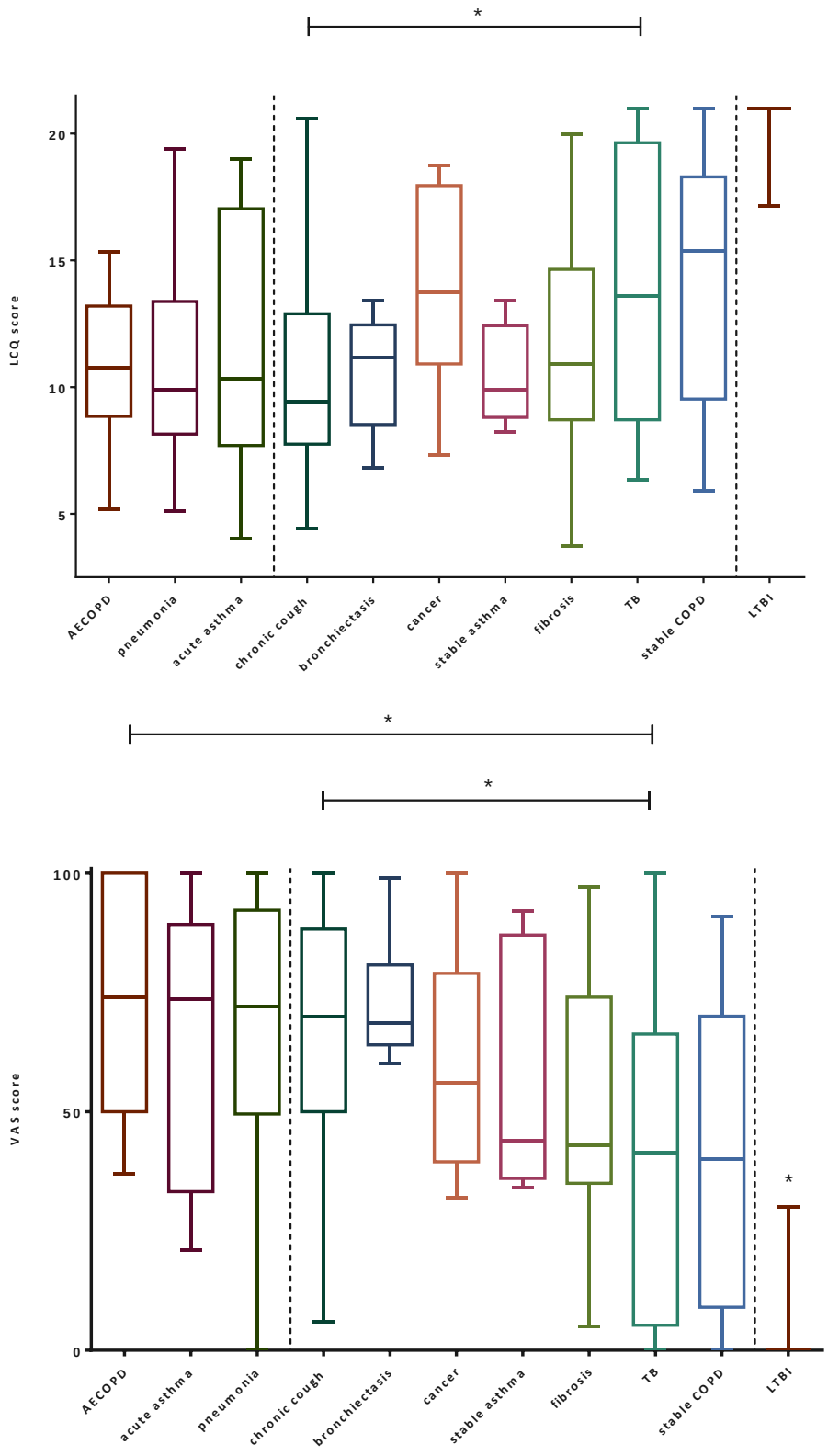
4.4.1.1 *Diagnosis, cough severity and cough-related quality of life*

Summary statistics for patient-reported cough severity (VAS score) and cough-related quality of life (total LCQ score) from the prospectively-recruited patient cohort are shown in Table 4.1 and Figure 4.1.

Table 4.1. Patient-reported cough severity and cough-related quality of life according to respiratory diagnosis.

Group	n	Cough severity (VAS)	Cough-related quality of life (LCQ score)			
			Physical	psychological	social	Total
Isolated chronic cough	143	70 (50-88)*	3.8 (3.1-4.8)	2.9 (1.9-4.0)	2.3 (3.0-4.5)	9.5 (7.9-12.9)*
Stable COPD	18	40 (9-70)	5.3 (3.4-5.6)	4.8 (3.0-6.4)	5.3 (3.4-6.5)	15.4 (9.5-18.3)
Stable asthma	5	44 (36-87)	3.1 (2.6-4.6)	3.3 (2.9-3.9)	3.5 (3.0-4.3)	9.9 (8.8-12.4)
Bronchiectasis	9	69 (64-81)	3.3 (2.9-4.3)	3.6 (2.8-4.2)	4.1 (2.5-4.6)	11.1 (8.5-12.5)
Fibrosis	13	43 (35-74)	4.5 (3.8-6.0)	3.4 (1.7-4.1)	3.0 (2.5-4.5)	10.9 (8.7-14.6)
Lung cancer	9	56 (40-63)	3.9 (3.4-5.9)	4.0 (3.3-5.6)	4.8 (3.8-6.5)	13.4 (10.9-18.0)
COPD exacerbation	25	74 (50-100)*	3.8 (3.0-4.2)	3.1 (2.6-3.8)	4.0 (2.6-5.1)	10.8 (8.8-13.2)
Acute asthma	18	74 (37-89)	3.4 (4.8-5.8)	3.7 (2.6-6.0)	3.0 (2.5-6.0)	10.3 (7.7-17.0)
Pneumonia	17	72 (50-92)	3.4 (2.4-4.8)	3.7 (2.3-4.3)	3.8 (2.3-4.5)	9.9 (8.1-13.4)
TB	44	40 (9.5-62.5)	4.5 (4.0-6.5)	4.4 (2.8-6.3)	5.3 (3.0-7.0)	13.6 (8.8-19.2)
LTBI	17	0 (0-0)*	7.0 (7.0-7.0)*	7.0 (7.0-7.0)*	7.0 (7.0-7.0)*	21 (21-21)*

Values are median (IQR).
*$p < 0.05$ for difference compared to TB



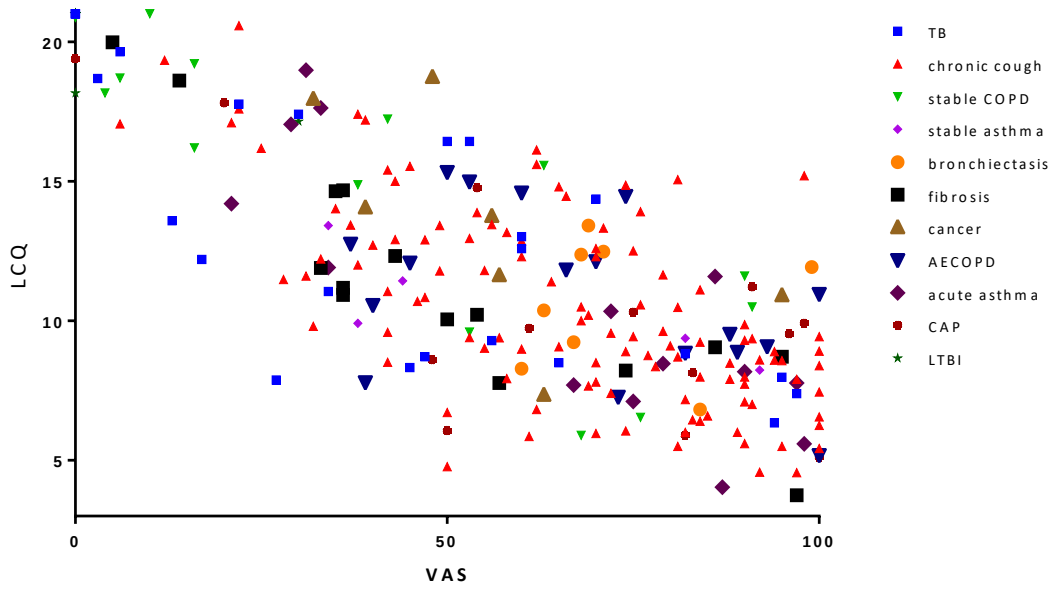
VAS – visual analogue scale (top); LCQ – total Leicester Cough Questionnaire score (bottom); LTBI – latent TB infection. AECOPD – COPD exacerbation. * $p<0.05$ for difference between groups (Dunn's multiple comparisons test; cough severity in LTBI significantly lower than in all other groups). Vertical dashed lines separate acute and chronic respiratory diseases and LTBI.

Figure 4.1. Patient-reported cough severity and cough-related quality of life and respiratory diagnosis.

Amongst the disease groups (i.e. excluding LTBI), there was evidence of a difference in cough severity (VAS score) only between TB and isolated chronic cough ($p < 0.05$), and between TB and COPD exacerbations ($p < 0.05$). For cough-related quality of life (LCQ score) there was evidence of difference only between TB and isolated chronic cough ($p < 0.05$; Figure 4.1). Cough-related quality of life and cough severity were worse in isolated chronic cough than tuberculosis. Cough symptoms were a lot milder in LTBI than in all the disease groups ($p < 0.05$ to < 0.0001); almost all participants with LTBI reported an absence of cough, although the participant with the likely upper respiratory tract infection (Section 3.5.10) had a cough VAS score of 30 and total LCQ score of 17.1.

Taking the three domains of the LCQ separately (physical, social and psychological), on multiple pairwise comparisons with Dunn's test there was evidence of a difference from the other groups only for LTBI, for all domains, and between isolated chronic cough and stable COPD, for the psychological domain, where quality of life was worse in isolated chronic cough (median scores 2.9 [1.9-4.0] *vs.* 4.8 [3.0-6.4]; $p<0.05$).

Self-rated cough severity (VAS scores) and cough-related quality of life (LCQ scores)



VAS – visual analogue scale; LCQ – total Leicester Cough Questionnaire score.

Figure 4.2. Correlation between subjective cough severity and cough-related quality of life.

overall were correlated (ρ = -0.73, 95% confidence interval -0.79 to -0.67, $p < 0.0001$; Figure 4.2).

4.4.1.2 *Non-disease related factors and cough symptoms*

Wilcoxon rank-sum tests revealed no statistically significant associations between gender, ethnicity, county of birth, smoking status, current ACE inhibitor or opiate use and either VAS or total LCQ scores across all disease groups overall (data not shown). Spearman's rank correlation coefficients (ρ) provided evidence of almost no association between age and cough severity (ρ = 0.13; $p = 0.039$), and no evidence of association between age and cough-related quality of life (ρ = -0.038, $p = 0.56$). Neither was there any evidence of an association between these variables and cough symptoms when looking at each of the disease groups individually, or together as chronic respiratory conditions (stable asthma, stable COPD, bronchiectasis, pulmonary fibrosis and lung cancer) or acute conditions (acute asthma, COPD exacerbation and pneumonia). This was even true in each of TB and isolated chronic cough, the groups of largest sample size displaying particularly wide variation in symptom scores, particularly VAS. Similarly, there was no evidence of association between the individual domains of the LCQ score and any of these same variables (analyses not shown).

4.4.1.3 *Cough and sputum*

There was no evidence of a difference in the self-reported effects of sputum production between diseases on any pairwise comparison (Tukey's multiple comparisons tests, all $p > 0.05$). The association between sputum symptoms and cough symptoms appeared strongest for lung cancer, fibrosis, stable asthma and stable COPD, although confidence intervals for Spearman's rank correlation coefficients were wide (Table 4.2).

Table 4.2. Sputum symptom scores from Leicester Cough Questionnaire and correlation with cough symptoms.

Group	n	Sputum symptom score	Correlation with cough severity (VAS score)		
			Spearman's rho	*p*	*P'*
TB	44	4.6 (±2.2)	-0.43 (-0.71 to -0.04)	0.028	0.276
Chronic cough	143	3.8 (±2.1)	-0.28 (-0.53 to 0.01)	0.055	
Stable COPD	18	4.1 (± 1.8)	-0.80 (-0.95 to -0.37)	0.0024	0.024
Stable asthma	5	3.8 (± 1.9)	-0.70*	0.233	
Bronchiectasis	9	2.6 (± 1.4)	-0.27*	0.477	
Fibrosis	13	4.4 (± 2.1)	-0.82 (-0.95 to -0.48)	0.0006	0.006
Lung cancer	9	3.1 (± 1.6)	-0.877*	<0.0001	<0.001
COPD exacerbation	25	3.1 (± 2.2)	-0.16 (-0.66 to 0.45)	0.553	
Acute asthma	18	3.9 (± 1.9)	-0.19 (-0.72 to 0.48)	0.528	
Pneumonia	17	3.7 (± 2.2)	-0.47 (-0.84 to 0.20)	0.132	

Values are mean (± SD).
p' – after Bonferroni correction for multiple comparisons * data sets too small for calculation of 95% confidence intervals.

Subjectively-described cough in tuberculosis

4.4.1.1.1 Reported cough in tuberculosis (retrospective data)

There were 197 cases of pulmonary tuberculosis treated at HUH between 2010 and 2013; clear details about cough were available for 176 of them (Table 4.4). 141 (80%) reported cough at initial presentation, of median duration at diagnosis 2 (1-5) months, which on univariate analysis was positively associated with smoking, sputum smear positivity, visible lung cavities, and radiographic extent of disease. Reported cough was negatively associated with coincident extrapulmonary disease, and screening as the initial source of referral to the TB clinic. On multivariate analysis, absence of coincident EPTB, sputum smear positivity, and referral from screening retained a significant relationship with the presence of cough.

Table 4.3. Factors associated with the reported presence of cough in pulmonary tuberculosis on clinical presentation (retrospective data set).

	Total no.*	No. (%) with cough	Univariate analysis		Multivariate analysis	
			odds ratio (95% CI)	*p* value	odds ratio (95% CI)	*p* value
Gender						
male	109	85 (78.0)	1			
female	67	56 (83.6)	1.43 (0.65-3.18)	0.368		
HIV status						
uninfected	107	83 (77.6)	1			
infected	9	8 (88.9)	2.31 (0.28-19.43)	0.681		
Current smoking						
no	99	72 (72.7)	1		1	
yes	54	48 (88.9)	3.00 (1.13-7.97)	0.021	2.64 (0.89-7.81)	0.079
Coincident EPTB						
no	155	129 (83.2)	1		1	
yes	21	12 (57.1)	0.27 (0.10-0.72)	0.005	0.24 (0.07-0.79)	0.019
Positive sputum smear						
no	90	62 (68.9)	1		1	
yes	81	75 (92.6)	5.65 (2.10-15.21)	0.0001	3.64 (1.28-10.40)	0.015
Positive sputum culture	45	34 (75.6)	1			
no	123	103 (83.7)	1.67 (0.72-3.85)	0.227		
yes						
***M. tuberculosis* strain**						
Beijing or Cameroon						
no	83	72 (86.7)	1			
yes	36	31 (86.1)	0.95 (0.30-2.96)	0.926		
Haarlem						
no	89	80 (89.9)	1			
yes	30	23 (76.7)	0.37 (0.12-1.10)	0.074		
Cavitary disease						
no	115	84 (73.0)	1			
yes	53	49 (92.5)	4.52 (1.46-14.01)	0.004		
Radiological extent						
0-1 zones	101	75 (74.3)	1			
≥2 zones	67	59 (88.1)	2.56 (1.08-6.06)	0.033		
Referral source						
other	140	118 (84.3)	1		1	
screening	25	12 (48.0)	0.17 (0.07-0.45)	<0.0001	0.15 (0.05-0.47)	0.001

*Missing data amongst these retrospectively-gathered data accounts for differences in patients included in each variable.

4.4.1.1.2 The Leicester Cough Questionnaire in pulmonary TB

The multi-disciplinary team and patients thought the LCQ to be relevant, well-written, comprehensive and useful in tuberculosis. The five patients were all non-native English speakers (having been born in Angola, Brazil, India, Pakistan and Somalia, respectively); two were female and two white, of median age 41 (range 27-50); four with a diagnosis of smear-positive disease, compared to one had smear-negative, culture positive TB. However, it was commented that there were no questions relevant to symptoms of

pulmonary TB other than cough (e.g. weight loss, haemoptysis), and that there were no items on the questionnaire relating to patients' concerns of infectiousness when coughing. Internal reliability of responses was high (Cronbach's $\alpha = 0.93$), and concurrent validity was shown by strong correlation between VAS and LCQ scores in pulmonary TB (Spearman's $\rho = -0.81$ [95% CI -0.91 to -0.62], $p < 0.0001$).

4.4.1.1.3 Predictors of cough symptom severity in tuberculosis (prospective data)

As in the other disease groups, there was wide variation in the cough symptom scores in pulmonary TB. On Mann-Whitney tests for associations between binary variables and cough symptoms, there was statistical evidence for an association of an absence of coincident extrapulmonary TB (EPTB) with LCQ score, and strong trends (with $p \leq 0.10$) of associations of sputum smear positivity, culture positivity, and absence of EPTB with VAS score, and of an absence of current smoking and culture negativity with LCQ score (Table 4.4). There were too few patients with HIV infection (n = 2) or who had been referred from screening (n = 2) to test for any effect of these variables on symptoms. On multivariate analysis, in a model including EPTB and sputum smear status there were independent trends for each variable of an association with VAS scores ($p \leq 0.20$), whereas for LCQ scores, there were trends for independent associations with EPTB and sputum culture status (Table 4.4).

Table 4.4. Cough symptoms and other variables in pulmonary tuberculosis (prospective dataset).

	n	VAS	*p* (uni-variate)	*p* (multi-variate)	LCQ	*p* (uni-variate)	*p* (multi-variate)
Current smoking							
no	20	28 (0-68)			17.1 (11.1-21.0)		
yes	23	51 (27-65)	0.348		12.6 (8.3-14.4)	0.075	
Coincident EPTB							
no	36	46 (17-70)			12.5 (8.5-17.8)		
yes	7	2(0-28)	0.076	0.200	19.8 (17.6-21.0)	0.043	0.164
Cough duration at presentation	27	55 (27-65)			12.6 (8.7-16.4)		
≤8 weeks	16	48 (34-94)	0.791		11.6 (8.2-15.4	0.687	
>8 weeks							
Positive sputum smear					17.8 (12.2-19.6)		
no	16	22 (3-38)			12.8 (8.5-17.4)	0.224	
yes	27	56 (13-82)	0.101	0.180			
Positive sputum culture	7	6 (3-22)			18.7 (17.8-19.6)		
no	36	47 (17-70)	0.101		12.4 (8.5-17.4)	0.052	0.104
yes							
M. tuberculosis* strain	7	65 (30-82)			8.8 (8.3-17.4)		
Haarlem	7	42 (0-56)			14.5 (10.6-21.0)		
Beijing or Cameroon	18[†]	49 (10-98)	0.464		13.0 (7.4-21.0)	0.595	
Other							
Cavitary disease							
no	26	27 (0-60)			16.4 (9-3-21.0)		
yes	17	50 (30-94)	0.142		13.3 (8.3-16.4)	0.267	
Radiological extent							
0-2 zones	33	36 (10-60)			13.0 (8.8-18.7)		
>2 zones	10	58 (0-70)	0.755		15.4 (8.5-21)	0.703	

Values are median (IQR).
Univariate *p* values relate to Mann-Whitney tests, apart from *, Kruskal-Wallis test. Multivariate *p* values relate to linear regression analysis.
[†] strain data unavailable for 11 patients (Section 3.5.10).

4.5 Discussion

This chapter set out to document cough symptoms in acute and chronic respiratory conditions. Wide variability in subjectively-measured cough in patients with respiratory disease was demonstrated which was significantly worse than in controls with latent TB infection. Cough symptoms in pulmonary TB were milder than in isolated chronic cough, but very few other differences were observed between diseases. There were no non-disease factors associated with cough symptoms across the group as a whole. In tuberculosis there were trends for the reported presence of cough with sputum smear and culture results, and for subjectively-described impact of cough with positive sputum microbiology and (an absence of) coincident extrapulmonary TB.

4.5.1 Cough symptoms and diagnosis

Cough symptoms in all disease groups were highly variable. This was particularly evident for stable COPD and TB, where the impact of cough in the majority of patients ranged from negligible to severe. A statistically significant difference was shown in both subjective measures of cough (visual analogue scale and Leicester Cough Questionnaire) only between isolated chronic cough and pulmonary TB.

The presence of an observed significant difference only between isolated chronic cough and TB could be due to greater statistical power than associated with other groups: these were the two largest disease groups (n = 143 and 44, respectively). Estimating appropriate sample sizes for investigating an association between diagnosis and cough symptoms *a priori* was difficult due to the lack of explicit data in the literature about the mean and variance of LCQ and cough VAS scores, particularly in conditions other than isolated chronic cough. It is now possible to retrospectively calculate the power of this part of study. For example in COPD (where both VAS and LCQ values approximated a normal distribution), the mean ± SD VAS and LCQ scores were 42 ± 33 mm and 14.2 ± 4.9, respectively. The relatively larger minimum important difference (MID) for VAS compared to LCQ scores (which has at least been stated for chronic cough) of 17 mm (ref [225]) *vs*. 1.3 (ref [230]) meant that small sample

sizes were more likely to show clinically meaningful differences between groups using VAS than LCQ scores. 224 patients would have been needed in each group to test for a difference in LCQ score of magnitude ≥1 x the MID compared to that observed in COPD with a power of 0.80; the equivalent sample size using VAS measurements would be 53 (assuming $\alpha = 0.05$).[309] However, 14 patients would be needed in each group to test for a difference in LCQ score of ≥4 x the MID (total score 14.2 *vs.* 9.0), or 9 patients for ≥5 x the MID (14.2 *vs.* 7.7). Conversely, two groups of 14 patients would be adequate with $\alpha = 0.05$ and 80% power to test for a difference in VAS score of ≥2 x the MID from that measured in COPD (41 *vs.* 7 mm).[309]

This study was therefore adequately powered in the large part to test for clinically meaningful differences in VAS scores between disease groups. Apart from stable asthma (n = 5), bronchiectasis (n = 9) and lung cancer (n = 9), where sample sizes were particularly small, the lack of large observed differences in VAS scores (≥2 x the MID) between disease groups are likely to be representative of what might be found in a larger study. However, a larger study would be required to exclude the possibility of a clinically significant difference in LCQ scores between disease groups.

These comparative observations between diseases are not dissimilar to those reported from the only other study of which I am aware to have compared cough symptoms between diagnostic groups. Polley *et al.* observed no differences in LCQ score between the included diseases, stable asthma (n = 20), bronchiectasis (n = 26), COPD (n = 18) and isolated chronic cough (n = 79).[83] However, that study was also likely to have been under-powered as the observed mean ± SD LCQ scores were very similar to those measured in the current work (e.g. 13.2 ± 4.4 in COPD in the previous study compared to 14.2 ± 4.9 in this Chapter).

Amongst the individuals with chronic respiratory diseases it is not unexpected that cough symptom scores were numerically most severe in isolated chronic cough, given that this group was defined solely by the subjective presence of cough.[2] In contrast, participants with other diagnoses were selected regardless of whether or not they

reported coughing. However, the particularly strong psychological impact in isolated chronic cough (at least in comparison to COPD) suggests that in this group there might have been a greater subjective awareness of coughing. This hypothesis will be tested in Chapter 6 by comparing the subjective impact of cough to objective measurements across disease groups (Section 7.4.3).

As expected, almost all participants with LTBI reported no cough symptoms; they were free of respiratory disease and in the majority were non-smokers. Indeed, LTBI was selected as a diagnostic group to act as a negative comparator to those with disease. However, the one participant in this group with an upper respiratory tract viral infection served as a reminder that coughs in any individual are not always necessarily caused by the main diagnosis of interest.

4.5.2 Cough and sputum

No difference was observed in self-reported sputum production between diseases, and an apparently stronger association between sputum production and cough symptoms in pulmonary fibrosis and lung cancer than in COPD exacerbations and isolated chronic cough. Bronchiectasis is strongly characterized by cough productive of sputum, and the cough of pulmonary fibrosis is classically described as dry.[319] Although there is a precedent for the method used in the current study for measuring sputum production (Question 2 of the Leicester Cough Questionnaire),[346] its use has not been validated, and it is not clear how responses to the question correlate with objectively-measured sputum production. The method of estimating sputum production could therefore be a reason for a failure to differentiate between diseases on the subjective sputum score. Another shortcoming with the use of Question 2 is that it cannot be easily compared with total LCQ score as the two variables are non-independent; the questionnaire has a high level of inter-relatedness between component items.[226] Sputum scores were therefore compared only against VAS scores, not the LCQ score.

In COPD, the use of an alternative method of estimating sputum production seems to be useful in the context of cough. Sumner *et al.* showed the response to the question

"Over the past 4 weeks I have brought up phlegm (sputum): not at all, only with respiratory infections, a few days a month, several days a week or most days a week?," from the St George's Respiratory Questionnaire[228] to be a predictor of 24-hour cough frequency.[78] Despite this, responses to this question similarly do not appear to have been compared to objective measures of sputum production in respiratory disease. In contrast, the UK Medical Research Council (MRC) validated another a set of questions used in epidemiological studies of chronic bronchitis in relation to objectively-measured morning sputum volumes and the proportion of mornings on which sputum is produced.[347] The MRC questions related to the number of months during the year in which 'phlegm' is usually brought up, and whether, if this occurs, just in the early morning, or during the whole day.

Cough and sputum are clearly closely associated, particularly in COPD,[348] bronchiectasis and TB, yet respiratory mucus has been researched relatively infrequently (Section 1.4.2).[105] It would be of interest to measure how self-reported sputum symptoms relate to objective measures of sputum in other conditions, and how objectively-measured sputum production is related to both cough symptoms and objectively-measured cough frequency across diseases.

4.5.3 Subjectively-reported cough in tuberculosis

There was no consistent evidence that coughing affected patients more or less in TB than in any other group, other than isolated chronic cough or latent TB infection. Given the wide variability in cough scores (Figure 4.1) it is unlikely that a larger study would show evidence for clinically significant differences between disease groups, at least for VAS scores (for which this study was more strongly powered compared to LCQ outcomes).

Although in TB there was a trend towards a worse cough-related quality of life amongst smokers than non-smokers, no general non-disease related factors were quantitatively associated with cough symptoms overall. This may again be due to limited statistical power. Based on the variability in LCQ score observed in TB (mean

14.2, standard deviation 5.2), with two samples of 22 patients (total n = 44) this study had a power of 0.13 to detect a difference in LCQ score associated with a particular binary variable between groups of ≥1 x the MID (as determined in chronic cough) with $\alpha = 0.05$, although a power of approximately 0.8 to detect a difference of ≥3 x the MID.[309]

For specific disease-related factors in pulmonary tuberculosis, the only significant association with the severity cough symptoms was an absence of coincident extra-pulmonary tuberculosis, at least on univariate analysis, for LCQ score. From the retrospective data, the reported presence of cough was independently associated with sputum smear positivity whereas an absence of cough was associated with screening as the source of patient referral and co-existent extra-pulmonary disease.

The relevance of EPTB and screening is presumably that the associated pulmonary disease in both cases was clinically mild, patients having presented either with symptoms related to other anatomical sites of disease (e.g. neck swellings in TB lymphadenitis, dyspnoea and systemic symptoms in pleural disease), or with only very few or no symptoms but an abnormal chest x-ray, respectively. The relative lack of a noticeable cough in screened patients may have been precisely why active medical care was not sought. Conversely, the associations of sputum smear and culture positivity with cough suggest that bacterial numbers are important in either the frequency or awareness of cough, or both. Other retrospective studies have also noted associations between sputum culture positivity, smear status and x-ray characteristics and the presence of cough, but it is not clear whether these associations were independent of each other.[305,349]

Only one other study of which I am aware has studied cough symptoms in tuberculosis quantitatively, with the LCQ and a VAS (although scaled from 1-10 rather than 1-100).[234] Amongst 124 smear positive patients in Brazil, cough symptoms were possibly associated with *M. tuberculosis* genotype: there was a non-statistically significant trend for weaker coughs amongst a cluster corresponding to the Haarlem strain, which

may have related to less extensive disease as assessed by chest radiograph. Although the authors of the study suggest a pattern of more severe cough in those with cavitary disease, they do not state whether this association was statistically significant. There was also a trend for worse cough symptoms in patients with lung cavities (albeit in a group with smear negative as well as smear positive disease) which was not statistically significant ($p = 0.14$ for VAS, $p = 0.27$ for LCQ).[234]

4.5.4 The measurement of cough symptoms in respiratory disease

The current study has shown that the use of the Leicester Cough Questionnaire and a cough severity visual analogue scale is feasible in a range of respiratory diseases. Both of them, particularly the VAS, are straightforward to use.

The Leicester Cough Questionnaire, as previously discussed, is probably the most widely-use and best-validated method of quantifying cough-related quality of life (Section 1.6.1).[225,227] Despite this, the large majority of the related published literature refers to isolated chronic cough rather than other diseases. The LCQ does not seem to have previously been used in lung cancer,[350] pneumonia, acute asthma or COPD exacerbations, and has only been used very rarely in pulmonary fibrosis[77] and tuberculosis.[234] The fact that good correlation with the VAS was demonstrated, particularly in TB, suggests concurrent validity of the LCQ in these other conditions.

Concurrent validity of the LCQ in TB was also demonstrated by the strong trend for worse QOL scores in patients with higher bacterial loads, seen in those with culture positive, compared to culture negative disease. Face validity was shown by MDT discussion and interviews with patient. The comments from some individuals that the questionnaire did not give opportunity to comment on other symptoms reflect the fact that the LCQ is a *cough* questionnaire, applicable to a range of diseases and allowing comparison of cough symptoms across different types of patient. The absence of questions about perceived infectiousness of coughs also reflect the fact that the LCQ is not a TB-specific tool. There is a need to develop a general quality of life tool specific to TB to quantify symptom burden and psychological impact of the disease.[351] This

study therefore provides justification for use of the LCQ in TB that was lacking in the only other study to have used the Questionnaire in the disease.[234]

The cough VAS and LCQ are complementary tools measuring crude subjective severity of cough, and a more detailed assessment of impact of cough on quality of life, respectively. The simplicity of the VAS is clearly an advantage but the LCQ has been more fully validated in cough against other clinical outcomes.[225]

The subjective measurement of cough has clear limitations. As in the documentation of any symptom, there are varied associations with underlying pathological phenomena. From the earliest studies it has been clear that there is poor correlation between objective cough counts and patients' awareness of coughing (Section 1.6.1).[88,240] Patients' awareness and interpretation of cough will depend on a variety of factors including personality, anxiety, experience, culture, education, social factors, and associated symptoms, both those resulting from coughing itself (pain, breathlessness, sputum production), and others caused by the underlying disease (including wheeze, fever, weight loss and malaise). Because it is almost impossible to separate objectively measurable phenomena from subjective experience when documenting symptoms on their own, I have made only limited conjecture on the reasons for an absence of differences in symptom scores between diseases (Section 4.4.1.1), or the lack of an association across diseases between cough severity and quality of life with demographic and other non-disease related factors (Section 4.4.1.2).

Rather the documenting only the subjective experience of cough, the *objective* measurement of cough should therefore be much more instructive for determining mechanisms, causative factors, associated phenomena and response to treatment. This is the subject of the following Chapters.

4.6 Conclusion

Coughing is common in many respiratory disorders and subjectively-measured cough is highly variable in all. Coughing was shown to be subjectively milder in TB than in

isolated chronic cough, but no overall clear differences between other diseases were demonstrated, and few general factors appeared to be associated with cough symptoms. However, an initial validation of the Leicester Cough Questionnaire in tuberculosis was provided, and cough symptoms in pulmonary TB were shown to be associated with sputum smear status and an absence of extrapulmonary disease. A detailed examination of cough in respiratory disease requires objective measurement, as shall be explored in Section 3 of this thesis.

SECTION THREE: OBJECTIVELY-DESCRIBED COUGH

5 The objective measurement of cough frequency

5.1 Introduction

While fully-automated cough monitors remain in development, counting coughs by ear remains the reference standard against which automated systems should be compared (Section 1.6.2.2.2).[252] However, defining coughs essentially depends on the subjective experience of the listener.[2] It is generally assumed the human ear can distinguish coughs from other sounds,[352] but this seems only to have been tested within pairs of individuals involved in the research of cough.[77,253,258] A broader consistency among larger numbers of people naïve to counting coughs has not been reported in the literature.

Recent research of cough frequency has often used computer software to visualize changes in amplitude in sound recordings to help identify and count coughs.[79,259,353,354] This is despite the recommendation that cough be defined by its sound,[2] and the effect of such visual assistance does not appear to have been evaluated.

Automated cough monitors would greatly facilitate the measurement of cough frequency. Of the recent attempts at developing cough monitors, PulmoTrack® (iSonea (formerly KarmelSonix), Haifa, Israel) is one of the least tested devices (Section 1.6.2.2.3.2),[262] yet at the time of this research it was marketed as a fully-automated ambulatory device for measuring respiratory sounds including cough.[261] It was the only automated cough monitor available to me for evaluation.

The following hypotheses were tested:

1. untrained observers agree when counting coughs;
2. visually representing audio data does not affect cough counting;
3. the automated PulmoTrack® cough monitor performs as well as human listeners.

5.2 Methods

5.2.1 Participants

A convenience sample of 13 patients from all those recruited to the wider study was selected (Chapter 3). All reported excessive coughing and had a range of diagnoses.

5.2.2 Automated cough monitor

Cough monitoring with PulmoTrack® took place in hospital over 16-24h. Clinic patients were admitted specifically for this purpose. The PulmoTrack® software (Version 6.5.0) used an algorithm to calculate cough counts expressed as 'cough events' and 'component coughs' per min. These terms were not clearly defined in the product literature but it was presumed that they equated to bouts of coughing and individual cough sounds, respectively. The system allowed playback for non-automated cough counting. To test repeatability the recordings were analyzed with the software twice.

5.2.3 Listeners

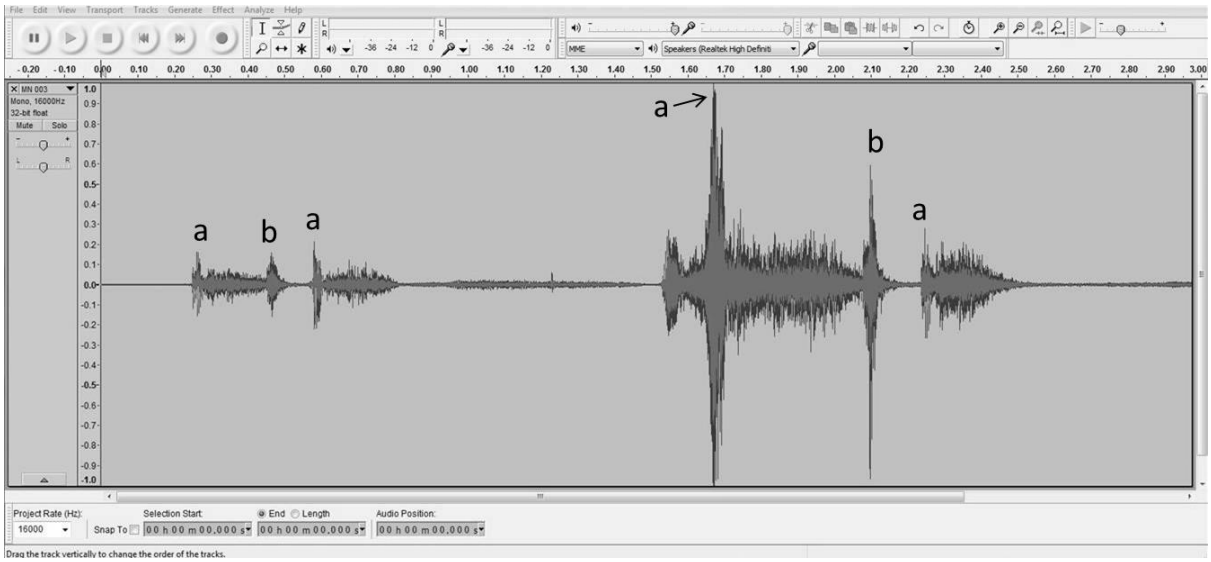
The author counted cough sounds in a 4-hour section from each of the recordings where PulmoTrack® indicated the presence of the greatest number of coughs. Three sequences were then selected lasting 14-22 minutes based on the presence of a high density of coughs and differing underlying pathologies. These short sequences were played to 15 respiratory physicians who were asked to count all cough sounds.

Listeners were asked about any known hearing problems and, in order to estimate their experience of listening to closely spaced sounds, the frequency with which they played a musical instrument, and their confidence in detecting fixed splitting of the second heart sound (minimum duration 0.02s[355]) when auscultating the praecordium during routine clinical examination. No specific training or instructions of how to count coughs were given other than asking listeners to count cough sounds, whether occurring in isolation or as part of a bout of prolonged coughing.

Listeners could pause and repeat playback of the sequences as desired. They were unaware of the cough counts of other auditors and of the interpretation of the machine.

The doctors listened to the sequences on three occasions at intervals of ≥4 weeks. Visual data were not shown on the first two occasion, but on the final occasion a simultaneous visual representation of sound amplitude was provided using Audacity® open source audio editing software (version 2.0.2; see Figure 5.1).[356] The method is summarized in

Figure 5.2.



Initial explosive phases (a) and final, voiced, phases (b) of each cough sound indicated where present (Section 1.6.2.4). [Reproduced with permission. Audacity® software is copyright (c) 1999-2016 Audacity Team. The name Audacity® is a registered trademark of Dominic Mazzoni.]

