UNIVERSITY OF HULL

Azithromycin, Oesophageal Function, and Cough in Chronic Respiratory Disease

being a Thesis submitted of the

requirements for the degree of

MD by Thesis

in The University of Hull and The University of York

by

Dominic Lee Sykes

Hull York Medical School

March 2025

Acknowledgements

I would like to thank all who made this work come to life. I believe it most fitting to begin by offering my thanks to all the patients who volunteered their time and effort for the studies completed as part of this thesis, without them none of this would have been possible.

I would like to thank my supervisors Professor Michael Crooks, Professor Simon Hart, and Professor Alyn Morice. The patience they have shown and wisdom they have shared with me throughout the journey of completing this work has been greatly appreciated. I am lucky to say that not only have a I gained knowledge and expertise through my time working with my supervisors, but I have also gained 3 good friends in the process.

I would like to thank Kayleigh Brindle, Jennifer Nielsen, and the rest of the team at the respiratory clinical trials unit in Castle Hill Hospital for all their help with multiple aspects of running this trial. I also thank Warren Jackson, John Gallagher, Elisabeth Kirton and all the staff at the GI physiology lab in Castle Hill for their generosity in performing all the HROM studies and somehow managing to put up with my pathological lack of organisation!

To my Mum, Dad, and sister Beth, thank you for instilling me with the belief that hard work, above all else, is key to any path we choose in life. Without your unwavering support, unadorned advice, and the example you have set, there is no way I would be where I am today. Thank you for putting up with me for 30 years, I am truly grateful for everything you have given me.

Finally, to my fiancée Nisha, thank you for sharing the emotional burden that this work has been. I am constantly in awe of your patience, intelligence, and the lengths you go to support the people you love. Thank you for supporting me through the countless breakdowns and tantrums that I have inflicted on myself over these past few years.
I love you and dedicate this work to you.

Publications and Conferences

Publications in peer-reviewed journals:

Sykes DL, Crooks MG, Hart SP, Jackson W, Gallagher J, Morice AH. Investigating the diagnostic utility of high-resolution oesophageal manometry in patients with refractory respiratory symptoms. Respiratory Medicine. 2022 Oct 1;202:106985.

Sykes DL, Mason P, Rahunathan N, Hart SP, Morice AH, Crooks MG. The effect of long-term azithromycin on objective and subjective cough in chronic respiratory disease: a systematic review and meta-analysis of randomised controlled trials and noncomparative studies. Lung. 2024 Oct;202(5):569-79.

Zhang M, Sykes DL, Brindle K, Sadofsky LR, Morice AH. Chronic cough—the limitation and advances in assessment techniques. Journal of Thoracic Disease. 2022 Dec;14(12):5097.

Zhang M, Sykes DL. Coughing our guts up: how do we diagnose reflux cough? ERJ Open Research. 2024 Jul 29;10(4):00406-2024.

Abstracts published after presentation at international conferences:

Sykes DL, Hart SP, Crooks MG, Jackson W, Gallagher J, Morice AH. Oesophageal Dysmotility: Does it Play a Role in Interstitial Lung Diseases? European Respiratory Journal.60(suppl 66):2129.

Sykes DL, Crooks MG, Hart SP, Jackson W, Gallagher J, Morice AH. Investigating the Prevalence of Oesophageal Dysmotility in Patients with Refractory Chronic Cough. European Respiratory Journal.60(suppl 66):2115.

Sykes DL, Menon R, Brindle K, Nielsen J, Jover L, Small P, et al. Is there a relationship between real-time continuous cough monitoring and subjective cough assessment? European Respiratory Journal.64(suppl 68):PA2139.

Abstract

Chronic cough is a disabling feature of many chronic respiratory diseases and is now recognised as a distinct disease. Despite being amongst the commonest causes for healthcare contact, there are no licenced treatments for chronic cough in the UK. The role of the gut-lung axis in respiratory disease is increasingly recognised. However, much of the current research has been centred around gastro-oesophageal reflux disease, with little research on the potential co-existence of oesophageal dysfunction in chronic respiratory conditions. The macrolide antibiotic azithromycin is used to prevent exacerbations in various respiratory diseases; however, its mechanism of action remains unclear. It is known that azithromycin improves gastrointestinal motility through agonism of motilin receptors. We hypothesise that this mechanism may contribute to the beneficial effects of azithromycin.

In this thesis we aimed to explore different elements of this hypothesis through multiple methodologies. First, we establish the prevalence of oesophageal dysmotility in respiratory disease through a retrospective analysis of high-resolution oesophageal manometry (HROM) studies in patients with persistent respiratory symptoms. We then perform a systematic review and meta-analysis to examine the effect of azithromycin on objective and subjective measures of cough in patients with chronic respiratory disease. Using the established methodology of a feasibility study, we evaluate the practicability of performing repeated HROM measures and continuous cough monitoring in patients with chronic respiratory disease. Finally, we perform an exploratory analysis of the effect of azithromycin on continuously recorded objective cough frequency using both traditional and novel methods of cough analysis.

We have addressed multiple aspects of our hypothesis with the studies conducted in this thesis. We have highlighted the high prevalence of oesophageal dysmotility in those with chronic cough and shown that azithromycin has shown early promise as a potential antitussive agent. Moreover, we demonstrate that using both HROM and continuous cough monitoring is feasible and acceptable to patients. This study provides rationale for a large-scale, randomised controlled trial using mixed methodologies to investigate azithromycin's potential as a treatment for chronic cough.

Contents

List of Figures

25. A) Displaying the change in median daily cough density from pre-treatment baseline per week in follow-up. B) Change in median daily relief of cough count from pre-treatment baseline per week in follow-up.

26. Displaying change in the mean proportion of monitoring time that participants were relieved of cough i.e. 0.8= 80% of monitored time relieved of cough.

27. Scores for each patient reported outcome measure at baseline and follow-up for all patients.

28. Scores for the SGRQ including A) Total score, B) Symptom domain, C) Activity domain, and D) Impact domain at baseline and follow-up for all patients.

29. Change in weekly mean cough severity VAS scores calculated from completed cough diaries

30. Daily VAS scores for each participant that completed the 4-week diary.

31. Scatter plot matrix for all participants that completed the 4-week cough diary.

List of Tables

Chapter 1 Background

Chronic respiratory diseases affect one in five people in the United Kingdom and now represent the third leading cause of death in England.[1] Symptoms such as chronic breathlessness and cough are extremely prevalent, with such complaints accounting for up to 15% of clinical contacts in primary care.[2, 3] Furthermore, chronic respiratory diseases account for 6.5% of all hospital admissions in the United Kingdom (UK) annually at the cost of £9 billion to the National Health Service (NHS), this is an issue that is becoming increasingly prevalent despite recent advances in therapeutics.[4, 5]

The predominant morbidity associated with chronic respiratory disease is the presence of uncontrolled respiratory symptoms, predominantly cough and breathlessness [6], that are refractory to conventional treatment strategies. These symptoms can precipitate significant deleterious effects on the physical, psychological, and social health of those who endure them. Patients with a chronic refractory cough are at higher risk of developing physical complications such as inguinal hernias, incontinence, and even the potentially life-threating disease of cough syncope.[7-9] Many patients with chronic respiratory disease also experience the daily disabling feeling of dyspnoea. This usually comes from the perceived inability to carry out physical activity due to symptoms, resulting in exercise limitation, muscle atrophy, anorexia, and sarcopenia.[10-12] This ultimately leads to a syndrome of frailty which is commonly observed in patients with end-stage chronic respiratory disease, particularly Chronic Obstructive Pulmonary Disease (COPD), Idiopathic Pulmonary Fibrosis (IPF) and other Progressive Fibrosing Interstitial Lung Diseases (PF-ILD).[13-15]

Compounding the experiences of these patients are the negative psychological and social impacts of living with chronic respiratory symptoms. The rates of anxiety and depression have been demonstrated to be as high as 33% and 16%, respectively, in patients with Chronic Cough (CC).[16] Rates of psychological morbidity have been shown to be as high as 62.4% in older patients with chronic breathlessness.[17] Furthermore, the majority of patients with CC and chronic breathlessness are known to have a significantly impaired quality of life.[18, 19] This is a finding that is consistently observed among people living with chronic respiratory disease.[20-22]

Despite the incontrovertible impact that refractory symptoms have on patients with chronic respiratory disease, many therapeutic agents are developed with the aim of reducing exacerbations or altering lung function. An exacerbation is defined as an acute deterioration of disease control, usually driven by a transient increase in symptom burden and reduced functional status, necessitating acute treatment. Many patients with chronic respiratory disease may have well controlled daily symptoms but still experience exacerbations triggered by infections, seasonal changes, and exposure to specific allergens. In airways diseases such as asthma, COPD, and bronchiectasis, the primary endpoints of trials are largely based on exacerbation reduction and effect on lung function.[23-25] In patients with IPF and PF-ILD, the largest trials of the therapeutic nintedanib utilised the annual rate of change of Forced Vital Capacity (FVC) as their primary endpoint.[26] Few large-scale clinical trials utilise objective or subjective measures of symptom control to evaluate the efficacy of novel therapeutics. This pattern of behaviour has also been repeated in CC clinical trials, with a large emphasis on audio-recorded 24-hour objective cough frequency as the 'gold standard' parameter for use as a primary endpoint when examining the efficacy of new therapeutics.[27] This is despite the fact that patients may experience their cough differently and there may be significant heterogeneity in the frequency of cough which may be perceived as bothersome between individual patients. However, data have shown that changes in objective cough frequency only moderately correlate with improvements in patient reported outcomes (PROs) of cough severity[28]. Evidently, fitting together the jigsaw of assessment of respiratory symptoms remains an arduous task. Moreover, this observed lack of association makes it difficult to prioritise clinical endpoints, i.e. objective cough frequency vs subjective cough assessment, in trials of novel antitussives.

The importance of the gastrointestinal tract in the development and progression of respiratory disease has been highlighted in numerous studies in recent years[29-31]. There is debate as to what aspect of the gastrointestinal tract is most pertinent in relation to respiratory disease, with many believing that gastro-oesophageal reflux disease (GORD) may be responsible. The hypothesis is that local damage to lung parenchyma is engendered by the retrograde transit of acidic gastric contents into the respiratory tree. However, thus far there is no convincing clinical trial evidence to support the use of protein-pump inhibitors (PPI), the cardinal therapeutic for GORD, for

respiratory diseases such as CC and asthma[32, 33]. The microbiological link between the gut and the lungs has also been implicated in the development of respiratory disease. Indeed, gut and lung 'dysbiosis', with differences in the microbiome of these two organ systems, has been suggested as a factor in the development of allergic/atopic asthma[34, 35]. The third theory, pertinent to this thesis, is that of micro-aspiration of non-acidic, gaseous refluxate. This mechanism has been termed 'Airway Reflux'[36], a process that involves chronic 'silent' aspiration of refluxed gastrointestinal gaseous content from the oesophagus.