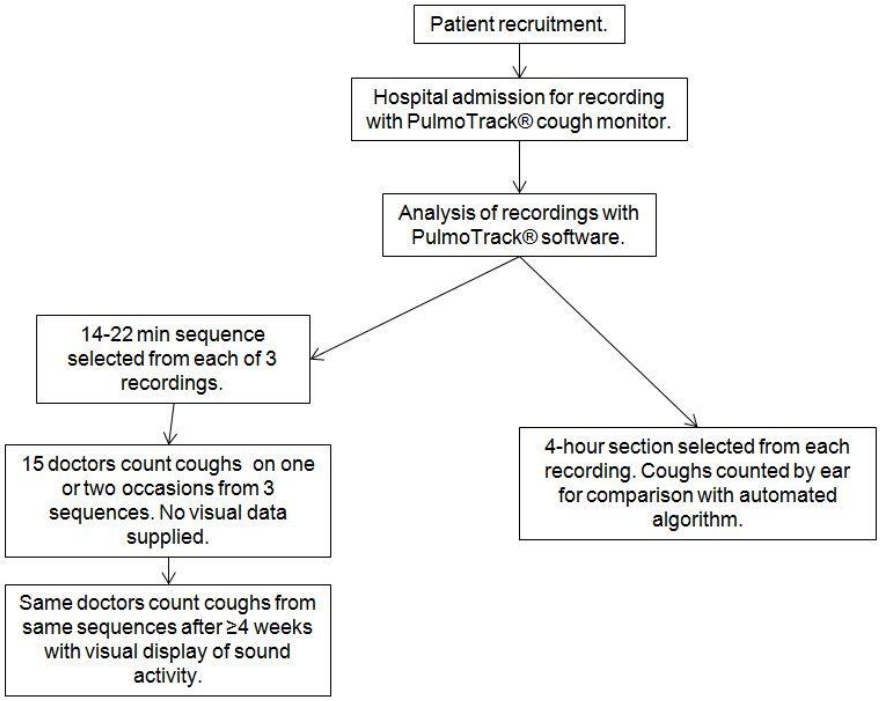
Figure 5.1. Audacity® audio editing software representing four cough sounds from patient with unexplained chronic cough.

5.2.4 Data analysis

Intra-class correlation coefficients were used to describe agreement between observers and to evaluate PulmoTrack®. Mixed effects regression models and a

likelihood ratio test were used to explore the variation associated with each non-automated counting method (using sound alone or sound with visual data). The two methods were also compared with a Bland-Altman plot.

The intended study size was 15 observers. From initial data this number would give 80% power at a significance level of 0.05 to detect a difference in total cough counts of 7% when comparing listening alone to listening with the addition of visual data, or 50% power to detect a difference of 5%.



See text for further explanation.

Figure 5.2. Investigation of methods for determining cough frequency: study overview.

5.3 Results

5.3.1 Participants

Thirteen patients were included with sarcoidosis (n = 1), acute asthma (n = 2) COPD exacerbation (n = 2), stable COPD (n = 2), tuberculosis (n = 2), idiopathic pulmonary fibrosis (IPF; n = 1), community acquired pneumonia (n = 1) and unexplained chronic cough (n = 2), who all reported coughing excessively.

5.3.2 Cough monitoring

The setup of PulmoTrack WHolter™ took an average of nine minutes. Recordings were inadequate on four occasions owing to battery failure, on one occasion, and disconnection of recording sensors on three separate attempts. One patient with chronic cough agreed to undergo a second attempt at monitoring. The median duration of the 10 successful recordings was 19.6 h (range 9.3 to 24.5 h).

Sequences from 3 patients were played to 15 doctors, to 7 of them on two occasions. O = counts by author. Error bars: means and SD. p-values are for paired 2-tailed t-tests for differences between listening attempts.

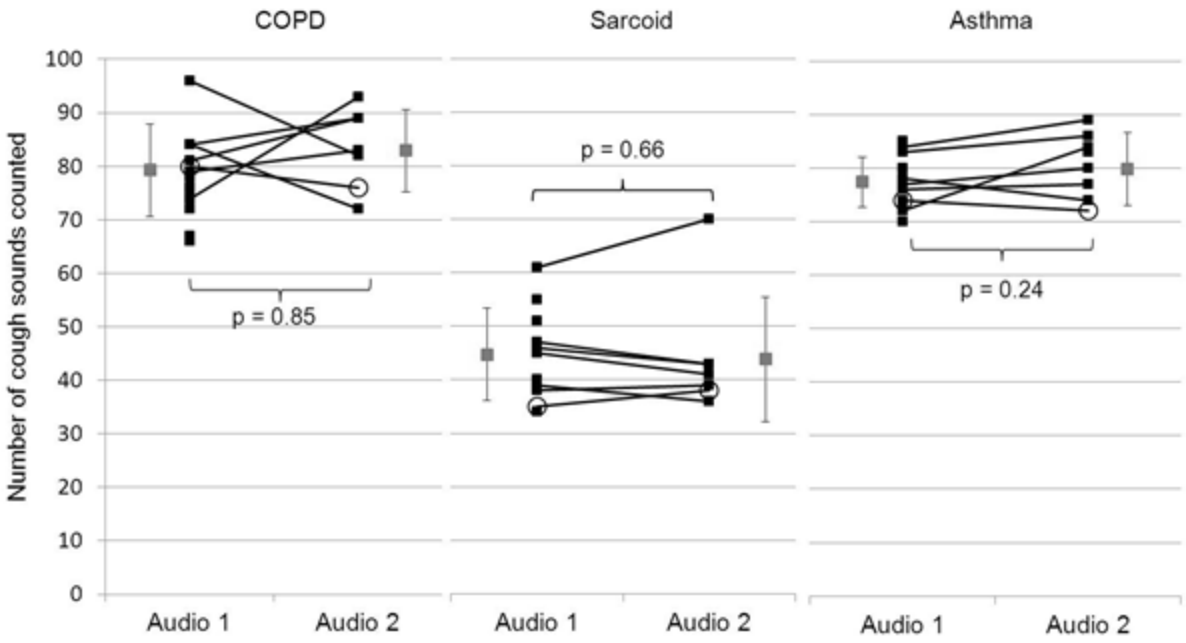


Figure 5.3. Intra- and inter-observer variation in cough counting with auditory information alone.

5.3.3 Intra- and inter-listener consistency

Cough counts of the three sequences using only auditory information are shown in Figure 5.3. The selected sequences were from patients with COPD, sarcoidosis and asthma and lasted exactly 19, 14 and 22 minutes respectively. On the first attempt mean (± SD) cough counts were 78.7 (± 8.1), 44.6 (± 7.5) and 77.3 (± 4.7) respectively (total count for all sequences: 200.7 ± 14.6).

Seven of the 15 doctors listened to the sequences on a second occasion without visual data. Intra-class correlation coefficients (ICCs) between individuals were 0.89 (95% confidence interval [CI], 0.65 to 1.00) on the first attempt and 0.86 (0.53 to 1.00) on the second. Within-individual ICCs were in the range of 0.96 to 0.99 between 1st and 2nd attempts with a mean of 0.7% more coughs counted on the second attempt (95% CI, -4.7 to 6.1).

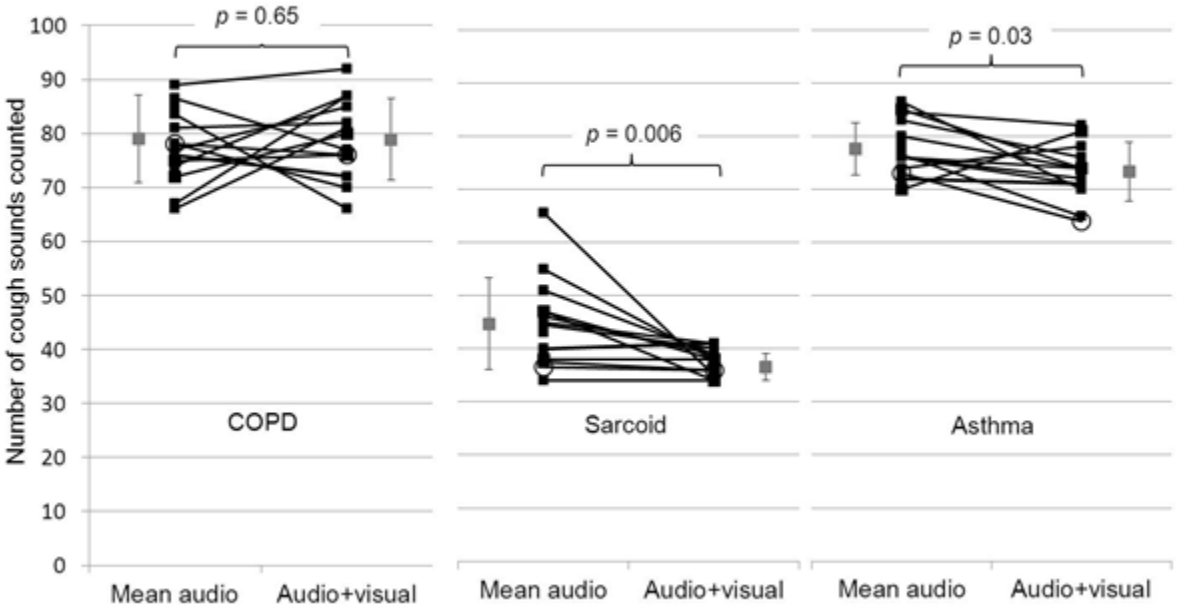
Sequences were also analyzed as one minute segments. Those segments in which coughs were counted by any observer were compared as two equal-sized groups: segments with lower variation in counts against those with higher variation (SD of mean <1.15 or >1.15). The only factor strongly associated with higher variation was a higher count of cough sounds (mean 7.3/minute in high variation periods, 3.2/min in low variation periods, $p = 0.03$). Eight and 5 segments contained speech in the higher and lower variation groups respectively ($p = 0.12$). Sounds which were considered by the author to represent throat clearing did not affect count variation although they were only present in 8 segments. There were even fewer background noises to test their influence on cough counting.

All doctors stated having normal hearing. Gender, doctor seniority, musical ability and ability to detect fixed splitting of the second heart sound did not affect the total numbers of coughs counted (Table 5.1).

Table 5.1. Association of listener-related factors with total cough counts across all sequences on first listening attempt.

		n	*Mean (±SD) total cough count*	*p*
Gender	Male	10	201.1 (± 14.1)	0.75
	Female	5	199.8 (± 17.1)	
Doctor seniority	Cons / SpR	10	202.3 (± 17.0)	0.56
	F1 / SHO	5	197.4 (± 8.76)	
Plays musical instrument	> monthly	4	205.5 (± 18.4)	0.46
	Rarely / never	11	198.9 (± 13.5)	
Ability to hear fixed splitting of 2nd heart sound	Yes	6	207.2 (± 15.6)	0.17
	No	9	196.3 (± 12.9)	

P-values from paired sample t-tests. Cons: consultant in respiratory medicine; SpR: specialist registrar in respiratory medicine; F1: Foundation Year one doctor (first year post-medical school qualification); SHO: senior house officer (1-4 years post medical qualification).

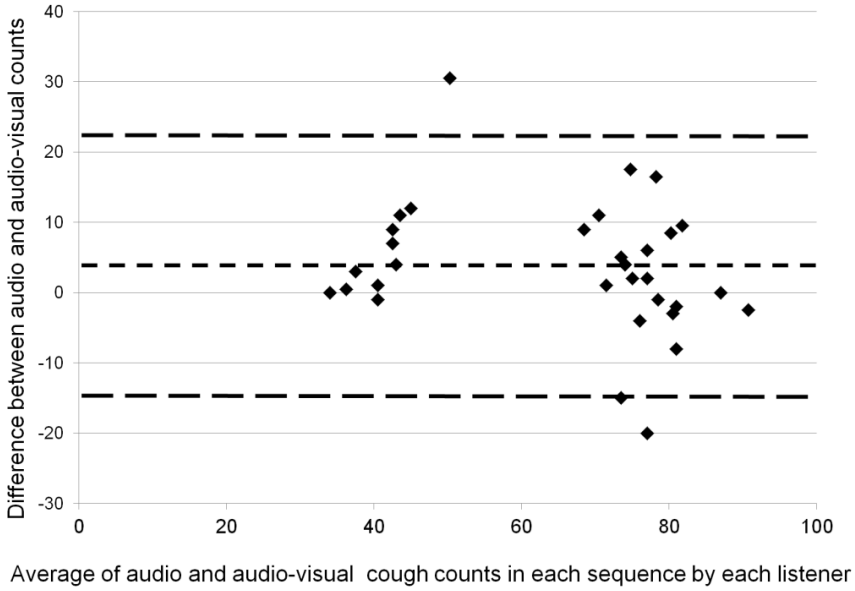


Sequences from three patients played to 15 doctors on two or three occasions. Mean cough counts from attempts using only auditory information compared to counts when visualizing a display of sound amplitude simultaneous to audio playback. O = counts by author. p-values from paired sample t-tests between means.

Figure 5.4. Comparison of auditory with visual and auditory cough counting.

5.3.4 Audio vs. audio-visual counting

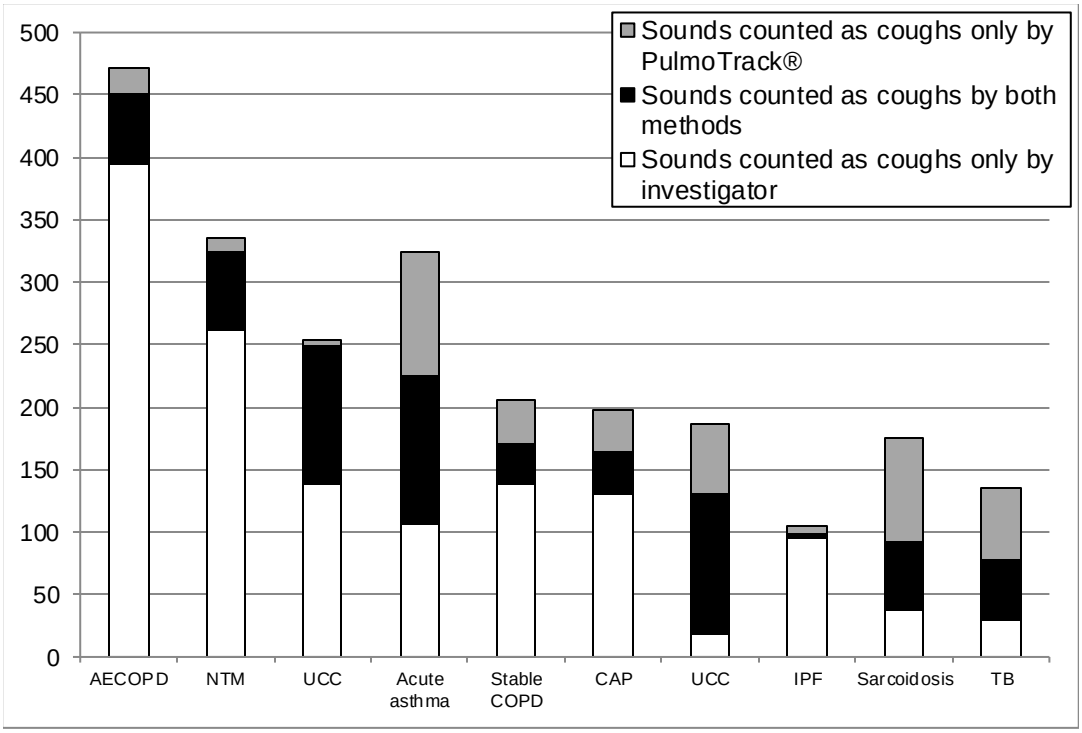
Thirteen of the 15 original listeners re-analyzed the sequences with simultaneous visual data. Mean counts of coughs sounds were lower than the corresponding average values from the two attempts without visual information, significantly so for the sarcoidosis and asthma sequences (Figure 5.4) ; mean [± SD] total cough count 190.2 [± 3.4]). A mean of 3.1 (4.8%) [95% CI: 0.3 to 9.2, (0.6 to 9.5%)] fewer coughs were counted in each sequence with the visual display than when listening without it ($p = 0.04$). For all but two of the counts the differences between methods were within two standard deviations of the mean (Figure 5.5). The agreement between the 13 doctors counting by ear and eye together was excellent (ICC=0.94, 95% CI 0.80-1.00). The apparent decrease in inter-observer variation between methods, as assessed by the regression analysis and likelihood ratio test, was not statistically significant ($p = 0.80$).



Mean count from listening alone minus count with additional visual data plotted against the average cough count from between methods for each listener for each sequence. Dotted lines: mean difference (3.1) ± 2SD (-15.3 to 21.5).

Figure 5.5. Bland-Altman plot comparing methods of non-automated cough counting.

The majority of participants stated that cough counting was quicker with the addition of the visual display.



Number of sounds counted as coughs by PulmoTrack® and a human observer in 4-hour sequences from 10 patients. Data presented in descending order of numbers of coughs counted by the investigator. AECOPD – acute exacerbation of COPD; NTM – non-tuberculous pulmonary mycobacterial infection; UCC – unexplained chronic cough; COPD – chronic obstructive pulmonary disease; CAP – community-acquired pneumonia; IPF – idiopathic pulmonary fibrosis; TB – pulmonary tuberculosis.

Figure 5.6. Comparison of PulmoTrack® and non-automated cough counting.

5.3.5 Automated cough counting

PulmoTrack® took approximately 7 h to process a 24 h recording. Automatically detected numbers and timings of coughs were identical the second time all sequences were analyzed. A comparison of the number of sounds counted as coughs by the machine and the human observer in the 4-hour recording segments is shown in Figure 5.6. Almost 2,000 coughs were counted by the author. Even ignoring agreement over individual sounds, overall crude counts of cough sounds between auditory and

automated methods substantially differed (ICC = -0.23, 95% CI, -0.51 to 0.34, p = 0.87). On non-automated counting, mean cough rates in these sequences ranged from 19 to 119 cough sounds/h. A median of 100 (range -29 to 465) fewer cough sounds were counted by PulmoTrack® than by the non-automated method in each 4-hour sequence.

A total of 39% of the cough sounds across sequences counted by PulmoTrack® were identified as such only by the machine (Figure 5.6). The author recognized these most commonly as speech, swallowing, microphone interference, breath sounds and background noise (Table 5.2).

Table 5.2. Non-cough sounds misclassified by Pulmotrack®.

Sound	n	% of total number of sounds classified as coughs
speech	149	14.4
swallow	71	6.9
microphone interference	58	5.6
background noise	28	2.7
breathing	27	2.6
throat clearing	25	2.4
no sound	24	2.3
sneezing	7	0.7
belching	7	0.7
Eating	4	0.4
Other	7	0.7
TOTAL	407	39.4

With non-automated cough counting as the reference standard, the overall positive predictive value (95% CI) of PulmoTrack® was 60.6 (57.5 to 63.6) % and sensitivity 32.1 (30.1 to 34.3) %.

5.4 Discussion

The findings of the current chapter have demonstrated that untrained listeners are consistent in counting cough sounds, that simultaneously visualizing audio sequences

led to lower counts, and that the Pulmotrack® automated cough monitor disagreed with human cough counting.

5.4.1 Consistency within and between listeners

To my knowledge this is the first work to test consistency in cough counting among >2 individuals. The overall relative standard deviation here of 7.3% (14.6/200.7) when listening alone and 6.4% (12.1/190.2) with both visual and auditory representation is of the same order of magnitude as studies comparing two listeners. Key *et al.* noted a 9.5% difference in cough counts between two individuals experienced in cough counting when coughs from 30 min sequences in 19 patients with IPF occurred at 9.4/h.[77] The same group also reported a difference between two people of about 2.6% when analyzing 24-hour recordings from 10 patients with chronic cough due to a variety of conditions.[258] The intra-class correlation coefficient for 11 listeners of 0.94 concurs well with the 0.98 reported for studies comparing just two listeners.[253]

Intra-observer consistency was high (ICC 0.96-0.99). Birring *et al.* reported an ICC of 0.99 when one cough researcher counted cough sounds in 2-hour recordings from 9 patients twice but the time interval between counting attempts was not stated.[253]

There appeared to be no listener factors which affected cough counting. Disagreement was associated with higher cough frequencies and possibly the presence of speech but not the rarer presence of throat clearing or background noise. Further comment on causes of inter-observer variation is limited as it was so low. However, 'unusual' cough sounds are probably a cause of disagreement. For example, I have demonstrated elsewhere that there is less consistency between listeners counting coughs during bronchoscopy procedures than in the current study; the median (IQR) difference in total cough counts by three respiratory physicians from 24 procedure recordings was 15.6 (6.1-31.0) %.[357] The sound produced by coughs during bronchoscopy is presumably altered because the endoscope prevents apposition of the vocal cords, but may also be muffled by the noise of suction apparatus.

The fact that untrained listeners demonstrated good agreement in the current study, and that there was no evidence for an effect of doctor seniority, suggests that experience and specific training in counting coughs might not be required. Not including listeners with experience of cough counting here allowed easier testing of the previously untested assumption that the 'characteristic sound'[2,352] of cough is universally distinguishable from other noises.

5.4.2 The effect of visual data on cough counting

Audio editing software simplified cough counting by eliminating the need to listen to periods of silence which are evident from visual inspection. Fewer coughs were counted with a visual display of sound amplitude simultaneous to audio playback. The appearance of the sound amplitude trace presumably led to dismissal of certain sounds which would have been counted by listening alone. This is suggested most clearly by the difference in cough counts between methods in the sequence from the patient with sarcoidosis (Figure 5.4). The finding that recognition of coughs depends partly on the appearance of the amplitude waveform has implications for the definition of a cough, about which there has been debate.[3,4]

It is not clear why there was only a significant difference in cough counts between methods of non-automated counting for two of the sequences (from the patients with asthma and sarcoidosis); there may be certain types of cough that are more consistently recognized by listening alone. The cause of the two outlying results in the comparison between methods (Figure 5.5) is uncertain. Distraction leading to missing coughs, double-counting or errors in transcribing results to the data sheet are possibilities. There was no other evidence that attention span or fatigue were significant problems, but these factors may become important with longer recordings.

Although the addition of visual data to audio sequences appeared to improve inter-observer agreement in cough counts this was not statistically significant. A type 2 error is possible as this part of the study was not powered to detect relatively small differences in variation between counting methods.

5.4.3 Pulmotrack® cough monitor

PulmoTrack® provided useful ambulatory recording and playback of patient data and showed perfect consistency on repeated analysis of the same sequences. However, there were technical problems and agreement between PulmoTrack® and non-automated cough counting was unsatisfactory.

As Figure 5.6 shows, agreement between the machine and the human cough counter was poor across all recordings, but the extent of this disagreement was variable. In some recordings almost all the coughs counted by the investigator were missed by PulmoTrack (e.g. from the patient with AECOPD and IPF), whereas in others (asthma, sarcoidosis) a substantial number of 'coughs' classified the machine were thought to be other sounds by the listener. In a sample of patients of this size, and without knowing the nature of the algorithm used by PulmoTrack to detect coughs, it is only possible to speculate on reasons for this lack of consistency. The fact that the 'sensitivity' of the machine for detecting coughs recognized by ear was substantially different in the two patients with unexplained chronic cough suggests that the nature of pathology might not be important for the functioning of the cough detection algorithm. There are a number of other factors which could have been important for cough detection, such as vocal characteristics, background noise, movement artefact causing interference with contact microphones, and features of the digital sound encoding technology and the microphones themselves.

One study to evaluate PulmoTrack® showed high agreement with non-automated cough counting, but recruited 12 healthy volunteers who were asked to make voluntary coughs and other noises during 25-minute recordings.[262] The evaluation undertaken in the current work was more representative of the circumstances in which such a system might be normally employed.

The development of a completely automated system for counting coughs has been slow.[86] The brain appears to identify a cough from both a complex distribution and pattern of sounds with ease (Figure 5.1), suggesting there may be an evolutionary

advantage to the recognition of coughs. The best tested automated or semi-automated cough monitors require human input either to help calibrate the system[253,265] or to actively count coughs in sequences that have been condensed to remove silences and non-cough sounds.[263,264] High cough rates, speech, background noise and the ability to perform across a range of types of patient and cough are particular challenges for automated cough monitors.

5.4.4 Limitations

The data are limited by the inclusion of only three short sequences for testing consistency between observers in cough counting. The roles of auditory fatigue or inattention in longer recordings were therefore not examined. However, the sequences were selected for their very high number of coughs, aiming to amplify any differences between counting methods or observers that might only have become evident by using longer sequences with lower cough frequencies. Short sequences were used deliberately to make involvement in the study more acceptable to the volunteering listeners.

I cannot comment on the recognition of coughs by non-clinicians, although the absence of an effect of doctor seniority suggests that clinical experience is not important for distinguishing coughs from other respiratory sounds and counting them. Neither were there enough data to describe the effects of types of cough as possibly influenced by pathology, gender, age and anatomy, or the effects of background recording conditions. Nevertheless, consistency in cough counting was examined between and within observers more thoroughly than any other study of which I are aware.

All of the recordings were made in a hospital inpatient setting due to limited access to the cough monitoring equipment. A more rigorous assessment of PulmoTrack® would also include recordings in the ambulatory setting to test the effect of background noise and the acceptability of wearing the device during routine activities. Cough were only counted in 4-hour sections of recordings from ten patients, but the very poor

agreement between the non-automated counts and those of the machine strongly suggests that a more extensive assessment is unlikely to have altered the conclusions. No other work of which I am aware has contradicted this.

The Leicester Cough Monitor (LCM)[80,253] and Vitalojak[263,264] are the two automated cough monitors about which there is the most published research (Section 1.6.2.2.3). Neither were commercially available at the time of writing. I was not able to obtain access to Vitalojak but did have access to the LCM through collaboration with its developers, and was the device used to collect data for much of the rest of this thesis.

5.5 Conclusion

Cough counting is consistent among and within doctors without specific training. Audio-editing software simplifies the process and leads to lower counts, which may be more accurate than counting by ear alone. The fully automated PulmoTrack® cough monitor agreed poorly with non-automated counting. The optimum method for objectively quantifying cough is yet to be defined but any technique should be clearly described and non-automated methods remain the reference standard.

6 Measurement of cough reflex sensitivity

6.1 Introduction

Inhalation cough challenges have been used for some time in research.[358,359] Their role has been to attempt to quantify the sensitivity of the cough reflex, particularly when evaluating the efficacy of treatments for cough (Section 1.6.2.1).[244] Capsaicin is the most widely used agent,[241] and the currently recommended protocol produces results which are repeatable within individuals in the stable state and responsive to treaments.[5] However, as it has been used, the capsaicin inhalational challenge is poor at distinguishing healthy subjects from those with respiratory disease, and measurements do not correlate well with daily cough frequency (Section 1.6.2.1).[5,70]

The most widely used endpoints to the capsaicin challenge test have been C2 and C5, the concentrations of capsaicin required to produce two or five coughs, respectively.[241] These cut-offs have been defined arbitrarily and largely recommended by opinion only.[5] It is not clear which of them should be preferred.[241] The recommended duration of time following the inhalation of capsaicin during which coughs should be counted to quantify the evoked response also seems to have been set arbitrarily, at 15 s.[5,241] The aim is to presumably count only coughs resulting from the capsaicin itself rather than coughs which would have occurred anyway due to underlying pathology, and other durations such as 10 s or 30 s have previously been chosen.[244,360] Another debate has been whether C2 or C5 should be taken as the actual lowest administered concentrations producing ≥2 and ≥5 coughs, respectively, or whether values should be interpolated from administered concentrations with log concentration-response data.[5,361]

An alternative approach to the capsaicin challenge recently proposed by Hilton *et al.* has produced results which separated patients from healthy volunteers, and were more predictive of 24-hour cough frequency.[85] It has been suggested that instead of

measuring C2 or C5, 'E_{max}' should be documented, the maximum number of coughs produced in response to any concentration of capsaicin.[85,246] However, this newer protocol appears to be more time-consuming than that previously proposed, involving the administration of up to 12 concentrations of capsaicin 4 times for each patient. Participant tolerance also seems to be a limiting factor, potentially preventing inhalation of the dose that would evoke the highest number of coughs.[85]

The highest concentration tolerated by the majority of patients in Hilton *et al*.'s study was 62.5 µM (16 of 20 with chronic cough, as opposed to only 8 who chose to proceed beyond this dose).[85] Measuring the number of coughs produced by the same relatively high dose of capsaicin without first going through a dose escalation protocol might be quicker, easier, and more tolerable to study participants, and produce data that are as valuable or nearly as valuable as measuring E_{max}.

I set out to evaluate alternative endpoints to the capsaicin inhalational challenge test with the following hypotheses:

1. '$E_{62.5}$', the number of coughs evoked by the inhalation of a 62.5 µM dose of capsaicin solution, is a new useful endpoint;
2. C5 is better correlated with 24-hour cough frequency in chronic cough than C2;
3. interpolated log-transformed data are not superior to using actual administered concentrations for the values of C2 and C5;
4. counting coughs within 15 s of administering capsaicin is more predictive of 24-hour cough frequency than counts performed over 30 s.

6.2 Methods

6.2.1 Setting and participants

The study was carried out at Kings College Hospital, South London. Sequential patients were recruited from a tertiary referral cough clinic, having been referred from both general practice and secondary care. All participants reported cough as the main or only presenting feature for ≥ 2 months. The cause of the cough was either

unexplained, unknown at the time of the study due to ongoing clinic work-up, or presumed secondary to an underlying cause. Chronic cough was only classed as unexplained following the exclusion of diagnoses known to cause cough (normal chest x-ray and ear nose and throat assessment, including nasendoscopy, a negative response to bronchodilator reversibility testing, and failed therapeutic trials of inhaled or oral corticosteroids and high dose proton-pump inhibitor therapy for ≥2 months [Section 3.2.1]).[2]

Demographic and routine clinical data were documented, including spirometry values taken within the preceding six months. Age <16 y, inability to provide written consent, current smoking (within the previous two months), a pre-existing respiratory diagnosis, recent coryzal symptoms (within the last one month), haemoptysis, recent use of angiotensin-converting enzyme (ACE) inhibitor medication (within the previous two months), and current use of opiate medication were patient exclusion criteria.

6.2.2 Capsaicin cough challenge

Capsaicin inhalation cough challenge was carried out as described in international guidelines.[5]

6.2.2.1 *Preparation and dilution of capsaicin solution*

30.5 mg capsaicin powder (Sigma-Aldrich, Gillingham, UK) was dissolved in 1 ml 100% alcohol and 1 ml polyoxethylene sorbitan (Tween 80, Sigma-Aldrich, Gillingham, UK), and then mixed with 8 mL 0.9% saline solution to produce 10 ml of 0.01 M stock solution. This was kept in a refrigerator at 4 °C for up to one month before being used or discarded. On the day of performing cough challenges, the stock solution was serially diluted with saline to produce solutions of doubling concentrations 0.49-1000 µM.

6.2.2.2 *Administration of capsaicin solution*

Capsaicin solutions were administered with a single breath method using a Koko DigiDoser (nSpire Health Inc., Louisville, CO, USA) in combination with an inspiratory

flow regulator valve, limiting flow to 0.5 L/s regardless of inspiratory force.[5,362] Patients were asked to "allow yourself to cough if you need to, and as much as you need to". The concentration of capsaicin was increased until inhalation of solution produced five or more coughs or until the maximum concentration solution (1000 µM) had been administered. Each concentration of solution was administered only once. 0.9% saline was interspersed once or twice between the escalating concentrations of capsaicin solution. If the 62.5 µM solution had not yet been administered after evoking ≥5 coughs, this was then given. An audio recording was made of the procedure (Philips DVT 3000 digital recorder and Philips ME15 microphone).

6.2.3 24-hour cough frequency measurement

24-hour cough frequency was measured with the Leicester Cough Monitor as further described later (Section 7.2.2).[253] The audio recorder was set to record several minutes after completing the cough challenge and the device returned by mail. It has been shown previously that the effect of capsaicin disappears rapidly and does not affect cough frequency in the following 24 h.[245]

6.2.4 Analysis of data

The number of coughs occurring within 15 s of each dose delivery were later counted with the assistance of Audacity audio editing software.[356] For the 62.5 µM solution, all coughs occurring within 30 s were also counted, giving values for the 15s-$E_{62.5}$ and 30s-$E_{62.5}$, respectively. C2 and C5 were defined in each of two ways, as the first actual administered concentrations producing ≥2 or ≥5 coughs respectively ($C2_a$, $C5_a$), or as values interpolated from log concentration-response curves ($C2_i$, $C5_i$). Cough 'latency' following inhalation of the 62.5 µM dose was also measured, defined as the time between the brief 'click' of the dosimeter indicating completion of dose nebulization and the start of the first cough sound.

The outcome variable was 24-hour cough frequency (CF24). The strength of correlation of CF24 with each explanatory variable was tested in turn ($C2_a$, $C5_a$, $C2_i$, $C5_i$, 15s-$E_{62.5}$, 30s-$E_{62.5}$ and cough latency). Pearson's *r* was used following normalization of the non-parametrically distributed variables with log transformation. If log-transformation did not successfully normalize data then Spearman's rank correlation was used.

6.3 Results

6.3.1 Study participants

57 patients were recruited, with underlying diagnoses unexplained chronic cough (n = 38), cough-variant asthma (n = 6), gastro-oesophageal reflux disease (n = 2), upper airways disease (n = 2), COPD (n = 1) and sarcoidosis (n = 1). In seven patients workup was ongoing at the time of writing. Participant characteristics are shown in Table 6.1.

Table 6.1. Characteristics of study participants.

	All patients (n = 57)	Unexplained chronic cough (n = 38)	Other diagnoses (n = 19)	*P*
Female	42 (73.7)	30 (78.9)	12 (63.2)	0.202
Ethnicity				
white	45 (78.9)	32 (84.2)	13 (68.4)	
other	12 (21.1)	6 (15.8)	6 (31.6)	0.168
Age	60 (54-66)	60 (54-65)	61 (40-69)	0.863
BMI (kg/m²)	27.6 (23.5-31.4)	27.8 (24.0-30.6)	27.4 (22.8-35.2)	0.863
Duration of cough (months)	4.5 (3.0-12.1)	9.0 (3.3-14.5)	3.3 (1.1-10.0)	0.014
FEV_1 (% predicted)	92.6 (±18.3)	89.4 (±16.7)	99.4 (±20.2)	0.375
FEV_1/FVC ratio	77.0 (73.4-81.9)	76.2 (72.6-82.0)	78.3 (72.9-81.7)	0.673

Values are n (%), median (IQR) or mean (±SD)

6.3.2 Capsaicin cough challenge

24-hour cough frequency and measured values from capsaicin challenges are shown in Table 6.2. There were trends for differences between unexplained chronic cough and other underlying diagnoses for $C5_a$, $C5_i$, 15s-E62.5 and 30s-E62.5, and a significant difference in 24-hour cough frequency between groups.

Table 6.2. 24-hour cough frequency and measured values from capsaicin inhalation challenge.

	All patients (n = 57)	Unexplained chronic cough (n = 38)	Other diagnoses (n = 19)	*P*
$C2_a$ (μM)	7.8 (2.0-11.7)	7.8 (1.7-9.8)	7.8 (2.0-15.6)	0.956
$C2_i$ (μM)	4.6 (1.3-8.2)	4.5 (1.1-8.0)	5.5 (1.6-9.1)	0.629
$C5_a$ (μM)	7.8 (7.8-31.2)	7.8 (3.9-15.6)	15.6 (7.8-31.3)	0.140
$C5_i$ (μM)	7.8 (3.9-15.6)	6.2 (3.8-12.5)	13.5 (5.9-24.4)	0.085
15s-$E_{62.5}$	10 (7-12)	10 (8-13)	8 (6-12)	0.120
30s-$E_{62.5}$*	11 (7-14)	12 (8-22)	8 (6-12)	0.052
cough latency (ms)	690 (501-989)	695 (509-937)	663 (481-1,165)	0.968
24-hour cough frequency	436 (181-700)	487 (309-780)	207 (109-617)	0.031

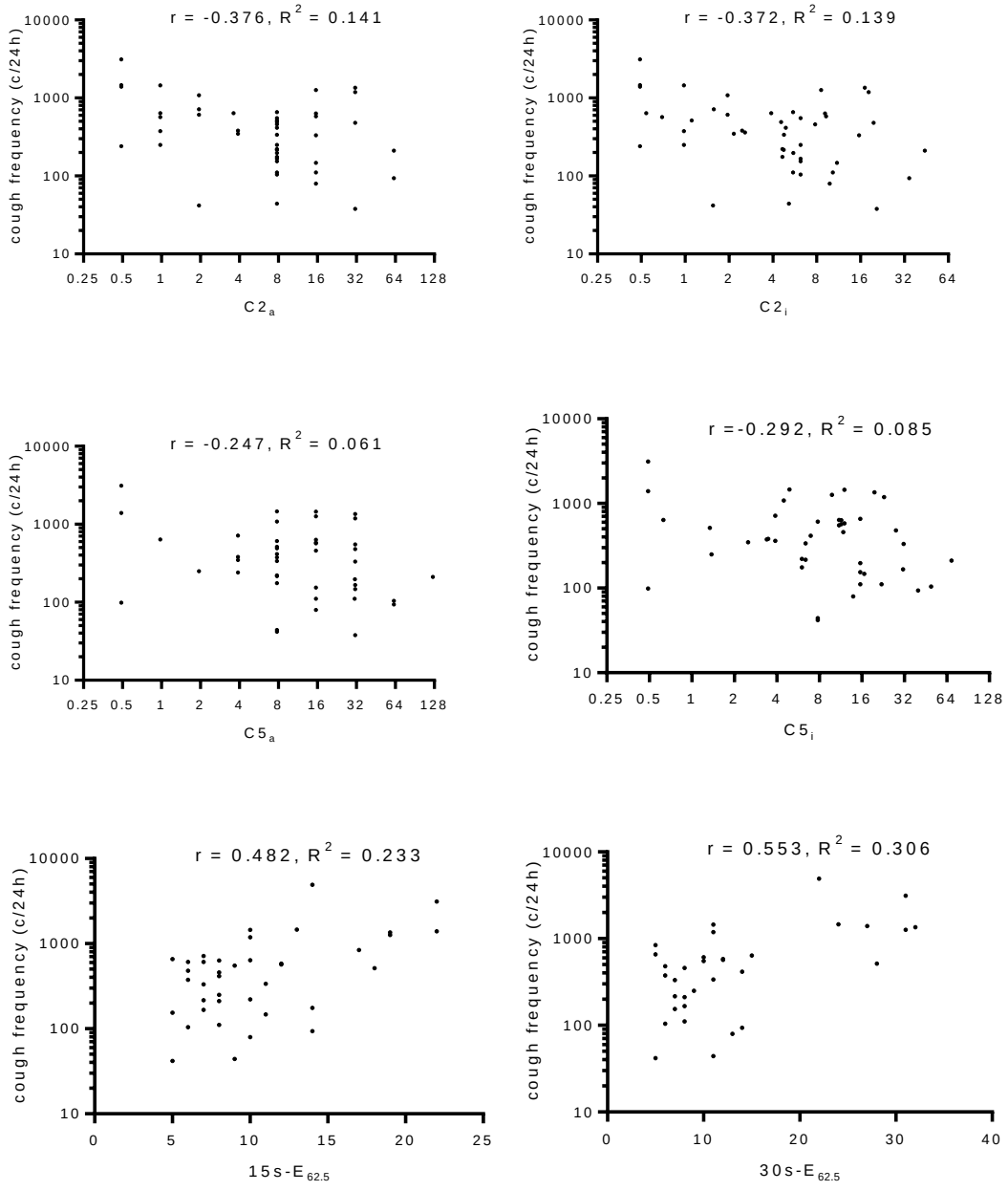
Values are median (IQR). * missing data.

Correlation coefficients and R^2 values for correlations between capsaicin challenge endpoints and 24-hour cough frequency are represented in Figure 6.1 and ranked in Table 6.3.

C2 appeared to correlate better with 24-hour cough frequency than C5. For C2, the actual administered dose, $C2_a$, seemed a better predictor of cough frequency than $C2_i$, the interpolated value; for $C5_i$ and $C5_a$ the converse was true.

Significantly more coughs were counted over 30 s than 15 s following inhalation of the 62.5 μM capsaicin solution (Wilcoxon matched-pairs signed rank test, $p < 0.0001$). 24-hour cough frequency seemed to correlate better with 30s-$E_{62.5}$ than 15s-$E_{62.5}$. However, there were wide confidence intervals for *r* values and also missing data for 30s-$E_{62.5}$ for 18 patients, due to a decision to make this measurement part way through the study. 23 of the 39 patients for whom there were data coughed 15-30 s after the administration of capsaicin; 16 coughed more than once (range 2-13 coughs).

Figure 6.1. Correlation between capsaicin cough challenge data and 24-hour cough frequency in chronic cough.



Subscripts $_a$ and $_i$ denote actual and interpolated values of C2 and C5, respectively. 15s - and 30s-$E_{62.5}$ – number of coughs elicited by 62.5 µM capsaicin in 15 and 30 s, respectively.

There was no evidence of correlation between 24-hour cough frequency and cough latency following the administration of 62.5 µM capsaicin solution, which was highly variable (range 0.32-5.63 s).

Table 6.3. Correlation between capsaicin cough challenge data and 24-hour cough frequency in chronic cough.

	Correlation coefficient (95% CI)	R^2	*P*
30s-$E_{62.5}$*	0.553 (0.259 to 0.753)[1]	0.306	0.0008
15s-$E_{62.5}$	0.482 (0.197 to 0.692)[1]	0.233	0.002
$C2_a$	-0.376 (-0.605 to -0.089)[1]	0.141	0.012
$C2_i$	-0.372 (-0.603 to -0.085)[1]	0.139	0.013
$C5_i$	-0.292 (-0.539 to 0.001)[1]	0.085	0.052
$C5_a$	-0.247 (-0.504 to 0.051)[1]	0.061	0.103
cough latency	-0.080 (-0.398 to 0.255)[2]	-	0.968

Values are median (IQR). [1] – Pearson's *r* (from log-transformed data); [2] – Spearman's ρ (from untransformed data). * missing data.

6.4 Discussion

This is one of very few studies to compare the two most widely-used endpoints in the capsaicin cough challenge, C2 and C5, with 24-hour cough frequency, and I am unaware of previous work to have compared the actual *vs*. interpolated values of these values with reference to 24-hour cough frequency. I have also tested two other parameters, $E_{62.5}$ and cough latency.

6.4.1 C2, C5 and cough frequency

In 62 patients with chronic cough of various causes Decalmer et al.[245] and reported a correlation coefficient of -0.45 between C5 and cough frequency. However, these data are difficult to compare to the current study as the authors used citric acid rather than capsaicin as the inhalation agent, quantified cough frequency for a 10-hour period rather than over 24 h for correlation with cough reflex sensitivity data, and reported cough frequency as time spent coughing rather than numbers of coughs. They did not report values of C2. In another study of seven patients with various causes of chronic cough, Birring et al.[84] reported correlations of daytime cough frequency over 6 h with capsaicin-related C2 and C5 of r = 0.8 and r = 0.9, respectively. Again, comparisons with the current study are limited, in this case due to the short duration over which cough frequency was determined and the very small number of patients. In the largest study comparing (citric acid) cough reflex sensitivity to cough frequency, of 100 patients with chronic unexplained cough, correlations of 24-hour cough frequency with C2 and C5 were r = -0.23 and r = -0.47, respectively.[75]

The current study is therefore the largest of which I am aware to compare C2 and C5 from the capsaicin cough challenge to 24-hour cough frequency in chronic cough. The wide confidence intervals in the reported *r* values give no evidence of a difference from correlations with cough frequency reported in other studies of a similar size in chronic cough using citric acid. The two agents, capsaicin and citric acid, are thought to act on the same pathway of the cough reflex,[244] and are both agonists of the receptor TRPV1.[27] There is therefore no difference to suggest *a priori* that cough challenge results from the two agents would have different relationships with 24-hour cough frequency. A larger study with more statistical power would be required to test this.

C2 appeared to have a stronger correlation with 24-hour cough frequency than C5, the opposite finding to that of Kelsall *et al*. However, the wide confidence intervals in the correlation coefficients again suggest this difference between C2 and C5 is not statistically significant. It is not clear which should be the preferred endpoint as there are conflicting data about which is the most reproducible endpoint over time.[2,242,243] However, there is some evidence in the current study that C5 might be superior to C2 in the fact that there was a trend for a difference in C5 but not C2 between patients with unexplained compared to other causes of chronic cough, coinciding with a significant difference in 24-hour cough frequency.

6.4.2 Interpolated values or administered concentrations?

Only one other study has compared the two methods of calculating data for C2 or C5, using actual administered concentrations or interpolating values from the log dose-response relationship.[361] Patients with chronic cough (n = 15) and healthy volunteers (n = 15) were recruited. As both methods were equally repeatable and the values strongly correlated, the authors concluded both methods to be equally valid.

The current study does not give clear support for superiority of either of the two standard approaches, given that interpolated and actual values for C2 and C5 led to similar correlation coefficients with 24-hour cough frequency. However, using interpolated rather than actual values probably produces more statistical power by

effectively creating a continuous variable from ordered categories (Figure 6.1). There is some support for this effect in the observed fall in *p* values for the comparisons against cough frequency of $C5_a$ *vs.* $C5_i$ or $C2_a$ *vs.* $C2_i$ between diagnostic groups (Table 6.2), and in comparing correlation coefficients for actual with interpolated values in Table 6.3.

6.4.3 $E_{62.5}$: a useful new endpoint?

E62.5, the number of coughs evoked by a 62.5 µM solution of capsaicin is a novel endpoint, and appeared to be superior to C2 or C5a as a predictor of 24-hour cough frequency.

In a study which set out to characterize the capsaicin pharmacodynamic dose-response in the cough challenge, Hilton *et al*. measured E_{max}, the maximum number of coughs produced by any concentration of capsaicin.[85] Patients with chronic cough (n = 20) and healthy volunteers (n = 20) underwent a dose-escalation protocol that increased the administered concentration of solution until no further doses were tolerated. Although E_{max} appeared to be more predictive of 24-hour cough frequency (r = 0.71) than $E_{62.5}$ was in the current study (r = 0.48 or 0.55), a meaningful comparison is limited because confidence intervals for r were not reported in the previous study.

E_{max} is presumed to equate to the 'maximum capacity of a subject to cough when inhaling increasingly potent stimuli'.[85] However, unlike in animal studies, E_{max} can only be estimated in human subjects due to the necessity to terminate the dose escalation protocol once the limits of an individual's tolerance of the test have been reached. This tolerance relates to coughing and throat irritation as no other adverse reactions have been previously reported during capsaicin cough challenges.[363] Presumably, the number of evoked coughs does not always level off with a protocol of this kind, although the authors did not state the proportion of subjects in which a plateau effect was observed.[85]

The highest tolerated dose in the majority of patients with chronic cough in the study of Hilton *et al*. was 62.5 µM, hence my choice of this dose as an alternative to estimate a value similar to 'E_{max}'. I hypothesized that $E_{62.5}$ might have a similar potential to

predict 24-hour cough frequency in chronic cough as E_{max} (as measured by Hilton *et al.*) but would have the advantages of relative simplicity of measurement and reduced dependence on subject tolerance.

Other than whether or not the plateau cough response had been reached in the current work and the study of Hilton *et al.*, participant characteristics may have accounted for the apparent better correlation of 24-hour cough frequency with E_{max} than $E_{62.5}$. In particular, including healthy volunteers and subjects with mild to moderate asthma as well as patients with chronic cough in the previous study produced a larger variation in 24-hour cough frequency than in the current work.

$E_{62.5}$ appears to be a useful endpoint to the capsaicin challenge. As well as the strength of correlation with 24-hour cough frequency, it seemed to differentiate unexplained chronic cough from patients with other underlying diagnoses better than most of the other capsaicin challenge endpoints evaluated. Further investigation is needed, with studies including healthy volunteers, and comparison to other measures which might reflect the maximum cough response more accurately, for example 'E_{125}'.

6.4.4 15 s *vs.* 30 s

It is recommended that only coughs occurring with 15 s of dose delivery should be counted in inhalational cough challenges. This is because the response is 'immediate and brief'.[2] However, this duration of time appears to be arbitrary and chosen by extrapolation from earlier work in healthy volunteers[359,364] or mild asthma,[365] in which all evoked coughs occurred within this time. Conversely, after administration of the 62.5 µM capsaicin solution in the current study of patients with chronic cough, a number of patients coughed between 15 and 30 s, with coughing bouts in several cases being truncated at the 15 s mark. 30s-$E_{62.5}$ was significantly higher than 15s-$E_{62.5}$, and for comparisons of values between patient groups there was an associated trend for a difference that almost reached statistical significance for 30s-$E_{62.5}$ but not 15s-$E_{62.5}$. 30s-$E_{62.5}$ also appeared to correlate better with 24-hour cough frequency than 15s-$E_{62.5}$.

It therefore appears that limiting the counting of coughs to 15 s may result in the loss of informative data. As counting coughs over 30 s is technically no more demanding than over 15 s, with the application of digital sound recording and playback with audio editing software (Section 5.3.4), I would suggest that cough responses be routinely measured over 30 as well as 15 s when performing inhalation cough challenges.

A reason for keeping the counting period as short as possible appears to be to exclude coughs not directly attributable to the inhalation of capsaicin. In the current study, the median cough rate of 487 c/24h in unexplained chronic cough equates to approximately one cough per 3 min. Ignoring the effects of the capsaicin challenge, the chance of a cough occurring as a result of the background pathological process over any 30 s period follows a Poisson distribution but is approximately 0.17 as opposed to 0.08 for any 15 s period, a small and probably clinically insignificant difference. It is also possible that extending the period of cough counting from 15 to 30 s increases the chance that some of the coughs following the inhalation of capsaicin are 'voluntary' rather than 'involuntary', thus reducing the reproducibility of the test. However, there is no evidence for this as far as I am aware, and voluntary effects on coughing can clearly occur within only 15 s of the administration of capsaicin.[364]

6.4.5 Cough latency

Latency of coughing in inhalational challenge tests has been rarely measured. In a previous study of healthy volunteers at the highest administered concentration of capsaicin, mean values were 1.65 ± 0.18 s, and longer with voluntary suppression (2.16 ± 0.34 s),[364] although comparison of the actual values with the current study is of limited value due to the different techniques of capsaicin administration. Coughing is probably triggered when capsaicin solution interacts with cough receptors in the larynx.[365] Once reaching the larynx, capsaicin must interact with cough receptors to generate an action potential in afferent airway nerves, which in turn is conducted along the vagus to the nucleus tractus solitarius, before stimulating the efferent limb of the pathway to trigger the respiratory muscles responsible for coughing. The time

taken for nebulized capsaicin solution to reach the larynx is likely to vary little between patients as the flow regulator valve of the Koko dosimeter limits the speed at which it is inhaled. Similarly, it is doubtful if signal transduction at the larynx and nerve transmission along afferent and efferent components varies by a measureable amount between individuals. Tachyphylaxis tends not to occur with increasing concentrations of capsaicin.[85,358] Differences in cough latency between subjects is therefore probably due to central processing, which in the current study exceeded 5 s.

The role of conscious and voluntary or semi-voluntary influences on coughing following capsaicin inhalation, and also more generally, is of particular interest.[20] As shown by the study of Hutchings *et al.*,[364] even when healthy volunteers are asked to actively suppress coughing, the effect on the latency and number of evoked coughs is very variable. Latency did not correlate with the number of coughs in 24 h in chronic cough, nor was there a difference between unexplained chronic cough and other causes. Further work in this area is required, for example by observing the effect on cough latency of behavioural training in chronic cough.[65,66]

6.4.6 Cough measurements and diagnosis

In a study of 134 healthy subjects and 88 patients with various diseases, C2 and C5 from capsaicin inhalational challenge varied substantially in healthy volunteers and in general distinguished poorly between health and disease.[70] There were, however, significant differences between patients with cough variant asthma, gastro-oesophageal reflux-related cough and unexplained chronic cough and healthy volunteers. In the current work there were trends for differences in $C5_a$, $C5_i$, 15s-$E_{62.5}$ and 30s- $E_{62.5}$ between unexplained and other causes of chronic cough. For 15s-$E_{62.5}$ the difference almost approached statistical significance ($p = 0.052$); a type 2 error may have occurred due to insufficient statistical power due to missing data.

$E_{62.5}$, like E_{max}, might therefore reflect the underlying physiological and pathological processes in cough more accurately than C5 and C2.[85] Comparison of $E_{62.5}$ between healthy volunteers and those with respiratory disease would be of interest.

6.4.7 Limitations

This was a pilot study to explore potential differences between capsaicin challenge endpoints and numbers of patients were relatively small. Also, for 30s-$E_{62.5}$, there were missing data.

The study did not include healthy volunteers. Doing so may have allowed the potential discriminatory ability of the capsaicin challenge endpoints to be evaluated with more certainty. However, the main reference point for comparison with capsaicin endpoints in this study was 24-hour frequency, rather than the ability to discriminate between diagnostic categories. Cough frequency is more variable in disease than in health (Section 7.4.2),[89] and it has been shown previously that although E_{max} correlates with 24-hour cough frequency in chronic cough,[85] this is not the case in healthy volunteers.[246] Clearly the physiological processes governing cough in health are different from pathological mechanisms in disease.

The data presented here suggest $E_{62.5}$ might be a useful measurement in capsaicin cough challenges. Clearly though, this is a novel endpoint and more data are required for a fuller validation. In particular, repeatability of the test needs to be established, both in healthy volunteers and in disease. However, the same could be said for other endpoints used in capsaicin challenges, not only for measures such as E_{max},[85] but also established endpoints such as C2 and C5, which have only been explicitly tested for repeatability in healthy volunteers.[242]

6.5 Conclusion

C2 and C5 are not necessarily the most relevant endpoints in cough inhalational challenges for investigating mechanisms of cough. Measuring the number of coughs evoked by a single relatively high concentration of capsaicin solution adds to the utility of this research tool. Further work is required in this area, also to establish the optimum duration over which coughs should be counted in inhalational challenges, and to investigate variation in cough latency.

7 Temporal patterns of coughing and respiratory disease

7.1 Introduction and objectives

The usually physiological and episodic phenomenon of coughing is increased in frequency in respiratory disease. Because of the only relatively recent development of portable cough monitors, temporal patterns of coughing in respiratory disease are only beginning to be investigated (Section 1.6.2.2). Very few studies have directly compared 24-hour coughing patterns in more than one type of disease.[89,91] There is no published work reporting 24-hour cough patterns in tuberculosis. Some of the varied underlying pathologies in other lung diseases are shared with TB (consolidation, granuloma formation, fibrosis and mucus hypersecretion) whilst others are not (bronchospasm and emphysema; Section 3.1). Further investigation in this area should reveal more about the mechanisms of cough, and whether this aspect of cough in TB is distinct or is similar to that in other disease.

In this Chapter the following hypotheses are addressed:

1. temporal cough patterns in respiratory disease are associated with general disease-independent factors related to participant (host) characteristics, such as sex, age and smoking;
2. temporal cough patterns in respiratory disease are associated with specific disease-related factors, such as diagnosis itself, markers of disease severity and, in tuberculosis and other infections, microbial factors;
3. cough in tuberculosis is less noticeable to the subject than in other conditions.

7.2 Methods

7.2.1 Patients

Patients were recruited with a range of respiratory diagnoses and routine clinical data were documented as described in (Section 3.2). In isolated chronic cough, where

patients were recruited to the study by the author whilst running a clinic, a sample of those completing cough symptom questionnaires also underwent 24-hour cough monitoring, selected by convenience.

7.2.2 Leicester Cough Monitor

As previously outlined (Section 1.6.2.2.3.3), the recording component of the Leicester Cough Monitor (LCM) consisted of a commercially-available small battery-powered MP3 digital audio recorder (in this study Philips DVT 3000, DVT 5000 or LFH 0865) and a lapel free-field microphone (Olympus ME-15 or Philips LFH 9173). The equipment was worn as shown in Figure 7.1 and set to record sound continuously with a sampling frequency of 22 kHz (DVT 3000, DVT 5000) or 16 kHz (LFH 0865) and an encoding rate of 64 kbit/s (DVT 3000, DVT 5000) or 48 kbit/s (LFH 0865). The devices were powered by two AAA batteries, Duracell Rechargeable, which supplied >24 h of continuous recording, or Varta Industrial, which allowed for recordings lasting >48 h.

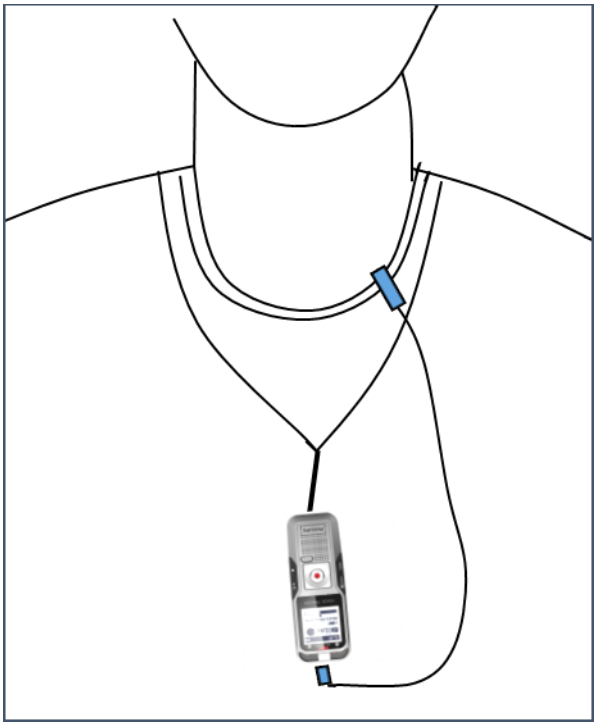


Figure 7.1. Leicester Cough Monitor recording equipment.

The resulting digital audio files were transferred to a computer for analysis with the Leicester Cough Monitor software which, through an algorithm similar to that used in automated speech recognition, identified cough sounds.[265] After initial automated analysis the software played back a selection of approximately 60-100 possible cough sounds for independent calibration by the operator (which in all cases was the author), before further refinement by the software. An automated report then displayed total cough frequency for each included 24-period, mean cough rate per hour, as well as similar data for cough bouts (defined as episodes of coughing in which the interval between individual cough sounds is <2 s).[251] The timings of each detected cough sound cough could also be easily extracted. The software calculated nocturnal and daytime cough counts, from 22:00 to 08:00 h and 08:00 and 22:00 h, respectively. Data were derived on cough clustering as proportions of all coughs distributed in bouts rather than 'lone' coughs (those separated from other cough sounds by ≥2 s), and numbers of coughs per 'episode' (i.e., a period of coughing consisting of either a either a lone cough or a bout of cough sounds separated by <2 s).

The Leicester Cough Monitor software was kindly supplied by the developers, Dr. Surinder Birring (King's College, London) and Dr. Sergio Matos (University of Aviero, Portugal). The developers and an independent investigator have evaluated the cough detection algorithm in isolated chronic cough and other diseases and reported it to be valid on comparison with auditory analysis as previously discussed (Section 1.6.2.2.3.3).[89,253,266] The cough monitor had not previously been used in tuberculosis.

Although further formal validation of the Leicester Cough Monitor was not undertaken, automated cough monitoring was evaluated in two ways. First, for recordings which appeared to contain long periods during waking hours with substantially fewer reported coughs than at other times of the day, the quality of recording was checked. Using Audacity® audio editing software (Section 5.2.3), visual inspection of the audio trace verified that sounds had been recorded throughout the entire 24-hour period, with confirmation in selected sections by ear.

The LCM had the potential to detect background coughs and falsely attribute them to the subject undergoing cough monitoring, a particular problem on an open respiratory ward (Section 1.6.2.2.3.3). This potential was minimized during operator calibration of the cough detection algorithm by choosing to ignore coughs which either sounded distant to the microphone (due to their relative quiet volume and distinct quality compared to the majority of other coughs in a recording), or were 'sex incongruent', i.e. had a voiced quality of someone of the opposite sex to subject being studied. The cough monitoring process was also evaluated in this regard by making background recordings. To observe the potential impact of unwittingly detecting coughs of other people, the cough monitor was left running for 24 h at various locations on the respiratory ward and the acute admissions ward at Homerton University Hospital to measure background 24-hour cough frequency.

7.2.3 Cough monitoring procedure

On patient recruitment, or on repeat measurement, patients were asked to wear the cough monitoring equipment for a period of at least 24 h. In individuals with acute respiratory disease, or early in the treatment of pulmonary tuberculosis, some recordings were made for 48 h. The LCM recorded sounds constantly throughout the period in which it was worn, during which patients were requested to continue normal activities (depending on the effects of the underlying disease) and to wear the monitor for the entire time, other than if showering or bathing. Study participants were either hospital inpatients or outpatients. If not hospitalized or not due to reattend hospital shortly after the monitoring period, subjects were asked to return the recording equipment by post at the end of the recording period in a pre-paid envelope.

Patients were informed that the recording device would pick up normal conversation and other sounds as well as coughs but that, due to the nature of the semi-automated process of analysis, it would be only rarely necessary to listen to sections of recordings not containing coughs.

7.3 Analysis of data

Temporal cough data (24-hour cough frequency and cough clustering) were compared across diagnostic groups with Kruskal-Wallis and one-way ANOVA tests for non-parametric and parametric distributions, respectively. Both LTBI and pulmonary TB were used as individual comparator groups. Each group was also separately compared with every other, allowing for multiple comparisons with Dunn's (non-parametric), or Dunnett's (parametric) tests.

For individual disease groups in which there were at least 15 participants, possible associations were explored between categorical disease-related variables and 24-hour cough frequency, initially with two-sample Wilcoxon rank-sum (Mann-Whitney) tests or, if >2 categories, Kruskal-Wallis tests. A Bonferroni correction was made for multiple two-sample comparisons if $p \leq 0.05$. For single variables which had a statistical association with cough frequency of $p \leq 0.15$, multivariate linear regression analysis was performed after log-transforming non-normally distributed data (Section 2.5). Cough frequency data were compared with continuous variables with Spearman rank correlation tests.

7.4 Results

7.4.1 Patients

The study participants who underwent 24-hour cough monitoring are described in Chapter 3 (Section 3.5). For all disease groups other than chronic cough, the patients are the same. The characteristics of the 59 patients with isolated chronic cough who underwent cough monitoring are shown in Table 7.1.

Compared to the whole group of patients with isolated chronic cough, the subgroup undergoing cough monitoring were a representative sample except they were more likely to have unexplained chronic cough or no diagnosis due to ongoing follow-up in the clinic. There were also non-significant trends for a diagnosis of asthma and a longer duration of symptoms prior to the first clinic visit in the monitored sample.

Table 7.1. Characteristics of patients with isolated chronic cough who underwent 24-hour cough monitoring

Characteristic	All patients with isolated chronic cough (n = 143)	Subgroup undergoing cough monitoring (n = 59)	*p*
Female sex	98 (68.5)	38 (64.4)	0.622
White ethnicity	54 (37.7)	25 (42.4)	0.635
West European-born	64 (44.8)	28 (47.5)	0.758
Age (y)	51 (39-65)	47 (38-61)	0.290
Current smokers	5 (3.5)	0	0.324
ACEi	13 (9.1)	2 (3.4)	0.239
Opiate use	4 (2.8)	3 (4.5)	0.419
Duration of cough (months)	6 (3-12)	8 (5-27)	0.093
Diagnosis			
asthma	44 (30.8)	10 (16.9)	0.054
gastro-oesophageal reflux	23 (16.1)	10 (16.9)	1.000
ACE inhibitor-associated	12 (8.4)	2 (3.4)	0.360
upper airway pathology	10 (7.0)	2 (3.4)	0.515
smoking	4 (2.8)	1 (1.7)	1.000
voluntary coughing / throat clearing	1 (0.7)	1 (1.7)	0.500
sarcoidosis	1 (0.7)	1 (1.7)	0.500
unexplained chronic cough	23 (16.1)	20 (33.9)	0.004
no diagnosis	18 (12.6)	15 (27.1)	0.035

Values are n (%) or median (IQR).
Note 3 patients (5.1%) had 2 diagnoses.

7.4.2 Baseline 24-hour cough patterns in respiratory diseases

Data on cough frequency, diurnal variation and cough clustering across disease groups are shown in Table 7.2.

Table 7.2. Cough frequency, diurnal variation and cough clustering in respiratory diseases.

Group	n	cough frequency			Diurnal variation*	Cough clustering†		
		Total (c/24h)	Day (c/h)	Night (c/h)		Cough episodes/ 24 h	coughs/ episode	% coughs in bouts
Chronic cough	59	422 (244-795)	23.1 (13.9-46.1)	9.3 (3.3-16.8)	22.6 (±11.8)	221 (154-331)	2.0 (1.6-2.5)	69.9 (±17.9)
Stable COPD	18	232 (112-385)	9.5 (5.3-20.4)	6.4 (2.6-12.5)	28.0 (±14.8)	154 (93-273)	1.5 (1.4-2.0)	55.4 (±20.4)
Stable asthma	5	70 (26-384)	4.7 (1.8-11.9)	0.6 (0.4-3.7)	14.1 (±10.8)	51 (19-105)	1.9 (1.3-2.5)	61.8 (±28.7)
Bronchiectasis	9	398 (194-1060)	24.4 (12.5-49.9)	7.0 (4.9-29.8)	23.9 (±11.9)	284 (157-388)	2.1 (1.6-2.3)	70.3 (±14.7)
Fibrosis	15	258 (182-458)	14.4 (12.3-24.1)	4.9 (3.4-9.4)	20.3 (±10.7)	164 (93-198)	1.9 (1.3-2.4)	64.9 (±22.4)
Lung cancer	9	341 (217-552)	14.2 (9.7-22.0)	9.9 (6.9-12.2)	32.3 (±11.9)	287 (175-304)	1.3 (1.2-1.5)	42.2 (±21.8)
AECOPD	25	573 (367-1028)	27.0 (17.2-46.3)	19.6 (13.2-27.8)	33.3 (±11.3)	290 (199-461)	1.8 (1.5-2.4)	64.8 (±18.1)
Acute asthma	18	389 (234-695)	16.4 (10.7-28.3)	11.5 (3.8-23.0)	28.8 (±16.6)	207 (131-322)	1.8 (1.5-2.2)	66.1 (±18.3)
Pneumonia	17	715 (411-967)	35.4 (21.0-45.6)	27.4 (16.0-34.2)	37.5 (±11.4)	332 (230-448)	2.0 (1.6-2.6)	71.9 (±19.6)
TB	44	203 (68-475)	9.7 (4.1-19.0)	3.8 (0.7-13.4)	23.1 (±17.9)	128 (44-253)	1.6 (1.4-1.9)	57.5 (±22.9)
LTBI	17	14 (8-53)	0.85 (0.5-2.9)	0.30 (0.1-0.7)	18.1 (±15.5)	14 (8-48)	1.2 (1.0-1.3)	25.8 (±21.8)
ALL	236	357 (128-664)	18 (8-33)	9 (3-20)	25.5 (±14.8)	200 (92-312)	1.7 (1.4-2.4)	62.8 (45.2-81.4)

Values are median (IQR) or mean (± SD).
*% of coughs from 24 h period occurring between 22.00 and 08.00
† bout: 2 or more coughs separated by < 2 s; cough episode: coughing activity consisting of either a lone cough or a cough bout, separated by ≥ 2 s from other coughs.

7.4.2.1 *Cough frequency*

24-hour cough frequency across respiratory diseases was highly variable (Table 7.2; Figure 7.2). Median cough rates differed between disease groups overall ($p < 0.0001$) and cough frequency in TB was significantly lower than in chronic cough ($p < 0.05$), COPD exacerbations ($p < 0.001$) and pneumonia ($p < 0.01$). Cough frequency in the chronic diseases COPD, asthma, bronchiectasis, pulmonary fibrosis and lung cancer together was significantly lower than in the combined group of COPD exacerbations, acute asthma and pneumonia (median 305 [IQR 128-450] *vs* 559 [IQR 340-949] c/24h,

respectively, $p < 0.0001$). The highest cough frequency, 2,347 c/24h, was measured in a patient with a COPD exacerbation.

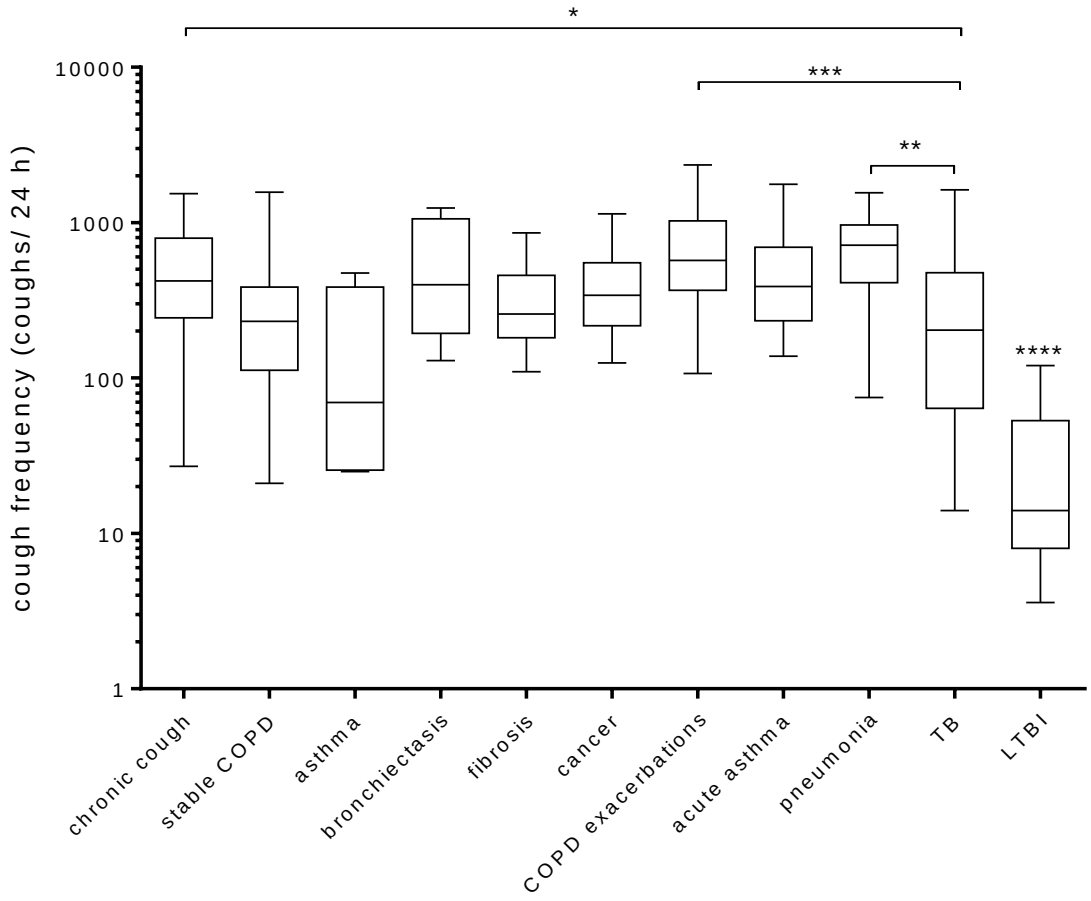


Figure 7.2. 24-hour cough frequency in respiratory diseases.

There was very little coughing detected in participants with latent TB, which was significantly less frequent than in every disease group. However, the individual with LTBI who reported a cough of onset several days prior to study recruitment (Section 3.2.11) coughed 120 times in 24 h.

Common to almost all the patient groups was a pronounced skewed distribution of cough frequencies, where small numbers of patients had relatively very high cough rates.

7.4.2.1.1 Non-disease related factors and cough frequency

Female sex was associated with cough frequency across disease groups (i.e. excluding LTBI); cough rates were almost 1.5 times higher in women than men. Cough frequency was higher in participants of white compared to other ethnicity, although this was not statistically significant following a Bonferroni correction for multiple comparisons (Table 7.3). There was overall no evidence for an effect of smoking, ACE inhibitor use, opiates or age (Figure 7.3).

Table 7.3. Cough frequency and non-disease-related variables across groups.

	n	24-hour cough frequency	*p*	*p'*
Sex				
male	104	312 (126-614)		
female	105	457 (248-795)	0.003	0.015
Ethnicity				
non-white	113	337 (129-580)		
white	96	413 (248-795)	0.014	0.070
Current smoking				
no	146	369 (189-691)		
yes	63	410 (162-731)	0.661	
Current ACE inhibitor use				
no	181	368 (182-697)		
yes	28	410 (251-718)	0.520	
Current opiate use				
no	187	368 (179-703)		
yes	20	491 (290-929)	0.081	

Values are median (IQR). *p*' – Bonferroni-corrected value.

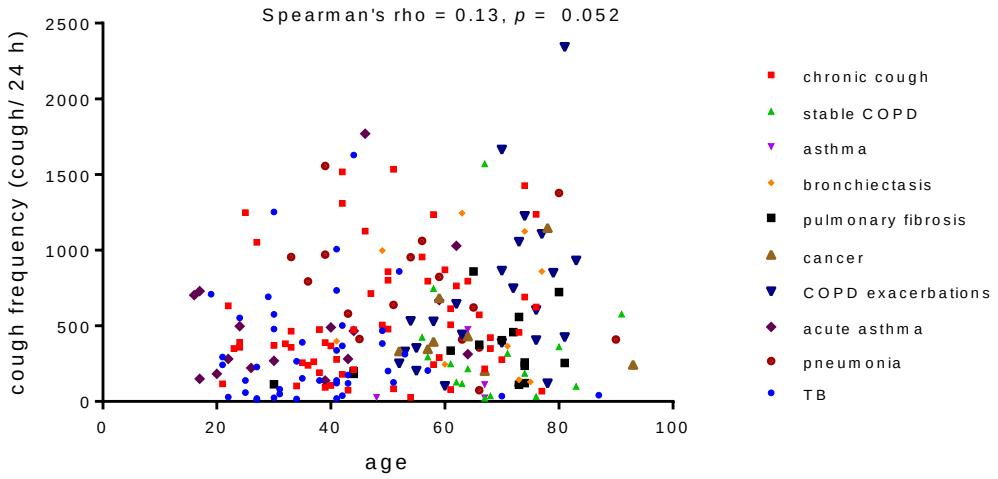


Figure 7.3. Age and cough frequency in respiratory diseases.

7.4.2.1.2 Isolated chronic cough

No association was observed between final diagnosis, sex or ethnicity and 24-hour cough frequency in isolated chronic cough (Table 7.4). Statistical analysis of the effects of ACE inhibitor medications was not performed due to very small sample sizes, but the two participants currently taking these drugs had relatively very high cough rates (Table 7.4). There were only three patients currently taking codeine but no evidence for an effect on cough frequency. There was no association between age or symptom duration and 24-hour cough frequency in chronic cough (Spearman's rho, ρ = 0.160, *p* = 0.226; ρ = 0.158, *p* = 0.397, respectively).

Table 7.4. Cough frequency and other clinical variables in chronic cough.

Variable	n	cough frequency (c/24h)	*p*
Sex			
male	21	357 (191-624)	
female	38	440 (277-763)	0.410
Ethnicity			
non-white	34	375 (191-632)	
white	25	457 (350-795)	0.315
ACE inhibitor use			
no	57	389 (239-690)	
yes	2	919 (713-1125)	-
Codeine use			
no	56	230 (390-738)	
yes	3	239 (690-1237)	0.427
Final diagnosis			
none	16	357 (256-478)	
asthma alone	8	376 (215-475)	
GORD alone	7	448 (244-710)	
other*	9	364 (244-713)	
unexplained chronic cough	20	652 (216-1165)	0.789

Cough frequency values are median (IQR). *p* – values from Wilcoxon signed rank tests, other than from Kruskal-Wallis test for final diagnosis.
* ACE-inhibitor use (n=2), upper airway pathology alone (n=1), behavioural/throat clearing (n=1), smoking (n=1), sarcoidosis (n=1), asthma and GORD (n=2), GORD and upper airways pathology (n=1).
n = 59.

In patients of the subgroup unexplained chronic cough, there was a significantly higher cough frequency in women (n = 13, median 795 [IQR 539-1241] c/24h) than men (n = 7, median 191 [IQR 94-614] c/24h, *p* = 0.036). There was no evidence for an effect of

age on cough frequency (ρ = 0.212, *p* = 0.369), but a trend for higher cough frequencies in white (779 [587-1450] c/24/h) than non-white (398 [161-882] c/24h) individuals (*p* = 0.063).

7.4.2.1.3 Stable COPD

In COPD there was a trend towards higher cough frequencies in current smokers than non-smokers (*p* = 0.10) but no observed association with sex, ethnicity, ACE-inhibitor use (Table 7.5), age (Spearman's rho, ρ = -0.202, *p* = 0.448), FEV_1 (ρ = 0.407, *p* = 0.168) or number of exacerbations per year (ρ = 0.426, *p* = 0.129). Only one patient was currently taking codeine and had a very high cough frequency of 1237 c/24h. Cough rates were relatively lower in the small number of patients taking antimuscarinic medication (n = 3); no effect of inhaled corticosteroids or long-acting β-agonists was observed.