The series of studies in this thesis aim to decipher the interactions between the respiratory and gastrointestinal systems in patients with chronic respiratory diseases. This work will also examine the use of azithromycin, a commonly used drug across several respiratory diseases, and attempt to examine its effect on oesophageal function and subjective, and objective measures of respiratory symptoms.

1.1 Disease Classification and Definitions

This work will include different respiratory diseases with a broad range of pathophysiological mechanisms; however, the core focus of the studies will be refractory cough and breathlessness. Here, the specific diseases of interest will be predefined, and their diagnostic criteria will be used throughout the studies that constitute this thesis.

1.1.1 Asthma

Despite the high prevalence of asthma diagnoses in the general population, with estimates of a prevalence of 5-10% in the UK[37]. However, asthma is broadly defined by the primary features of breathlessness, wheeze, chest tightness, and cough together with airway inflammation, variable airflow obstruction, and reversibility of said obstruction with bronchodilating agents, such as salbutamol.[38]

The current National Institute for Health and Care Excellence (NICE) guidelines in the UK recommend diagnosing asthma in adults who have symptoms suggestive of asthma and one of the following sets of parameters[39]:

- A fractional exhaled nitric oxide (FeNO), a marker of airway inflammation, level of 50 parts per billion or a blood eosinophil count above the laboratory reference range.
- If FeNO or blood eosinophils are not raised, bronchodilator reversibility (BDR) with spirometry is recommended. A diagnosis of asthma can be made if the FEV1 increase is 12% or more and 200 ml or more from the pre-bronchodilator measurement (or if the FEV1 increase is 10% or more of the predicted normal FEV1).
- If spirometry is not available, repeated peak expiratory flow measurements showing >20% variability is adequate to diagnose asthma.

1.1.2 COPD

For the purposes of this work COPD will be defined as per the Global Initiative for Chronic Obstructive Lung Disease (GOLD) definition, updated in 2023 to best describe it's diverse characteristics: 'a heterogeneous lung condition characterized by chronic respiratory symptoms (dyspnea, cough, expectoration, and/or exacerbations) due to abnormalities of the airways (bronchitis, bronchiolitis) and/or alveoli (emphysema) that cause persistent, often progressive, airflow obstruction'.[40]

The GOLD framework also extends to the taxonomy of the potential 'aiteotypes' for COPD, which include COPD/ asthma overlap, environmental COPD, and genetically determined COPD. Furthermore, they also subclass COPD into severity based on their GOLD ABE model, this takes into account the severity of airflow obstruction (measured by % of predicted Forced Expiratory Volume in 1 second [FEV1]), exacerbation frequency, as well as symptom burden (measured using the modified Medical Research Council Dyspnoea Scale [mMRC] and the COPD Assessment Test [CAT]).

Due to burdens on modern-day healthcare systems, many patients are treated as 'physician diagnosed' COPD, without formal lung function testing or subjective assessment with the aforementioned tools. Some patients included in the studies that constitute this thesis, particularly in data that has been

retrospectively collected, will not be precisely categorised as per the 2023 GOLD framework. Despite this, all patients that are included in the analyses of this thesis will have been diagnosed as having COPD by a consultant respiratory physician.

1.1.3 Chronic Cough

Historically, the definition of CC has used the arbitrary cut-off of a troublesome cough that lasts over 8 weeks.[27] Despite this, more recent guidelines have moved away from this definition, as the natural history of CC can vary widely between patients; some may cough daily for a number of years whereas others may experience a relapsing-remitting course. The current European Respiratory Society (ERS) guidance therefore recommends a diagnosis based on a global clinical assessment of the patient, considering recognised phenotypes of the disease.

The key distinct phenotypes that are highlighted by the ERS are[41]:

- Asthmatic cough/ eosinophilic bronchitis: a disease defined by eosinophilic airway inflammation and a cough-predominant symptomatology
- Reflux cough: A syndrome of GORD, oesophageal dysmotility, and cough where there may be other key clinical features such as voice change, nasal symptoms, and dysgeusia.
- Post-nasal drip cough/ upper airways cough syndrome: a controversial classification, however, thought to be due to laryngo-pharyngeal reflux.
- Iatrogenic cough: a disease that is caused by pharmacological agents, most frequently angiotensin converting enzyme (ACE) inhibitors.

The establishment of the paradigm of Cough Hypersensitivity Syndrome (CHS), has prompted many clinicians to believe that the above phenotypes are different aetiologies that cause a similar disease process of neural hypersensitivity causing a chronic non-productive cough.[42] A pathophysiology of CHS is believed to be sensitisation of vagal afferent nerves in the upper and

lower airways. Chronic airway inflammation, caused by the aforementioned processes may precipitate this sensitisation, shifting the tussigen-cough response curve to the left, leading to increased cough sensitivity to normally innocuous stimuli.[43]

1.1.4 Interstitial Lung Disease

Interstitial lung disease (ILD) is a collective term that includes a wide range of disorders, many of which primarily impact the pulmonary parenchyma, though not all. [44]The interstitial space, situated between the alveolar epithelium and the capillary endothelium, contains occasional fibroblasts, lymphatic vessels, and the proteins which encompass the extracellular matrix. Patients with ILD have a thickening of the interstitium which in turn impairs gas exchange. This leads to a clinical syndrome of breathlessness, cough, and hypoxia which often times progresses rapidly, precipitating early morbidity and mortality [45].

Although they are grouped together, many of the over 200 recognised ILDs have significantly different aetiologies and pathophysiology. Broadly, these diseases can be split into inflammatory and fibrotic processes. Inflammatory diseases such as hypersensitivity pneumonitis are characterised by ground glass opacification on computerised tomography (CT) imaging and are usually responsive to anti-inflammatory therapy such as corticosteroids.[46] Fibrotic ILDs, on the other hand, usually respond poorly to anti-inflammatory therapy and are usually caused by early cell senescence and overactivation of fibroblasts.[47] The commonest fibrotic ILD is Idiopathic Pulmonary Fibrosis (IPF). This often-devastating disease has a worse 5 year survival rate than most cancers, with a median survival of 3-5 years.[48] It is characterised by reticulation, honeycombing, and architectural distortion leading to traction bronchiectasis on CT scan. Such findings are reliable indicators of the typical usual interstitial pneumonia (UIP) histopathological pattern which can be seen on tissue sampling.[49]

1.2 Epidemiology of Chronic Cough

Almost everyone in their lifetime will suffer from a troublesome cough for a period of time. In most cases this will be short-lived and will be due to an upper or lower respiratory tract infection. However, some will experience persistent symptoms that can have profoundly impact their quality if life. There have been a plethora of studies from around the world that have attempted to quantify the prevalence of chronic cough. The reported prevalence from such studies has been quite broad, ranging between 9-33%[50, 51]. There will, of course, be great differences in estimates based on the populations of patients that one is studying or questioning regarding their cough. For example, current smokers are much more likely to suffer with a persistent cough which may be attributed to their smoking i.e. a 'smokers cough'[52].

There is increasing evidence that both indoor and outdoor air pollution are associated with higher prevalence of chronic cough[53]. Outdoor pollutants such as diesel fumes have been shown to be associated with an increased prevalence of cough and wheeze, particularly in children.[54] Results from a large study of 50 care homes from across Europe showed that elevated levels of particulate matter, defined as particles with a 50% cut-off aerodynamic diameter of <10μm (PM10), and nitrous oxide (NO2) were associated with significantly greater risk of cough and breathlessness in elderly people residing in such homes.[55] The growing problem of poor indoor air quality and increased indoor air pollution has led to the paradigm of the 'sick building syndrome', which is characterised by atopy, bronchial hyperresponsiveness, and chronic cough.[56] This is thought to be mainly attributable to people living in areas with poor ventilation and heightened exposure to volatile organic compounds, such as, formaldehyde, benzene, and toluene.[57] The phenomenon of escalating urbanisation, coupled with the sustained population growth in lower-middle-income countries, is poised to exacerbate levels of both indoor and outdoor air pollution. This environmental shift is likely to lead to a significant increase in the prevalence of

chronic cough and related respiratory ailments over the coming decades. The proportion of patients for which cough is their cardinal symptom is also highly variable between distinct diseases.

Cough in asthma

There are no robust data to accurately define the prevalence of cough in patients with asthma, however, some data has shown the prevalence to be over 50%[58]. This may be because cough is one of the hallmarks of the clinical syndrome of asthma i.e. cough, wheeze, chest tightness, and breathlessness. This is supported by the fact that in a recent Korean study of 498 patients with severe asthma, it was shown that the mean rating for cough severity on the visual analogue scale (VAS) was significantly higher than it was for wheeze severity.[59] This study also showed that cough VAS was significantly associated with asthma control and health-related quality of life. These findings indicate that cough is a critical symptom impacting the physical, psychological, and social dimensions of asthma.

Cough in COPD

Traditionally, patients with COPD have been categorised into one of two distinct phenotypes. The colloquially named ‘blue bloaters’ are patients who have chronic bronchitis, usually associated frequent productive cough. In contrast to, the 'pink puffer' phenotype typically presents with more dyspnoea, primarily driven by emphysema. Patients with on the chronic bronchitis end of the COPD spectrum typically report a chronic cough and sputum production due to chronic airway inflammation. It is vital that this addressed as it has been reported that frequent cough has been associated with a greater exacerbation frequency and disease burden [60]. In a cohort study of over 400 patients with COPD, it was shown that 74% reported cough and sputum production. This study also showed that the prevalence of smoking was significantly higher in those who reported a cough [61]. However, a more recent large-scale population-based study from *Landt et al.* found that only 10% of COPD patients reported concomitant chronic

cough [62]. Such discrepancies between the reported prevalence of cough will differ amongst studies due to differences in symptom assessment. For example, large population studies will rely on self-reported symptoms as opposed to rigorous clinical assessment using the standard definition of chronic cough or validated questionnaires with cough-specific domains. Despite the differences in measuring and reporting the presence of chronic cough, both aforementioned studies and others report that presence of cough is associated with higher risk of exacerbation and death in patients with COPD [63].

1.3 Objective and Subjective Measures of Chronic Respiratory Symptoms

Over the past twenty years, significant progress has been made in the understanding of the mechanisms that drive the cough reflex and the pathophysiology of chronic coughing. This progress in both clinical and preclinical cough research has been facilitated by the creation and implementation of new metrics for assessing cough both objectively and subjectively.[64, 65]

Various validated methods have been developed to measure cough severity, which can be categorized into subjective and objective approaches. Subjective assessments concentrate on the patient's personal perception of the cough and its effect on their cough-related quality of life (QoL).[66] However, subjective patient-reported outcomes (PROs) show only a moderate correlation with objective cough counting.[28] The method of 24-hour cough counting, which is considered a semi-objective approach, has been proposed as the most accurate way to quantify cough and is currently the ‘gold standard’ approach testing new therapeutics for cough[27]. Nevertheless, due to its labour-intensive and time-consuming nature, this method is primarily used within research environments. In addition to these measures of cough severity, there are also assessments of cough reflex sensitivity. These include inhalation challenges using noxious stimuli known to trigger coughing, such as capsaicin and citric acid.[67] These will not be explored here but are mentioned in Figure 1 in combination with other common methods of cough assessment.

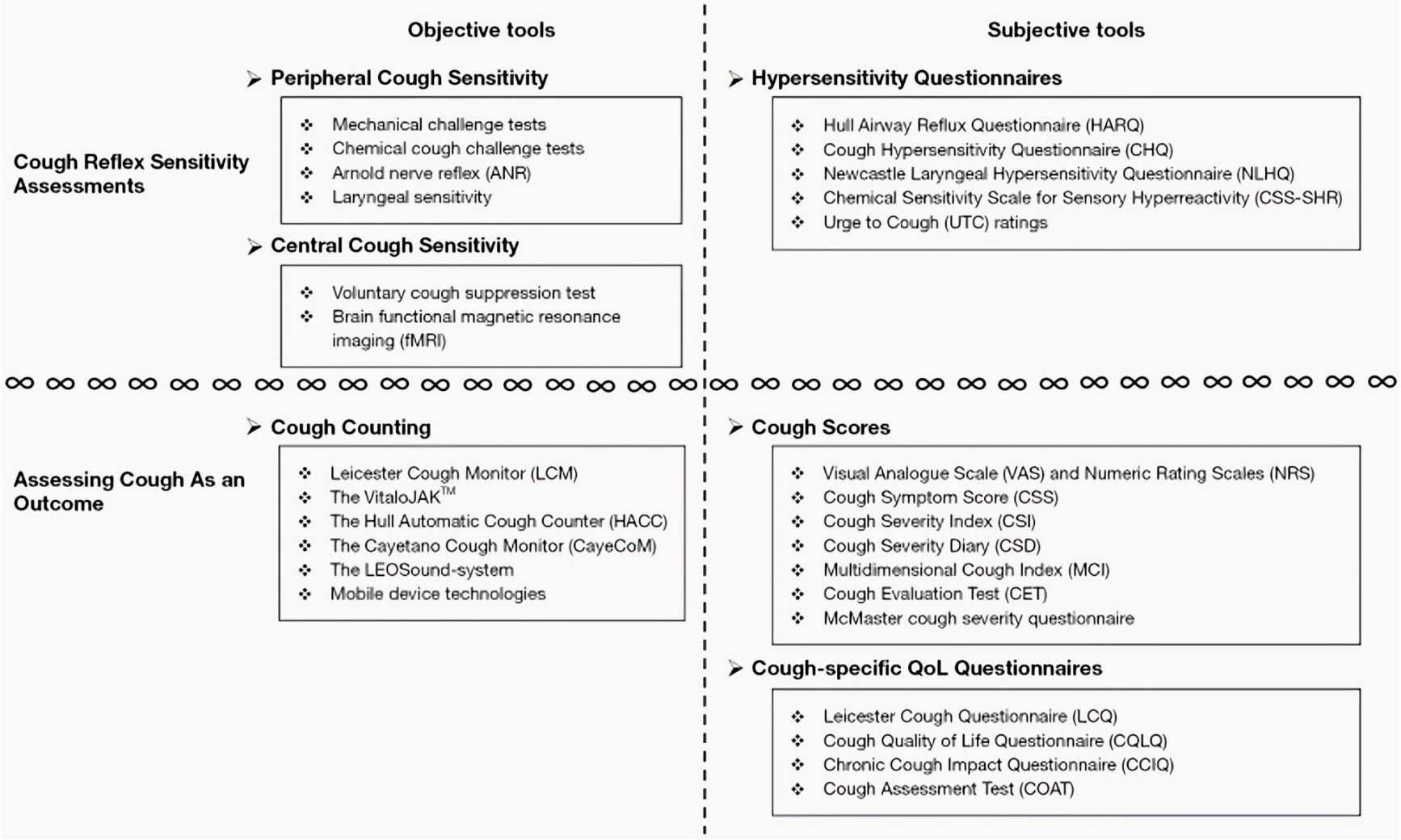


Figure 1. *Schematic depicting the various methods of assessment of cough*

1.3.1 Objective measures of cough

The exact number of times a patient coughs in a particular period is known as the objective cough frequency, which is typically measured by 24-hour cough counting. This is only a partially objective measure of cough frequency as it is extremely difficult to differentiate between a patient voluntarily coughing, for example whilst clearing their throat, and a true reflex-driven cough.

There has been considerable debate over the seemingly simple task of defining a cough. Traditionally, physiologists defined it as an inspiration followed by a forceful expiration, implying that the resulting burst of sounds were not coughs but rather expiration reflexes (ERs)[68]. Today, it is generally accepted that each individual sound event is considered a cough, composed of explosive, intermediate, and voiced phases[69]. Various parameters have been proposed for counting coughs, including individual cough sounds, cough epochs [70], and cough seconds [71].

In a study of patients with unexplained chronic cough, these three units exhibited a strong correlation with each other and a moderate association with subjective assessments and quality of life (QoL), although cough epochs were found to be a less satisfactory option (64). In tuberculosis (TB) treatment research, measuring the total time spent coughing (seconds per hour) proved to be a better predictor of disease severity and response to microbiologic treatment than cough epochs [72]. However, in a prospective observational study comparing nocturnal cough across healthy subjects, patients with cystic fibrosis (CF), and those with primary ciliary dyskinesia, the repeatability of cough epochs per hour was higher than that of coughs per hour, with an intraclass correlation coefficient (ICC) of 0.75 versus 0.49 [73]. Additionally, a study of a novel therapeutic targeting peripheral gamma-aminobutyric acid type B ($GABA_B$) receptors, was shown to reduce the cough response to capsaicin in patients with RCC. This reduction was noted in the number of cough bouts per hour but not in total coughs per hour, compared to a placebo[74]. The researchers attributed

this finding to the specific role of $GABA_B$ receptors in initiating cough bouts, with minimal impact on the duration of each bout. Although most contemporary studies use individual explosive cough sounds as the unit for counting coughs, simply averaging the number of coughs per hour over a set period does not strongly correlate with the patient's experience. This leaves significant uncertainty regarding the optimal method of measuring cough to assess the efficacy of new treatments.

As of now, no universal standardized methods exist for counting coughs. Manual cough counting, which is non-automated, does demonstrate significant agreement between distinct listeners. This is the primary reason why manual cough counting is as widely used in large-scale clinical trials[75, 76].

The quest to design the perfect cough counting device has continued since the turn of the millennium. In 2001, *Pavesi et al.* demonstrated the utility of a computerised cough monitoring system which recognised cough audio vibrations signals and transmitted the the audio signal with electromagnetic waves to a piece of hardware in a locked box, without requiring a wired connection[77]. Although the data collection was able to be fully computerised, the individual cough data points for each participant had to be counted by humans. This unfortunate stumbling block led to the innovation of automated cough monitoring devices.

There are many challenges when approaching the design of a cough monitor. Ideally, it must be easy-to-use, non-obstructive, robust, and lightweight whilst having the capacity to record for a minimum of 24 hours. It is also essential that such monitors are able to identify as many coughs as possible and differentiating them from shouting, laughter, sneezing, and other explosive noises encountered in everyday life. The two most frequently used devices that have been developed are the VitaloJAK™ and the Leicester Cough Monitor (LCM). Both devices have been validated and are used in clinical studies, however, they have not been widely used for routine clinical practice.

Traditional Cough Counting Tools

VitaloJAK™

This semi-automated cough monitoring system was co-developed by clinical academics and the respiratory medical device company Vitalograph®. To date, it is the only cough monitor which is approved by regulatory bodies to be used in clinical trials to evaluate the effectiveness of antitussive agents.[78] The device consists of a digital acoustic sound recording device which has both a contact microphone, which is attached to the sternum, and a lapel microphone. Accompanying this is a web-based digital portal for transfer of data, and a digital signal processing algorithm which filters out all other sounds and leaves only possible cough sounds. After this algorithm is applied, a compressed audio file is then produced and sent to a specifically trained analyst who manually counts coughs.[64]

The initial validation of this device was performed in a study of 20 patients which investigated the capability of the VitaloJAK™ device to filter out background noise and non-cough sounds. The results of this study demonstrated that 98% of non-cough and irrelevant sounds could be filtered out using the machine's algorithim whilst allowing 100% of cough sound recordings to be preserved.[79, 80] This has since been further validated in a larger cohort of 143 patients. This found the sensitivity of the algorithim to be very close to 100% in a broad range of respiratory diseases including chronic cough, IPF, bronchiectasis, and asthma. The only disease where sensitivity was marginally lower was in COPD subjects, where the sensitivity was found to be 97.8%.

The VitaloJAK™ system has been the pre-eminent tool utilised in large-scale clinical trials of novel antitussive therapeutics over the past decade. It was used to measure the primary endpoint of 24-hour cough frequency in the COUGH-1 and COUGH-2 phase 3 trials of the P2X3 receptor antagonist gefapixant for patients with refractory and/or unexplained chronic cough.[81] It has also been

used in other disease areas, including in a phase 2 trial of the mixed opioid agonist/antagonist nalbuphine in IPF patients with severe cough.[82]

Leicester Cough Monitor

The Leicester Cough Monitor (LCM), developed and initially validated by Birring et al. in 2008, is an automated ambulatory cough monitor that records sound data. It consists of a portable, battery-powered digital recorder and a flip-collar microphone.[83] The data from these sound recordings are then analysed using the Leicester Cough Algorithm, which takes 6-24 hours of sound data and compresses it into adjoining 10 second segments. It then classes them as either potential cough events or irrelevant background sounds. This study then manually validated the cough sounds and irrelevant noises, taking 5 minutes per 24-hour recording period. A single cough in this analysis was defined as an isolated explosive sound, regardless of whether it was a stand-alone cough or occurred in a bout of coughs. The sensitivity for the LCM to capture cough events was 91% and the specificity 99% for one-hour recordings, they also reported a false positive rate of 2.5 coughs per hour.