Table 7.5. Cough frequency and other clinical variables in stable COPD.

Variable	n	cough frequency (c/24h)	*p*
Sex			
male	9	201 (108-327)	
female	9	283 (82-585)	0.529
Ethnicity			
non-white	3	116 (100-128)	
white	15	293 (186-423)	0.315
Current smoking			
no	12	157 (100-293)	
yes	6	370 (248-747)	0.104
ACE inhibitor use			
no	12	255 (36-361)	
yes	6	188 (116-423)	1.000
Long-acting β-agonist			
no	6	248 (216-361)	
yes	12	186 (36-423)	0.366
Long-acting antimuscarinic			
no	3	33 (21-100)	
yes	15	293 (186-423)	0.013
Inhaled corticosteroid			
no	4	289 (172-554)	
yes	14	217 (68-370)	0.396

Values are median (IQR). n =18.

7.4.2.1.4 Pulmonary fibrosis

No association of sex, ethnicity, current smoking, diagnosis or ACE inhibitor use with cough frequency in pulmonary fibrosis was observed (Table 7.6). Neither was there a correlation of age (Spearman's rho, ρ = 0.167, *p* = 0.549), forced vital capacity (ρ = 0.036, *p* = 0.899) or total lung capacity (ρ = 0.224, *p* = 0.533), with cough frequency. However, there was a trend for an inverse correlation of cough rates with total diffusing capacity of the lung for carbon monoxide (DLCO; ρ = -0.467, *p* = 0.108), and a significant inverse correlation between diffusing capacity of the lung for carbon monoxide per unit alveolar volume (KCO) and cough frequency (ρ = -0.666 [CI -0.888 to -0.193], *p* = 0.011; Figure 7.4).

Table 7.6. Cough frequency and other clinical variables in pulmonary fibrosis.

Variable	n	cough frequency (c/24h)	*p*
Sex			
male	9	336 (182-405)	
female	6	245 (200-458)	0.724
Ethnicity			
non-white	10	356 (200-559)	
white	5	254(114-258)	0.221
Current smoking			
no	13	336 (200-458)	
yes	2	184 (114-254)	-
Diagnosis			
IPF	9	375 (254-559)	
connective tissue disease	3	336 (235-458)	0.130
sarcoidosis	3	122 (114-200)	
ACE inhibitor use			
no	10	247 (182-458)	
yes	5	336 (254-375)	0.713

Values are median (IQR).

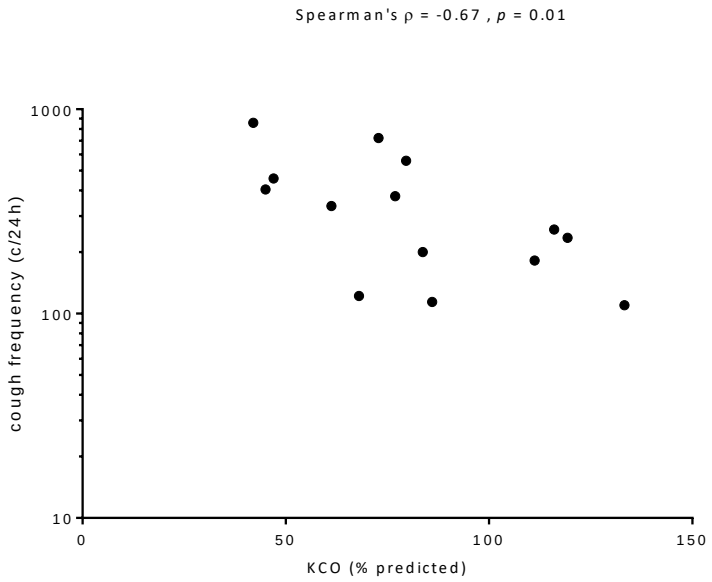


Figure 7.4. Cough frequency and carbon monoxide transfer factor in pulmonary fibrosis.

7.4.2.1.5 COPD exacerbations

None of the examined categorical variables were significantly associated with cough frequency in COPD exacerbations (Table 7.7). Neither was there an observed effect of annual exacerbation frequency (Spearman's rho, ρ = -0.229, *p* = 0.331), baseline FEV_1 (ρ = -0.179, *p* = 0.463) or CRP (ρ = 0.286, *p* = 0.175). There was a trend for higher cough frequencies in patients with a longer onset of symptoms prior to hospitalization (ρ = 0.398 [95% CI -0.019 to 0.967], *p* = 0.054; Figure 7.5) and a significant association with age (ρ = 0.497 [0.094 to 0.760], *p* = 0.016). There was a trend for an association between age and duration of pre-hospital symptom onset (ρ = 0.378, *p* = 0.068).

Table 7.7. Cough frequency and other clinical variables in COPD exacerbations

Variable	n	cough frequency (c/24h)	*p*
Sex			
male	12	573 (346-957)	
female	13	592 (411-1083)	0.729
Ethnicity			
non-white	6	346 (124-936)	
white	19	629 (430-1059)	0.125
Current smoking			
no	13	420 (232-896)	
yes	12	701 (491-1144)	0.149
ACE inhibitor use			
no	18	608 (392-936)	
yes	7	447 (358-1059)	0.727
Codeine use			
no	20	650 (401-998)	
yes	5	447 (358-534)	0.414
Day of hospital admission			
1	10	536 (392-752)	
2	10	650 (256-1059)	
3	5	855 (430-1669)	0.657

Values are median (IQR).
n = 25.

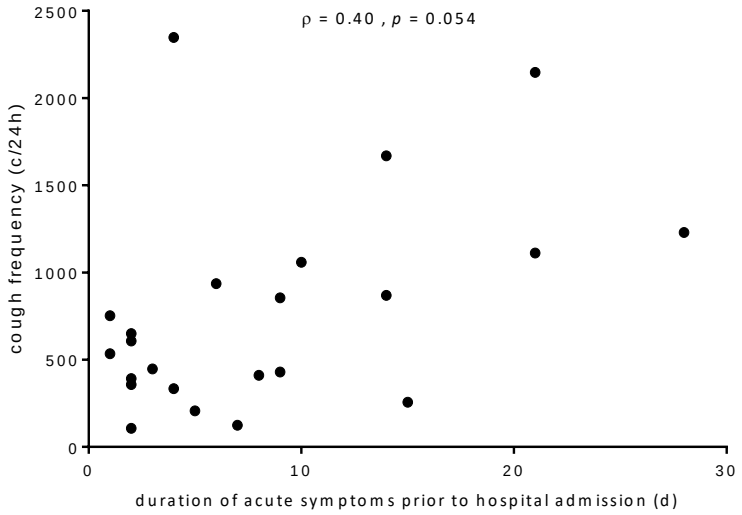


Figure 7.5. Cough frequency and duration of acute symptoms in COPD exacerbations.

7.4.2.1.6 Acute asthma

In acute asthma there was no evidence of an effect of admission peak expiratory flow reading (Spearman's rho, ρ = 0.160, *p* = 0.553), duration of acute symptoms prior to

hospital presentation (ρ = -0.124, *p* = 0.659) or annual number of exacerbations (ρ = 0.137, *p* = 0.642) on 24-hour cough frequency. Neither was there a significant association of cough rates with the categorical variables examined (Table 7.8), or with age (ρ = 0.274, *p* = 0.302), although there was a trend for higher cough rates in women than men (*p* = 0.070). Only one patient was taking ACE inhibitor mediation and had a high cough rate of 1029 c/24h.

Table 7.8. Cough frequency and other clinical variables in acute asthma.

Variable	n	cough frequency (c/24h)	*p*
Sex			
male	5	182 (150-281)	
female	13	490 (281-703)	0.070
Ethnicity			
non-white	13	281 (222-497)	
white	5	703 (312-1029)	0.156
Current smoking			
no	12	281 (222-497)	
yes	6	596 (466-729)	0.158
Day of hospital admission			
1	8	312 (222-497)	
2	8	466 (150-703)	
3	2	1025 (281-1769)	0.670

Values are median (IQR).
n = 18.

7.4.2.1.7 Community-acquired pneumonia

No relationship was detected between CRP (Spearman's rho, ρ = -0.357, *p* = 0.192), neutrophil count (ρ = -0.380, *p* = 0.169), or symptom duration (ρ = 0.118, *p* = 0.965) and cough frequency in pneumonia. Neither was there any observed association of cough frequency with age (ρ = -0.436, *p* = 0.091), sex, ethnicity, current smoking status, ACE inhibitor medication, time since hospital admission or other baseline markers of pneumonia severity (Table 7.9).

Table 7.9. Cough frequency and other clinical variables in pneumonia.

Variable	n	cough frequency (c/24h)	*p*
Sex			
male	9	629 (384-873)	
female	8	889 (497-1219)	0.172
Ethnicity			
non-white	5	1008 (768-1219)	
white	12	629 (410-888)	0.115
Current smoking			
no	7	793 (357-1377)	
yes	10	638 (413-955)	0.958
ACE inhibitor medication			
no	5	888 (517-1016)	
yes	12	495 (41-609)	0.115
Day of hospital admission of baseline cough monitoring			
1	3	953 (75-1377)	
2	9	629 (452-816)	0.774
3	5	955 (412-1016)	
Number of chest x-ray zones involved			
1	12	808 (410-967)	
2	5	629 (212-956)	0.625
Bilateral consolidation			
no	13	793 (413-955)	
yes	4	621 (75-1061)	0.638
CURB-65 score*			
0 or 1	8	793 (580-953)	
2 or 3	8	621 (357-1377)	0.749

Values are median (IQR).
* missing data for one patient.
n = 17.

7.4.2.1.8 Pulmonary tuberculosis

On univariate analysis there was some evidence for an association between sputum smear positivity and 24-hour cough frequency in pulmonary TB ($p = 0.056$), and a trend for higher cough frequency in males than females ($p = 0.132$).

There was, however, no evidence for an effect of the other documented clinical variables, including pulmonary cavitation, extent of radiographic disease involvement, and organism strain (Table 7.10). There were insufficient data to test for the effect of the Beijing strain alone, from only two patients, whose baseline cough frequencies were 242 and 391 c/24h respectively. The two patients who had been referred from

screening had substantially lower cough rates than patients who had been identified by other means. Both were sputum smear negative. Age and cough frequency were not correlated in TB (Spearman's rho, ρ = 0.042, *p* = 0.787).

Table 7.10. Cough frequency and other clinical variables in pulmonary TB.

Variable	n	cough frequency (c/24h)	*p* (univariate analysis)
Sex			
male	31	383 (152-502)	
female	13	157 (58-367)	0.132
Ethnicity			
non-white	34	152 (58-478)	
white	10	289 (201-468)	0.358
Current smoking			
no	20	364 (46-633)	
yes	24	174 (81-312)	0.210
Opiate medication use			
none	40	203 (64-496)	
methadone	4	253 (49-447)	0.795
Co-incident EPTB			
no	37	215 (70-485)	
yes	7	205 (49-478)	0.869
Sputum smear			
negative	16	139 (32-352)	
positive	28	266 (120-691)	0.056
Source of patient referral			
other	42	217 (110-484)	
screening	2	17 (14-19)	-
Sputum culture			
negative	7	139 (36-312)	
positive	37	235 (101-490)	0.250
Organism strain*			
other	18	190 (75-604)	
Beijing or Cameroon	7	242 (138-391)	0.776
Haarlem	7	294 (122-859)	
Chest x-ray involvement			
<2 zones	24	215 (129-496)	
≥2 zones	20	187 (39-447)	0.563
Pulmonary cavitation			
no	27	178 (42-478)	
yes	17	228 (120-468)	0.510

Values are median (IQR).
n = 44.

7.4.2.1.9 Latent tuberculosis

The 17 individuals with LTBI had a median cough frequency of 14 (8-53) coughs/ 24 h. The participant with the upper respiratory tract infection (URTI) coughed 120 times in 24 h; cough rates in the remainder were 13 (8-45) c/24h. Cough rates in the two

smokers were 7 and 56 c/24h, respectively, and in the non-smokers without URTI, 13 (IQR 8-41, geometric mean 16.5) c/24h.

7.4.2.2 *Diurnal variation*

Hourly cough frequency in all diagnostic groups was lower overnight than during the day. The pattern shown in Figure 7.6 for pulmonary TB was typical across diseases. On visual inspection there were no obvious peaks in coughing at particular times of day unique to certain diseases (Figure 7.7 compares the three acute respiratory diseases). However, a significantly larger proportion of total coughs occurred overnight in pneumonia than in TB, chronic cough or LTBI ($p < 0.05$), and in AECOPD than in TB or LTBI ($p < 0.05$; Table 7.2). The number of coughs between the hours of 22:00 and 08:00 was strongly correlated with 24-hour cough frequency across diseases (Figure 7.8).

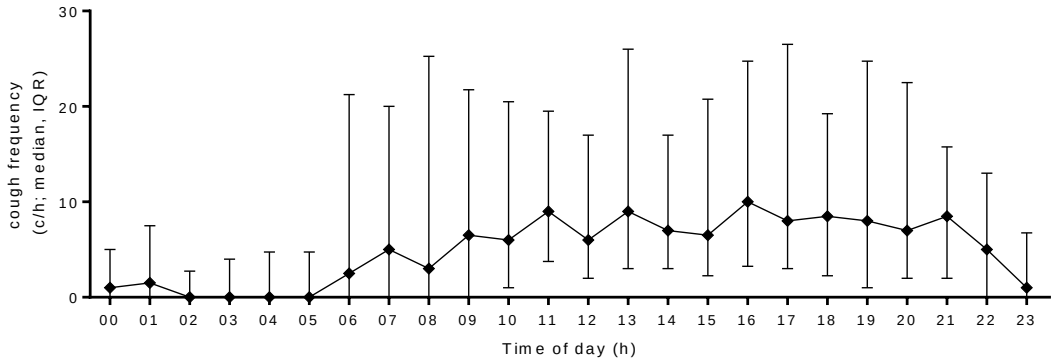
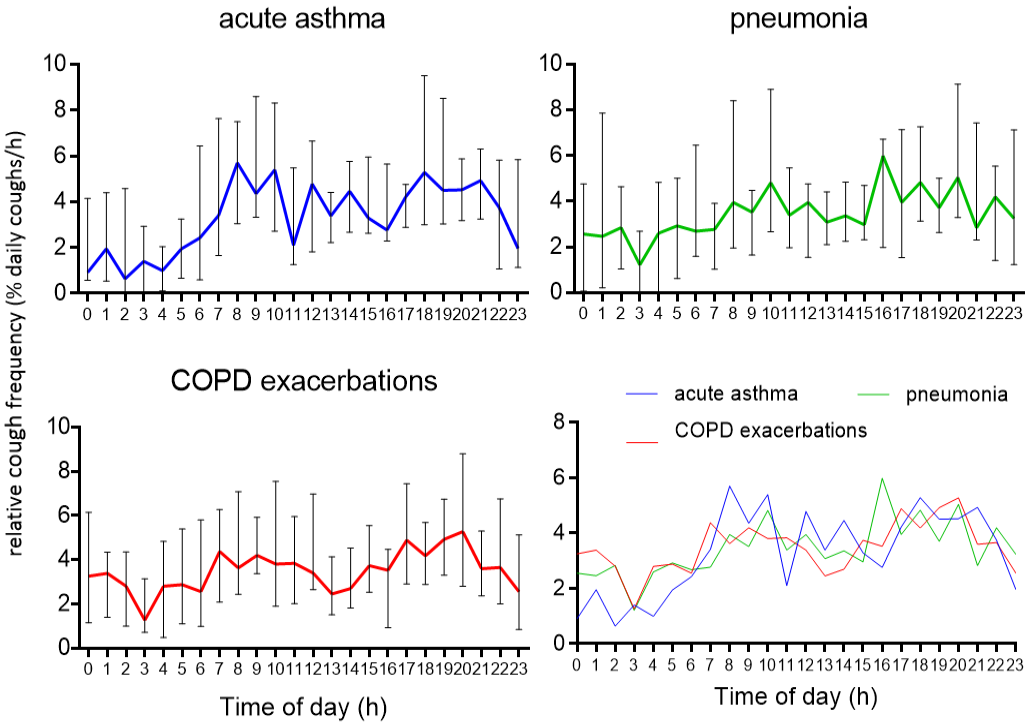


Figure 7.6. Diurnal variation in hourly cough frequency in pulmonary tuberculosis.



Error bars: median ± IQR

Figure 7.7. Diurnal variation in cough frequency in acute respiratory diseases: relative number of coughs/h.

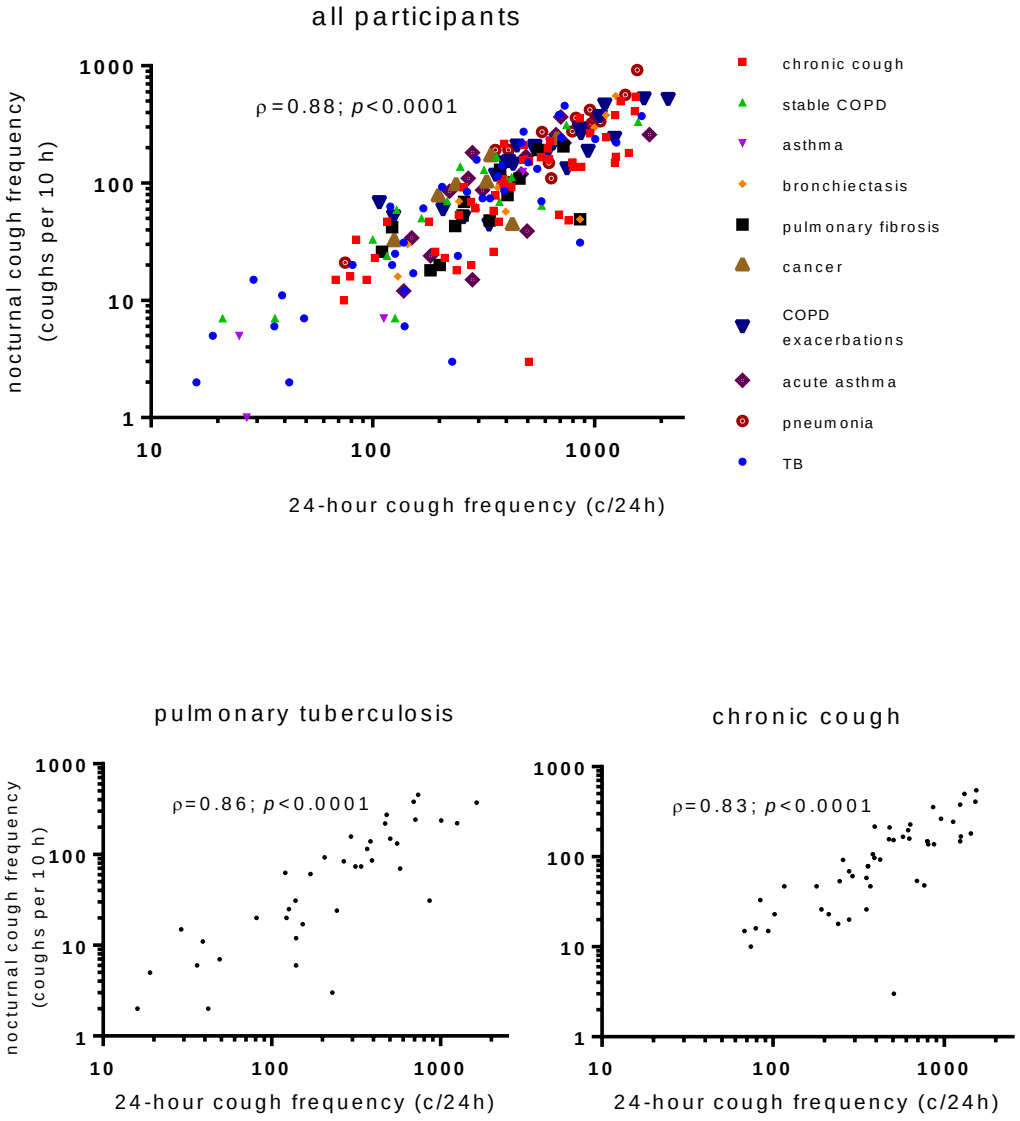


Figure 7.8. Correlation between nocturnal and 24-hour cough frequency in respiratory diseases.

7.4.2.3 *Cough clustering*

Significantly fewer coughs were clustered into bouts in TB (mean 57.5 [±22.9] %) than in chronic cough (69.9 [±17.9] %, $p < 0.05$; Table 7.2). Coughs in LTBI were significantly less likely to be clustered into bouts than in all other groups except lung cancer. There was no other difference in this measure of cough clustering upon comparing each of the other groups to TB. The proportion of coughs in bouts and 24-hour cough

frequency were correlated across all participants (Spearman's rho, ρ = 0.616 [0.520-0.697], $p < 0.0001$).

The median number of coughs per episode was variable within groups and not significantly different between diseases, although lower in LTBI than in all diseases except asthma ($p < 0.01$; Table 7.2**Error! Reference source not found.**).

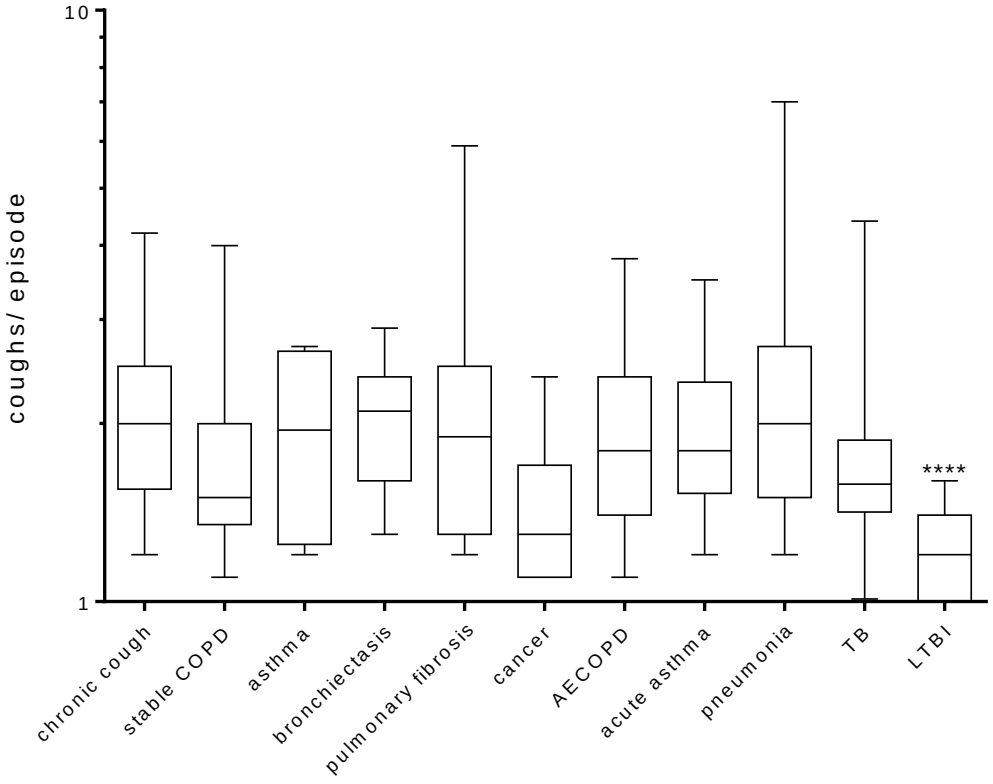


Figure 7.10. Cough clustering in respiratory diseases

7.4.2.4 *Power*

The log-transformed value for mean cough frequency in TB was 2.24 and standard deviation 1.91 coughs/24h. With 44 patients and two-sided statistical significance (α) 0.05, the power to detect a difference in cough frequency of magnitude 15% from another disease group of a similar size was 82%. Amongst patients with TB, to compare 24-hour cough frequency in any two equal-sized groups of 22 patients there was a 53% power to detect a difference of 15%, although a power of 80% to detect a difference of 21%.

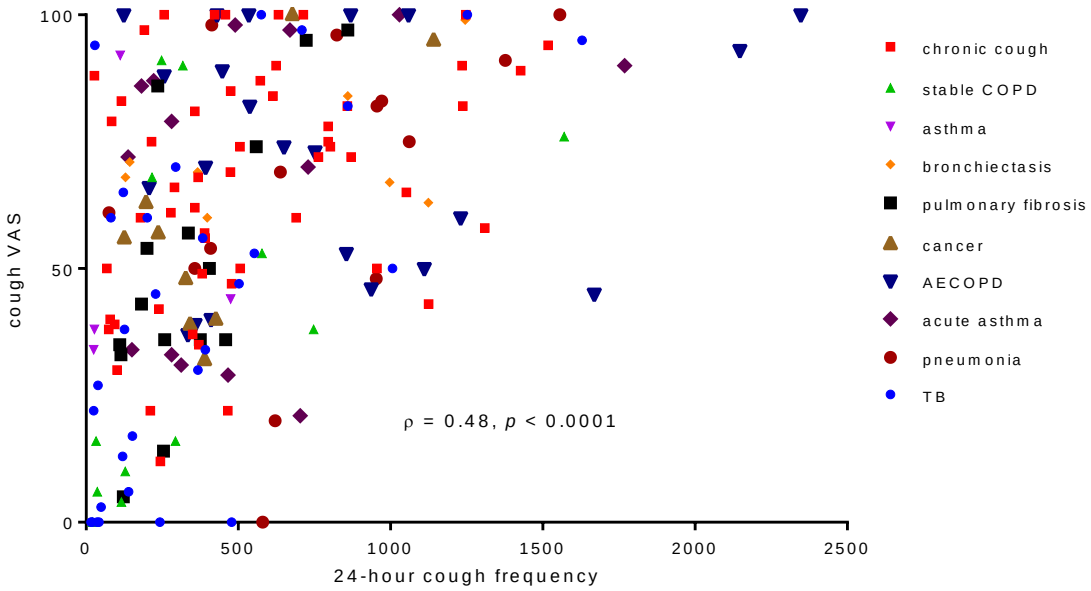


Figure 7.11. Cough frequency and subjective cough severity in respiratory diseases.

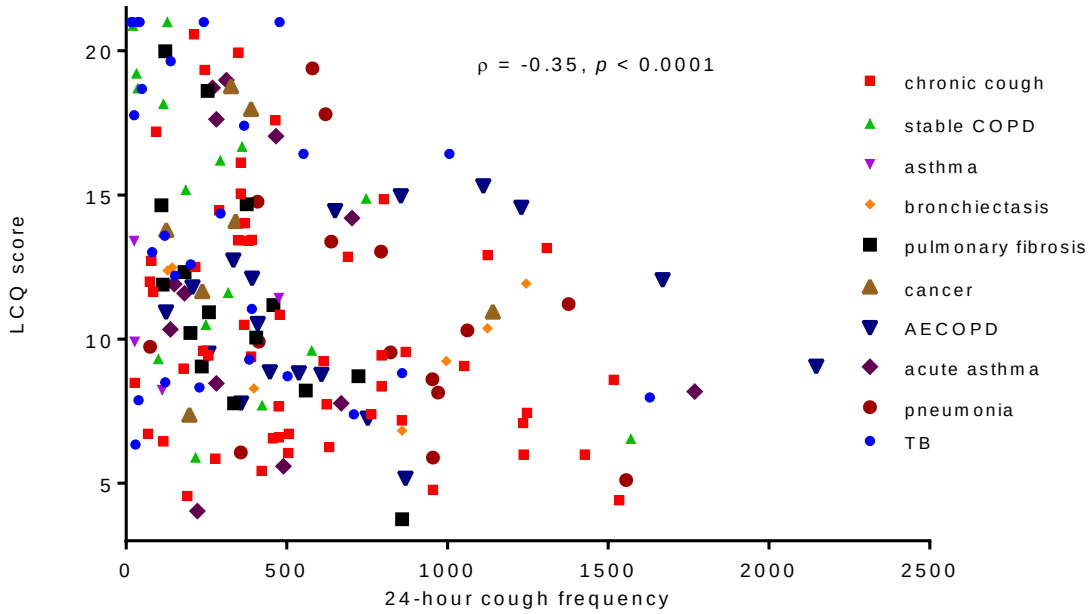


Figure 7.12. Cough frequency and cough-related quality of life in respiratory diseases.

7.4.3 Correlation between objectively-measured cough patterns and the subjective appreciation of cough

Overall there was moderate correlation for 24-hour cough frequency with cough severity visual analogue scale (VAS) scores (Spearman's rho, ρ = 0.48 [95% CI 0.35 to 0.59], p < 0.0001; Figure 7.11), and weak correlation with Leicester Cough Questionnaire (LCQ) scores (Spearman's ρ = -0.35 [-0.48 to -0.21], p < 0.0001; Figure 7.12).

Each of the domains of the LCQ was similarly correlated with cough frequency across diseases (Spearman's rank correlation coefficients -0.321 [-0.451 to -0.278, p < 0.0001], -0.318 [-0.448 to -0.174, p < 0.0001] and -0.345 [-0.473 to -0.204] for associations between CF24 and the physical, psychological and social domains, respectively).

Table 7.11 shows that within diagnostic groups the strength of correlation between objective and subjective measures of cough varied and was perhaps stronger in COPD and pulmonary fibrosis than in other diseases, although there were wide confidence intervals for correlation coefficients.

Table 7.11. Correlation between 24-hour cough frequency and patient-reported cough measures in respiratory diseases.

	VAS score		LCQ score	
	Spearman's ρ (95% CI)	*p*	Spearman's ρ (95% CI)	*p*
TB	0.588 (0.278 to 0.788)	0.0006	-0.337 (-0.643 to 0.061)	0.0853
Chronic cough	0.351 (0.083 to 0.571)	0.009	-0.315 (-0.545 to -0.0403)	0.0217
Stable COPD	0.701 (0.193 to 0.912)	0.014	-0.609 (-0.8539 to -0.146)	0.0141
Stable asthma	0.800*	0.333	-0.400*	0.750
Bronchiectasis	0.095*	0.840	-0.452*	0.268
Fibrosis	0.652 (0.194 to 0.877)	0.010	-0.643 (-0.873 to -0.179)	0.0116
Lung cancer	0.150*	0.708	0.214*	0.662
COPD exacerbations	0.100 (-0.347 to 0.511)	0.657	0.117 (-0.383 to 0.564)	0.645
Acute asthma	0.244 (-0.345 to 0.695)	0.397	-0.105 (-0.631 to 0.488)	0.727
Pneumonia	0.517 (-0.037 to 0.827)	0.062	-0.354 (-0.741 to 0.210)	0.196
ALL	0.479 (0.355 to 0.587)	<0.0001	-0.350 (-0.476 to -0.208)	<0.0001

* no. of values too small for calculation of 95% confidence interval.

The relative physical, psychological and social burden of cough symptoms in relation to cough frequency in TB was similar to that observed in the other chronic disease groups for which there were adequate numbers of participants to make a comparison (Table 7.12). The possible exception is in pulmonary fibrosis where LCQ physical domain scores were less well correlated than psychological and social domain scores with cough frequency, all though confidence intervals for Spearman's ρ were wide.

Table 7.12. Correlation between 24-hour cough frequency and domains of the Leicester Cough Questionnaire in TB and chronic respiratory diseases

	Physical		***Psychological***		***Social***	
	Spearman's ρ (95% CI)	*p*	Spearman's ρ (95% CI)	*p*	Spearman's ρ (95% CI)	*p*
TB	-0.230 (-0.569 to 0.176)	0.249	-0.371 (-0.665 to 0.023)	0.057	-0.399 (-0.683 to 0.011)	0.039
Chronic cough	-0.295 (-0.530 to -0.019)	0.032	-0.268 (-0.508 to -0.010)	0.052	-0.357 (-0.578 to -0.088)	0.009
Stable COPD	-0.629 (-0.862 to -0.178)	0.010	-0.636 (-0.865 to -0.190)	0.009	-0.653 (-0.872 to 0.217)	0.007
Fibrosis	-0.345 (-0.736 to 0.219)	0.204	-0.605 (-0.857 to -0.118)	0.018	-0.702 (-0.897 to -0.281)	0.004

Overall, and within diagnosis groups, subjective cough scores were also correlated with 24-hour cough *episodes*, to a similar degree as with the frequency of individual coughs (ρ = 0.44 [95% CI 0.31 to 0.56], $p < 0.0001$ for correlation of cough episodes/ 24 h across disease groups with cough severity VAS score; ρ = -0.29 [95% CI -0.43 to -0.13] for correlation with LCQ score, $p = 0.0003$).

7.4.4 The Leicester Cough Monitor and background coughing

All audio recordings from participants with COPD exacerbations, acute asthma and pneumonia were made during inpatient hospital admission, as were a number from patients with TB (n = 21), lung cancer (n = 7) and pulmonary fibrosis (n = 4). In TB all inpatient recordings were made in separate single patient side-rooms, whilst in acute asthma and COPD exacerbations, cough monitoring for the majority took place on open wards. In lung cancer and pulmonary fibrosis the numbers of patients in individual side-rooms was four and one, respectively.

In pneumonia, 7 of 18 patients were in side-rooms and the remainder located on open wards during cough monitoring. Measured baseline cough frequency did not significantly differ between the two groups: 687 (396-959) *vs.* 731 (412-1140) c/24h, respectively, $p = 0.535$.

Nine background 24-hour audio recordings were made, from six different locations on the respiratory and acute admissions wards at Homerton University Hospital. Median background cough frequencies detected by the Leicester Cough Monitor were 78 (35-86) c/24h (3.3 [2.5-3.3] c/h). This compares with the median measured cough frequency for all patients of 559 (340-949) c/24h. There was no obvious differences between background cough counts on the two wards ($p = 0.290$).

7.5 Discussion

In this Chapter the temporal patterns of cough have been explored in a wide range of respiratory diseases to an extent that has not previously been reported. I set out to investigate general and specific factors associated with objective measurements of coughing. General factors would be disease-independent, and specific factors would be related to pathology. In particular, the aim was to identify any host or micro-organism-related variables associated with coughing in tuberculosis, which due to its role in transmission may have unique temporal characteristics compared to other diseases.

Wide variation in 24-hour cough frequency in respiratory diseases was observed, and rates in TB were lower than in isolated chronic cough and acute respiratory diseases. Cough frequency across diseases was associated with female sex and, in TB, with sputum smear positivity (although $p = 0.056$). In pulmonary fibrosis cough frequency was associated with transfer factor for carbon monoxide, and in COPD exacerbation with duration of symptom onset, both novel findings. In all diseases cough frequency reduced overnight, but less so in patients hospitalized with pneumonia or COPD exacerbation than in some of the other conditions. Clustering of coughs occurred less

in TB than in chronic cough, and in healthy individuals (with LTBI) the majority of the little coughing that occurred was as lone coughs rather than as bouts, the converse of the findings in disease.

Across a range of respiratory diseases there was only a weak to moderate correlation between patient-reported cough symptom scores and objectively-measured cough frequency, and greater for the cough severity visual analogue scale than the Leicester Cough Questionnaire.

7.5.1 Temporal cough patterns and respiratory disease

There was wide variation in 24-hour cough frequency within all diagnostic categories and few differences between diseases. Cough frequencies were amongst the highest in isolated chronic cough, a condition defined solely on the basis of patient-reported excessive coughing,[2] and lowest in latent TB, a group specifically chosen as a negative control. Patients in other disease groups were not selected on the basis of cough symptoms yet in some cases had cough frequencies as high as in isolated chronic cough.

The pronounced skewed distribution of cough frequencies in almost all groups, which did not necessarily equate with markers of disease severity, suggests that small numbers of individuals are particularly prone to coughing in certain contexts for largely unknown, but presumably disease-independent (host-related) reasons. These individuals could perhaps be the super-spreaders or 'tuberculosis disseminators' in pulmonary TB.[186] This phenomenon is also exemplified by the concept of unexplained chronic cough, or "cough hypersensitivity syndrome", in which otherwise healthy individuals have very higher cough rates in response to seemingly innocuous stimuli.[62]

7.5.1.1 *'Normal' cough frequency and latent tuberculosis*

'Normal' cough frequency has not been well defined. Latent tuberculosis, by definition, does not cause cough or any other clinical manifestations, but differentiating true 'latent' infection from subclinical disease can be very difficult in practice.[295,366] It was

assumed that the observed cough frequency in the participants with LTBI would reflect cough frequency in health, but comparisons with the literature are limited due to the paucity of evidence of what constitutes 'normal' cough frequency.

Only three studies appear to have reported 24-hour cough frequency in healthy volunteers. Yousaf *et al*. reported a geometric mean cough frequency of 18.6 c/24h using the Leicester Cough Monitor in 44 healthy non-smokers,[89] and in another small study using the LCM, mean cough rates in the included nine healthy volunteers were 2 (SD ±1) per hour.[84] The Manchester cough group reported median 24-hour cough frequencies in 12 healthy non-smokers using their Vitalojak cough monitor: 16.8 (range 3-103) c/24h.[78] They also investigated coughing frequency in another study which included healthy volunteers but quantified coughs as time spent coughing rather than numbers of coughs,[76] complicating comparisons with the current study. The limited published data (from a total of 65 patients), therefore, reports very similar values for normal cough frequency to those observed in latent TB in the present study (16.5 c/24h for the 14 non-smokers without URTI).

The finding of normal cough frequencies in latent TB is valuable for two reasons. Firstly, it supports the notion that latent TB is a condition without clinical manifestations which can be recognized as such by experienced physicians. Secondly, it adds to the limited data available data on 'normal' 24-hour cough frequency. As stated by Robert Loudon in 1965, "the development of cough-counting devices measuring cough frequency in individual patients has made it important to estimate the frequency with which cough occurs in the general population".[6] This remains true 50 years on: further data on cough frequency in health are needed to advance cough research. Normal ranges need to be established in different population groups in different situations to be able to interpret cough frequency values in disease and measure the effects of treatment. For example, if cough frequency measurement were to be used to try and separate latent from active tuberculosis, no clear upper limit of normal currently exists.

7.5.1.2 *Demographic predictors of cough frequency*

7.5.1.2.1 Smoking

It is evident anecdotally, from patient surveys,[367,368] through direct observation of people smoking,[72] and ambulatory cough monitoring,[78,89] that smokers generally cough more frequently than non-smokers. This is probably due to the direct irritant effects of tobacco smoke, mucus hypersecretion and other mechanisms.[78] However, despite the current observations of trends for associations between cough frequency and smoking in stable COPD (p = 0.104), COPD exacerbations (p = 0.149) and acute asthma (p = 0.158), there was no observed association of smoking with cough frequency across all disease groups overall. As there were relatively few patients who were current smokers, it is possible that the study was underpowered in several cases to detect an effect of smoking. However, in pulmonary fibrosis, pneumonia and tuberculosis, cough frequencies were numerically lower in smokers than in non-smokers, with relatively large numbers in each group in TB (n = 20 *vs.* n = 24, respectively), which goes against an artefactual lack of effect of smoking in those conditions.