The automated and manual observer cough counts were also validated for longer recordings, with an intraclass correlation co-efficient of 0.93. Although, the sensitivity of the automated system did drop to 86% when it analysed 6 hour recordings.[84]

The LCM has been used to assess the patterns of cough in various diseases, including acute cough[85], vocal cord dysfunction[86], asthma[87], and bronchiectasis[88]. It has also been used as a measure of objective cough frequency in randomised controlled trials of the neuromodulating drugs pregabalin and gabapentin.[89, 90] In addition to the use of LCM as a research tool, more recent data has shown that the LCM was acceptable to patients in routine clinical practice. A retrospective study analysing the use of the LCM showed that 75% of patients reported they were able to understand the instructions and use the monitors with relative ease. This study also claimed that

the LCM was able to detect real-world response to therapy and that there was a moderate correlation between changes in cough frequency and changes in subjective measures of cough severity such as the Laryngeal Hypersensitivity Questionnaire (LHQ) and Leicester Cough Questionnaire (LCQ), which will be explored later in this chapter.[91]

Hull Automatic Cough Counter

The Hull Automatic Cough Counter (HACC) was created to automatically recognize and analyse cough and non-cough sounds, excluding the silence period . It underwent preliminary validation by counting coughs one hour after waking up in 33 actively smoking participants. This approach resulted in a 97.5% reduction in counting time, achieving a sensitivity of 80%, specificity of 96%, and a reproducibility rate of 100%.[92] In a further validation study, HACC demonstrated a strong correlation with manueal human cough counting (r=0.87); however, it consistently missed approximately 15% of actual coughs [28]. As a result, a hybrid version of the HACC/LCM system was implemented to replace the HACC in subsequent studies. This system was initially implemented in a study to observe the changes in cough frequency in COPD patients convalescing from acute exacerbations.[93] It has also been utilised to study the effect of cystic fibrosis transmembrane conductance regulator (CFTR) modulators on cough in patients with cystic fibrosis.[94]

Novel Continuous Cough Monitors

In recent years there has been a shift in the paradigm of cough counting, with many now favouring the use of continuous cough monitors that can be worn for many days, weeks, and even months. The obvious advantage of this is the ability to collect more data, which better informs both patients and clinicians regarding responses to experimental and non-experimental therapy. Despite the relative novelty of such systems, there is some early data to demonstrate their use in both the research and real-world settings.

HyfeAI© Cough Monitoring

Hyfe© are a digital technology company which uses artificial intelligence (AI) to analyse explosive cough sounds. It utilises a cough monitoring application which is installed on an accompanying smart watch. It uses the smart watch's microphone to record and encrypt ambient 0.5 second sounds when an explosive 'cough-like' sound is detected. This sound recording is then classified as either a cough or non-cough by the app's algorithm. The captured data, including individual time stamps and cough duration, is then uploaded to *Hyfe's* data cloud via Wi-Fi whilst the smart watch is charging. In a non-peer-reviewed preprint validation study the AI-reported coughs were compared to manually-reviewed continuous recordings. This reported a 90.4% sensitivity over the 546 hours that were monitored, with a Pearson correlation coefficient of 0.99 between the manual recording and the AI systems. [95]

This technology has also been utilised in a smart phone application, which contains the same software as that in the smart watch. A feasibility study by *Lee et al.* demonstrated a median adherence to monitoring of 91% over an average of 13.9 days. This study also reported that 86% of users found the application acceptable and that 84% found it easy to use.[96] The smartphone based application has been evaluated in a number of studies in a broad range of disease areas, including tuberculosis (TB)[97], bronchiectasis[98], and COVID-19[99]. The *Hyfe* software has also been used in smart watches to measure the primary endpoint of daily cough rate in a clinical trial of inhaled alkaline hypervalent salts[100].

SIVA Health ™ Wearable Cough Monitor

The SIVA cough monitor is a small, lightweight monitor with a 'pebble-like' appearance that is worn as a pendant on the end of a necklace and sits on the centre of a patient's chest. The cough detector captures audio and movement data, pre-processing it to store only relevant information, specifically sound segments that surpass a predetermined loudness threshold. The cough-detection algorithm then analyses this data, producing a stream of time-

stamped cough events that do not enable the reconstruction of conversations or other audio details.

The algorithm incorporated in the SIVA device was evaluated by comparing manual cough counts over a 24-hour period in 27 patients. This study showed that the algorithm could detect coughs with an 88.5% sensitivity and close to 100% specificity. It also demonstrated excellent agreement between manual coughs counted per hour and coughs per hour detected by the algorithm, with a correlation coefficient of 0.95. When asked to respond to the statement 'I found wearing the cough detector was comfortable', 64% of patients stated that they either 'agreed' or 'rather agreed'[101].

While many other cough counting systems exist, including those integrated into multiparameter monitoring devices, those mentioned here are most frequently reported in the literature.

1.3.2 Subjective measures of cough

Cough Severity Patient Reported Outcomes

Cough Visual Analogue Scale and Numeric Rating Scale

Both the Visual Analogue Scale (VAS) and Numeric Rating Scale (NRS) are simple, standardised measures for assessing a patient's cough. The VAS is a 100mm long linear scale with two extremes of cough, usually represented as 'no cough at all' and 'worst cough imaginable', or other similar statements. A patient then places a mark on how troubling their cough is on a particular day. The NRS is very similar to this, however, the patient completing the NRS is usually asked 'On a scale of 0-10, how bad is your cough today?' and they are asked to circle a number between 0-10 on a linear scale.[102] Moreover, there are typically anchor statements underneath the numerical values on the scale indicating severity.

Thanks to its usability and practicality, the Visual Analogue Scale (VAS) is one of the most employed tools in both research and clinical settings for evaluating cough severity across various diseases[103-105]. Reports indicate good repeatability, with an Intraclass Correlation Coefficient (ICC) of 0.604 for chronic cough [28] and an ICC of 0.87 for COPD [106]. When used as an outcome measure in clinical studies, it has been shown that the minimally clinically important difference is ≥30mm[107].

Multidimensional Cough Index

The Multidimensional Cough Index (MCI) is a user-friendly scale consisting of nine items that assess various elements of cough including: cough intensity, frequency, physical impact, and sputum burden. The first four components are rated on a scale from 0 to 20. The MCI's performance has been validated, demonstrating robust psychometric properties with a intraclass correlation coefficient of 0.779. It has been shown to have significant correlations with cough VAS frequency (r = 0.651) and cough VAS intensity (r = 0.543),. A Cough Index of ≥4 effectively distinguishes chronic cough patients from healthy individuals, achieving a sensitivity of 80% and a specificity of 85%.[108]

Cough Symptom Score

The Cough Symptom Score (CSS) is a simple and practical tool that comprises two questions regarding cough frequency in both the day and nighttime. Both of the questions have scores ranging from 0 to 5 and the total score is from 0-10, with 0 representing no cough at all, and 10 representing the worst cough imaginable.[109] There has only been a formal validation of the Korean-language version of this score, which demonstrated a high repeatability (intraclass correlation coefficient of 0.75)[110].

McMaster Cough Severity Questionnaire

The McMaster Cough Severity Questionnaire is a newly validated questionnaire which consists of 8 questions which patients' rate on a 0-6 Likert scale. The

questions included in this tool explore multiple aspects of the cough, including the urge-to-cough, cough triggers, coughing whilst sleeping, and cough bouts. When tested on 100 patients with chronic cough the MCSQ demonstrated high level of internal consistency, with a Cronbach's alpha of 0.89. It also correlated well with the cough VAS, with a Pearson's correlation coefficient of 0.76.[111]

Cough Hypersensitivity Patient Reported Outcomes

Cough Hypersensitivity Questionnaire

The initial version of the Cough Hypersensitivity Questionnaire (CHQ) was a self-reported questionnaire featuring 35 items that evaluate the presence and severity of cough triggers and laryngeal sensations using a Likert scale, with total scores ranging from 0 to 150. Preliminary results indicated its potential to differentiate chronic hypersensitive cough (CHS) from healthy subjects; however, it lacked reliability and repeatability data.[112]

The revised version of the CHQ is made up of 23 items, 16 questions relating to cough triggers and 7 related laryngeal sensations, with yes/no answers. This was used in a pulmonary sarcoidosis study and demonstrated good internal consistency (Cronbach's α=0.90). It was also found to have a moderate correlation with health status (r=-0.68) and cough severity (r=0.57).

Hull Airways Reflux Questionnaire

The Hull Airways Reflux Questionnaire (HARQ) is a tool consisting of 14 items, designed to determine the likelihood of airway reflux based on the typical consequences of gaseous, non-acidic reflux from the gastrointestinal tract. Each item on the HARQ is scored from 0 (no problem) to 5 (serious problem), with higher scores reflecting more severe cough-related symptoms. The HARQ is validated and recommended as a diagnostic tool [41] and demonstrates good internal consistency (Cronbach's α = 0.81) and reproducibility (r = 0.78).

Normal scores for healthy individuals range from 0 to 13. It has shown high sensitivity (94%) and specificity (95%) for detecting chronic cough and it has a MCID of 16.[113] To further validate this questionnaire in patients with chronic cough, it was applied to over 2000 patients with refractory and/or unexplained chronic cough in the COUGH-1 and COUGH-2 trials for gefapixant, this showed that 95% of respondents had a score above the upper limit of normal (14/70), with a mean HARQ score of 40.[114]

Cough-specific Quality of Life Patient Reported Outcomes

Leicester Cough Questionnaire

The Leicester Cough Questionnaire (LCQ) is a multifaceted tool that explores the 3 broad domains of physical, psychological, and social effects of cough on a patient's quality of life. It is composed of 19 questions which are scored from 1 to 7, with higher scores indicating a better quality of life. It has been shown to have a have good internal reliability (Cronbach's α=0.89) and high repeatability (intraclass correlation coefficient of 0.96) in chronic cough patients and strongly correlated with cough VAS.[115] This tool has been shown in multiple studies to correlate well with cough frequency in patients with chronic cough[28, 71, 116]. The MCID for the LCQ is 1.3 for the total score, 0.2 for both the physical and social domains, and 0.8 for the psychological domain[117].

The LCQ has been the most popular cough-related quality of life questionnaire for many years and is often used in large-scale clinical trials of novel cough therapeutics. It has also been used in a number of different diseases including COPD[118], Cystic Fibrosis [119], and asthma[120].