In otherwise healthy volunteers, cough frequencies seem to be about twice those in smokers compared to non-smokers, although the evidence for this is only from two small studies, and the numerical difference in daily coughs in small (geometric mean 33 *vs.* 19 c/24 h,[89] or median [range] 5.3 [0.20–13.9] *vs.* 0.7 [0.13–4.3] c/h,[78] respectively). 24-hour cough frequency has very rarely been investigated in smokers, and only once before in patients with respiratory disease as far as I am aware; in COPD median cough frequency in current smokers was 9.0 c/h compared to 4.9 c/h in ex-smokers.[78] Current smokers have generally been excluded from other studies involving 24-hour cough monitoring,[75–77,79,80] so there are no other data on 24-hour cough rates in smokers with diseases other than COPD apart from those in the current study. However, on 8-hour overnight cough recordings, Loudon and Brown in the 1960's observed no effect of usual smoking habit on cough frequency in pneumonia (n = 48) and pulmonary tuberculosis (n = 96).[88]

It could be the case that smoking is more likely to have an effect on cough frequency in diseases of the airways (COPD, asthma) than in diseases of the lung parenchyma (tuberculosis, fibrosis, pneumonia). This might be due to direct contact of cigarette smoke with the site of disease and amplification of the (largely unknown) mechanisms of cough in COPD and asthma, perhaps involving airway inflammation[78,95] and sputum production.[78] The reason that no effect of smoking on cough frequency was observed, either overall, or in tuberculosis, pneumonia and pulmonary fibrosis individually, could be that any effect of smoking was small (perhaps equivalent to only an extra 14 coughs per day compared to non-smokers, as in healthy volunteers[89]), and dwarfed by the much larger effect on cough frequency of the underlying disease itself.

Clearly, more data are needed to understand the role of smoking in the mechanism of cough in disease, but these, perhaps unexpected, findings have suggested that in disease of the lung parenchyma smoking may not always be an important determinant of cough frequency. This has implications in tuberculosis where, if not an important determinant of coughing, smoking might not affect infectiousness. Indeed Fennelly *et al*. have shown that when individuals with smear positive TB disease do cough there seems to be no effect of smoking status on the release of airborne bacilli.[199] Also in isolated chronic cough, smoking is usually cited as an aetiological factor,[9] yet there is no evidence as far as I am aware that it is usual for smoking on its own to cause cough frequencies in the pathological range. Just as gastroesophageal reflux probably does not cause significantly elevated cough rates in the majority, those that develop isolated chronic cough who are smokers may well have another reason for a significantly elevated cough frequency, for example hypersensitivity of airway nerves.[62]

7.5.1.2.2 Sex

Median cough frequencies were about 1.5 times higher in women than men across diagnostic groups overall. However, this difference was not statistically significant in any of the disease groups individually, apart from unexplained chronic cough and possibly acute asthma. In TB, there was a trend for cough frequency to be higher in

men than women (p = 0.13). Evidence for an effect of sex in stable asthma, bronchiectasis and lung cancer was not sought due to small sample sizes.

Yousaf *et al.* using the LCM similarly showed higher cough frequencies in women than men overall across a range of (slightly different) diagnostic categories, a difference of 1.9-fold.[89] However, on overnight recordings in patients with COPD, pneumonia and pulmonary TB, Loudon and Brown observed no effect of sex.[88] As in the current study, 24-hour cough frequency has been reported elsewhere to be higher in women than men In unexplained chronic cough[75] and asthma.[76] Also in keeping with my findings, other work has similarly failed to show an effect of sex on cough rates in COPD,[78] idiopathic pulmonary fibrosis[77] and isolated chronic cough due to a variety of causes.[369] Data in URTI are conflicting.[79,80]

Therefore, the current findings of sex and cough frequency are consistent with those in the literature. There may be no association between the two variables other than in idiopathic chronic cough, and perhaps asthma. In idiopathic chronic cough, reasons for a sex difference in cough frequency are not entirely clear and require further evaluation (Section 1.4), although differences in airway inflammation, peripheral neuronal sensitivity or central sensory processing between men and women may be important.[9,75,81,340]

7.5.1.2.3 Other factors

There was no significant effect of ACE inhibitor use on cough frequency in any disease group, although in isolated chronic cough there were only two patients taking the medication, both of whom had very high cough rates. ACE inhibitors are clearly a cause of isolated chronic cough,[74] and appear to alter the threshold of the cough reflex in healthy individuals.[14] However, cough reflex sensitivity (as it is usually measured) at best correlates only moderately with cough frequency,[84] and there are very few data in the literature on objectively-measured 24-hour cough rates in individuals taking these medications, possibly from a maximum of five subjects.[89,369] Although chronic cough has been reported to occur in 5-35% of individuals taking ACE inhibitors, it remains

unclear how frequently ACE inhibitors affect objectively-measured cough frequency in health and disease. Genotype may have an important influence.[53]

In keeping with the lack of convincing evidence for codeine as an effective antitussive,[17] no association of the use of the use of this medication with cough frequency was seen. Similarly, there is little convincing previous evidence on the action of methadone as an antitussive,[370] and the current findings do not support an effect of this drug on cough frequency.

The current study found no correlation of cough frequency with age across all participants as a whole, and a correlation within disease groups individually (which have different age profiles [Section 3.5]) only for COPD exacerbations. Previous work has not found an association of cough frequency with age in COPD,[78] chronic cough due to a various causes,[89,369] IPF,[77] URTI,[79] pneumonia and TB,[88] although other studies in idiopathic chronic cough[75] and asthma[76] have reported higher cough rates in older individuals. The apparent correlation of cough frequency with age in the current onset might be due to the association of age with duration of pre-hospital symptom onset (Section 7.5.1.3.4), although why older individuals might have symptoms for more days prior to admission with a COPD exacerbation is unclear. The general question of how the tendency to cough through life might change remains unresolved.[82]

Across all patients overall cough rates were higher in white than non-white participants. Although there was no evidence for a significant association of ethnicity with cough frequency within diagnostic groups individually, there was a trend towards higher cough frequencies amongst white participants in unexplained chronic cough. Part of the difference overall might have been due to an overrepresentation of white participants in the groups with COPD exacerbation and pneumonia (Section 3.5), both groups with relatively high cough rates. However, an effect of ethnicity on cough frequency cannot be excluded. There does not appear to be previous work examining possible associations of ethnicity with cough frequency in health or disease, although

one study found no difference between healthy volunteers of Indian, Chinese or European descent in capsaicin cough reflex sensitivity.[371]

7.5.1.3 *Baseline cough frequency and disease-related factors*

7.5.1.3.1 Isolated chronic cough

None of the variables investigated, other than perhaps ethnicity and sex (as discussed above) were associated with cough frequency in isolated chronic cough. Only one other previous study appears to have looked at predictors of cough frequency in isolated chronic cough, all in patients with unexplained symptoms, and found sex and age to be important.[75] As also discussed above, this correlation with age has not been supported by other work. Understanding of the direct causative mechanisms of coughing in chronic cough remains limited although neuronal hypersensitivity is increasingly thought to be important.[62]

7.5.1.3.2 Stable COPD

The Manchester cough group have looked in the most detail at cough frequency in COPD, finding current smoking, smoking history, reported sputum production, and sputum neutrophilia, but not lung function, to be predictors.[78] Similarly, in the current work there was a trend for an association with current smoking and no evidence for an effect of FEV_1.

The observed association of cough frequency with long-acting muscarinic medication (tiotropium) could be a chance finding given small numbers involved. Alternatively, worse cough could have been a reason for an increased tendency to prescribe this commonly-used medication in COPD. However, there is some limited evidence for the beneficial effect of the short-acting anti-muscarinic agent ipratropium bromide on cough severity in isolated chronic cough,[372] and tiotropium has been shown to reduce capsaicin-induced cough via action on TRPV1 receptors.[373] An effect of tiotropium on cough frequency has not been previously investigated.

7.5.1.3.3 Pulmonary fibrosis

Only one study of which I am aware has looked at objective cough frequency in pulmonary fibrosis, again from the Manchester group, involving a total of 19 patients with IPF.[77] Median (range) cough rates in the previous work were very similar to the current study, 226 (36-946) c/24h *vs.* 258 (110-859), respectively. The authors documented age, sex, body mass index, lung function, and current steroid use, and found no evidence for an influence of any of the variables on cough frequency. It is therefore of interest that in the current work there was a correlation between cough frequency and KCO, albeit in a group of mixed underlying diagnoses (n = 15). Lung function and other patient characteristics in the Manchester cohort seem very similar to those in the current study, although the former did not report ethnicity. The discrepancy between the two studies on KCO could reflect differences in underlying causes of fibrosis; further evaluation is required.

The mechanisms of cough in pulmonary fibrosis, as in many disease are largely unknown.[77,374,375] Changes in transfer factor could relate to cough frequency via an independent process indicating general progression of disease. Alternatively the alveolar destruction with which the loss of transfer factor is associated could in some way be accompanied by increased inflammation and activation of cough receptors in distal airways. Positron emission tomography studies have shown fibrotic lung tissue in ILD to be a lot more metabolically active than might have been assumed.[376] Cough mechanoreceptors might also be more sensitive as a result of lung stretching following architectural distortion and traction bronchial dilatation. The airways can also be directly involved in both sarcoidosis and connective tissue disease which may more readily explain cough in these conditions,[374] and perhaps an association with KCO as disease progresses.

No attempt was made to measure the radiological extent of disease in patients with pulmonary fibrosis in the current study. Doing so may have shown a correlation with

KCO and cough frequency although the scoring systems that exist for this purpose have not been extensively validated.[377,378]

7.5.1.3.4 COPD exacerbations, acute asthma and pneumonia

There are no published reports of 24-hour cough frequency in COPD exacerbations, acute asthma and pneumonia of which I am aware. In Loudon and Brown's overnight cough recordings the authors did not look for any correlates with cough rates in patients hospitalized with COPD and pneumonia, other than with sex and smoking status, which were not found.

In the current study there was a trend for higher cough frequencies, in COPD exacerbations, with duration of symptom onset, and, in acute asthma, with female sex, but no other predictors of cough frequency were identified in these conditions or in pneumonia, including markers of disease severity such as CRP and CURB-65 score, or peak expiratory flow in asthma. Other work has similarly failed to show an association between lung function measures and cough frequency in asthma, albeit in stable disease.[76] Associations with sex have been discussed (section 7.5.1.2), but the possible association with symptom duration is novel. It is possible that there are 'cough-predominant' COPD exacerbations, better tolerated in the outset than other exacerbations, perhaps related to certain viral aetiologies and less associated with systemic inflammation (CRP) than more severe types.[379] Such exacerbations may start with cough and slowly build up before patients seek medical intervention for accompanying 'major' symptoms,[380] by which time cough frequency has reached higher levels than in other, 'dyspnoea-predominant' episodes.

The causes of excessive coughing in these three conditions have not been specifically investigated. In COPD exacerbations and acute asthma airway inflammation may be relevant, as has been shown in stable COPD,[78] although not in stable asthma.[76] COPD exacerbations have classically been termed 'infective' or 'non-infective', based mainly on the presence or absence, respectively, of increased sputum purulence, and the observation that purulent sputum is more likely to culture bacteria.[381] This distinction

is difficult to make clinically, and no attempt was made to do so in the current study. Furthermore, molecular techniques have revealed viruses may be involved more frequently than previously recognized, in exacerbations of both COPD and asthma,[382] often acting in tandem with bacteria.[383] Viral infections probably contribute to excessive coughing in a large proportion of exacerbations of airway disease, acting directly or indirectly on airway nerves.[68,384]

7.5.1.3.5 Tuberculosis

To my knowledge, this is the first work to have carried out 24-hour cough frequency measurement in pulmonary tuberculosis. Cough frequency was shown to be highly variable in TB, as in other diseases. The variable for which there was the best evidence for an association with 24-hour cough frequency was sputum smear status ($p = 0.054$). There was a suggestion that sex may also have been important ($p = 0.132$), but associations for none of the variables met the pre-specified level of statistical significance ($p < 0.05$).

The only other studies of cough monitoring in TB of which I am aware are by Loudon from the 1960s, and owing to limitations of technology at the time, were constrained to overnight bedside recordings lasting up to 8 h.[88,125] The authors made recordings in 96 patients with pulmonary TB and reported a mean overnight cough frequency of approximately 8 c/h. This is about twice the median nocturnal rate for TB in the current study, although considerable variation was reported in the previous work, with rates <3 c/h in 50% of patients.[88] The difference from the current study could reflect differences in patient characteristics. This is however difficult to ascertain as the earlier study report gives little detail other than that 35 patients had 'far advanced' disease, 44 'moderately advanced' and 17 'minimal pulmonary tuberculosis', without defining these categories, which seem to refer to extent of radiographic change.[88] Treatment and microbiological status was not stated. However, in keeping with the current work, cough frequency in TB was lower than in patients hospitalized with pneumonia and COPD, but in TB was also increased with the 'roentgenographic extent of disease'.[88]

High variation of cough frequency in TB was therefore a common observation of the current work and the previous study of Loudon. All cough monitor recordings were started before commencing anti-tuberculous treatment in the current work, and even in smear-positive disease, 24-hour cough rates overlapped with those in latent TB.

No other studies apart from that of Loudon and Brown[88] have reported predictors of objectively-measured cough frequency in tuberculosis. In Chapter 4 an association of the *presence* of subjectively-reported cough in TB was reported with sputum smear positivity, absence of coincident extrapulmonary TB, and referral from screening. Associations with the *severity and impact* of cough symptoms were noted with absence of EPTB and possibly sputum smear and culture status (Section 4.4.1.1). Other work has associated patient-reported cough with sputum and culture status, and radiological extent of disease in TB.[305,349]

These observations on subjective and objective measures of cough in TB are therefore complementary but a weak correlation between the two fits with findings in other diseases. For example, the independent trend for an association of the presence of coexistent EPTB with better cough severity and cough-related quality of life (Section 4.4.1.1.3) was not coupled with a correlation with lower objective cough frequency. It therefore seems that in the presence of coexistent extrapulmonary disease, cough frequencies in pulmonary TB are no lower than in the absence of EPTB (as reported here), but that coughing is less noticeable (as described in Chapter 2), presumably due to the presence of other symptoms of greater importance to the patient (e.g. neck lumps in lymph node disease or dyspnoea in pleural TB).

Sex remains as a possible associated factor with cough frequency in TB, although there was only a trend for an association between the two variables ($p = 0.13$; Section 7.5.1.2.2). This possible association is not explainable by sputum smear status or radiological extent of disease, which were not significantly different between the two sexes (Section 3.5.10).

There was no evidence to support the hypothesis that *M. tuberculosis s*train type might influence cough frequency, although there were only two patients with the Beijing strain (of particular interest due to reports of increased transmissibility[182]). Neither was there evidence for an association of the presence of visible cavities with cough frequency in TB. However, chest radiographs are insensitive to small cavities compared to computed tomography.[385] Mechanistically, cavities could be important, as sites of high bacterial numbers and routes of access of *M. tuberculosis* to the airways,[386] the location of the majority of cough receptors.

An association passive rather than active case finding (screening), and possibly smear status, with cough frequency in TB, supports the idea that coughing becomes more prominent as the disease progresses.[88,387] In general symptoms in pulmonary TB are probably more common and severe in sputum smear-positive compared to smear-negative tuberculosis.[388] The presence of larger numbers of bacteria in the sputum, and therefore the airways, presumably directly and indirectly triggers the cough reflex, by triggering inflammation involving neutrophils and other mediators,[389] possibly involving secreted TB antigens. There is further discussion about possible mechanisms of cough in TB in Section 8.5.2.

The quantity and characteristics of sputum are also probably important in TB (Section 4.5.2),[102] but were not explored.

Other insights are reported in Chapter 8 with further discussion (Section 8.4.2), but the determinants of cough frequency in TB remain largely obscure. Because of implications for transmission of the disease this is a particular area in demand of further research.

7.5.1.4 *Diurnal variation in cough*

This is the first reported demonstration that objectively-measured cough frequency is reduced overnight in TB, lung cancer, COPD exacerbation, acute asthma and pneumonia. This has already been shown to be the case in isolated chronic cough,[75,369] stable COPD,[78] asthma,[76] pulmonary fibrosis,[77] and bronchiectasis.[390] There were no

unique patterns in any of the disease groups, in common with other work showing no significant differences in diurnal variation of cough between asthma, acute cough and chronic cough.[91] However, overnight reductions in coughing were less pronounced in patients hospitalized with pneumonia and COPD exacerbations than in other study participants.

Nocturnal cough rates were strongly correlated with total 24-hour cough frequencies across diseases. A similar finding has been reported elsewhere in chronic cough,[369] but with perhaps a less strong association (Spearman's $\rho = 0.62$, compared to $\rho = 0.83$ in the current study). I am unaware of other data on this in other diseases. If the current findings are replicated elsewhere, and indeed nocturnal cough counts are a strong predictor of cough frequency throughout the 24-hour period in respiratory diseases, then for patient convenience, cough monitoring for research and clinical purposes could be done overnight rather than during a whole day. This might increase the utility of cough monitoring, and would also reduce interference from background noise.

Sleep appears to suppress coughing; the large majority of overnight coughs probably only occur during episodes of wakefullness.[391] Patients were not specifically asked to record the approximate times of going to sleep and waking, but in one study of outpatient recordings in chronic cough, doing so did not make a significant difference to estimations of nocturnal cough rates compared to using the arbitrary times of night as 22:00 to 06:00 h as in the current study.[369] Nocturnal cough rates were possibly relatively higher in patients with acute respiratory disease due to excessive noise and other factors in the hospital environment limiting sleep. Also the greater severity of accompanying symptoms (fever, dyspnoea, pain) compared to in chronic conditions presumably also affected sleep quality.

Only one study (of 10 patients with COPD) appears to have coincided nocturnal cough monitoring with electroencephalogram data, showing coughs during true sleep to be rare;[391] correlations between levels of central arousal and cough frequency have been little investigated. It is not clear how often nocturnal waking occurs because of an urge

to cough or whether the majority of nocturnal coughing occurs during periods where wakefulness has resulted from other causes. Sensitivity to an inhaled tussive agent has been shown to be suppressed during sleep.[392] Investigating central mechanisms suppressing the cough reflex during sleep could lead to new insights for the development of antitussive therapies.[393]

An overnight reduction of cough frequency in TB has implications for the transmission of disease. If coughing is infrequent overnight then sharing a bedroom with a potential source case of pulmonary tuberculosis may not represent any more of a significant risk for infection than other types of household contact. However, the risk may of course still be high depending on the duration of the total exposure and the nature of daytime contact.

7.5.1.5 *Cough clustering*

The temporal clustering of coughs was of interest as any difference between diseases may suggest differences in the mechanism or the purpose of coughing. Spacing of coughs closely together, for example, might increase the efficiency of clearing secretions,[93] or aerosolizing pathogens (Section 1.4.2). This does not appear to have been investigated previously in any detail. In one study of spontaneous coughing in healthy individuals, the numbers of coughs per expiratory phase were counted and no differences were found based on smoking or sex.[72] There were no differences between diseases in the current work other than a reduced proportion of clustering of coughs into bouts in TB compared to chronic cough. This is the opposite of what might be expected if cough clustering was strongly associated with the presence of sputum or bacterial aerosolization.

The converse may therefore be the case, that a relatively lower number of cough bouts, and a high proportion of lone coughs for the same overall cough frequency could be associated with more efficient aerosolization of airway secretions. The first cough of an expiratory phase is usually the most powerful in terms of peak flow and volume of air expelled, whereas later coughs in the same breath are much less so due

to lower volumes of air remaining in the lungs.[94] There may also remain a relatively much smaller amount of aerosolizable matter within in the airways after the first cough within a certain time period, meaning that second and subsequent coughs of a bout are less relevant for the expulsion of bacteria and other material into the air. As a consequence, the number of cough 'episodes', might be a better predictor of efficient aerosolization than the total number of coughs *per se*. Meanwhile the clustering of coughs into bouts might perhaps be more noticeable and therefore, as seen in the current work, more characteristic of isolated chronic cough, a condition defined by its irritating symptom, and associated with worse subjective scores than TB (Section 4.4.1.1).

Clustering of coughs and daily cough frequency are, however, correlated (Spearman's $\rho = 0.62$ in the current study). In health (latent TB) only 26% of the few coughs were clustered into bouts compared to 72% in pneumonia, the disease with the highest median cough frequency. The only differences observed between diseases in clustering, between isolated chronic cough and TB, therefore are most probably a result only of differences in overall cough frequency between the conditions, and there is no evidence of independent variation between groups in cough clustering.

The current study also provided no evidence that numbers of cough episodes are a better predictor of symptoms than numbers of individual coughs (although there were wide confidence intervals associated correlation coefficients). Other work in chronic cough has similarly shown numbers of cough bouts (or epochs) in chronic cough and the frequency of individual coughs to be closely correlated with one another and similarly related to quality of life.[87]

7.5.2 Objective and subjective measures

There was an overall weak to moderate correlation between objectively-measured 24-hour cough frequency and subjective measures of cough (cough severity visual analogue scale score and Leicester Cough Questionnaire score). This has been noted for correlations with LCQ score in recent studies in chronic cough (correlation

coefficient -0.6 [ref [84]], -0.53 [ref [87]]), COPD (ρ = -0.64),[78] asthma (ρ = -0.55 to -0.45),[76] bronchiectasis (ρ = -0.54)[390] and pulmonary fibrosis (ρ = -0.80).[77] Similar findings have been shown for correlations of cough frequency with VAS in chronic cough (Pearson's *r* [for normalized data] = 0.45 [for daytime cough]; *r* = to 0.67 [nocturnal cough]),[87] COPD (ρ = 0.48 [nocturnal cough]; ρ = 0.66 [daytime]),[78] asthma (ρ = 0.44),[76] bronchiectasis (ρ = 0.56)[390] and pulmonary fibrosis (ρ = -0.80).[77] No previous studies have looked at correlations of 24-hour cough counts with subjective measures in COPD exacerbations, acute asthma, pneumonia, cancer or tuberculosis as far as I am aware. However, on their study of overnight coughing in a mixture of patients, mainly with TB, pneumonia and COPD, Loudon and Brown found a very poor correlation between cough frequency and the response to the question "Do you have a cough at the present time?", but did not present the findings by diagnosis.[88]

As previously discussed, 24-hour cough frequency is probably a poor predictor of patients' subjective appreciation of coughing for a number of reasons (Section 1.6.1; Section 4.5.4). The findings did not support the hypothesis that cough might be less noticeable in tuberculosis than in other conditions, perhaps due to the action of the pathogen promoting its own transmission; correlation between objective and subjective measures of cough in TB was similar or stronger to that seen in the majority of other disease groups. Wide confidence intervals limited any meaningful comparisons of the relative social and psychological impacts of symptoms (based on LCQ domain scores) across disease in relation to cough frequency, which in TB would be relevant for social mixing and the spread of disease, but correlation coefficients were very similar in the two largest groups, TB and chronic cough (Table 7.3).

The weakest correlations between objective and subjective measures of cough were in COPD exacerbations and acute asthma. The reasons for this are unclear, but perhaps reflect the relative lesser subjective importance of cough in these conditions compared to other symptoms, particularly acute breathlessness. For example in COPD exacerbations, increased cough is classed as a 'minor' symptom compared to the 'major' symptoms of increased breathlessness, sputum quantity and quality.[380] In

acute severe asthma, breathlessness is also probably the most important symptom, perhaps making the perception of coughing less troublesome. The novelty of cough in otherwise healthy patients with pneumonia might also make it more noticeable, compared to in acute asthma and COPD, where patients experience respiratory symptoms on a more frequent basis.

Overall the Leicester Cough Questionnaire scores seemed to correlate less well with cough frequency than the visual analogue scale score, although confidence intervals in correlation coefficients overlapped. This is the largest study to perform this comparison. This finding is perhaps not unexpected. The VAS is a subjective marker specifically of the severity of cough, whereas the LCQ measures cough-related quality of life. The LCQ includes questions about concern of underlying illness, embarrassment of coughing and feelings of frustration, i.e. psychological and social impact rather than just physical effects, the latter of which might relate better to cough frequency.

Subjective measures, although obviously very important for quantifying symptoms, are therefore poor surrogates for objective measurements. 24-hour cough frequency measurements are clearly more relevant for studying disease mechanisms, the efficacy of treatments and potential infectiousness in tuberculosis.

7.5.3 The Leicester Cough Monitor

The Leicester Cough Monitor was found to be useful. Although lacking previous evaluation in certain contexts, for example tuberculosis and acute respiratory disease, it provided meaningful data. It has probably been peer reviewed to a greater extent than any other cough monitoring system (Section 1.6.2.2.3.3).[253,265,266] The demonstration of responsiveness in cough frequency data to treatment of tuberculosis presented in Chapter 8 (Section 8.4.2) provides further validation of the LCM in general, as well as specific validation in TB, as do the data correlating cough frequency with symptom scores in TB.

There is no reason to suspect that the cough monitor should not be as accurate in TB as in other conditions; there is no strong evidence that coughs sounds vary any more

between than within diseases.[98,272] Furthermore, the correlations between measured nocturnal and 24-hour cough frequency were as strong in TB as in other conditions, and the comparisons to subjective measures of cough similar to in other diseases, suggesting the device performed equally across patient groups.

The recording device was simple and unobtrusive, perhaps less so than other systems,[90] and was acceptable to patients, with few refusing to undergo monitoring. Outpatient recordings were simplified by the ability to send digital recorders through the post, made possible by their small size and relative low cost (£40-50). As technology progresses, the recording component of the Leicester Cough Monitor should improve, perhaps involving smartphones and transfer of audio files by the internet.

It is unlikely that any automated or semi-automated cough monitoring system would report all coughs with complete accuracy. As demonstrated in Chapter 5 (Section 5.3.3), even the reference standard of auditory cough counting has a degree of error, perhaps due to classification of 'atypical' cough sounds. Compared to auditory cough counts, the sensitivity and specificity of the Leicester Cough Monitor has been

Table 7.13. Cough frequency measurements in disease from other studies.

	24-hour cough frequency			
	Leicester Cough Monitor			Vitalojak
	current study		previous work	
Unexplained chronic cough	652 (216-1165)	480 (±0.5)	477 (±0.3)[a]	381 (206-552) [b]
COPD	232 (112-385)	194 (±0.5)	213 (±0.3) [a]	170 (9.6-648) * [c]
Pulmonary fibrosis	258 (110-859)	286 (±0.3)	-	226 (36-946) [d]
Healthy non-smokers	13 (8-14)	17 (±0.5)	19 (±0.5)[a]	17 (3-103) * [c]

Values are: median (IQR or *range); geometric mean (±$\log_{10}$SD)
c – ref [78]; a – ref [89]; d – ref [77]; b - ref [87]

reported to be 88 and 99%, respectively.[256] There was therefore a small degree of error associated with cough frequency measurements in this study, as there would have been making measurements of any variable with any measurement tool. Because the inherent error of the LCM in most cases is probably random rather than systematic it should not qualitatively influence the findings.

The main shortcoming of the cough monitoring system was a potential inability to exclude background coughs. In most cases this should not be a significant problem, but it influences comparisons of absolute cough frequencies reported from different environments. A crude method of assessing the degree of 'cough contamination' on hospital ward recordings was used, and probably overestimated the number of background coughs (median 559 c/24 h) that would have been picked up during a routine patient recording. The background recordings included all captured coughs, a lot of which if present in patient recordings would have been filtered out by the LCM software. This would have occurred at the operator calibration stage by the ability to recognize background coughs as such by comparison to the louder, more frequent, and often qualitatively different cough sounds of the subject being studied. The lack of a significant difference in cough counts in pneumonia between patients in side rooms and on the open ward is reassuring that cough contamination might not be a significant problem in ward recordings. Also, it is less of a concern for measuring response to treatment in serial recordings made in similar conditions where relative change is more important than absolute change in numbers (Chapter 8).

Table 7.13 shows other recent reports of 24-hour cough frequency in several diseases, using either Vitalojak, by the Manchester cough group, or the Leicester Cough Monitor. Comparisons between studies are of only limited value due to patient differences, but relative cough rates between diseases and variation within groups are similar between studies. This suggests that the two cough monitors produce very similar results. It would be of interest to compare these two systems directly, currently the two main cough monitors being used in clinical research.[225]

7.5.4 Limitations

The main limitation for at least some of the investigations described in this chapter was statistical power. The limited available data on variation on cough frequency across diseases made an *a priori* estimate of adequate sample sizes difficult. However, the very nature of the study was that it was exploratory, to look for obvious

differences between diseases and large effects of particular variables on cough that might direct further investigation. In fact, the study was adequately powered to detect a 15% or greater difference in cough frequency between TB and isolated chronic cough, as was demonstrated. Conversely, the lack of statistically significant differences in cough frequencies between some groups despite numerical differences in medians (e.g. stable asthma and acute asthma) may well have been due to small numbers of patients.

Sputum production was not measured, although, at least when assessed subjectively, it has been shown to be an important predictor of cough frequency in COPD.[78] The measurement of sputum production was attempted, by responses to Question 2 of the Leicester Cough Questionnaire, as previously described, but the method was shown to be problematic (Section 4.5.2). The relationship between objectively-measured quantitative and qualitative characteristics of respiratory mucus and cough frequency awaits description, particularly in diseases of the airways (COPD, bronchiectasis and asthma).

A further consideration is repeatability of 24-hour cough monitoring. Intra-individual day-to-day variability in cough frequency was not formally assessed, and could impact further on sample sizes required for detecting differences between diseases. This would be of most interest in the stable state (chronic cough, fibrosis, COPD, asthma, lung cancer and TB pre-treatment) in order to quantify differences due to the method of measuring cough frequency and random biological processes. Day-to-day differences in cough frequency in acute respiratory disease would also reflect the evolution of the underlying pathology and the response to treatment, and are addressed in Chapter 8. No other researcher seems to have explicitly assessed the repeatability of 24-hour cough monitoring in the stable state in the same patient. Some of the novel findings, such a possible effect of KCO on cough frequency in pulmonary fibrosis, and the association between symptom duration and cough frequency in COPD exacerbations, should be explored in larger studies. Equally, the

absence of particular associations, such as that between organism strain and coughing in TB, also need confirmation.

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The negative control group in this Chapter was latent TB infection. The rationale for this was that participants in this category should be as similar to the principal group of interest as possible, pulmonary TB, but for the fact that they do not have the disease. As discussed above (Section 7.5.1.1), the assumption was that 'latent' infection would not cause coughing, but the possibility exists that some individuals could have had subclinical disease causing subtle effects on cough frequency. However, while this is of interest and warrants further investigation, such subtle effects would not impact on the observation presented here of a much lower cough frequency in LTBI than in respiratory disease. Although cough frequency in LTBI reported here was very similar to values reported elsewhere for healthy volunteers (Section 7.5.1.1), inferences from observed cough frequencies in the control group should also take account of the ethnic profile (minority white), relative young age and low smoking prevalence compared to other study groups (Table 3.1).

7.6 Conclusion

There was little evidence to support the hypothesis that temporal patterns of coughing vary with disease, and that any disease (particularly tuberculosis) might display a distinct pattern. However, the investigation of cough frequency in respiratory diseases in the stable state and has produced novel findings and hypotheses. The wide as-yet

unexplained variation in cough frequency in all diseases groups suggest 'host'-related factors are important. At the same time, the differential impact of variables across diseases (e.g. sex, smoking) suggests different mechanisms are of different relative importance across conditions. Investigation of the differential rates in which cough resolves with treatment between and within diseases, might give further important insights, and shall follow in Chapter 8.

8 The response of cough to the treatment of respiratory disease

8.1 Introduction and objectives

Cough is a prominent feature of respiratory disease and usually improves with resolution of the underlying cause. The rate of improvement is, however, unclear. Serial objective cough measurements have been made infrequently and in a limited number of contexts.[79,80,125] Knowledge of the rate of improvement of cough in respiratory conditions would be of interest for several reasons. Firstly, it would provide clinicians with evidence to inform patients what to expect with treatment. Secondly, the relative rate of change in cough frequency in comparison to changes in other clinical parameters might add to research into the causes of cough. Differences in the timing of improvements between types of pathology (e.g. pathogen *vs.* host-related, airway-centred *vs.* parenchymal disease) might lead to further hypotheses about cough mechanisms. Thirdly, cough could be used as a novel biomarker of disease activity in respiratory disease with serial monitoring providing an objectively measure of clinical response to treatment. Finally, in TB, improvements in cough frequency might help with estimations of continued infectiousness and decisions regarding infection control.

The following hypotheses were explored:

1. The rate of change in 24-hour cough frequency in response to treatment of the underlying cause varies with respiratory disease;
2. Improvements in cough frequency in respiratory conditions correlate with other markers of disease resolution.

8.2 Methods

Serial cough monitoring was performed with the Leicester Cough Monitor in study participants with tuberculosis and the acute respiratory conditions (COPD

exacerbation, acute asthma and pneumonia; Section 3.5) as previously described (Section 7.2.2). Patients with the acute respiratory diseases were invited to participate in this part of the study if it was predicted they would remain hospitalized for >24 h after the initial cough monitoring period. Repeat monitoring was completed prior to hospital discharge.

In TB, repeat cough monitoring was set up during initial hospitalization (where applicable) and later in treatment, by convenience to coincide with routine clinic attendance or directly observed therapy (DOT). Routine follow up clinic appointments in TB at HUH were scheduled for two weeks, two months and six months after initiation of therapy for uncomplicated cases.

In order to magnify any overall responses of cough frequency to treatment in TB, patients were invited to undergo repeat cough monitoring only if the baseline cough rate was >100 coughs/24 h (c/24h), a cough frequency which probably coincides with the upper limit of the normal range for healthy non-smokers.[78]

8.3 Analysis of data

For the repeated measures analysis of the non-normally distributed cough frequency data, Wilcoxon matched-pairs signed rank tests were used.

8.4 Results

8.4.1 Acute respiratory disease

In total, 26 patients with acute respiratory disease (COPD exacerbation, n = 10; acute asthma, n = 5; pneumonia, n = 11) underwent repeat cough monitoring during hospital admission, a median of 2 (IQR 1-4) days after the first. There was overall a non-significant trend for a reduction in cough frequency over this time, from a median of 629 (358-954) to 487 (255 to 718) c/24h (p = 0.071; Figure 8.1, top / middle).

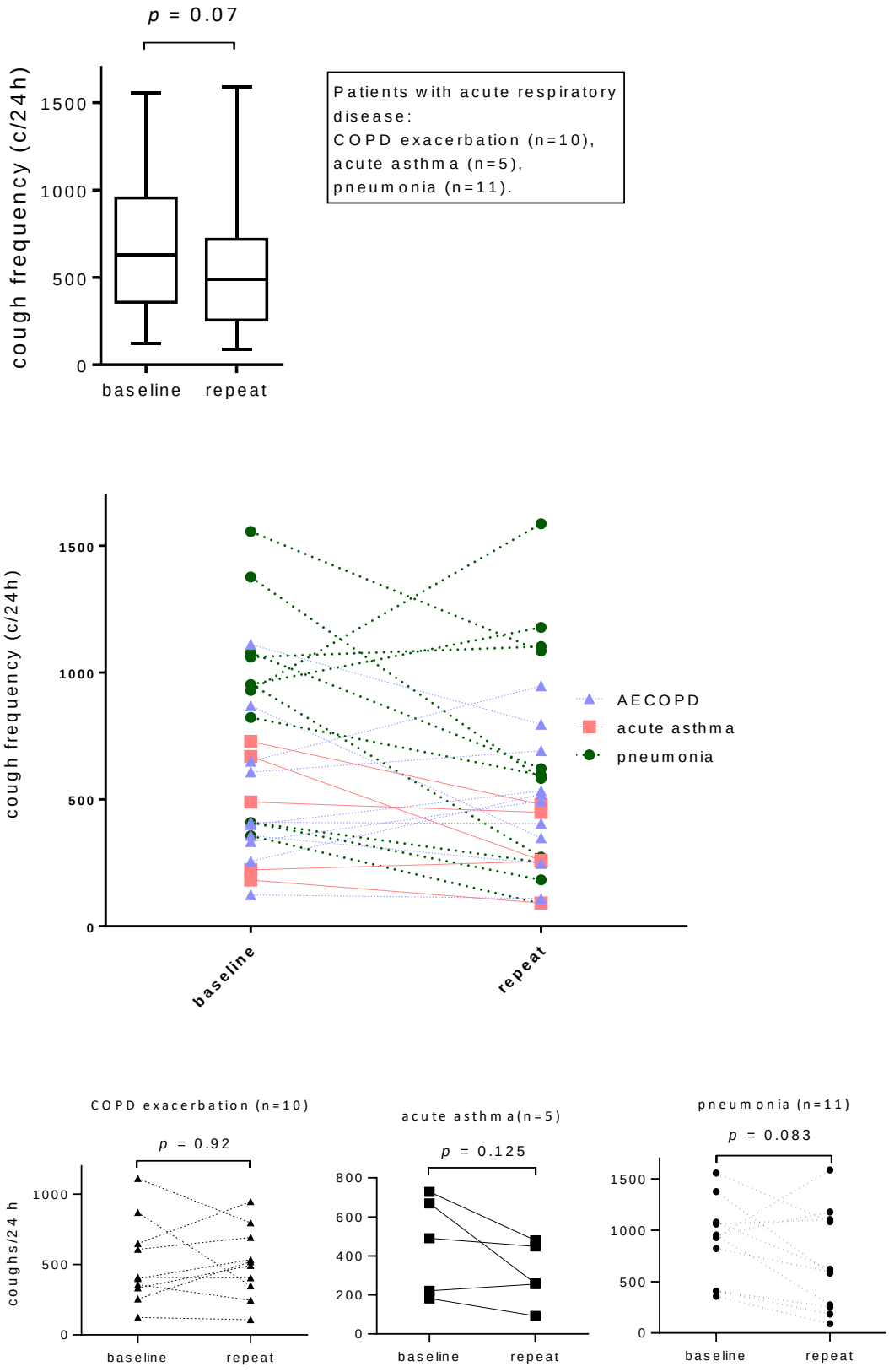


Figure 8.1. Change in cough frequency in acute respiratory diseases over a median of two days

8.4.1.1 *COPD exacerbation*

Patients with COPD exacerbations demonstrated no change in overall cough frequency on repeat monitoring (from median 405 [315-705] c/24h to 507 [323-718] $p = 0.92$; Figure 8.1, bottom left). Two patients showed substantial rises, and two patients substantial decreases in cough frequency. There was no obvious differences between these two pairs of patients in terms of final outcome and duration of hospital admission, treatment, duration of symptoms prior to hospitalization, baseline numbers of exacerbations per year, smoking status, ACE inhibitor use, or interval between recordings (data not shown). However, the two patients who showed the most rapid improvement in cough frequency had amongst the least severe lung function deficits, FEV_1 83 % and 82 % predicted, respectively.

Over the same interval between repeated cough frequency measurements there was a trend for CRP to improve but missing data (n = 8; 45 [10-117] mg/L at baseline *vs.* 20 [8-39] mg/L on repeated measurement; $p = 0.078$). There was no correlation between relative changes in cough frequency and CRP ($\rho = -0.179$, $p = 0.713$).

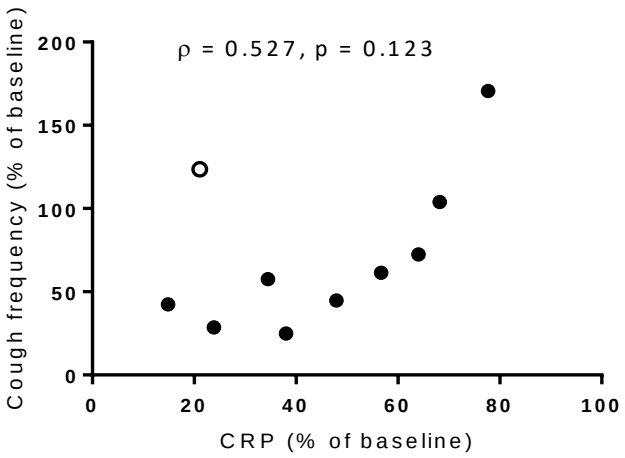
8.4.1.2 *Acute asthma*

Four of the five patients with acute asthma who underwent repeat cough monitoring showed an improvement in cough frequency ($p = 0.125$ for overall change in cough frequency in group). There was no obvious difference in the two patients who failed to improve in cough frequency by at least 20 % from those that did show an improvement (Figure 8.1, bottom middle), in terms of smoking status, duration of acute symptoms, timing of cough monitoring or PEFR at hospital presentation, treatment and final outcome (data not shown). However, the two non-responders were both female and both pregnant.

8.4.1.3 *Pneumonia*

All but three of the 11 patients with pneumonia and repeat cough monitoring data showed reductions in cough frequency (median 953 [IQR 410-1079] c/24h to 596 [252-1103], $p = 0.083$; Figure 8.1, bottom right). All three non-improvers were smokers,

compared to 4 of the other 8 patients with pneumonia, although this difference was not statistically significant ($p = 0.236$). There was a trend for a correlation between



Outlying result marked with open circle.

Figure 8.2. Change in cough frequency and decline in CRP during treatment of pneumonia.

change in cough frequency and the fall in plasma CRP levels between the same time points: apart from one outlier, the percentage fall in CRP seemed predictive of the change in cough rate (overall ρ = 0.527, $p = 0.123$; Figure 8.2). There was an interval of 6 days between cough monitoring periods for the patient with the largest rise in cough frequency, and a drop in CRP of only 233 to 181 mg/L over the same period. He subsequently required surgical intervention for a loculated empyema. The other two patients with pneumonia and rises in cough frequency were discharged from hospital two days after repeated monitoring and, as with all other patients with repeat cough frequency results, had good clinical outcomes. There was therefore no obvious reason for the increase in cough frequency despite a significant fall in CRP in the outlying patient in Figure 8.2. Time since admission, duration of symptoms, ACE inhibitor medication, initial CURB-65 score and radiological extent of disease had no obvious association with improvement in cough with treatment (data not shown).

8.4.2 *Pulmonary tuberculosis*

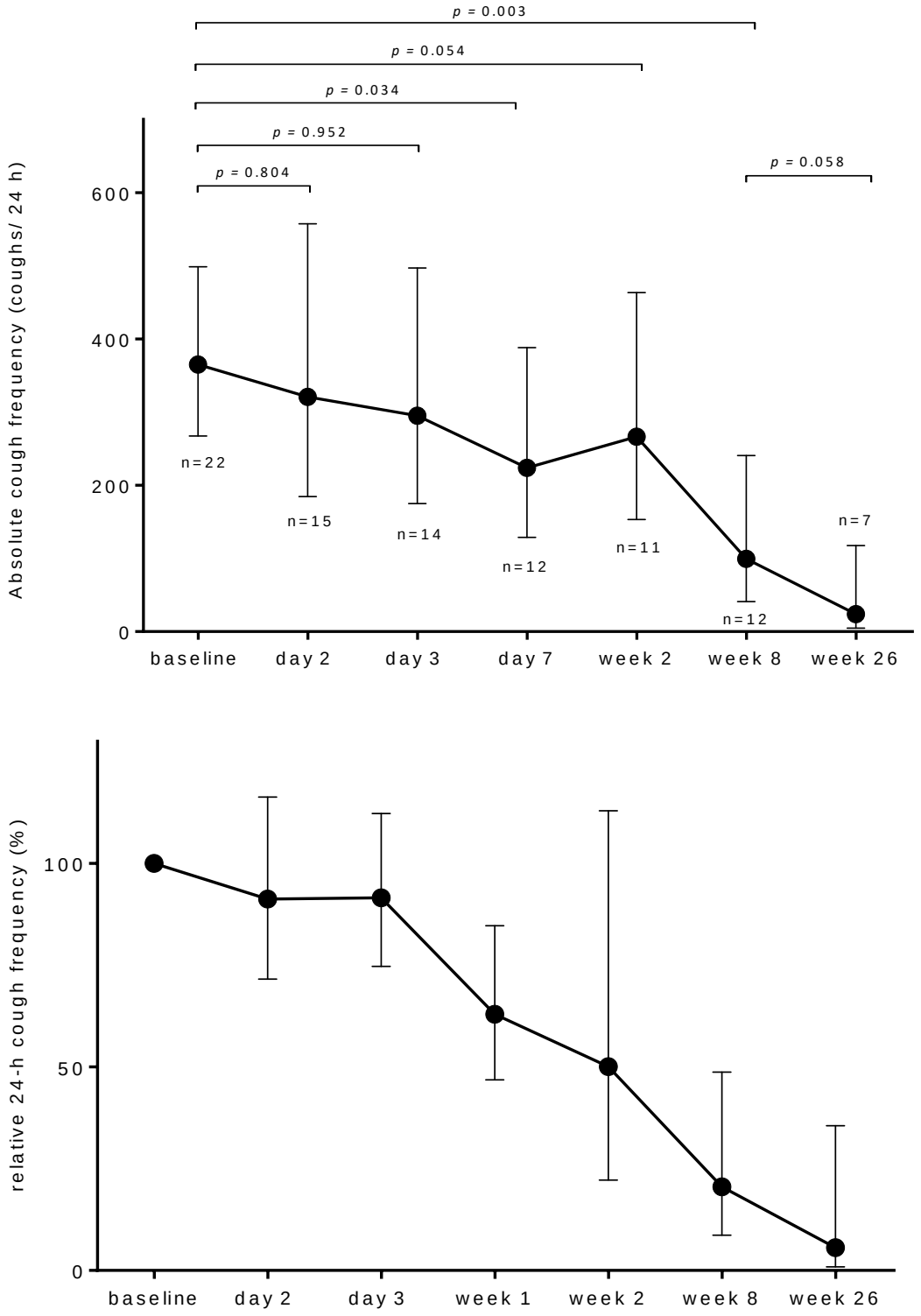
Of the 44 patients with TB, baseline cough frequency was >100 c/24h in 32 (72.7%). Twenty-two were willing and available to undergo serial cough monitoring for at least 48 h. In clinical and demographic characteristics, these individuals were comparable to the remaining 10 participants, apart from the fact that they were more likely to be sputum smear-positive and have evidence of cavitary disease (Table 8.1). All patients were eventually treated successfully. The median baseline cough frequency of these 22 patients was 375 (222-695) c/24h.

Table 8.1. Characteristics of patients with TB and baseline cough frequency >100 c/24h: comparison of participants undergoing serial cough monitoring with the rest of the group.

Variable	Participants with TB and baseline cough frequency >100 c/24h		
	without serial cough monitoring	with serial monitoring	*p*
n	10	22	
Sex (female)	3 (30%)	7 (32%)	1.00
Age	40 (31-43)	41 (30-48)	0.83
Current smokers	5 (50%)	13 (59%)	0.71
Other resp dis	1 (1%)	0	0.31
HIV	2 (20%)	1 (2%)	0.22
Smear-positive	4 (40%)	18 (82%)	0.037
Cavities	1 (10%)	12 (55%)	0.024
Isoniazid-resistant	2 (20%)	3 (15%)	0.64

Values are n (%) or median (IQR).

As shown in Figure 8.3, 24-hour cough frequency in the majority of patients with TB declined consistently during therapy with an overall significant improvement of 37.0% in the geometric mean value by one week (95% confidence interval 15.3 to 53.2%, p = 0.034). However, there was substantial variation; at Week 2 and/or Week 8, five of the 15 monitored patients had a higher cough frequency than at baseline. Amongst these slow responders there was poor adherence to medication with ongoing weight loss (n = 1), a paradoxical reaction to treatment with the development of a paraspinal abscess



Top - absolute cough frequency, bottom – relative cough frequency (% of baseline for patients included at time point). Data points: geometric mean ± 95% confidence interval.

Figure 8.3. Objectively-measured 24-hour cough frequency during treatment of TB.

(n = 1), extensive radiographic change at baseline (n = 1), and, in the patient with HIV, a persistently positive sputum smear at 8 weeks with minimal radiographic improvement. This was despite directly-observed therapy and a therapeutic peak blood rifampicin level (>5 mg/L [ref [394]]). One other patient had a highly variable cough frequency during the first 8 weeks of treatment. There was no evidence for an effect of isoniazid resistance, cavitary disease, smear status or smoking on rates of cough resolution (data not shown).

Due to a withdrawal of consent to repeatedly undergo cough monitoring and constraints on the time of the investigator, there was loss to follow up and missing data for certain time points as indicated in Figure 8.3.

There was no evidence for a change in diurnal patterns of coughing with treatment in this sample but there was large variation: at baseline a median of 23.2 (13.5-43.0) % of coughs occurred overnight compared to 16.0 (2.7-53.4) % at week 2 (p = 0.844).

8.5 Discussion

Repeated 24-hour cough monitoring with treatment or natural resolution of respiratory disease has been only carried performed previously in isolated chronic cough[43,66] and acute cough[79,80] as far as I am aware. TB and acute respiratory diseases were chosen in the current study as they are tractable conditions in which a response to treatment was expected. This appears to be the first documentation of the resolution in 24-hour cough frequency during the entire course of treatment of pulmonary tuberculosis.

In patients hospitalized with acute respiratory conditions, reductions in cough frequency after several days' treatment were investigated and were shown to be more likely to occur in successfully-treated pneumonia than in acute asthma or COPD exacerbation, with a possible correlation with changes in CRP in pneumonia.

8.5.1 Acute asthma, COPD exacerbations and pneumonia

Over a small time interval (median two days) a significant decrease in cough frequency was shown in pneumonia in patients with other indicators of response to treatment, an improvement in acute asthma in 3 of 5 patients, and overall no change in patients with COPD exacerbations, despite overall clinical improvements in the majority. Cough monitoring over this interval therefore seemed to be most useful as a predictor of overall treatment response in pneumonia. This was particularly demonstrated by the relative correlation of changes in cough frequency to response in CRP in pneumonia compared to COPD exacerbations, a marker which in both conditions has been shown to reflect disease activity.[325,379,395]

The apparently more rapid response to treatment of cough in pneumonia compared to the other two acute respiratory diseases is of interest. Differences in the nature of the pathology might be important. For example, asthma and COPD are diseases of the airway, the site of cough receptors, whereas pneumonia causes parenchymal lung changes. Aetiological factors of the acute disease could also be of relevance to resolution of cough: bacteria are responsible for the large majority of community-acquired pneumonia,[325] whereas viruses are more frequently implicated in acute asthma and COPD exacerbations.[68,382,396]

Of probable relevance is the fact that patients with pneumonia in this study otherwise had no other known lung disease. Conversely, patients with exacerbations of COPD and asthma by definition had chronic respiratory diseases, which in the stable state have been objectively shown to be associated with coughing (Section 0).[76,78] The baseline physiological state in respect to cough will therefore be very different in COPD and asthma compared to individuals without chronic respiratory disease who develop pneumonia. In this regard it is noteworthy that two of the patients with only mild lung function deficits in COPD had the more rapid improvements in cough frequency.

There were too few patients with acute asthma (n = 5) to make any meaningful interpretations of the data of serial cough monitoring. However, the lack of

improvement in cough frequency in the two pregnant women, despite an overall clinical response to treatment is of interest. The relationship between asthma and pregnancy is complex,[397] and the frequency of exacerbations is probably increased.[398] Asthma exacerbations have also been reported to be prolonged in pregnancy, although this may have been a result of unnecessary excessive caution with the use of corticosteroid treatment in pregnant women in previous practice.[399] There was no evidence of this in the current study. It has been suggested that the cough reflex may be increased in pregnancy, as an adaptation to prevent aspiration.[340] Alternatively coughing might be more frequent in pregnancy *because* of (micro-) aspiration and gastro-oesophageal reflux. This does not appear to have been investigated, but it may be relevant to these current observations.

The sample size was too small to detect whether current smoking habit was associated with a lack of improvement in cough frequency in pneumonia with treatment. However, as well as being a risk factor for pneumonia, current smoking is also associated with a negative outcome of the disease, independently of disease severity and comorbidities.[400] The mechanism through which this occurs could also influence cough. In the current study it is possible that some of the current smokers with pneumonia also had undiagnosed COPD, with an additional influence on cough frequency resolution.

There are no other data on objectively-measured serial cough counts in these three conditions with which to compare the current data. Reports of the resolution of subjectively-reported cough symptoms in acute respiratory disease are few and varied. The duration and resolution of symptom-defined COPD exacerbations has been investigated,[315,401–403] although cough, classed as a 'minor' symptom,[380] appears to have been researched infrequently in this context. The median time for symptom scores to return to baseline following a COPD exacerbation in the community is probably 7 or 8 days.[315,404] From daily diary cards, cough symptoms (rated by patients as 'increased' or 'not increased',[315] or on a severity scale of 0-3 [ref [403]]) probably take three or four weeks to return to baseline. In acute severe asthma treated with oral

corticosteroids symptoms seem to take 7-10 days to fully resolve,[405] but there do not appear to be studies looking at resolution of cough in asthma specifically.

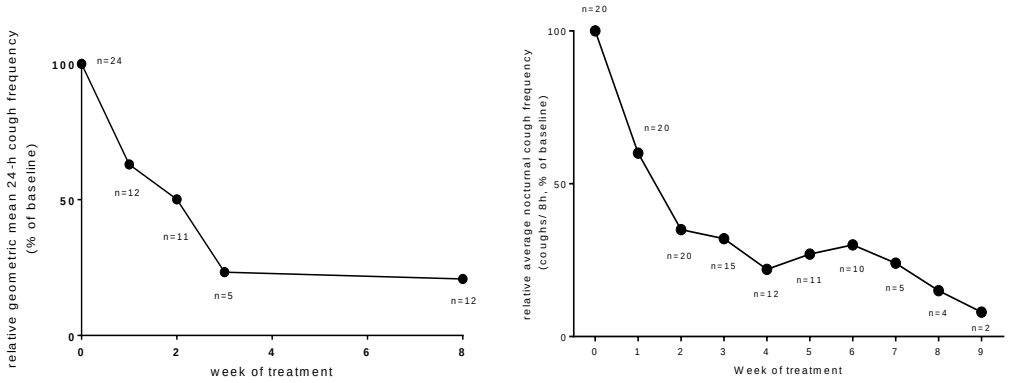
In community-acquired pneumonia a small number of studies, reviewed elsewhere,[324] have prospectively documented the persistence of symptoms following the initial diagnosis and appropriate treatment. In one study which regularly assessed symptoms following diagnosis in a mix patients who had been either hospitalized or treated in the community for pneumonia, cough was reported to take a median of 14 (range, 7-21) days to resolve.[406] In other series cough persisted in 50% at 30 days,[407] and in 32% of patients at 90 days.[408] The relative severity of cough at particular time points following treatment of pneumonia was not reported in any of these studies.

The limited literature on patient-reported cough therefore suggest a more protracted course of symptoms in pneumonia than acute asthma or COPD exacerbations. This difference was not reflected in the results of cough monitoring over the first few days. Early changes in cough frequency may not reflect the total duration of excessive coughing. However, as discussed in Chapter 7, symptoms correlate poorly with objective cough frequency in these conditions, particularly acute asthma and COPD exacerbations. Patients with no chronic lung disease recovering from pneumonia may be more likely to notice frequent coughing which, for them, is a comparatively novel symptom. More data from serial cough monitoring in acute respiratory disease over multiple time points could be very informative.

8.5.2 Tuberculosis

In pulmonary tuberculosis Loudon and Spohn investigated the effect of treatment on overnight cough frequency (Section 1.6.4).[125] Comparisons between the current study and this earlier work are limited by differences in cough monitoring technology and the omission of patient characteristics from the short study report.[125] However, the current study has shown that objective 24-hour cough counts respond to treatment of TB in a very similar way to the 8-hour nocturnal counts reported previously (Figure

8.4). Cough frequency improved at a similar rate in both studies (by approximately 40% and 80% after 1 and 8 weeks, respectively).



Left: current study; data points are geometric mean. Right: from Loudon and Spohn (ref [125]). Data points are means.

Figure 8.4. Rate of resolution of cough frequency during treatment of TB in current study compared to previous work.

Although there was variability, nocturnal cough rates maintained a relatively constant relationship to 24-hour cough frequency after two weeks of TB treatment in the current work. As was shown in Chapter 7 for measuring baseline cough frequency (Section 7.4.2.2), cough recordings overnight in TB might therefore be a more convenient surrogate for 24-hour cough frequency measurement to monitor response to treatment.

8.5.2.1 *Stability in cough frequency during days 0-2*

No significant change in cough frequency was observed from the first monitoring period, started before commencing treatment, and the second period, completed within 48 h of receiving the first TB medications, where this occurred. This has two implications. The first is practical; for serial cough monitoring a baseline, 'pre-treatment' cough frequency measurement could probably be made at any point within the first 48 h of starting treatment with similar results. The fact that the cough recording device does not have to be available at the precise point of receiving the first dose of medication might increase the utility of serial cough monitoring as a clinical

and research tool in tuberculosis, as long as a recording can be made within the first two days.

The second implication is mechanistic; the lack of change in cough within the first 48 h suggests that numbers of live bacteria might not be necessarily directly related to coughing in TB. Serial sputum early bactericidal activity analyses during initial treatment in drug-sensitive disease,[409,410] and the rapid drop in infectiousness immediately following the start of treatment[118,218] suggest that the majority of mycobacteria are killed with the first 24-48 h of TB medication. If Mtb is isoniazid-sensitive, approximately >90% are probably killed within the first two days (although the rate of killing slows down over the following 12 days).[411,412] Physical and chemical components of bacteria, or substances they release before and after death, along with the lung's inflammatory response might therefore be more important in the (unknown) mechanisms of cough in tuberculosis than the direct action of live bacteria.

Consistent with this idea was the observation that cough resolution was no slower in individuals with isoniazid-resistant TB than in those who were isoniazid-sensitive, despite a presumably slower decline in numbers of live bacteria without the action of this highly bactericidal agent.[409] Furthermore, the drug with the most potent bactericidal action in standard current regimens during the third to fifth days of treatment is rifampicin.[411] The availability of this drug in the current study seemed to make little difference to the rate of cough resolution over the first week of treatment compared to that seen in the older work from the pre-rifampicin era.[125]

A large number of secreted proteins and other molecules have been shown to be produced by *Mycobacterium tuberculosis.*[413,414] The role of many of these is unknown and a role in cough is possible. Secreted antigenic peptides would be of greater interest due to a longer half-life than volatile organic compounds given the relatively slow resolution of cough in TB compared to the time frame demonstrated here for pneumonia. Lipopolysaccharides might have a longer half-life still.

Markers of pulmonary or systemic inflammation were not routinely measured in TB in the current study. Although the data in pneumonia showed that the decline in CRP and cough frequency might occur over a similar time frame (Section 8.4.1.3), more detailed observations would clearly be required to test for a direct role of the host response in the mechanism of cough in TB.

The more rapid decline in cough frequency observed here in pneumonia than in TB suggests different mechanisms for cough in the two conditions. Live bacteria, for example, may have a greater (direct or indirect) effect on triggering the cough reflex in pneumonia.

8.5.2.2 *Variability in cough frequency resolution*

A delayed improvement of cough frequency was seen in some patients with TB in the current work (e.g. a third of the monitored individuals had a cough frequency higher than at baseline by Week 2 and/or Week 8), and possible reasons for this were identified. Loudon and Spohn, in the only other similar previous work to the current study, did not discuss possible causes of variation in improvements in objectively-measured cough frequency with treatment of TB.[125] However, a previous retrospective study of subjectively-reported cough involving close to 2,000 patients found that almost 20% of patients still complained of a productive cough after 24 weeks of treatment for pulmonary TB.[305] Some had failed treatment or later relapsed, but the authors speculate that in the majority this was due to smoking, coexistent chronic respiratory disease or irreversible TB-related lung damage. In the current work there was no evidence for smoking as either a predictor of baseline cough frequency in TB (Section 7.5.1.2.1), or of a cough frequency response to treatment.

The same previous study estimated the rate of improvement of cough symptoms, complete resolution in 50 % of patients after about six weeks.[305] Elsewhere, the median time to disappearance of subject-reported cough was about 70 days.[349] This figure fits with an extrapolation from Figure 8.3 in this Chapter; by 70 days, median cough frequency would have been <100 c/h, i.e. probably within the normal range for

healthy individuals (7.4.2.1.9). However, comparing the two studies is problematic given the limited correlation that exists between subjective and objective descriptions of coughing (Section 7.4.3).

8.5.2.3 *Further implications of rates of improvement in cough*

There is an urgent need for new biomarkers in tuberculosis, for use as endpoints in clinical trials of new tuberculosis drugs and regimens,[415] and for individualizing treatment, particularly in drug resistant-TB.[416] Cough frequency measurement could be a useful laboratory-free method of measuring the response to treatment.[259] It seems to detect resolving disease a lot more quickly than the currently most frequently-used clinical measures of treatment response, weight gain,[417] radiographic improvement[418] and sputum microbiological conversion.[304] Cough frequency in the current study was also sensitive to poor treatment adherence.

More data are required to correlate changes in cough frequency with other established markers of treatment response. A limitation of cough monitoring is that not all patients with tuberculosis cough more frequently than would be expected in health (only 73% in the current study). However, serial cough frequency might still be valuable form a lower baseline, but day-to-day random variation and the performance of the cough detection algorithm would become relatively more important. From a public health perspective, patients with the highest cough frequencies would in any case be the priority for measuring treatment response. Due to increased coughing itself, and the possible association with sputum smear positive disease (Section 7.5.1.3.5), these patients are presumably the most infectious.

8.5.3 The serial measurement of cough frequency

Repeated cough monitoring could be a useful and novel tool for measuring responses to treatment in respiratory disease. More data are required to take this forward. One of the next steps will be to establish variability in day-to-day cough frequency in the stable state. This appears to have been done rarely in any context, including isolated chronic cough, the most investigated condition. Loudon and Brown[88] measured intra-

individual variation in cough frequency in a group of inpatients with tuberculosis, pneumonia or chronic bronchitis. On overnight cough recordings on three consecutive nights there was much less variation within than between patients, although it was more marked in COPD. These data are however of limited value, being from only overnight recordings, and presumably not reflecting a stable state as the participants were hospitalized for treatment of their acute lung disease. From baseline variability it will also be necessary to establish the minimal important difference in cough frequency in lung diseases that correlate with meaningful clinical improvements, as has been done in acute cough (by correlation with the main endpoint of symptom scores in that context).[80]

8.5.4 Limitations

The need to establish the repeatability of cough monitoring in the stable state before embarking on larger investigations of changes in cough frequency with treatment has been discussed (Section 8.5.3).

This exploratory work is limited by small sample sizes in the strengths of its conclusions, particularly for acute asthma with only 5 patients. The missing data from serial cough monitoring in with TB also precluded more than a speculative consideration of reasons for the apparent variability in the rate of change in cough frequency. However, an acknowledgement of expected loss to follow up will be useful in future studies investigating improvement in cough in TB and other contexts.

8.6 Conclusion

Serial cough monitoring could be a useful novel approach to measuring treatment response. The varying timeframes over which cough improves with treatment of underlying disease may also reflect how mechanisms of cough differ with pathology.

9 *TRPV1* polymorphism and phenotype in chronic cough

9.1 Background and aim

The previous chapters have demonstrated significant variation in objectively measured cough frequency and cough reflex sensitivity between individuals, within as much as between disease groups. This strongly suggests the presence of as yet uncertain factors influencing cough independent of pathological processes. One such factor could relate to inherent genetic differences between people.

The genetic influences on cough patterns have been investigated rarely. Angiotensin converting enzyme receptor polymorphism may be important for developing ACE inhibitor-induced cough[51,419,420] but probably not for chronic cough of other causes,[52] although it may affect cough reflex sensitivity.[53] Polymorphism in the tachykinin gene *NK-2R* has been associated with cough reflex sensitivity to capsaicin but not with symptoms of chronic cough.[54] There do not appear to have been other studies comparing objectively-measured cough to genetic variation.

There has been interest in the TRPV1 (transient receptor potential vannilloid 1) channel for its role in the cough reflex (Section 1.3).[9,27] The pro-tussive compound capsaicin acts through this channel and TRPV1 antagonists reduces sensitivity of the cough reflex in human studies.[29,30,40] A recent study has identified single nucleotide polymorphisms (SNPs) of *TRPV1* associated with cough symptoms in a large multi-centre population.[55] Six SNPs located on chromosome 17p13.3 appeared to be of particular interest (rs161365, rs17706630, rs2277675, rs222741, rs150854 and rs224498): all but two were associated with chronic cough (defined as coughing during the day or night on most days for as much as 3 months per year, rs161365 and rs224498 were associated only with nocturnal cough), and all but rs222741 were associated with cough independently in each of the two large adult cohorts included in the study (Epidemiological Study on the Genetics and Environment of Asthma,

EGEA,[421] and the European Community Respiratory Health Survey, ECRHS[422]). The putative effects of these polymorphisms in objectively-measured cough is not known.

This Chapter will address the hypothesis that SNPs of *TRPV1* are associated with cough reflex sensitivity and 24-hour cough frequency in chronic cough.

9.2 Methods

This was an observational cross-sectional study carried out at King's College Hospital, London. Participants were patients with chronic cough who had been referred to a tertiary care cough clinic by other respiratory specialists or a general practitioner; recruitment criteria and routine demographic measurements are described in Section 6.3.1.

The primary outcome variable was cough reflex sensitivity to capsaicin. This was measured as C5, the minimum concentration of capsaicin solution required to elicit five coughs (C5). Secondary outcome variables were cough reflex sensitivity measured as the number of coughs produced following inhalation of 62.5 µM capsaicin solution ($E_{62.5}$; Section 6.1), 24-hour cough frequency (CF-24) and cough symptoms (Leicester Cough Questionnaire [LCQ] score and cough visual analogue scale [VAS], score). The key predictor variable was *TRPV1* genotype.

9.2.1 TRPV1 genotype

A saliva sample of approximately 2 ml was taken from each study participant and stored at room temperature using Oragene® collection kits (DNA Genotek, Ottowa, Canada).[423] DNA extraction and analysis were carried out by the Barts and the London Genome Centre (London, UK) in order to genotype the six SNPs of *TRPV1* of interest, rs161365, rs17706630, rs2277675, rs222741, rs150854 and rs224498.[55]

DNA extraction was carried out according to the Genome Centre's protocol, following the recommendations of DNA Genotek.[424] Oragene/saliva samples were incubated at 50 °C before a 1000-µL aliquot was taken from each to be mixed with Oragene Purifier. After incubation on ice and centrifugation, the supernatants were mixed with 95%

ethanol to precipitate out DNA, which was separated out by further centrifugation before being dissolved in standard buffer solution.

For SNP genotyping, purified DNA was run through TaqMan® probe-based polymerase chain reaction (PCR) amplification and allelic discrimination assays in accordance with the manufacturer's instructions (Applied Biosystems, Foster City, California).[425] A fluorogenic 5' nuclease chemistry technique was used to detect specific PCR product following its accumulation during PCR cycles with the use of sequence-specific primers and probes corresponding to the five *TRPV1* SNPs.[426] Automated fluorescence measurements from PCR wells revealed the identity of alleles within in each sample.

9.2.2 Cough variables

Capsaicin inhalation cough challenge was carried out following international guidelines,[5] and as described in Section 6.2.2). The values of C5 were calculated by the method of interpolation. 24-hour cough frequency (CF24) was measured with the Leicester Cough Monitor (LCM), worn by each patient for 24 h (Section 7.2.2), starting after the capsaicin challenge. Before capsaicin challenge testing, participants completed the LCQ (for measurement of cough-related quality of life) and rated the severity of their cough in the previous two weeks with a visual analogue scale (Section 4.2.2).

9.2.3 Statistical methods

A priori estimation of sample sizes for detecting an association between *TRPV1* polymorphism and cough sensitivity and frequency was difficult without knowledge of the expected effect of allele frequency on cough characteristics. From previous work the background minor allele frequency for all the SNPs of interest was assumed to be approximately 0.35.[55] In accordance with the Hardy-Weinberg principle, homozygotes for the minor allele genotype for each SNP were assumed to be present at a frequency of 0.12, heterozygotes 0.46, and major allele homozygotes 0.42 in the background population. For planning the study a working figure of 60 patients was used. Under the

null hypothesis that the *TRPV1* SNP genotype distribution in patients with chronic cough is no different from that in the background population, it was therefore estimated that about 28 major allele homozygotes for each SNP would be recruited, a total of 32 individuals with the two other genotypes (heterozygous or minor allele homozygous).

The minimally clinically important difference in C5 has not been explicitly defined. Despite this, on repeat testing with a standard capsaicin challenge protocol of serial doubling concentrations of solution, 85% of healthy individuals produce values for C5 within one doubling concentration of the initial value.[242] A difference of >1 doubling concentration is therefore probably clinically important. Practically, due to the way in which the test is performed, a difference of less than this would be difficult to detect in any case.

The mean and SD of C5 in isolated chronic cough were not explicit in the existing literature, but other studies using C5 as an outcome variable in chronic cough have used sample sizes of 20-30 in each comparator group.[40,267,427] The power of the study to detect a difference in C5 between genotypes for each SNP was therefore unknown *a priori*. The sample size of 60 was chosen primarily because of feasibility, time and resources for this pilot study.

Univariate analysis was performed for each SNP in two ways. For a between-group comparison, patients were divided into three groups according to genotype (minor allele homozygous, heterozygous or major allele homozygous), and the non-normally distributed outcome variables were compared to look for differences with Kruskal-Wallis tests. Univariate linear regression analysis was also performed for each SNP after normalising data (where necessary) with either a square root or logarithmic transformation, as appropriate. This tested for a dose response of numbers of copies of the minor allele for each SNP under the assumption their effects were additive.[55] A possible confounding effect of ethnicity was tested by including this variable (white *vs*. non-white) into regression analyses.

After genotype data had been provided by the Barts and the London Genome Centre, all further data analysis was carried out by the author.

9.3 Results

57 patients were recruited with chronic cough; 38 with unexplained chronic cough and 19 with cough secondary to other diagnoses. Participant characteristics are described further in Chapter 6 (Section 6.3.1, Table 6.1).

The process of saliva collection, DNA purification and genotyping on the whole was successful. All 57 study participants donated saliva; adequate DNA samples were extracted for analysis of at least the majority of SNPs from 54 patients. Genotyping results were achieved for the following numbers of individuals for each SNP: rs161365, 54; rs17706630, 54; rs2277675, 54; rs222741, 53; rs150854, 51 and rs224498, 54.

Table 9.1. Capsaicin sensitivity, 24-hour cough frequency *TRPV1* genotype; between-group analysis.

	n	C5 (μM/L)	*p*	$E_{62.5}$	*p*	CF24	*P*
Sex							
male	15	15.6 (6.4-16.6)		10 (6-14)		197 (147-611)	
female	42	6.4 (3.4-13.0)	0.091	9 (8-12)	0.694	482 (250-777)	0.101
SNP							
rs161365							
CC	29	10.4 (5.0-19.1)		9 (7-12)		499 (260-840)	
CT	18	6.9 (2.5-12.0)		10 (8-12)		458 (111-636)	
TT	7	3.9 (1.4-16.6)	0.501	8 (8-11)	0.278	251 (147-362)	0.217
rs17706630							
GG	37	7.8 (4.9-15.6)		9 (7-13)		436 (197-636)	
GA	16	5.7 (1.3-15.6)		10 (8-12)		362 (120-976)	
AA	1	15.6 (n/a)	0.379	5 (n/a)	0.303	154 (n/a)	0.583
rs2277675							
TT	26	9.41 (5.3-15.6)		9 (7-12)		499 (205-635)	
TC	25	5.9 (1.9-13.8)		10 (8-13)		362 (154-1187)	
CC	3	22.1 (3.9-49.6)	0.231	8 (6-15)	0.666	104 (42-285)	0.141
rs222741							
AA	28	7.8 (3.9-15.6)		10 (6-13)		338 (111-636)	
AG	18	5.4 (2.5-23.0)		9 (7-12)		425 (292-738)	
GG	7	12.0 (7.8-16.6)	0.244	9 (7-11)	0.758	567 (212-611)	0.664
rs150854							
TT	19	11.0 (5.5-16.6)		8 (6-10)		458 (147-611)	
TG	28	6.1 (3.4-17.1)		10 (8-13)		399 (166-1081)	
GG	4	9.8 (6.0-11.0)	0.699	10 (7-15)	0.225	448 (241-949)	0.778
rs224498							
TT	26	9.8 (5.1-19.7)		10 (8-12)		410 (209-636)	
TG	22	7.8 (3.4-15.6)		9 (7-12)		380 (120-634)	
GG	6	6.7 (0.5-11.0)	0.531	9 (7-17)	0.927	695 (441-1985)	0.389

Values are median (IQR).

9.3.1 Genotype and objective measures of cough

There was no evidence for an association between *TRPV1* polymorphism and either the primary endpoint, C5, or the secondary objective endpoints, $E_{62.5}$ or CF24. This was the case both for Kruskal-Wallis analyses of differences between pairs of genotypes at an individual locus (Table 9.1), and regression analyses to test for a dose response of number of copies of each minor allele (Table 9.2). There were trends for lower cough reflex threshold and higher CF24 in women than men (Table 9.1). Similar results were observed by including ethnicity in the regression analyses.

Table 9.2.Capsaicin sensitivity, 24-hour cough frequency and *TRPV1* minor allele status; regression analysis.

SNP	C5		$E_{62.5}$		CF24	
	Regression coefficient (95% CI)	*p*	Regression coefficient (95% CI)	*p*	Regression coefficient (95% CI)	*P*
rs161365	-0.38 (-1.07 to 0.31)	0.277	-0.05 (-0.24 to 0.14)	0.601	-0.24 (-0.68 to 0.19)	0.263
rs17706630	-0.34 (-1.31 to 0.63)	0.487	-0.14 (-0.40 to 0.12)	0.288	0.02 (-0.59 to 0.63)	0.943
rs2277675	0.10 (-0.68 to 0.89)	0.795	-0.005 (-0.22 to 0.21)	0.964	-0.25 (-0.74 to 0.25)	0.321
rs222741	0.38 (-0.31 to 1.08)	0.274	-0.06 (-0.24 to 0.12)	0.58	-0.14 (-0.31 to 0.59)	0.534
rs150854	-0.29 (-1.18 to 0.61)	0.525	0.04 (-0.18 to 0.26)	0.714	0.32 (-0.22 to 0.85)	0.237
rs224498	-0.47 (-1.19 to 0.25)	0.198	0.08 (0.11 to 0.28)	0.401	0.10 (-0.38 to 0.57)	0.684

Values from logistic regression analyses testing for relationships between each variable and the number of copies of each minor allele. C5 data normalised with square root transformation, $E_{62.5}$ and CF24 normalised with log transformations.

9.3.2 Genotype and cough symptoms

There was no evidence for an association between *TRPV1* polymorphism and cough-related quality of life (LCQ scores) or subjective cough severity (VAS scores; Table 9.3, Table 9.4).

Table 9.3. LCQ scores, VAS scores and TRPV1 genotype; between-group analysis.

	n	Cough VAS score	*p*	LCQ score	*p*
Sex					
male	15	64 (23-85)		11.7 (9.4-15.6)	
female	42	65 (40-74)	0.986	10.8 (8.4-13.2)	0.451
SNP					
rs161365					
CC	29	65 (32-84)		10.4 (7.6-13.2)	
CT	18	56 (39-73)		10.9 (9.1-12.9)	
TT	7	35 (25-56)	0.379	12.4 (10.3-14.9)	0.689
rs17706630					
GG	37	65 (32-80)		11.1 (8.8-12.8)	
GA	16	50 (34-72)		10.7 (8.5-15.0)	
AA	1	n/a	0.518	n/a	0.681
rs2277675					
TT	26	56 (22-80)		11.5 (8.9-13.0)	
TC	25	65 (40-73)		10.3 (8.3-13.1)	
CC	3	62 (n/a)	0.785	13.3 (n/a)	0.596
rs222741					
AA	28	62 (34-72)		10.0 (8.2-13.2)	
AG	18	67 (40-77)		10.3 (8.5-12.8)	
GG	7	48 (20-86)	0.570	12.9 (11.5-13.7)	0.351
rs150854					
TT	19	62 (39-86)		11.5 (8.9-12.9)	
TG	28	65 (30-77)		10.7 (7.5-15.1)	
GG	4	50 (34-65)	0.734	10.7 (7.8-13.7)	0.947
rs224498					
TT	26	65 (50-80)		10.5 (8.3-12.7)	
TG	22	49 (22-73)		11.7 (9.1-14.3)	
GG	6	56 (25-77)	0.407	11.1 (8.9-12.9)	0.529

Values are median (IQR).