Cough Quality of Life Questionnaire

The Cough Quality of Life Questionnaire (CQLQ) is a 28-item tool which is comprised of six domains: psychosocial issues, functional abilities, physical complaints, extreme physical complaints, personal safety fears, and emotional well-being. All questions are answered on a 4 point Likert scale and lower scores

signal better quality of life[121]. It has demonstrated validity and reliability in chronic cough with a Cronbach's α=0.92 and an intraclass correlation coefficient of 0.89[122]. The MCID for the CQLQ was found to be 22 out of a total of 112 points.

The CQLQ has also been shown to correlate moderately with the LCQ before treatment (r=-0.42) and strongly after treatment (r=-0.60) in patients with chronic cough. It has also had validation studies performed in IPF and COPD patients[123, 124].

All the tools listed here attempt to understand the patient's experience of cough in both research and clinical settings To align with common practice in large-scale clinical trials, this thesis will predominantly utilize the LCQ and Cough Severity VAS. The HARQ will also be used as it is the only questionnaire which assesses the impact of reflux on cough hypersensitivity.

1.3.3 Subjective measures of breathlessness

As with cough, there is a plethora of subjective tools to measure breathlessness in a broad range of diseases. Many of these tools are not exclusively designed for those with chronic respiratory disease, as they are also administered to those with advanced cancer and cardiovascular diseases such as heart failure.[125] It is generally accepted that subjective measures of breathlessness are superior to simple physiological parameters that are associated with an increased breathing frequency. This is because a patient's experience of breathlessness does not correlate well with objective measures such as respiratory rate, forced expiratory volume in 1 second, and peripheral oxygen saturation[126]. The reason for this is thought to be due to the complex, multifactorial nature of breathlessness. It is thought that dyspnoea is not a single sensation as it includes several sensations that are distinct from one another including: work/effort of breathing, chest tightness, and air hunger or unsatisfied inspiration. These sensations are also believed to have distinct physiological, neurological, and psychological mechanisms.[127] Thus, only patient-centered, subjective

measures of breathlessness that are applicable to patients with chronic respiratory disease will be utilised in this thesis.

1.4 Measuring Gastro-oeosphageal Reflux and Oesophageal Function

The field of gastrointestinal physiology has advanced significantly over the last few decades. Predominantly working in conjunction with gastroenterologists, there are various testing modalities physiologists can employ which help clinicians gain nuanced insight into patients with a broad range of conditions, including GORD and dysphagia [128]. Traditional methods for investigating reflux-related disease, such as oesophagogastroduodenoscopy (OGD), rely on direct visualisation of the oesophagus to detect tissue damage, such as is present with reflux oesophagitis[129]. Such techniques may be very user-dependent and do not provide any information regarding the function of the gastrointestinal tract and the transit of solid and fluid boluses along the tract. The development of specially designed trans-nasally inserted catheters has provided us with the means of measuring pressure within the oesophagus. Such tools utilise either solid-state and/or water-perfused pressure transducers, which have the ability to translate changes in oesophageal pressure into electrical signals[130]. The development of such methods of testing oesophageal function have greatly advanced the ability to diagnose gastrointestinal dysmotility, which I hypothesise may play a role in the development and progression of respiratory disease.

1.4.1 Multichannel Impedance-pH Monitoring

The requirement to comprehensively assess the nature of gastro-oesophageal reflux has led to the development of multichannel impedance-pH monitoring (MII-pH). This tool enables full assessment of the acidity of refluxate as well as quantifying the frequency of reflux events. Moreover, as it is placed and kept in situ for up to 24 hours, this method of testing enables patients to record symptom diaries, allowing for temporal association of symptom onset and reflux

events[131].

Impedance is a unit of resistance that expresses the measure of opposition to current flow between two or more electrodes. The impedance of the conductor surrounding the catheter consists of the fluid or solid bolus, the walls of the oesophagus, and bodily fluids which each have characteristic recorded values[132]. This allows for specific values to be attached to differing types of refluxate, providing a qualitative description of an individual patient's GORD. Using this technique, classical, liquid refluxate will be demonstrated by a high electrical conductivity compared to that of the smooth muscle of the oesophagus, thus impedance is decreased. Conversely, there is very little conductivity with the passage of gaseous refluxate, leading to increased impedance[133]. In clinical practice, measurements are taken with a small diameter (~2mm), flexible, polyurethane catheter which has 6 to 8 electrodes spaced at 17, 15, 9, 7, 5, and 3 centimetres from the lower oeosphgaeal sphincter (LOS) [134]. The tip of the catheter consists of the pH sensor, which is usually made of antimony, and is placed roughly 5cm above the LOS. This sensor is calibrated to detect rapid reductions in pH below 4.0. Normal oeosphageal pH is believed to be around 7.0. The reason that a cut-off of 4.0 is used is because the gastric enzyme pepsin is inactive above this pH [135].

Despite the valuable information regarding the nature and frequency of reflux that MII-pH can provide, it does not yield any information on the contractility of the oesophagus. It can only determine whether patients experience typical acid reflux associated with GORD or if they have increased gaseous reflux events. As a result, one may mistakenly assume that if neither of these are present, the patient does not have gastrointestinal pathology. However, a normal MII-pH study does not exclude oesophageal dysmotility.

1.4.2 High Resolution Oesophageal Manometry

The measurement of oesophageal contractile vigour using conventional line-tracing manometry has been available since the early 1970's. This technique was performed utilising silicone catheters which had 3 -8 external volume-displacement transducers spaced roughly 3-5cm apart [136]. During each study, the pressure that is generated by oesophageal smooth muscle contraction distorts the transducer and consequently changes the electrical resistance of the sensor. The changes for the sensors are then recorded in real time and specific line graphs are generated for each individual sensor [137]. The resulting polygraph would originally have been printed on recording paper with hand-written annotations of the positions of each catheter. With the advent of advanced computer software programming, such polygraphs were able to be generated and analysed automatically as we approached the turn of the 21st century.

The watershed moment of oesophageal manometry testing came in the 1990's when a gastrointestinal physiologist named Ray Clouse pioneered the development of High Resolution Oesophageal Manometry (HROM) [138]. By vastly increasing the numbers of pressure transducers in the manometry catheter we are now able to assess oesophageal function in much more granular detail. Most modern HROM catheters possess 36 pressure sensors spaced 1cm apart. Each sensor having 12 pressure sensitive segments which are averaged for each location, enabling a circumferential measurement of pressure along the entire length of the oesophagus [139].

The abundance of pressure readings that are produced by HROM allow the production of colour topography traces (Figure 2). Such traces reflect the contractile vigour of each section of the oesophagus, allowing for a space-time reading of oesophageal contractility. Computer software assigns a colour code to specific measurements of pressure, creating a 'colour bar' such as that seen in Figure 3. Individual colour bars are then generated for each of the pressure

readings recorded by all 36 sensors. Data gaps between sensors are filled in using interpolation, a process in which an analytical software calculates the estimated pressures located between sensors [140]. This yields a spatial continuum of contractility values across the length of the oesophagus which is then mapped onto the topography trace.

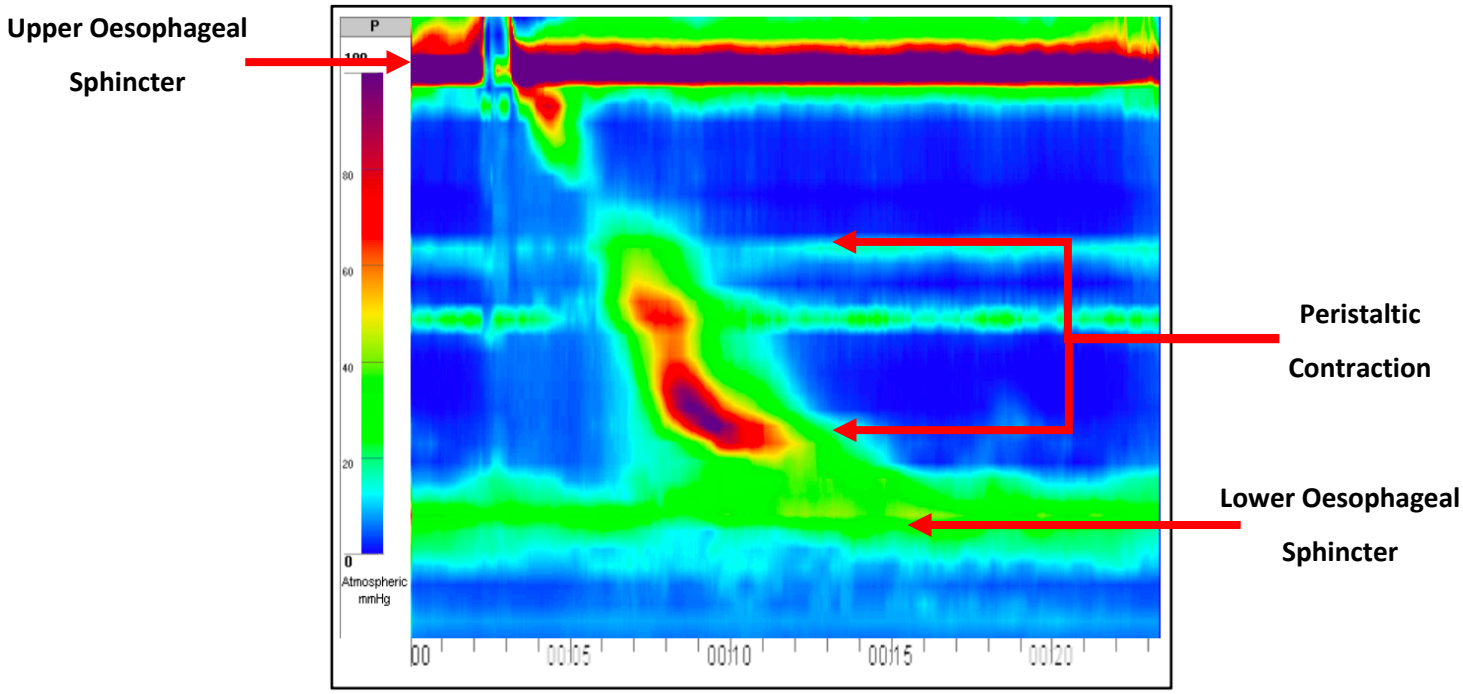


Figure 2. *An example of a colour topography trace with labels denoting the position of the oesophagus, upper oesophageal sphincter, and lower oesophageal sphincter*

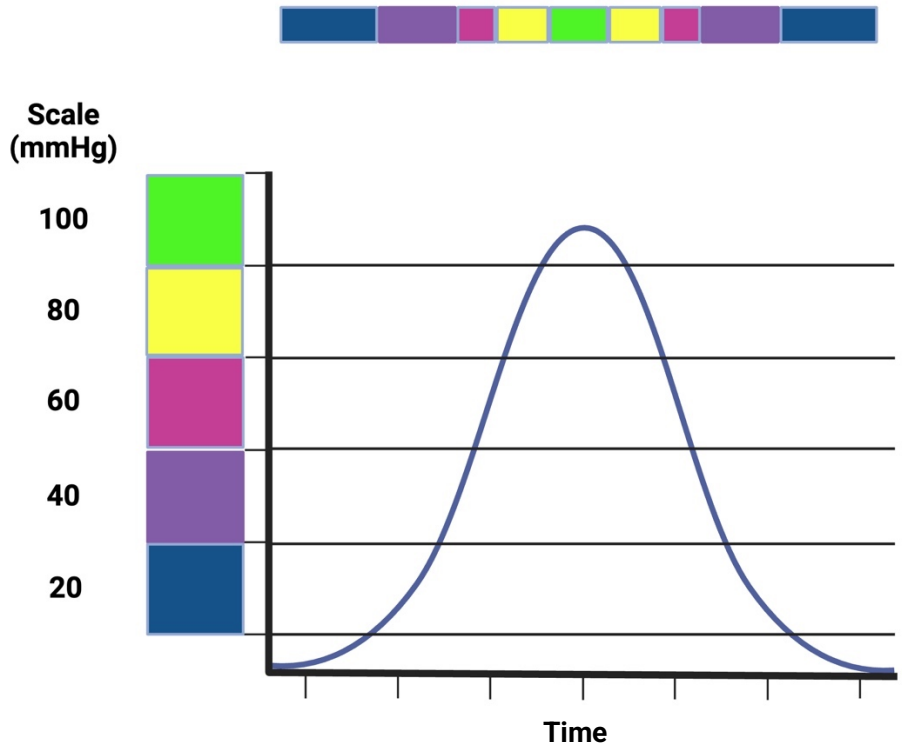


Figure 3. *An example of a recording from a single pressure sensor within the oesophagus during a peristaltic contraction. The colour bar at the top of the diagram indicates the pressure recorded at the specific time point on the x-axis.*

One of the predominant advantages of HROM over conventional manometry is the ability to visualise the function of both the upper oesophageal sphincter (UOS) and LOS[141]. This allows us to detect pathologies with both sphincters such as excessive transient LOS relaxations (TLOSR), in which relaxations may occur independently of swallowing leading to retrograde transit of gastric contents. A phenomenon that may contribute to the development of GORD[142].

The British Society of Gastroenterology have specific guidelines for the technical aspects of performing HROM[143]. Patients are to be fasted for at least 4 hours prior to the procedure and medications such as calcium channel blockers, opiates, and prokinetics are to be stopped for 48 hours prior to the investigation. The catheter is passed transnasally, in a similar manner to

nasogastric feeding tubes, and it is positioned under real-time manometric guidance. This allows for accurate visualisation of both the UOS and LOS. The catheter is positioned so at least 5 sensors are present in the stomach during testing, once this has been achieved the probe is secured before the swallow tests are performed. The physiologist conducting the test will then guide the patient through a series of swallowing tests, using both water and solid food (usually bread) as boluses in upright and supine positions. The exact quantity, volumes, and positions used will vary based on the suspected diagnosis, patient tolerance, and physiologist's experience[144].

The arrival of standardised colour topography plots and metrics of measuring oesophageal motility led to the development of the Chicago Classification of Motility Disorders [145]. The following HROM metrics are vital to classifying motility disorders[146]:

- Distal Contractile Integral (DCI), the primary measure of oesophageal contractility, expressed in mmHg/cm/s
- LOS resting pressure (LOSP), expressed in mmHg
- Distal Latency (DL), the measurement of the time taken for the initiation of oesophageal contraction following a swallow, expressed in seconds
- Integrated relaxation pressure (IRP) , the measure of how well the oesophagogastric junction relaxes during swallowing.

Specific cut-off values are then applied to diagnose a multitude of different motility disorders including gastro-oesophageal outflow obstruction, achalasia, ineffective oesophageal motility, and hypercontractile oesophagus. The development of this classification system has revolutionised the diagnosis of dysphagia and other associated swallowing disorders in the field of gastroenterology. This thesis will explore how these established principles may be applied to the world of respiratory medicine, specifically how we can utilise this detailed, minimally invasive investigation to better understand the role of the oesophagus in patients with refractory respiratory symptoms.

1.5 The Gastrointestinal Tract and Respiratory Disease: The Gut-Lung Axis

The interaction between the digestive and respiratory systems has been described for many years. Indeed, one of the earliest observations of the effect of the gastrointestinal tract on cough was made by Dr Thomas Sydenham, a famous English physician who lived in the 17th century. In his 1668 treatise *Tussi*s he observed that there was a distinct phenotype of patient who described a chronic cough, which he referred to as *tussis callida per accidens*. He described such patients to be '*of more robust and sanguine consitutions'* and advised that *'strong wines or vinous spirits should be avoided'*. To people who may suffer from particularly bad heartburn after a full-bodied glass of red wine, it may be deduced that this was one of the earliest descriptions of GORD-related chronic cough [147]. Almost 400 years later there have been descriptions of the impact of reflux on the development and progression of various respiratory diseases, however, the exact mechanism remains undescribed.

1.5.1 Reflux and Chronic Cough

As previously stated, the traditional belief was that GORD was responsible for the development of chronic cough due to the deleterious effects of gastric acid on pulmonary epithelium [148]. Indeed, it was suggested that proximal oesophageal reflux was the sole driver of reflux cough, however, a study by *Decalmer et al.* demonstrated that although patients with chronic had a significantly higher number of total reflux events, there were no differences in the number of events extending to the proximal oesophagus [149]. An alternative hypothesis centred around the 'oesopahgeal-bronchial reflex' has also been detailed. The cough reflex may be directly triggered from the distal oesophagus being irritated by acidic refluxate [150]. Animal studies have shown that this may be caused by crosstalk of vagal neurones at the nucleus tractus solitarius that converge from both the airways and oesophagus. The irritation

caused by acid exposure produces a referred initiation of the cough reflex via this vagal pathway, moreover it has also been demonstrated that vagotomy reduces airway inflammation in guinea pigs repeatedly infused with intra-oesophageal hydrochloric acid [151, 152]. These laboratory findings have been corroborated by a clinical study by *Javorkova et al.* where hydrochloric acid was infused into the oesophagus if patients with chronic cough and healthy controls. Using capsaicin inhalation challenges immediately post-infusion the authors demonstrated a significant increase in cough-reflex sensitivity in cough patients but no change in the healthy volunteers [153]. Despite the evidence for the oesophageal-bronchial reflex, as stated above, there have been mixed results to support the use of anti-acid therapy for chronic cough, even in those with concomitant GORD [154, 155].

Whilst it appears that typical acidic GORD plays a role in chronic cough, of tantamount importance is non-acid reflux. Non-acidic liquid and gaseous reflux events occur in a large proportion of the healthy population [156]. The paradigm of airway reflux was first described in 2013 and suggested that natural anatomical faults that were a result of humans becoming bipedal as well as our natural laryngeal apparatus allowing for aspiration into the airways [157]. Non-acid reflux is thought to be primarily mediated by disorders of the oesophagus, indeed *Vardar et al.* found that patients who reported cough had a significantly lower number of oesophageal contractions and a higher LOS pressure than healthy controls [158]. Moreover, a small retrospective review of patients that underwent HROM for investigation of their chronic cough demonstrated that weak oesophageal peristalsis with large breaks (WPLBs) occurred in over one-third of patients. This was more than double the 12% prevalence of WPLBs found in control patients who reported dyspepsia but no cough [159].

Advanced imaging techniques, specifically reflux scintigraphy, have provided additional evidence of both acid and non-acid reflux and their impact on the respiratory system. This radiological method enables direct visualization of refluxed particles entering the lungs by using technetium-tagged water [160]. Dynamic imaging for a prolonged period of time, usually up to 30 minutes, in

order to visualise the direction of travel of refluxate, this imaging is then performed again 2 hour later to assess for pulmonary aspiration. A scintigraphic study by *Burton et al.* demonstrated that oesophageal dysmotility, measured by manometry, was significantly associated with increased presence of pharyngeal and pulmonary aspiration [161]. There is a relative dearth of studies examining this technique in patients with chronic cough, however, one study by *Songur et al.* reported that although over two-thirds of patients had distal reflux, only 6% of patients had pulmonary aspiration. However, this study did use dated analytic methods. More studies are required to accurately measure the prevalence of pulmonary aspiration in patients with chronic cough, moreover the technique of reflux scintigraphy needs to be further evaluated as a diagnostic tool for patients with reflux cough.

1.5.2 Reflux and Idiopathic Pulmonary Fibrosis

The relationship between GORD and IPF is somewhat a 'chicken and egg' situation, as it is unclear whether decreased lung compliance in the fibrotic lung causes increases in pleural pressure, consequently causing a reduction in LOS pressure, and enabling reflux of gastric contents [162]. Despite this, it has long been known that there is a strong association between GORD and IPF. Indeed, a prospective study of 133 patients with IPF *Raghu et al. (*31) demonstrated that 87% had abnormal acid reflux, however, only a half of patients reported dyspeptic symptoms [163]. Moreover, the association between IPF and hiatus hernia is well described, with it being observed concomitantly in up to 40% of patients [164]. Additionally, it has been observed that the presence of a hiatus hernia, regardless of acid reflux, is associated with disease progression and death in IPF [164]. The role of non-acid reflux has also been examined in IPF patients, with *Savarino et al.* demonstrating significantly more frequent weakly acid reflux events using 24-hour multi-channel impedance monitoring. This study also reported a significantly higher concentration of pepsin, the gastric digestive enzyme, in the BAL and sputum in IPF patients compared to non-IPF controls. Finally, the authors demonstrated a significant association between reflux

events, BAL pepsin concentration, and the radiological severity of fibrosis on HRCT [165].

Despite first being described over half a century ago, the pathophysiology of IPF remains incompletely understood. Indeed, until recently it was thought to be an inflammatory disease which could be treated with immunosuppression. This was comprehensively rejected by the PANTHER trial which showed that anti-inflammatory drugs including corticosteroids and azathioprine increased mortality [166]. However, it is known that in patients with the usual interstitial pneumonia (UIP) histological pattern, the primary driver of fibrosis is the presence of fibroblastic foci. In a study by *Bois et al.,* it was found that in IPF patients who had a hiatus hernia, there were significantly higher concentrations of airway-centred fibroblastic foci. The authors suggested that this may reflect chronic microaspiration of gastric contents due to the positioning of the foci [167].