Table 9.4. Cough symptom scores and *TRPV1* minor allele status; regression analysis.

SNP	Cough VAS score		LCQ score	
	Regression coefficient (95% CI)	*p*	Regression coefficient (95% CI)	*p*
rs161365	-6.71 (-18.58 to 5.17)	0.261	0.41 (-1.09 to 1.91)	0.583
rs17706630	-3.63 (-20.98 to 13.72)	0.676	-0.14 (-2.28 to 1.99)	0.894
rs2277675	5.18 (-8.31 to 18.66)	0.443	-0.23 (-1.92 to 1.46)	0.788
rs222741	-1.58 (-12.5 to 9.31)	0.771	0.86 (-0.50 to 2.21)	0.211
rs150854	-5.14 (-19.4 to 9.08)	0.469	0.46 (-2.06 to 1.15)	0.570
rs224498	-6.12 (-17.3 to 5.02)	0.274	0.49 (-0.90 to 1.88)	0.481

Values from logistic regression analyses testing for relationships between each variable and the number of copies of each minor allele. Both untransformed VAS and LCQ data are approximately normally distributed.

9.4 Discussion

These data do not support the hypothesis that *TRPV1* polymorphisms influences either cough reflex sensitivity to capsaicin, 24-hour cough frequency or cough symptoms in patients with chronic cough. However, the findings need to be examined in view of the power of the study.

9.4.1 Statistical power

Mean and SD $\log_{10}$ C5 values of all patients in the current study were 0.901 and 0.502, respectively (corresponding to 7.96 and 3.18 µM). For a two-sided α value of 0.05 and a power of 0.80, 44 patients would be required in each comparator group to detect a difference at least as large as one doubling concentration of C5 between them.[309]

The mean frequency of minor allele homozygotes in the current study was 0.09. Therefore, on the assumption that the current study sample was representative of all patients with chronic cough, in order to expect approximately 44 patients to be minor allele homozygous for each of the *TRPV1* SNPs of interest, 489 study participants with chronic cough would be required. However, based on the observed overall mean minor allele frequency of 0.41 and assumptions of the Hardy-Weinberg principle, the expected frequency of minor allele homozygotes was 0.16 (not statistically different from that observed; Fisher's exact test, $p = 0.391$). This would suggest a minimum necessary total sample size of 275 participants.[309]

Therefore for simple statistical tests of comparisons between groups, the current study was underpowered to exclude the possibility of an association between C5 and the minor allele homozygous state for each of the SNPs. For the mean sample size of 5 minor allele homozygous the power was 0.05 for $\alpha = 0.05$, although 0.40 for $\alpha = 0.23$ (corresponding to the lowest value of *p* from Kruskal-Wallis tests of differences in C5 according to genotype).[309]

Despite this, due to the high mean *p*-values ($\alpha = 0.23$) for tests of differences in C5 amongst patients grouped by genotype, the power of the study was probably

adequate to compare the major allele dominant homozygous with the heterozygous genotype for each SNP: 0.79 for the mean group sample size of 24.[309])

Similar conclusions can be made for the other study endpoints – despite relatively small sample sizes, the large *p*-values suggest that a clinically meaningful difference in $E_{62.5}$, 24 cough-frequency, LCQ and VAS scores between heterozygotes and individuals homozygous for each of the major alleles of the SNPs studied is unlikely, but very small numbers limit interpretation of the possible effects of the minor allele homozygous states.

However, if the assumptions of other authors are followed that any phenotypic effects of TRP minor alleles are additive,[55,428,429] the current study does give evidence against a large effect of the minor allele homozygous states; the confidence intervals associated with the regression coefficients on testing for an additive dose effect of each minor allele on cough measurements were wide and all include zero (Table 9.2, Table 9.4)

9.4.2 *TRPV1* polymorphism and cough

Why an association between subjectively-assessed cough and *TRPV1* genotype was observed in the previous study but not in the current work may be either due to either limitations of statistical power, differences in the study samples, or differences in the methods of assessing symptoms. The study of Smit *et* al. included a large population sample made up of a mixture of individuals with asthma and healthy volunteers in whom chronic cough was relatively rare,[55] compared to a relatively small number of patients from a hospital clinic who all had chronic cough in the current work.

Several simple binary measures were the outcome for symptoms in the previous study; the presence or absence of 'usual' cough, nocturnal cough and chronic cough. The presence of chronic cough was defined as a positive response to the question "Do you cough first thing in the morning on most days for as much as three months each year?".[55] Such a question would have produced a positive response in the large majority in the current investigation, which instead used measures from a validated

questionnaire (the LCQ[226]) and a cough visual analogue scale to quantify the personal impact and severity of cough symptoms.

Although the current findings are constrained by small sample sizes, inherent reasons why *TRPV1* variation may not be associated with objectively-measured characteristics of cough, at least amongst patients with chronic cough, should be considered, particularly in light of an association with the presence or absence of cough symptoms in a large population study.[55]

Firstly, cough symptoms have been noted for a long time to have a weak correlation with objectively-measured characteristics of coughing (Section 4.5.4). It was reported almost 50 years ago that there was poor association between cough frequency in respiratory disease and response to the question "do you have a cough at the present time?".[88] *TRPV1* polymorphism may therefore affect the noticeability or memorability of coughing in a manner that is independent of the objective variables measured in the current study. *TRPV1* polymorphism may, for example, impact on the force of coughing, or may even have no impact on the physical characteristics of coughing at all, only on the subjective awareness of cough. The absence of any observed association between genotype and the severity of symptoms however makes this unlikely.

Alternatively, *TRPV1* polymorphism could affect cough in manner which is subtle. Such effects may not be detectable in a group of individuals who experience cough on a frequent basis, due to other underlying influences on cough, but may only become apparent in general population cohorts.

A problem with studying genetic influences on cough is that phenotypes are not static over time. Isolated chronic cough usually responds to treatment[335] and can resolve spontaneously[64] or in response to placebo[67] and behavioural training.[65] TRPV1 receptors are upregulated in chronic cough,[28] but also in response to rhinovirus[34] and allergic inflammation.[430] Objective measurements of cough may therefore need to be

measured over multiple time points in the investigation of potential genetic influences on the response of the cough reflex to different stimuli and respiratory insults.

There has recently been a decline in the interest of several years ago into the role of TRPV1 in the cough reflex.[31] Although TRPV1 antagonists appear to reduce cough reflex sensitivity to capsaicin in patients with chronic cough, this has not been coupled with an effect on cough symptoms or objectively-measured cough frequency.[40] Despite the fact that TRPV1 receptors are involved in the cough reflex, at least when triggered experimentally, their exact role in the complexity of spontaneous coughing is unclear.

Angiotensin-converting enzyme receptor polymorphism seems to be important for developing ACE-inhibitor-induced cough.[51,419,420] Other genetic influences on cough have been demonstrated rarely, although effects on cough reflex sensitivity to capsaicin are similarly not necessarily associated with differences in the amount of noticeable coughing.[54] Study of the genetic variation in cough is important as it may lead to improved understanding of mechanisms and novel approaches to controlling cough. The study of Smit *et al.* is the only of which I am aware which has attempted to compare polymorphisms affecting known components of the cough reflex to reported cough in a large population cohort. SNPs in *TRPV4* and *TRPA1* were also investigated in the same study but were shown not to be associated with the reported presence of cough.[55]

Further large epidemiological studies of potentially important polymorphisms should be undertaken to identify other SNPs which could be tested against objective cough measures in smaller clinical studies. One gene of interest might be *P2X3*, coding for an ATP-gated ion channel, the blocking of which appears to be very effective in reducing coughing in some, but not all patients with unexplained chronic cough.[43,431] Alternatively, large case-control genome-wide association studies could be performed to search for novel genetic loci associated with chronic cough, as has been done in asthma.[432,433]

9.4.3 Limitations

Small sample size has been discussed, which led to a paucity of individuals in the study homozygous for the minor allele variants of each of the *TRPV1* SNPs, and therefore a limitation on any conclusion about associations with these genotypes.

The inclusion only of patients with isolated chronic cough was also a limitation. Cough in other diseases, such as tuberculosis, may have different underlying mechanisms, perhaps of much greater relevance for *TRPV1* polymorphism. It is possible that secreted antigens from *Mycobacterium tuberculosis* (Section 8.5.2.3) act via the TRPV1 receptor, although this has not been investigated. Performing cough challenges in tuberculosis would be more complicated due to problems of infection control, and the need to account for any effects of treatment on cough in TB.

The inclusion of healthy volunteers may have added power to the study and would have allowed the presence of cough to be used as a binary outcome variable, as in the earlier population study.[55] However due to predicted very low 24-hour cough frequency, a very large sample would be required to test for influences on this outcome variable in healthy subjects.

Although C5 has been used as an outcome variable in many other studies, is recommended for this purpose by guidelines,[5] and is stable over time in healthy volunteers[242] its use is limited by a lack of evaluation of repeatability in chronic cough, and it has been criticised for a lack of discrimination between health and disease (Section 6.1).[85] 24-hour cough frequency as an endpoint similarly does not appear to have been assessed for repeatability over time in the stable state in chronic cough and chronic cough (Section 8.5.3). $E_{62.5}$ is a novel study endpoint introduced in this thesis with possible advantages over C5 but awaits further evaluation (Section 6.3.2).

Technical problems led to an inability to obtain any genetic data from the samples of three study participants and a partial lack of data from a further four. However, this yield is probably similar or higher than might have been expected if blood or buccal mucosa samples had been used rather than saliva (C. Mein, personal communication).

Compared to sampling blood, collecting saliva is convenient and painless and has been reported to result in extracted DNA of similar quality and quantity to that from sampling blood.[434]

9.5 Conclusion

This is the first study to compare *TRPV1* polymorphism to objectively-measured cough variables, and one of very few pieces of work to investigate the genetic determinants of cough. Although the number of participants was small, in chronic cough no relationship was observed between each of six SNPs of *TRPV1* and objectively-measured cough phenotypes. *TRPV1* polymorphism is unlikely to have a large clinically important effect amongst patients with isolated chronic cough. This pilot work should be repeated in a further study involving a larger number of participants with and without cough symptoms.

SECTION FIVE:
THE INFECTIOUSNESS OF COUGHS

10 The measurement of airborne particle concentrations during coughing and other respiratory actions

10.1 Background and aims

Tuberculosis is the archetypical aerially-transmitted infectious disease and coughing is assumed to be the way in which the majority of infectious particles are produced. However, the comparison of coughing with other respiratory actions has been rarely investigated in the same study. Comparing studies is difficult due to the variety of techniques used for particle measurement (Section 1.5.2). The pattern of coughing may also be relevant, for example whether coughs occur singly or as part of a prolonged coughing bout, as may physiological parameters, in particular peak expiratory flow rates and the duration of the expiratory cough phase. These variables have not previously been assessed in relation to the generation of exhaled particles as far as I am aware.

The particle size of interest is 0.5-10 µm as this is the theoretical diameter required to access the alveoli upon inhalation and is also thought to be the size that will remain in airborne suspension without being affected by gravity (Section 1.5.2.1).

For logistical and ethical reasons, the direct observation of the aerosolization of *Mycobacterium tuberculosis* from the respiratory tract in patients with pulmonary tuberculosis is difficult as previously discussed (Section 1.6.2.5). Apart from the complexity of directly detecting airborne Mtb,[202] studying this phenomenon would need to involve patients with untreated smear-positive pulmonary tuberculosis since effective antibiotics seem to render TB non-infectious very quickly.[435,436] Although possible,[126] involving such patients entails a risk of cross infection, and deliberately delaying treatment for the purpose of research has potential ethical difficulties. The

large majority of studies on the production of airborne respiratory droplets or droplet nuclei (from here on together referred to as particles)involve healthy volunteers only, on the assumption that in infectious disease at least some of these particles would contain microbes.[127]

Various methods have been employed to attempt to measure airborne particles expelled from the mouth, but among the most reliable seem to be modern techniques which involve laser particle counting equipment (Section 1.6.2.5.4).[127] A simple method recently used by Lai *et al.* used a handheld laser particle counter (Lighthouse 5016) in a room to demonstrate that far higher numbers of airborne particles are produced by blowing on a vuvuzela, a type of plastic horn, than by shouting.[163]

The objectives were,

1. to investigate the potential of a simple technique for measuring airborne particles produced during respiratory actions which would be applicable to a normal clinical environment and,
2. if meeting the first objective, to compare the concentrations of particles released from the mouth during coughing, and from different types of cough, with those released during other actions at different distances form the subject.

The key hypothesis being tested was that coughing releases more respiratory particles than other actions.

10.2 Methods

10.2.1 Study design, setting and participants

This was an observational pilot study carried out at Homerton University Hospital, London. The participants were healthy adults free from respiratory symptoms.

10.2.2 Variables

The concentration of airborne particles measured at a controlled distance from the mouth of diameter >0.3 µm was the outcome variable. The main explanatory variable was nature of the expiratory activity: silent breathing, speech at normal conversational volume and at high volume, and several patterns of coughing. Potential confounding variables were sex, age, smoking and lung function.

10.2.3 Data sources

10.2.3.1 *Particle counter*

Airborne particles were measured with a portable laser diffraction counter, Lighthouse 3016 (Lighthouse Worldwide Solutions, California, USA). The apparatus met the International Organization for Standardization standard ISO 21501-4 concerning the detection and sizing of particles measuring 0.1-10.0 µm.[437] It measured in the range 0.3-25.0 µm using the following diameter range categories: ≥0.3 to <0.5, ≥0.5 to <1.0, ≥1.0 to <3.0, ≥3.0 to <5.0, ≥5.0 to <10.0, and ≥10 µm. Although the device was used routinely for measuring the level of airborne particulate contamination in industrial cleanrooms, a very similar piece of equipment (Lighthouse 5016) has been used in a previous study for measuring exhaled particles close to the subject during shouting and blowing on a vuvuzela as mentioned above.[163] The Lighthouse 3016 and 5016 are identical pieces of equipment apart from the fact that the 3016 has a lower minimum particle detection limit, 0.3 compared to 0.5 µm for the 5016. To my knowledge there has been no comparison of this device with other methods of measuring particles produced during respiratory activities.

10.2.3.2 *Room particle measurement*

The particle counter was placed at a sitting head height in the centre of a clinic room of length, width and height 4.28, 2.30 and 2.62 m, respectively, with the study subject (this author) sitting facing the machine at a distance of 1.7 m from it (Figure 10.1). This distance was chosen to exclude larger droplets released from the mouth which should have fallen to the ground, but to also remain within the predicted propulsive range of

the cough air jet, assuming a cough velocity of approximately 10 m/s, and the other conditions discussed in Section 1.5.4, including RH 50% and ambient temperature 20 °C (Section 1.5.4.2). The machine took measurements every 2 s over a one-minute (30-cycle) period on three occasions whilst the subject carried out the following actions:

1. silently sitting with mouth closed;
2. silently walking around room;
3. loudly talking (counting) whilst seated;
4. openly coughing repeatedly into room;
5. coughing repeatedly into his hand;
6. coughing repeatedly whilst wearing a surgical mask;
7. coughing repeatedly while wearing a filtering face protector (FFP)-3 mask.

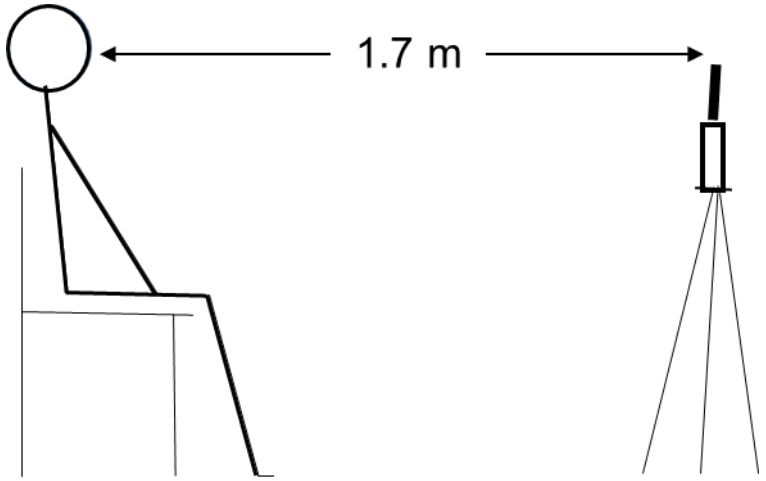


Figure 10.1. Positioning of subject and particle counter during measurement of room airborne particles.

Between each one-minute measurement period the total room air volume was changed by vacating the room and running the negative pressure ventilation (operating at 12 air changes/h) for 5 min. The subject repeated all the actions once. As control measurements, particle counts were also taken from the empty room (after changing the room air volume), and during the nebulization of normal saline, using a Medix AC2000 nebulizer (Clement Clarke International, England). According to the manufacturer,[438] the median aerodynamic diameter of nebulized particles was 3.4 µm

with 72% of particles <5 µm. Repeat measurements were taken from the empty room several hours after the first. Mean room particle concentrations were calculated for each corresponding activity.

10.2.3.3 *Near-subject particle measurement*

Whilst seated, participants carried out each of the actions listed below for 5 s. On every occasion, the participant held up the smaller end of a horizontal truncated hollow paper cone to their mouth. The particle counter was positioned at the other end of the cone and it took one measurement every second. In between actions the subject removed the cone from the mouth for several seconds. After reviewing interim data, the size of the cone was changed part way through this study in order to try and amplify any differences between activities. The first was of length 45.0 cm, diameter of smaller opening 10.0 cm, and diameter of wider opening 26.0 cm, the second cone had dimensions 14.0 x 1.6 x 5.0 cm, respectively (Figure 10.2).

The cycle of actions was performed three times for each subject, maintaining the cone configuration the same throughout. Particle measurements were also taken for 5 s from the end of the empty cone in between cycles. The instructions to the participants were as follows:

1. sit silently and breathe through your nose (for larger cone) or mouth (for smaller cone);
2. count upwards from one at a normal speaking volume;
3. count upwards from one at a loud speaking volume;
4. cough four times, taking a full inspiration between each one;

5. cough four times in one full breath.

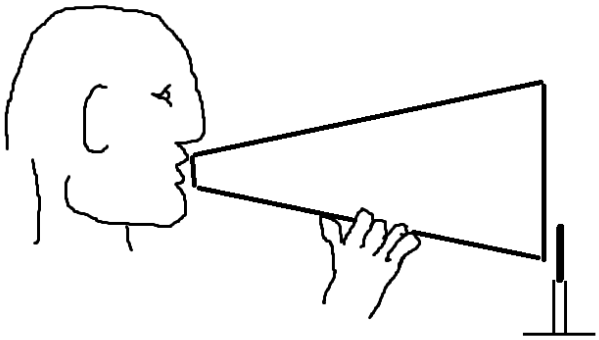


Figure 10.2. Positioning of truncated cone and particle counter during near-subject measurement of airborne particles from respiratory actions.

The peak, rather than the mean, airborne particle count during each action was taken for analysis. This was on the basis that the mean particle concentration during the 5 s activity may have included measurements, particularly at the beginning of the of the episode during nasal or mouth breathing, which did not include particles released from the respiratory tract, due to the time taken to travel down the cone.

10.2.3.4 *Other measurements*

Study participants wore a digital audio recorder and microphone during particle measurement in order to count the number of coughs. Subjects' peak expiratory flow rate (PEFR) and peak cough flow rate (PCFR) were measured with a portable peak flow meter (Mini-Wright Standard Range EU Scale, Clement Clarke International, England)[439,440] before proceeding with the particle measurements. The ambient temperature and relative humidity were also recorded with the in-built thermometer and hygrometer of the particle counter.

10.2.3.5 *Bias*

Blinding the participants to the type of activity they were performing was not possible, nor was blinding the investigator.

10.2.3.6 *Study size*

There were few data on which to estimate variation for calculating a minimum necessary sample size for this part of the study. Based on the closest similar study to this[163] I planned to involve a minimum of eight participants.

10.2.3.7 *Statistical methods*

For the data from room particle measurement, particle counts for pairs of scenarios were compared with two-sample t-tests, after verifying that data were normally distributed.

For near-subject particle measurements, the mean peak readings from the three attempts at each activity were taken in each subject for each cone. The mean difference in this value between pairs of actions across participants was calculated and tested for a statistically significant difference from zero in each case with one sample t-tests after testing for a normal distribution in the data.

10.3 Results

10.3.1 Room particle measurement

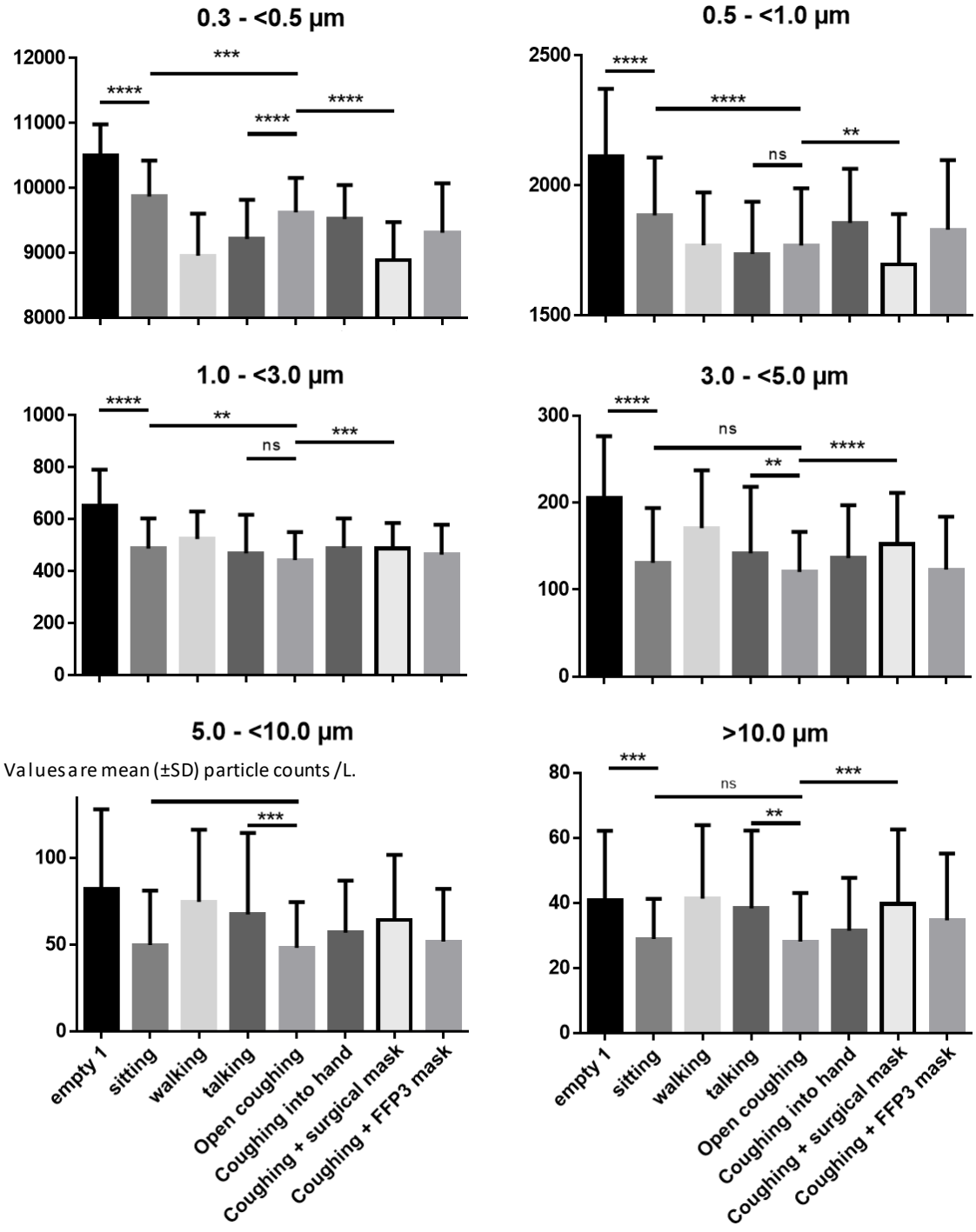
Comparing room particle concentrations during open coughing and quiet sitting there was no difference for three of the particle size ranges (Figure 10.3, Table 10.1). For each of the ranges >0.3 to ≤0.5 µm, >0.5 to ≤1.0 µm and >1.0 to ≤3.0 µm, however, airborne particle counts were significantly lower during open coughing to quiet sitting. There was no significant difference in counts on comparing coughing to talking for two size ranges. For each of the >3.0 to ≤5.0 µm, >5.0 to ≤10.0 µm and >10 µm size ranges, however, room particles counts were lower during coughing, and for >0.3 to ≤0.5 µm counts, were higher whilst the subject was coughing compared to during talking.

For all size ranges, measured room particle concentrations were significantly lower in the presence of the sitting subject compared to in the empty room. Wearing a surgical mask was associated with lower room particle concentrations during coughing for >0.3

- ≤0.5 µm and >0.5 - ≤1.0 µm particles and higher concentrations for all other particle size ranges compared to coughing openly into the room (Table 10.1, Figure 10.3).

Counts were substantially raised during the nebulisation of saline for all particle sizes ($p < 0.001$, Table 10.1). Repeat measurements from the empty room several hours after the first revealed a large increase in particle concentrations ($p < 0.001$; Table 10.1). The room temperature ranged from 23.0 to 24.6 °C. Ambient humidity also varied, from 39.8%, when the room was empty, to 50.0% during the nebulisation of saline. The peak cough flow rate of the subject was 410 L/min (6,833 cm^3/s). As the cylindrical mouthpiece of the peak flow meter was of diameter 2.8 cm, the corresponding cough flow velocity through the mouthpiece was 11.1 m/s.

The variation in room particle measurements was high during each activity. This was particularly the case for particle ranges ≥1.0 µm, where the relative standard deviation (SD: mean ratio) was often >0.25, and for ranges ≥5.0 µm, >0.50 (Table 10.1).



Values are mean (±SD) particle counts /L.

Counts given per L.

Figure 10.3. Mean (SD) Room particle counts during expiratory manoeuvres from one subject.

Table 10.1. Room particle counts during expiratory manoeuvres by one subject.

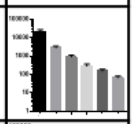
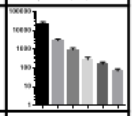
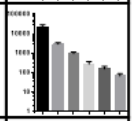
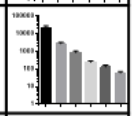
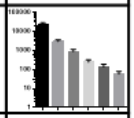
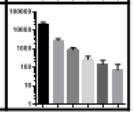
Particle size (µm):	≥0.3-<0.5	≥0.5-<1.0	≥1.0-<3.0	≥3.0-<5.0	≥5.0-<10.0	≥10	Total
Empty room (1)	10,497 (±486)	2,113 (±260)	651.6 (±139.8)	205.9 (±70.8)	81.88 (±46.13)	40.83 (±21.39)	13,575 (±810)
Sitting	9,870 (±554)	1,885 (±222)	487.5 (±116.1)	130.9 (±63.1)	49.78 (±31.36)	28.96 (±12.32)	12,355 (±1,284)
Walking	8,977 (±623)	1,778 (±186)	527.5 (±101.6	172.0 (±65.6)	75.15 (±41.47)	41.59 (±22.65)	11,467 (±1,224)
Talking	9,219 (±601)	1,737 (±201)	469.9 (±147.7)	143.2 (±46.3)	67.40 (±46.99)	38.37 (±23.96)	11,573 (±1,229)
Open coughing	9,627 (±530)	1,770 (±220)	443.7 (±107.2)	120.4 (±46.3)	48.69 (±26.06)	29.01 (±14.30)	11,940 (±1,262)
Coughing into hand	9,526 (±521)	1,856 (±208)	490.3 (±113.1)	136.3 (±60.7)	56.96 (±29.92)	32.34 (±15.66)	12,003 (±1,239)
Coughing + surgical mask	8,895 (±583)	1,697 (±194)	489.1 (±97.0)	152.6 (±58.9)	64.31 (±37.50)	39.67 (±22.94)	11,247 (±1,244)
Coughing + FFP3 mask	9,315 (±761)	1,831 (±267)	464.4 (±114.9)	123.3 (±60.6)	51.77 (±30.43)	34.67 (±20.57)	11,729 (±1,462)
Empty room (2)	20,394 (±1,003)	4,775 (±402)	2,163.6 (±268.1)	969.7 (±159.3)	421.83 (±105.28)	113.31 (±48.25)	28,836 (±1,632)
Nebulised saline	498,685 (±130,742)	211,149 (±64,396)	49,299.4 (±15,922.9)	2,582.9 (±823.9)	118.27 (±53.01)	30.71 (±15.88)	762,020 (±211,130)

10.3.2 Near-subject particle measurement

The larger cone was used by 11 participants (2 current smokers; 4 female) and the smaller cone by 8 (no current smokers; 5 female). For the participants using the larger cone, median (IQR) age was 31 (27-33) years, mean (SD) PEFR 591 (155) L/min and PCFR 390 (124) L/min. For the smaller cone experiments these values were 37 (31-54) years, 523 (217) L/min and 322 (135) L/min, respectively. Measured mean PCFR was lower than PEFR (p = 0.0005 and 0.004, for subjects using the large cone and small cone, respectively). The mean peak particle counts at the exit of the cones are shown in Table 10.2 and Table 10.3. A crude visual inspection of the data gives no indication of a difference in the distribution of particle sizes between respiratory activities. Only a small minority of the observed differences in mean peak particle counts between activities were statistically significant (Figure 10.4). For example, counts were lower for the majority of particle size ranges when coughing four times in separate breaths

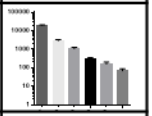
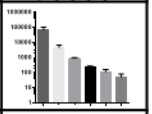
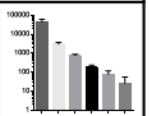
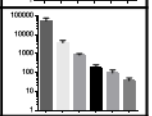
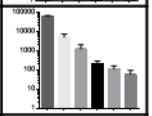
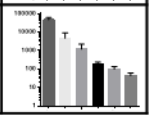
compared to counting loudly through the larger cone, and significantly fewer particles were counted for half of the size ranges during counting at normal volume than during breathing into the smaller cone.

Table 10.2. Peak airborne particle counts at exit of larger cone during respiratory actions.

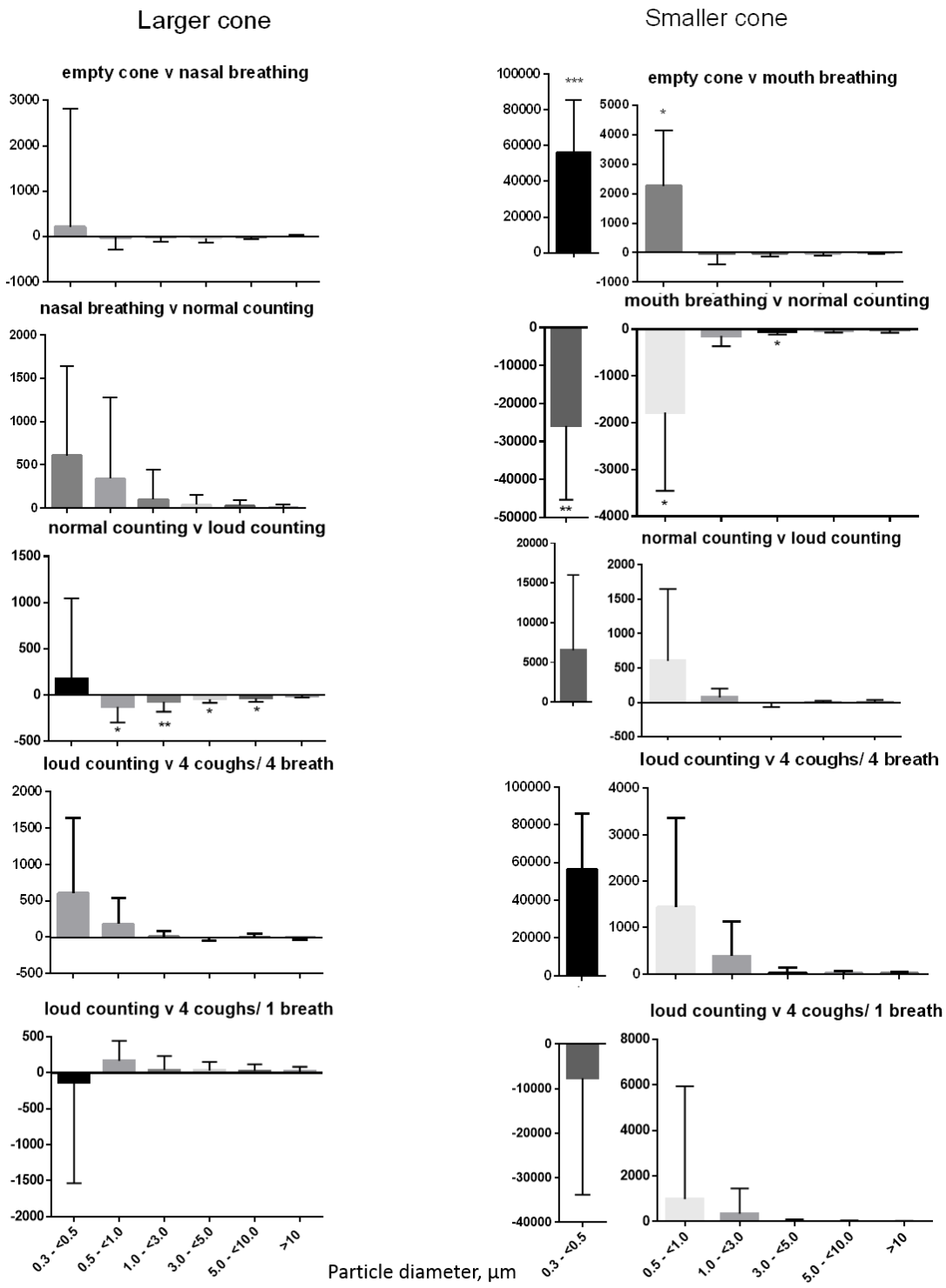
Particle size (µm):	***≥0.3- <0.5***	***≥0.5- <1.0***	***≥1.0- <3.0***	***≥3.0- <5.0***	***≥5.0- <10.0***	***≥10***	***Total***	
Empty cone	22,097 (5,851)	2,902 (647)	928.5 (225.2)	287.7 (77.6)	157.95 (42.76)	68.06 (14.22)	**26,440 (6,589)**	
Nasal breathing	22,703 (5,766)	2,935 (519)	955.8 (216.9)	282.5 (95.7)	159.24 (52.51)	68.70 (21.90)	**27,103 (6,242)**	
Counting at normal volume	22,277 (5,446)	2,964 (593)	964.4 (246.1)	294.7 (76.0)	170.15 (39.63)	71.27 (18.27)	**26,742 (6,088)**	
Loud counting	22,452 (6,098)	2,835 (526)	890.6 (195.9)	249.1 (50.1)	134.20 (38.69)	61.64 (13.06)	**26,623 (6,640)**	
4 coughs, 4 breaths	22,987 (5,990)	3,018 (654)	898.7 (234.2)	248.6 (81.2)	136.64 (50.63)	57.79 (21.38)	**27,346 (6,759)**	
4 coughs, 1 breath	22,187 (5,534)	3,009 (640)	941.2 (267.7)	285.3 (126.8)	163.09 (88.97)	80.52 (76.67)	**27,070 (6,431)**	

Values are mean (±SD) particle counts /L from measurements in 11 volunteers. Bars on right correspond to the same particle size ranges as in the columns and ordered in the same sequence with a logarithmic scale on the y-axis.

Table 10.3. Peak airborne particle counts at exit of smaller cone during respiratory actions.

Particle size (µm):	***≥0.3-<0.5***	***≥0.5-<1.0***	***≥1.0-<3.0***	***≥3.0-<5.0***	***≥5.0-<10.0***	***≥10***	***Total***	
Empty cone	19,137 (1,599)	2,951 (345)	1,082.4 (195.6)	298.9 (51.0)	159.80 (42.87)	68.86 (20.59)	23,697 (1,800)	
Mouth breathing	73,035 (32,014)	4,874 (2,093)	910.2 (114.0)	238.8 (48.6)	119.63 (47.62)	51.65 (29.78)	79,229 (33,934)	
Counting at normal volume	47,145 (16,736)	3,094 (706)	772.1 (149.1)	187.6 (58.2)	90.94 (30.31)	46.62 (27.63)	51,319 (17,295)	
Loud counting	53,682 (20,988)	3,699 (1,280)	845.8 (166.1)	186.7 (69.4)	95.79 (39.12)	37.52 (14.86)	58,546 (21,848)	
4 coughs, 4 breaths	60,153 (10,449)	5,140 (2,154)	1,235.1 (799.0)	219.0 (72.5)	113.01 (49.22)	58.71 (35.66)	66,919 (12,062)	
4 coughs, 1 breath	46,073 (15,514)	4,682 (4,506)	1,198.5 (1,034.5)	190.7 (47.5)	98.44 (35.94)	43.70 (16.66)	52,286 (19,899)	

Values are mean (±SD) particle counts /L from measurements in 8 volunteers. Bars on right correspond to the same particle size ranges as in the columns and ordered in the same sequence with a logarithmic scale on the y-axis.



Counts given per L. Differences not statistically significant unless indicated (* $p < 0.05$; ** $p < 0.01$).

Figure 10.4. Mean (SD) differences in peak airborne particle counts at exit of cone during selected pairs of respiratory activities.

10.4 Discussion

10.4.1 Room particle measurement

The significant differences observed in room particle concentrations at different times are difficult to explain by considering only the type of action performed by the subject. The finding that concentrations of particles measuring <3.0 µm decreased during coughing compared to sitting in silence was unexpected. On comparing coughing to talking, there was either a reduction or no significant difference in particle concentrations; only for the ≥0.3 to <0.5 µm range was coughing associated with higher particle concentrations. This also appears counter-intuitive, although Morawska *et al.* found exhaled particle concentrations to be higher during talking to coughing.[150] However, in that study the type of cough was described as a mild throat clearing rather than a loud open cough, as in the current work.

Wearing a surgical mask was associated with a statistically significant increase in room particle counts during coughing for the four larger particle size ranges. I would not necessarily expect surgical masks to affect the release of particles of a smaller size, as they are carried in air leaks around the edge of the mask,[290,441] but surgical masks should eliminate some of the larger particles released while coughing, as they have been proven to reduce the transmission of tuberculosis from infectious source cases.[222]

Compared to readings taken from the empty room, the observed drop in the concentration of airborne particles associated with the presence of the subject could have been due to electrostatic attraction between particles and the subject or his clothes.[442] Electrostatic forces could also be relevant specifically for the aerial movement of microorganisms yet have received little attention.[443,444] For example, the amount of clothing worn and types of fabric used might have an impact on the airborne transmission of infection, in turn influenced by climate, housing, socio-economic status, culture and gender.

These apparent paradoxical findings could all be explained by fluctuations in the background particle count, rather than by the nature of the activity performed. The large increase in particle counts with nebulized saline supports the reliability of the measurement device, yet the difference in particle counts in the empty room at different times suggests large variability in ambient particle concentrations. The clinic room in which the measurements were taken was situated at the front of the hospital near the car park in a large city. Changing the air in the room in between activities during the study with the negative pressure ventilation, although intended to remove any particles generated by the subject, would have accelerated the movement of particles into the room. Airborne particles generated by vehicles and other sources outside the room therefore presumably had a strong influence on the observed wide variability in particle counts.

The method of attempting to measure particles released from the mouth in a clinic room at a distance of 1.7 m away therefore seems to have been flawed, mainly due to the dilution by the much larger concentrations of particles already in the room, which appeared to fluctuate due to external influences.

10.4.2 Near-subject particle measurement

Attempting to measure released airborne particles closer to a subject should have been a more sensitive method due to greater concentrations compared to measurements taken further away. However, few significant differences in peak concentrations of any airborne particle size range close to the mouth were detected when comparing pairs of respiratory actions across a group of volunteers. The majority of these significant differences were also counterintuitive. For example, it appeared that, for most size ranges, fewer particles were released from the larger cone when counting loudly compared to counting at normal volume, and that fewer particles were released from the smaller cone when counting at normal volume than during quiet breathing. Also, for the smaller cone, the highest counts of particles of diameter >1.0 µm were from the empty cone, whilst those for particles measuring <1.0 µm occurred

during breathing. However, due to a lack of adjustment for multiple statistical comparisons (e.g. with a Bonferroni correction), it would be expected that some of the observed differences would have appeared significantly different at the 5% level by chance alone.

These findings are in contrast to other recent work, most notably that of Morawska et al.,[150] but also of others,[127,153,163] who have shown talking and coughing to produce higher particle concentrations than breathing. The methodology used in the current study was different to the majority of other work. In particular, background aerosol particles were not eliminated. This has been done in other work using high-efficiency particulate arrestance (HEPA)-filtered air,[445] a specially-constructed experimental rig,[150] a biological safety cabinet,[153] or a clean room.[283] Consequently, particle concentrations of diameter 0.3-20 µm measured during coughing by Morawska *et al*.[150] were almost 60 times lower than those measured in one part of the current study for the same size range (c. 460/L compared to 27,070/L at the exit of the larger cone). As with the room measurements, the near-patient airborne particle counts would have been influenced by fluctuations in background particle concentrations.

However, other work which has not eliminated background air particle counts has achieved positive results. In particular, the study of Lai *et al.* used a technique almost identical to the current work with a very similar particle counter (Lighthouse 5016) for measuring peak particle concentrations whilst shouting into a paper cone compared to blowing through a vuvuzela.[163] The study environment was a 'closed room free from drafts', with no mention of removing background particulate matter with a HEPA filter, and the laser particle counter was placed at the exit of the vuvuzela or cone. It was this earlier study which inspired to the methodology for the current work. Mean peak particle concentrations in the size range 0.5-25.0 µm were >175 times greater when participants blew on the vuvuzela than when shouting (658,000 *vs.* 3,700/L).[163] The reason that the methodology worked for Lai *et al*. was presumably simply because the number of particles produced by the vuvuzela was so great that the method of measurement to demonstrate this could afford to be relatively crude. The data from

the current study actually add weight to the findings of Lai *et al*. by suggesting that the vuvuzela can produce more particles than coughing or shouting; the same method which showed a large difference in particle production between shouting and blowing the vuvuzela failed to show a difference between loud talking and coughing. By contrast, in order to demonstrate differences in particle productions between simpler respiratory actions, a more sensitive measurement technique is presumably required, namely one that accounts for background aerial room particles. For example the difference in airborne particle counts released into a rig containing HEPA-filtered air from nasal breathing and coughing was approximately 30 *vs.* 460/L in the study of Morawska *et al.*[150] (a factor of 15.3), whereas the total background airborne particle count in one part of the current study was almost 27,000/L with a standard deviation of 6,750/L.

Because the initial objective of the study was not met, to validate the handheld particle counter for measuring particles released from the respiratory tract in a normal clinical environment, I did not attempt to investigate the effects of cough flow rate, participant gender, age and smoking on particle production. Other methodology, such as the HEPA-filtered experimental rig of Morawska's *et al.*, would be more suitable for this purpose.[150] As that system is enclosed from the surrounding environment it also has the advantage of having fewer problems with infection control than an open room if directly studying potentially transmissible disease. The same research group have used the apparatus on patients as well as healthy volunteers, specifically to observe the aerosolization of *Pseudomonas aeruginosa* in cystic fibrosis.[446] This could serve as a very useful model for studying the patterns of cough and other respiratory actions in relation to the aerial release of other pathogens, and could potentially be adapted directly to study tuberculosis, although airborne Mtb is more difficult to detect than *Pseudomonas*,[202,447] particularly when the average rate of airborne release infectious particles in pulmonary tuberculosis is perhaps only 1.25/h.[218,221]

In contrast to other work with healthy volunteers, peak cough flow rates in this study appeared lower than peak expiratory flows rather than the other way round.[448] This is probably an artefact of the measurement technique; recent work has suggested that hand-held peak flow meters underestimate cough flow peak rates when compared to readings made with a pneumotachograph.[440] Accelerations in flow rates during the expulsion of air are greater during a cough than during forced expiration.[440,448] The inertia and friction acting against movement of the sliding pointer of the Mini Wright peak flow meters is therefore more likely to be a problem during a cough than during forced expiration.

The results of this study, in common with a number of others investigating airborne particle production during respiratory actions, are only applicable to healthy volunteers. However, this does not detract from my main conclusion that a detailed investigation of this type should attempt to eliminate background aerial particles, whether using patients or healthy controls.

10.5 Conclusions

In the majority of circumstances, a simple hand-held laser counter was not suited for the measurement of particles released from the respiratory tract into ambient air by healthy volunteers. For further investigation into the role of respiratory activities on the aerosolization of material from the respiratory tract, closed systems which eliminate background airborne particles are likely to be necessary.

11 Cough in pulmonary tuberculosis and household infection

11.1 Background and objectives

The transmission of tuberculosis is determined by a complex interaction between a number of factors affecting the infectiousness of the source case, the chance of the environment permitting the aerial transit of viable *M. tuberculosis* (Mtb), and the susceptibility of others to new infection. Other than the bacterial status of the sputum, data relating to the determinants of source case infectiousness are few.[200] The subject of this thesis, coughing, is assumed to be important in the transmission of TB, but this has not been directly proven. The single previous study involving cough frequency monitoring was limited by measuring only nocturnal cough counts. It found only a statistically insignificant trend for a measure of infectiousness with cough frequency which was not necessarily independent of the stronger predictors of infectiousness, sputum smear and culture status, and radiological extent of disease.[125] Another study has shown that subjectively more severe coughing is associated with Mtb transmission.[234] However, as has been demonstrated in earlier chapters (Section 7.4.3) subjective interpretations of coughing correlate poorly with objective measurements.

This chapter with describe the investigation of the following hypotheses:

- index case-reported cough in TB is a predictor of Mtb exposure amongst household contacts;

- 24-hour cough frequency is a determinant of the infectiousness of tuberculosis.

11.2 Methods

This was an observational study, part retrospective and part prospective, carried out at Homerton University Hospital, Hackney.

11.2.1 Household contacts

The household contacts of patients notified with TB were screened for Mtb infection at Homerton University Hospital as part of routine care as recommended by national guidelines.[327] Their data were extracted from medical records retrospectively. Household contacts were defined as individuals living in the same house as the index case and sharing a bedroom, kitchen, bathroom or sitting room.[327] Demographic characteristics were noted, particularly those with previously-reported associations with risk of acquiring Mtb infection, including ethnicity, age, country of birth, HIV infection, and BCG vaccination status.[449] Age was categorized as <5 y, 5–14 y and ≥15 y in line with other work,[196,450] and corresponding to differences in TB epidemiology.[451] Country of birth was classified as high or low TB incidence as defined by the World Health Organization with a cut off national incidence of 40 cases per 100,000 population.[452,453]

Mtb infection status was estimated by the response to Mantoux testing and/ or interferon-γ release assay (IGRA; usually QuantiFERON® [QFT; Cellestis, Australia]), the tests performed according to standard procedures.[327,328] Routine practice at the Homerton University Hospital TB clinic was for both tests to be carried out in contacts aged 14-35 yrs. Children <14 years were usually tested with Mantoux alone, followed by IGRA if positive. Individuals >35 y were offered an IGRA and a chest x-ray without Mantoux testing. Because of the usual delay in developing immune sensitivity following Mtb infection, tests were repeated after 6 weeks if negative at baseline in case of recent transmission events. The Mantoux test was considered positive if the skin induration diameter measured ≥5 mm 48-72 h after tuberculin administration in BCG-naïve individuals or ≥15 mm following previous BCG vaccination, or had increased by >5 mm on re-testing.[327] Indication of infection with Mtb was followed by investigation for active TB.

11.2.2 Patients

Retrospective data

All patients notified to Public Health England (formerly the Health Protection Authority) as cases of tuberculosis at Homerton University Hospital during 2012 and 2013 were included.

Prospective data

Patients with tuberculosis were prospectively recruited for 24-hour cough monitoring before or on starting anti-TB medication. In order to increase the probability of observing recent TB transmission events, only patients with sputum smear-positive pulmonary disease were included. Routine clinical, radiological and microbiological data were collected for all patients as previously described (Section 3.3).

11.3 Analysis of data

The primary outcome measure was presumed infection with Mtb as determined by Mantoux and/or IGRA as just described. Univariate analysis of candidate categorical and continuous predictor variables was performed with Mantel-Haenszel tests and logistic regression, respectively. Variables possibly associated with Mtb infection ($p < 0.15$) were entered into multivariate logistic regression models as previously described (Chapter 2), fitted using a generalized estimating equation in Stata to control for clustering of subjects within households. It was decided *a priori* to add the presence of cough (retrospective dataset) or cough frequency (prospective data) to the models, being the main variables of interest. Adjusted odds ratios were derived from the logit models by raising *e* to the power of the logistic coefficient. This was done separately for the retrospective and prospective datasets.

11.4 Results

11.4.1 Retrospective data

470 individuals were screened for Mtb infection as contacts of 299 notified cases of TB during 2012-13. Associations of characteristics of these individuals and the index cases are shown in Table 11.1 and Table 11.2. On adjusted univariate analysis, contact characteristics associated with Mtb infection were TB incidence in the country of birth and age. Insufficient data on smoking in contacts were collected for analysis and there were only five patients with HIV to investigate its role as a risk factor for Mtb infection. Index characteristics associated with infection in contacts included site of disease (pulmonary, as opposed to extra-pulmonary tuberculosis). Amongst contacts of pulmonary TB, on adjusted analysis the presence of cough in the index case, sputum smear status of the index case, and index disease due to a Haarlem strain of organism appeared important. There were also independent trends for associations of index case sputum smear grade, radiological extent of disease, and the presence of visible cavities with Mtb infection in contacts.

On multi-variate analysis of all contacts, TB incidence in contact country of birth (OR 1.9 [1.1-3.1], $p = 0.02$) and site of index case disease (pulmonary *vs*. extrapulmonary, OR 2.0 [1.2-3.4], $p = 0.01$) were the only variables with an independent significant association with Mtb infection. For Mtb infection in contacts of pulmonary TB, only TB incidence in contact country of birth and sputum smear status appeared independent important explanatory variables (Table 11.2), with the presence of cough not being significant. Similarly, reported cough was not an independent predictor of Mtb infection looking only at contacts of smear positive pulmonary TB (data not shown).

Amongst the screened household contacts there were 10 co-prevalent cases of tuberculosis. Only two of these had smear-positive pulmonary disease; both were contacts of different cases of smear positive index TB. One was the only screened contact of the associated index case, whereas the other was one of four screened household contacts, the other three of whom had latent infection.

Table 11.1. Univariate analysis of variables associated with M. tuberculosis infection in household contacts of tuberculosis (retrospective cohort).

	CONTACTS		Univariate unadjusted analysis		Univariate adjusted analysis*	
	Total no.*	No. (%) infected	OR (95% CI)	*p*	OR (95% CI)	*p*
CONTACT CHARACTERISTICS						
TB incidence in country of birth						
<40/100,000	276	59 (21.4)	1		1	
≥40/100,000	132	50 (37.9)	2.24 (1.41-3.56)	0.0004	2.72 (1.63-4.15)	0.0001
Ethnicity						
white	125	34 (27.2)	1			
non-white	337	92 (27.3)	1.00 (0.64-1.56)	0.987		
Sex						
male	226	66 (29.2)	1			
female	242	61 (25.2)	0.82 (0.54-1.23)	0.332		
Age (y)						
<5	55	9 (16.4)	1		1	
5-14	98	19 (19.4)	1.23 (0.51-2.94)	0.643	1.21 (0.51-2.88)	0.671
≥15	316	99 (31.3)	2.33 (1.10-4.95)	0.028	2.41 (1.13-5.11)	0.022
Previous BCG						
no	57	17 (29.8)	1			
yes	381	99 (26.0)	0.83 (0.45-1.52)	0.540		
Diabetes mellitus						
no	457	122 (26.7)	1			
yes	13	5 (38.5)	1.44 (0.50-4.12)	0.496		
Household contact						
no	61	17 (27.8)	1			
yes	379	104 (27.4)	0.98 (0.53-1.79)	0.945		
INDEX CASE CHARACTERISTICS – CONTACTS OF ALL PATIENTS						
Case subsequently denotified						
no	431	120 (27.8)	1			
yes	39	7 (17.9)	0.64 (0.28-1.48)	0.300		
Site of disease						
extrapulmonary	132	24 (18.2)	1		1	
pulmonary	299	96 (32.1)	1.77 (1.08-2.89)	0.023	2.08 (1.19-3.63)	0.010
INDEX CASE CHARACTERISTICS – CONTACTS OF PULMONARY TB ONLY (n = 299)						
Sex						
male	208	68 (32.7)	1			
female	91	28 (30.8)	0.92 (0.54-1.56)	0.744		
Current smoking						
no	175	56 (32.0)	1			
yes	88	27 (30.7)	0.87 (0.30-2.50)	0.795		
Sputum smear status						
negative	110	28 (25.5)	1		1	
positive	187	68 (36.4)	1.67 (0.99-2.83)	0.053	1.92 (1.02-3.60)	0.043
Sputum smear grade						
+/-, 1+, or 2+	130	39 (30.0)	1		1	
3+	57	29 (50.9)	2.42 (1.25-4.65)	0.006	2.10 (0.98-4.50)	0.056
M. tuberculosis* strain						
Beijing						
no	225	77 (34.2)	1			
yes	10	5 (50.0)	1.92 (0.54-6.88)	0.307		
Haarlem						
no	197	62 (31.5)	1		1	
yes	38	20 (52.6)	2.42 (1.18-4.95)	0.012	2.62 (1.08-6.32)	0.033
Visible cavities						
no	196	56 (28.6)	1		1	
yes	103	40 (38.8)	1.59 (0.96-2.63)	0.071	1.80 (0.96-3.39)	0.069
Radiological extent						
0-1 zones	148	38 (25.7)	1		1	
>1 zones	151	58 (38.4)	1.81 (1.10-2.97)	0.019	1.62 (0.92-2.86)	0.095
Cough reported						
no	38	6 (15.8)	1			
yes	247	88 (35.6)	2.95 (1.18-7.42)	0.016	2.83 (1.02-7.86)	0.046
Cough duration (per month)			1.02	0.177		

Table 11.2. Multivariate analysis of variables associated with M. tuberculosis infection in household contacts of pulmonary tuberculosis (retrospective cohort).

	CONTACTS		Multivariate adjusted analysis	
	Total no.*	No. (%) infected	OR (95% CI)	*p*
TB incidence in country of birth				
<40/100,000	166	48 (28.9)	1	
≥40/100,000	73	29 (39.7)	2.33 (1.23-4.45)	0.010
Sputum smear status				
negative	99	24 (24.2)	1	
positive	142	53 (37.3)	2.60 (1.17-5.80)	0.019
Cough reported				
no	33	6 (18.2)	1	
yes	206	71 (34.5)	1.70 (0.55-5.30)	0.359

Background rates of Mtb infection amongst the individuals screened can be estimated from rates of infection in contacts of extrapulmonary TB and of denotified cases, 18.2% and 17.9%, respectively.

11.4.2 Prospective data

There were 28 patients with smear positive tuberculosis who underwent 24-hour cough monitoring at or before starting treatment (Section 3.5.10). 16 had household contacts who attended for screening at Homerton University Hospital. There were a total of 63 screened contacts, a median of 4 per index case (range 1-10). 23 screened contacts (37.1%) were deemed to have been infected with Mtb on the basis of Mantoux testing and/or IGRA. A Mantoux-/IGRA+ was considered significant in an adult and two Mantoux+/IGRA- were considered positive in children; the remainder were concordant. There were no cases of co-prevalent active TB.

There were only two contacts with HIV, one of whom was infected with Mtb. Only one had diabetes. There were no specific data on features of the housing type or proximity of sleeping overnight, and details of alcohol consumption were inconsistently documented.

Table 11.3. Univariate analysis of variables associated with *M. tuberculosis* infection in household contacts of smear positive tuberculosis (prospective cohort).

	CONTACTS		Univariate unadjusted analysis		Univariate adjusted analysis	
	Total no.*	No. (%) infected	OR (95% CI)	*p*	OR (95% CI)	*p*
CONTACT CHARACTERISTICS						
TB incidence in country of birth						
<40/100,000	33	14 (42.2)	1			
≥40/100,000	30	9 (33.0)	0.58 (0.20-1.67)	0.310		
Ethnicity						
white	19	7 (36.8)	1			
non-white	44	16 (36.4)	0.97 (0.32-3.02)	0.971		
Sex						
male	33	10 (30.3)	1			
female	30	13 (43.3)	1.75 (0.61-5.04)	0.287		
Age (y)						
<5	6	3 (50.0)	1			
5-14	18	8 (44.4)	0.80 (0.13-5.09)	0.813		
≥15	39	12 (30.8)	0.44 (0.08-2.53)	0.361		
Previous BCG						
no	7	3 (42.9)	1			
yes	54	19 (35.2)	0.72 (0.14-3.63)	0.693		
Current smoker*						
no	49	20 (40.8)	1			
yes	7	2 (28.6)	0.58 (0.10-3.36)	0.539		
INDEX CASE CHARACTERISTICS						
Sex						
male	34	12 (35.3)	1			
female	29	11 (37.9)	1.12 (0.40-3.16)	0.830		
Current smoking						
no	37	14 (37.8)	1			
yes	26	9 (34.6)	0.87 (0.30-2.50)	0.795		
Sputum smear grade						
+/-, 1+, or 2+	46	14 (30.4)	1		1	
3+	17	9 (52.9)	2.57 (0.79-8.33)	0.102	2.60 (0.84-8.00)	0.096
M. tuberculosis* strain						
Beijing or Cameroon						
no	52	20 (38.5)	1			
yes	7	2 (28.6)	0.64 (0.11-3.69)	0.615		
Haarlem						
no	34	13 (38.2)	1			
yes	25	9 (36.0)	0.91 (0.31-2.67)	0.862		
Visible cavities						
no	21	8 (38.1)	1			
yes	42	15 (35.7)	0.90 (0.30-2.69)	0.854		
Radiological extent						
0-1 zones	33	10 (30.3)	1			
>1 zones	30	13 (43.3)	1.76 (0.61-5.04)	0.287		
Cough characteristics:						
duration (per month)			1.02 (0.95-1.11)	0.546		
frequency (per 10 c/h)			1.22 (0.93-1.58)	0.148	1.22 (0.94-1.58)	0.142

*missing data

On univariate analyses there were trends ($p < 0.15$) for associations of sputum smear grade and cough frequency with household Mtb infection (Table 11.3). No other variables appeared to be important. There was no evidence that clustering of contacts within households affected these associations; adjusted statistical analysis revealed very similar results. However, on multivariate analysis, both sputum smear grade and baseline cough frequency (Figure 11.1) of the index case were both associated independently and significantly with Mtb infection in contacts (Table 11.4).

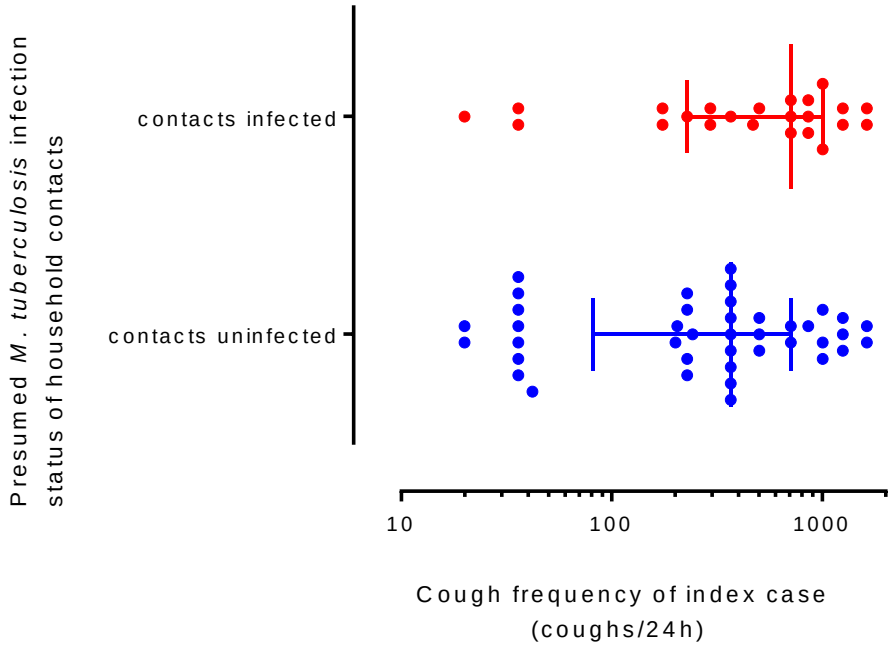


Figure 11.1. Cough frequency in smear positive tuberculosis and TB infection in household contacts.

Table 11.4. The association of index case cough frequency and sputum bacillary with *M. tuberculosis* infection in contacts on logistic regression analysis.

	CONTACTS		Multivariate adjusted analysis	
	Total no.*	No. (%) infected	OR (95% CI)	*p*
Sputum smear grade				
Scanty/ 1+/ 2+	46	14 (30.4)	1	
3+	17	9 (52.9)	3.53 (1.29-9.65)	0.014
Cough frequency (per 10 c/h)			1.30 (1.04-1.64)	0.022

11.5 Discussion

This is the first work to show an association of 24-hour cough frequency in TB with rates of Mtb infection amongst contacts. It is also the first work to show a significant association of objective cough frequency measured over any duration with Mtb infection. In contrast the subjectively-reported presence of cough was not independently associated with this measure of transmission.

11.5.1 Retrospective data: the presence of cough and household infection

Coughing has long been assumed to be important for the transmission of tuberculosis but direct data supporting this have been mainly circumstantial (Section 1.5).[119] Few studies appear to have investigated patient-reported cough as an independent risk factor for infectiousness.[234]

Although apparently important on univariate analysis, the current work did not find reported cough in the index case to be an independent risk factor for Mtb infection in household contacts.[234] This is in contrast to the recent study of Jones-López which measured cough symptoms in TB and Mtb infection in households.[234] However, in that study, symptoms were measured quantitatively (visual analogue scale and the Leicester Cough Questionnaire), rather than by the simple approach of cough present *vs.* cough absent, presumably resulting in greater power to detect an effect of cough symptoms.[234] There were relatively few index cases of pulmonary TB in the retrospective part of the study here who denied cough; information on the severity of cough symptoms was not available. In the (relatively small) prospective part of the study I chose not to investigate an association between cough symptoms and household Mtb infection due to the limited correlation that exists between subjective assessments of cough and the variable of interest, objectively-determined cough frequency in TB (Section 7.4.3).

Reported cough duration in the source case was not shown to be associated with Mtb infection in household contacts. This appears to have been rarely examined,[454,455] but individuals who have been coughing for a longer duration of time prior to diagnosis

and commencing treatment will presumably have given rise to more opportunities for transmission of infection. The current study may have been underpowered to detect this effect. However, as well as there being a poor correlation between objective cough frequency and the subjective appreciation of coughing, recall of onset of symptoms is probably variable. Patient-reported estimates of duration of cough might therefore not be an accurate representation of the objectively-measured time for which cough frequency has been increased.

In the retrospective dataset, the Haarlem strain of organism in the index case was associated with higher rates of presumed infection in household contacts. This was not supported by the prospective findings. Neither am I aware of reports from the literature suggesting an association of increased infectiousness with the Haarlem clade. Conversely, Jones-López *et al*. reported that a genetic cluster corresponding to the Haarlem spoligotype was associated with households in which there was a lower prevalence of Mtb infection on contact tracing.[234] There is clearly a need for further investigation into the potential role of Mtb strain type in transmission of infection.[184]

11.5.2 Prospective data: objectively-measured cough frequency and household TB infection

Regarding objective cough frequency in TB, the only previous relevant study involved 130 childhood household contacts, including 88 contacts of smear positive disease. In contrast to the current work which found a significant association between index case cough frequency and household rates of Mtb infection, the relationship between these two variables in the previous study was not statistically significant ($p < 0.20$).[125]

A reason for the discrepancies between the findings of the current work and this previous study might relate to the duration of cough monitoring. As previously discussed (Section 1.5.3.3), Loudon and Spohn performed only overnight recordings lasting 8 h,[125] as opposed to 24-hour recordings in the current work. However, although 24-hour monitoring produces a full picture of the amount of coughing on a daily basis, data presented earlier in this thesis (Section 7.4.2.2) demonstrate that

nocturnal cough counts are a good surrogate for 24-hour monitoring. Other differences between the current work and that of Loudon and Spohn include the method of determining infection with Mtb, the age of the contact cases, and, possibly, patient characteristics, strains of Mtb, and environmental factors. The earlier study used only the tuberculin skin test (TST) for determining infection, having taken place several decades prior to the introduction of IGRAs. A positive test was defined as a resulting skin induration of diameter ≥10 mm, as opposed ≥5 or ≥15 mm in the current study, depending on BCG vaccination status. The sensitivity of the test decreases with a larger minimum induration size.[297]

Previous BCG vaccination status affects interpretation of the result and was not stated in the previous paper, although few or no contacts are likely to have been vaccinated, the study having taken place in the USA, where routine BCG vaccination has never been recommended.[456,457] The performance of the TST in the earlier study may also have been adversely affected by the relatively very high levels of exposure to non-TB mycobacterial antigens which occur in Texas and other Southern States.[366,458] One of the shortcomings of the TST is its low specificity compared to currently available IGRAs.[459]

Unlike in the current study, Loudon recruited only children aged ≤14 years for estimating recent household Mtb transmission, to reduce the chance that a presumed infection had occurred at some other point in time, the opportunities for such events being lower with younger age. In the current work, like any using immunological markers as a surrogate for recent transmission, there was no way of confirming that the sources of presumed infection were the TB index cases of interest.

However, several additional findings add weight to assumption of additional transmission events amongst the screened contacts. Firstly, the rate of Mtb infection amongst this group (37%) was higher than the local background prevalence of 18% amongst contacts of presumed non-infectious individuals ($p = 0.007$, Fisher's exact test; Table 11.1). Secondly, the observation that characteristics relating to sputum

microscopy were also associated with rates of Mtb infection amongst household contacts suggests the occurrence of transmission from the source cases of interest, sputum smear status being the most commonly-used predictor of infectiousness in pulmonary TB (Section 1.5.3.1).[119,200] Thirdly, 50% of children aged <5 years demonstrated immune responses to Mtb antigen, and, finally, birth in a high TB-incidence country was not associated with LTBI amongst household contacts in the prospective cohort. These observations are all difficult to explain without recent transmission events, at least some of which were presumably associated with the index cases of interest.

There had presumably been far fewer recent transmission events amongst the retrospective cohort of household contacts; a large proportion of the index cases were likely to have been non-infectious, having either smear negative pulmonary disease, extra-pulmonary TB or no TB diagnosis at all (denotified cases; Table 11.1). As a consequence, in that cohort, only 16% of children aged <5 years demonstrated evidence of TB infection, and birth in a high TB-incidence was the strongest predictor of Mtb infection amongst all contacts overall, a likely result of a the relative importance of distant over recent transmission.

It is not possible to comment on differences between individual characteristics of the index cases and household contacts between the current work and that of Loudon, nor on differences in environmental characteristics as they were either not measured or not reported in one or both studies.

Although sputum bacterial content of the index case has repeatedly been shown to predict rates of infection in household contacts,[119] relatively few studies have specifically investigated the effect of sputum smear grade amongst smear positive source cases of TB.[460,461] In this context, the findings reported here of an association between smear grade and household transmission is of particular interest.

There was no evidence for an association between Mtb infection and any characteristic of the source case or contacts in the prospectively-recruited cohort

other than cough frequency and sputum smear grade. This included two of the apparently strongest risks for infection, contact birth place in a high TB-prevalence country,[450] and visible cavities on the chest x-ray of the index case. However, it is unclear whether the presence of cavities is a risk factor for transmission independent of smear positivity (Section 1.5.3.1). And it is possible that relatively high rates of recent infection were more important than previous background Mtb transmission events, masking the apparent effect of country of birth. Larger studies with more statistical power would be required for this to be explored further.

11.5.3 Limitations

There were no data on several variables which may have been relevant for transmission, for example details of the nature and duration of contact between index case and contacts,[119] and environmental features such as household ventilation.[181] However, this study was essentially exploratory and to control for other potentially important variables would have required a larger investigation.

The role of exposure to cases of TB other than the index case of interest was taken into account only by documenting co-prevalent smear positive pulmonary tuberculosis. However, there were no such cases in the prospective cohort, and only one of relevance (where there were other household contacts) in the retrospective series. Household co-prevalent active TB was therefore not a significant problem for the current work. The elimination of all other, possibly unknown, relevant source cases of Mtb infection, over a longer period for older screened individuals, is of course impossible. It is a general limitation of using immune responses as a marker of transmission, and has been discussed (previous Section and Section 1.6.2.5.5).

However, none of the variables potentially associated with observed rates of Mtb infection was likely to have been also related to coughing, so should not have confounded the observed association between cough frequency and the transmission of infection.

Because only smear positive TB was studied no comment can be made on the role of cough frequency on infectiousness in patients who were sputum smear negative for AFB. Owing to the much lower likelihood of transmission with smear-negative disease,[462] a larger study would be needed to investigate this.

Cough monitoring covered only one 24-hour period prior to starting treatment. This is only a surrogate for estimating the rates of aerosolization of TB bacilli during the total period of infectiousness prior to treatment. It would be difficult, ethically and logistically, to organize a study involving multiple periods of 24-hour cough monitoring before starting treatment in a potentially infectious source case. However, as observed in Chapter 8 (Section 8.5.2.1), the lack of change in 24-cough frequency within the first 48 h of TB treatment suggests relatively stability in cough counts for at least several days prior to the cough monitoring period.

11.6 Conclusions

The demonstration of an association between 24-hour cough frequency in smear positive tuberculosis and rates of household infection is novel. Any method of estimating the infectiousness of TB should be welcome for directing infection control measures and contact tracing efforts, particularly with drug-resistant disease in areas of high TB burden and low economic resources.

SECTION FIVE: SUMMARY AND CONCLUSIONS

12 Summary, conclusions and final remarks

From an initial idea of researching coughing in tuberculosis it soon became apparent that there were many gaps in knowledge about cough, not only in TB, but in respiratory disease in general. Some of these uncertainties were fundamental and seemingly basic, such as how to best define a cough. Identification of the gaps in the literature, led to a series of specific interlinking research questions.

I started by assessing whether cough impacts on patients to a greater or lesser degree in some respiratory conditions than others, i.e. whether there are subjective differences in cough between diseases (Chapter 4). Wide variation was observed both within as well as between respiratory conditions, with few significant differences overall between diseases. In the process of doing this I validated the Leicester cough Questionnaire for use in tuberculosis.

Following this confirmation that a reliance on subjective measures would be insufficient for research into cough, I addressed how best to measure cough frequency objectively (Chapter 5). I confirmed that coughs are easily recognizable by the human ear, providing justification for counting coughs based on their 'characteristic sound'.[2] This also provided a method for evaluating an automated cough monitor. In contrast with an earlier report,[262] PulmoTrack™ showed poor agreement with the human ear, and was not be suited for the remainder of the study (Chapter 5). I therefore used the Leicester Cough Monitor from then on which, although like any cough monitor currently used in research has limitations, has been more extensively validated than any other device.

Chapter 6 addressed the other common approach to the objective clinical evaluation of cough, the capsaicin cough challenge. In particular I provided further data to suggest that C5 and C2 may not be necessarily be the most useful endpoints for measuring

cough reflex sensitivity, and show that a novel measure, $E_{62.5}$, could have merit due to a correlation with 24-hour cough frequency.

Chapter 7 compared 24-hour temporal cough patterns in a large group of patients with different diseases, including several in which no such measurements have been reported previously. There was much variation in cough frequency within and between diseases, and nothing to suggest a unique temporal pattern of cough in any disease. In particular, nocturnal suppression of coughing appears universal across respiratory conditions, and there was a remarkably constant relationship between nocturnal cough rates and those during the day. This strongly suggests the existence of common basic mechanisms driving cough across varying contexts.

Chapter 7 also examined variables associated with coughing in respiratory diseases which have not been investigated previously. While some of the novel findings are probably merely hypothesis-generating due to small sample sizes, others have corroborated those of earlier work from overnight monitoring. Such findings include an association (albeit with $p = 0.054$) sputum smear status and 24-cough frequency in TB, and a (perhaps surprising) lack of general overall correlation between current smoking and cough frequency in respiratory disease.

The improvement in 24-hour cough frequency during treatment of acute respiratory conditions and tuberculosis was the rarely-studied subject of Chapter 8. Although only relatively small numbers of patients were included, the slower rates of decrease in coughing in acute asthma and COPD exacerbations compared to pneumonia despite otherwise successful recovery from illness were of interest and led to speculation about mechanistic factors. The pilot data on the rates of improvement in 24-hour cough frequency with treatment of pulmonary tuberculosis were remarkably similar to observations made almost 50 years ago in the pre-rifampicin era with overnight cough recordings. A slower response to treatment was associated with explanatory clinical factors, suggesting that 24-hour cough frequency might be a novel biomarker of treatment response in TB and other conditions.

An attempt to explain some of the poorly understood variability in baseline cough patterns in respiratory disease was made in Chapter 9 by measuring *TRPV1* polymorphism. Despite much interest in TRPV1, there was no evidence that such genetic variability substantially affects objectively-measured features of chronic cough.

The following chapters addressed the potential infectiousness of coughing in tuberculosis. Section 1.5.4 had taken a theoretical approach to discuss the movement of respiratory droplets on leaving the mouth and made predictions based on variables including droplet velocity, size and composition and ambient conditions including temperature and relative humidity. Chapter 10 attempted to measure such droplets with the aims of testing these predictions on comparing different respiratory actions and types of cough in aerosolizing respiratory secretions. Unfortunately the device tested was shown to be not sufficiently sensitive for this purpose. Finally, Chapter 11 assessed 24-cough frequency in tuberculosis along with other variables to try and explain variation in rates of household infection. I demonstrated, for probably the first time, a significant independent association between cough frequency and rates of household infection. Objectively-measured cough seemed a much more sensitive predictor of infectiousness than the documented presence or absence of coughing.

Any piece of clinical research has constraints, due to time, resources, expertise, and availability of study subjects. This is particularly the case with a doctoral thesis. The main limitation in the current work has been patient sample sizes, which, as a consequence, mean that many of the findings presented here should be seen as preliminary. The novel conclusions in particular should be confirmed in other contexts and in larger studies.

Another limitation of this research has been the lack of scope to explore other areas initially identified in Chapter 1 as being worthy of study. This includes the issue of whether cough sounds are different depending on underlying pathology, which remains unresolved. Initial data which I have not yet reported, in keeping with those of others,[272] suggests that the duration of phases of coughs vary at least as much within

as between diagnostic groups. I did not perform analysis of the sound frequency composition of coughs. Only one study seems to suggest that cough sounds may have diagnostic value,[100] and should be replicated.

Much still remains to be discovered about the mechanisms of cough. Genetic influences may still be important, if not involving TRPV1, then perhaps involving other cough receptors (such as P2X3), mucus production, the central processing of cough, or other complex determinants of coughing. The measurement of volatile organic compounds in exhaled breath in health and disease and during treatment may reveal novel mediators for cough.[463,464]

The interaction between taste sensation and the cough reflex is worthy of study. Observations made in the process of this research, but not reported, suggest the ability to taste phenylthiocarbamide (PTC) was not related to cough. However, a more detailed examination of the pathways involved in detecting taste in the context of cough would now seem to be even more fruitful following the recent demonstration of the efficacy of a P2X3 antagonist in chronic cough,[43,431] P2X receptors being integral to gustation.[50]

Finally, a much more detailed assessment of how coughing produces infectious airborne particles should be undertaken, in TB and other contexts. This will require a complicated approach, both to measure particles[283] and to study the mechanics of coughing.

Few pieces of research are definitive. The minimum I hope to have achieved in working towards this thesis is to have better defined areas of ignorance that exist within the literature,[465] and to have put forward new hypotheses which will form the basis for further work.

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Appendix

The Leicester Cough Questionnaire. See reference [226]. Reproduced with permission of the authors.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Thank you for completing this questionnaire.