The above evidence of the association between both acid and non-acid reflux and IPF is convincing and is supported by the small amount of evidence available for anti-reflux treatments in this patient group. A recent systematic review by *Fidler et al.* included 8 observational studies in a meta-analysis examining the effect of pharmacological anti-reflux therapy on IPF patients. The authors found that treatment of GORD was associated with a significant reduction in IPF-related mortality but not all-cause mortality [168]. However, a more recent real-world population-based study by *Tran et al.* demonstrated no mortality or hospitalisation benefit with PPI use compared with non-PPI users as controls [169]. One may argue that this does not consider the reason for PPI use and the study was not enriched for those with GORD only, meaning that it cannot disprove the efficacy of PPIs in a select group of patients. What is abundantly clear is the need for a large randomised controlled trial to plug this evidence gap, hopefully the results of the ongoing TIPAL trial should provide us with a definitive answer regarding the efficacy of PPIs in IPF patients.

1.5.3 Reflux and Airways Disease

The data for the role of reflux in both asthma and COPD are scarcer than that for both IPF and chronic cough. In a small study performed almost 30 years ago, it was shown that over two-thirds of patients with asthma had an oesophageal motility disorder. Moreover, it showed that a large proportion of patients had prolonged oesophageal acid exposure [170]. Such findings have been corroborated in a further study by *Amarasiri et al.* The investigators also observed that oesophageal motility in this cohort was associated with vagal hyperreactivity. This interesting finding further evidences the role between the neuronal disease of cough hypersensitivity, vagal pathology, and oesophageal dysmotility [171]. A novel experiment by *Jack et al.* many years ago simultaneously measured the oesophageal and tracheal pH in a group of asthmatic patients. This study demonstrated a small number of reflux events that lasted over 5 minutes, leading to a fall in tracheal pH and peak expiratory flow rate [172]. This suggests that there may be a role for acidic GORD in the pathogenesis of asthma. Despite this, there is only limited evidence for the role of anti-acid and prokinetic medications in asthma. One small study in 30 patients demonstrated lung function improvements with omeprazole and domperidone, but did not report any data on clinical parameters such as exacerbation frequency or symptom burden. However, a subsequent study that again used omeprazole and domperidone reported a significant reduction in SABA use as well as an improvement in symptoms and lung function [173].

Akin to IPF, the mechanism by which reflux may occur in COPD is believed to be structural in nature. Lung hyperinflation, a key feature of emphysema, causes diaphragmatic flattening leading to reduced LOS tone. This was evidenced by *Gadel et al.* who demonstrated that greater indices of hyperinflation was associated with reduced LOSP and UOSP [174]. A further small study examined the prevalence of GORD in COPD compared to healthy controls with fascinating results. The authors found that patients with COPD had a significantly higher DeMeester score compared to controls, indicating higher oesophageal acid

exposure. Moreover, it was found that over 70% of COPD patients had oesophageal dysmotility and that extent of proximal reflux was associated with an increased number of COPD exacerbations per year [175]. This has been corroborated by the large ECLIPSE cohort study, which observed that self-reported GORD increased risk of moderate and severe exacerbations [176]. However, distinguishing between correlation and causation in COPD is more difficult. Greater hyperinflation is associated with more advanced disease and therefore higher risk of exacerbation. Thus, a greater degree of structural distortion of the LOS may lead to increased reflux and dysmotility but may not necessarily drive the progression of COPD.

1.5.4 Measurement of Airway Reflux

Although a thorough clinical history, radiological, and physiological investigation will raise suspicions of the presence of refluxate in the respiratory system objective tests to confirm this are highly sought after. This desire led to the development of Peptest (RD Biomed Ltd, Cottingham, UK), which utilises lateral flow technology to analyse sputum samples. This includes two distinct anti-pepsin human monoclonal antibodies—one for detection and one for capture of pepsin within a clinical sample. The intensity of the pepsin ‘test’ line, located within the lateral flow device's window, is measured using a Peptest cube reader. This measured intensity is automatically converted into a pepsin concentration (ng/ml) [177].

One study by Strugala et al. investigated the prevalence of a positive sputum Peptest in a cohort of patients with chronic cough who were also believed to have an element of airway reflux. Pepsin positivity was detected in 80% of a cohort of 93 patientswho had a mean HARQ score of 31.9. This was significantly higher than the pepsin positivity identified in a group of 87 healthy subjects (37%)[178] . More recent data bu Gu et al. has demonstrated that Peptest has an 83.6% sensitivity and 82.7% specificity in diagnosing reflux cough using a pepsin cutoff value of 76.10ng/mL. They found that the salivary pepsin demonstrated comparable diagnostic value to the GerdQ questionnaire in diagnosing an acid-reflux cough and superior value in diagnosing a non-acid reflux cough. This is a rather expected result as this questionnaire is no more effective than flipping a coin in predicting non-acid reflux[179] , probably due to the fact it primarily

focuses on typical manifestations of acid reflux which are rarely seen in non-acid reflux [180], and therefore its utility in the cough clinic is questionable.

Despite the evidence to demonstrate the utility of Peptest in reflux cough it remains solidly in the category of 'research tool'. Integrating Peptest into the diagnostic algorithm may enhance diagnostic accuracy and enable targeted treatment strategies tailored to the underlying cause, thereby reducing unnecessary use of medications. It is important to consider the timing of saliva sampling for Peptest, either after cough attacks or in the morning and after meals, as coughing is often an episodic phenomenon with a temporal pattern. Peptest perhaps should be incorporated into clinical practice, but only as part of a more intricate workup in which the discovering the nature of the reflux is as important as identifying its presence.

1.6 Therapeutic Targets of the Gut-Lung Axis

The wealth of evidence supporting the gastrointestinal tract as a therapeutic target in chronic respiratory disease has led to numerous clinical studies evaluating the impact of both anti-acid and prokinetic agents. In the previous subchapter we have discussed the conflicting evidence for anti-acid medications such as PPIs and the need for large-scale randomised controlled trials to definitively assess their efficacy. Here we will explore the efficacy of medications which are believed to reduce reflux and improve gastrointestinal motility.

1.6.1 GABA receptor agonists

The LOS is innervated primarily by the vagus nerve. One of the important receptors responsible the relaxation of this sphincter, giving rise to reflux, is the gamma-aminobutyric acid (GABA) receptor. The GABAergic pathway is one of the cardinal inhibitory functions in the central and peripheral nervous system. $GABA_B$ receptors have been shown to be present in the enteric nervous system and importantly inhibit gastro-oesophageal mechano-sensitive vagal afferents [181]. The first drug that was utilised to help reduce reflux by targeting the GABAergic pathway was baclofen. This agent, which was initially used for spasticity in diseases such as multiple sclerosis, was shown to increase LOS tone and reduce the frequency of spontaneous relaxations, particularly in the post-prandial period [182]. Similar findings were also demonstrated in a cohort of patients with GORD by *Zhang et al.* Using oesophageal manometry, this study demonstrated that baclofen reduced reflux events by 40% and increased the basal LOSP but had no effect on oesophageal acid exposure time [183]. There have been studies examining the effect of baclofen in chronic cough, however, there is no placebo-controlled trial demonstrating its efficacy in respiratory disease [184].

There is stronger evidence supporting the use of an alternative GABA-receptor agonist; gabapentin. The first randomised-controlled trial demonstrating its safety and efficacy was reported by *Ryan et al.* which demonstrated a significant improvement in quality of life, measured by the LCQ, compared with placebo [89]. However, as gabapentin has been shown to improve anxiety disorders, the assessment of quality of life could be confounded by this effect. However, since this initial study, further trial and observational data have also reported significant improvements in cough-specific QoL, cough frequency, and cough severity [185]. It has also been shown that gabapentin demonstrated similar efficacy in chronic cough patients to other neuromodulators, but is better tolerated [186].

Recent advances have led to the development of an agent that works exclusively on peripheral $GABA_B$ receptors. This agent, lesogaberan, has a much more palatable side effect profile as its effects within the central nervous system are minimal [187]. Doubts were cast over this agent's role as an antitussive as early studies demonstrated it had no effect on cough-reflex sensitivity in healthy subjects [188]. However, this can be attributed to the fact that lesogaberan will not suppress the central cough reflex and in healthy volunteers, who do not have pathological reflux or vagal hypersensitivity, there will be no effect. Promisingly, a subsequent small trial reported improvements in cough reflex sensitivity and cough bout frequency [74]. This study did not, however, select patients based on reflux symptoms which suggests that a larger study of patients with reflux cough is required.

Unfortunately, there is very little data examining the effect of neuromodulators on other chronic respiratory diseases. Baclofen has been studied in asthmatic subjects, however, this has been focused on the effects on bronchial hyperresponsiveness rather than the sequalae of gastrointestinal reflux [189].

1.6.2 Dopamine receptor antagonists

The dopaminergic pathway has been implicated as a significant player in gastrointestinal motility. Animal studies have demonstrated that intravenous infusion of dopamine completely inhibits contractility of both the stomach and lower oesophageal sphincter. Moreover, in the same model, it was found that the dopamine receptor antagonists, metoclopramide and domperidone, restored digestive contractions [190]. Metoclopramide, a potent D2 receptor antagonist, has been used as a prokinetic in reflux disease and as an antiemetic for many years. It has been shown to reduce post-prandial reflux episodes, increase LOSP, and improved oesophageal contractility in healthy controls [191]. Such improvements have also been observed in patients with systemic sclerosis, a disease which can be associated with the development of interstitial lung disease [192]. Domperidone is an alternative D2 receptor antagonist, also used as an antiemetic, which possesses a lower side effect profile due to its inability to pass the blood-brain barrier. An early study of domperidone demonstrated its ability to rapidly raise the LOSP to almost 300% of its basal resting pressure, suggesting that it may also have an anti-reflux effect [193].

Unfortunately, due to safety concerns of prolonged exposure to drugs that act on the dopaminergic pathway, there are very few clinical studies that have examined their efficacy in chronic respiratory disease. One study of asthmatic children demonstrated that domperidone, when given alongside esomeprazole, led to improvements in asthma control and lung function [194]. The prokinetic effects of metoclopramide for preventing pneumonia was described in a randomised controlled trial by *Warusevitane et al.,* which showed a significant reduction in aspiration in a group of stroke patients treated with nasogastric tube feeding regimes [195]. There has also been one clinical study exploring the effects of domperidone on cough and airways inflammation. *Liu et al.* demonstrated a reduction in self-reported cough scores in conjunction with a reduction in sputum IL-8 and substance P concentrations [196]. However, the patients in this non-controlled study concomitantly received a PPI, making it

difficult to determine the true effect of domperidone. A high-quality randomised controlled trial of anti-dopaminergic agents is required, but, due to the non-selective nature of currently available drugs, such a trial is unlikely to occur.

1.6.3 Macrolides as prokinetic agents

Macrolide antibiotics, including azithromycin which is the main subject of this thesis, have been shown to improve gastrointestinal motility in several studies. When given as antibacterial agents, it is known that macrolides can engender gastrointestinal side effects such as abdominal pain, diarrhoea, and bloating. The cause of this was found to be due to their effect on the migrating motor complex. In a human study, *Tomomasa et al.* demonstrated that administration of erythromycin resulted in significantly longer durations of gastrointestinal contractions [197]. Moreover, as has been observed with the other prokinetic agents discussed above, erythromycin has been shown to increase LOSP in patients with GORD [198]. Macrolides have been shown to act on motilin receptors, which are expressed on enteric neurons in the smooth muscle of the stomach, duodenum, and colon [199]. Moreover, motilin agonists have been shown to improve oesophageal motility and LOS pressures, suggesting motilin receptors may also be expressed in the upper gastrointestinal tract [200]. It was initially believed that macrolides increased the endogenous production of motilin. However, in-vitro studies disproved this but demonstrated that erythromycin had differential effects between species. *Peeters et al.* reported that erythromycin displaced motilin at the receptor and led to contraction of duodenal smooth muscle in rabbits and humans, but not in rats and dogs [201]. Such findings were reproduced by *Broad et al.* with azithromycin, which displaced motilin on human gastric antrum cells [202].

Clinical studies have demonstrated the efficacy of the prokinetic properties of macrolides. Azithromycin was shown to reduce the number of acid reflux events and postprandial acid exposure in a study of patients with GORD. Interestingly, this study also reported a reduction in hiatus hernia size after treatment with

azithromycin [203]. These findings were also recorded in a group of patients who had received a lung transplant [204]. This finding is interesting because GORD has been implicated in chronic transplant rejection, and azithromycin has since been shown be of benefit for bronchiolitis obliterans in such patients [205]. The utility of macrolides will be discussed in the next chapter; however, no clinical studies have explored the relationship between the prokinetic effects of azithromycin and clinical outcomes. This is a blind spot that this thesis hopes to shed some light on.

1.7 Azithromycin and Chronic Respiratory Disease

Azithromycin is a second generation macrolide antibiotic which was first synthesised in the former Yugoslavia (modern-day Croatia) in 1980 [206]. It is a semi-synthetic derivative of the first generation macrolide erythromycin, the addition of a methyl-substituted nitrogen ion resulted in azithromycin becoming the first 15-membered lactone ring azalide[207]. This led to a superior oral bioavailability and tissue selectivity compared to other macrolides, enabling the drug's concentration to persistently stay above the minimal inhibitory concentration (MIC) of bacteria for many days. It has been shown to be particularly enduring in the gynaecological tissue, prostate, and respiratory tract [208]. Moreover, it's long half-life allows for single doses to be given in large quantities providing a prolonged bacteriostatic effect for many days [209].

Azithromycin, as with other macrolide antibiotics, primarily exerts its antimicrobial effect by binding to the large 50S subunit of the ribosome in bacterial cells. The occupation by macrolides in the region of the peptidyl transferase centre inhibits cell protein synthesis and consequently causes cell growth arrest [210]. Thus, azithromycin is classed as a bacteriostatic antibiotic, as its effects halt the process of bacterial cell reproduction, allowing the host immune system to eliminate the infection. What sets azithromycin apart from older macrolide antibiotics is its broad spectrum of activity. Not only is it highly

active against pyogenic bacteria such as *Streptococci* and *Staphylococci*, but azithromycin has also been shown to have good inhibitory activity against gram negative bacteria such as *Haemophilus Influenzae* and *Enterobacteriaceae* [211]. This is in stark contrast to erythromycin, which has little or no effect on such gram-negative bacteria. This broad spectrum of efficacy is particularly useful for infections in patients with chronic respiratory disease, such as COPD, where such pathogens are more prevalent [212, 213]. Due to its proven efficacy against a wide range of infectious diseases, azithromycin has been widely adopted as a first line treatment for various conditions, including sexually transmitted infections, upper and lower respiratory tract infections, diarrhoeal diseases, and ophthalmological infections [214]. Hence it is no surprise that the World Health Organisation (WHO) has recommended mass administration of azithromycin in areas of high infant mortality to prevent infection-related deaths [215].

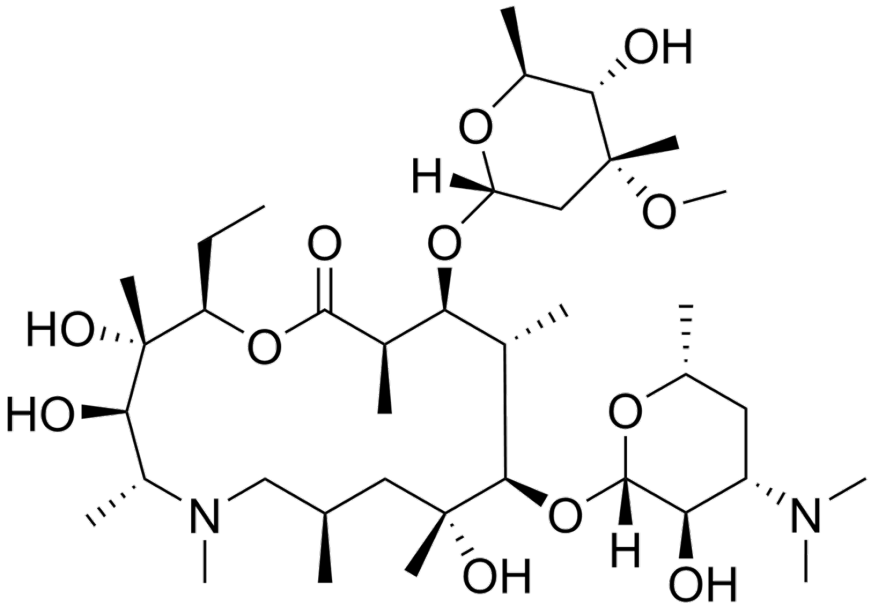


Figure 4. *Chemical structure of azithromycin*

1.7.1 Long-term azithromycin therapy

Over the past 25 years, much attention has been drawn to the repurposing of azithromycin as a long-term medication to treat chronic respiratory disease. The first disease where azithromycin was trialled in this capacity was Cystic Fibrosis

(CF) [216]. This initial trial performed by *Equi et al.* demonstrated a significant reduction in the need for courses of antibiotics in children with CF. This finding was then replicated by *Wolter et al.* in adult patients with additional data suggesting that azithromycin reduced the rate of lung function decline and improved quality of life [217]. The colonisation of the airways with *Pseudomonas auerginosa* and it's subsequent domination of microbiome is a prominent feature in this group of patients. Thus, it was believed that azithromycin, although previously thought to be ineffective against *Pseudomonas*, may inhibit the deleterious effects of this pathogen in such patients. Various mechanisms have been suggested, including the formation of an immune complex to disrupt the *Pseudomonal biofilms* and direct anti-*pseudomonal* effects [218, 219]. Despite some in vitro evidence, larger clinical trials in CF patients with known *Pseudomonas* colonisation showed no reduction in bacterial load with azithromycin, although similar clinical improvements were observed [220].

The findings in CF patients inspired others to test the efficacy of azithromycin for other chronic respiratory conditions. The first large randomised-controlled trial for azithromycin in COPD, reported *Albert et al.,* mirrored earlier successes as it showed an approximately 30% reduction in exacerbation frequency when compared to placebo [221]. Such findings were repeated in the COLUMBUS trial, which exclusively enrolled frequent COPD exacerbators [222].
This was then followed by two large trials, BAT and EMBRACE, examining the effect of azithromycin in non-CF bronchiectasis. Both trials found a significant reduction in exacerbation frequency in this patient group; however, there was no convincing effect on lung function or health-related quality of life [223, 224]. Moreover, microbiology data from the BAT trial once again found no difference in the burden of *Pseudomonas* found in sputum culture. However, this study did find that macrolide resistance was dramatically increased among all pathogens (including *Haemophilus Influenzae, Streptococcus Pneumoniae, and Moraxella Catarrhalis*) in those treated with long-term azithromycin, with a resistance rate of 88%.
The most recent discovery of the utility of azithromycin has come in patients

with severe, uncontrolled, asthma. The initial AZISAST study found that there was no effect of azithromycin on exacerbations in all patients. However, in a pre-specified subgroup analysis the authors demonstrated a significant benefit for those with a low type-2 inflammatory profile (i.e. non-eosinophilic asthma) [225]. This trial also demonstrated a significant improvement in asthma-related quality of life, measured using the Asthma Quality of Life Questionnaire (AQLQ). In the more recent, larger AMAZES clinical trial, a significant reduction in exacerbation was observed in both eosinophilic and non-eosinophilic patient groups [226]. Data from a systematic review and meta-analyses of the literature have also suggested that azithromycin may improve lung function and bronchial hyper-responsiveness [227].

The wealth of large-scale clinical trial evidence demonstrating the efficacy of long-term azithromycin, as well as other macrolide antibiotics, across a spectrum of respiratory diseases, casts major doubt on the original hypothesis that benefits arise from their anti-bacterial or anti-*pseudomonas* activity. The breadth of azithromycin's utility in diseases with differing pathophysiology suggests that other mechanisms may be at play in exerting its effects. The role of azithromycin as a prokinetic agent, a mechanism with potential cross-disease relevance, will be the primary focus of this thesis and will be discussed in further detail in following chapters. However, it is important to note that it is possible that azithromycin exerts its clinical effects in multiple ways, which may indeed work synergistically to improve the lives of patients with chronic respiratory disease.

1.7.2 Antimicrobial effects of long-term azithromycin

Long-term treatment with macrolides, including azithromycin, is usually prescribed at a dose that is thought to be subtherapeutic in terms of antibacterial activity. However, a microbiological substudy from the AMAZES trial demonstrated a reduction in a bacterial diversity of the lower respiratory tract microbiome after azithromycin treatment. Moreover, this study reported a

significant reduction in the load of *Haemophilus influenze* found in sputum samples [228]. A similar effect of long-term macrolide therapy was also shown in both adults and children with bronchiectasis as well as adults with COPD [229-231]. The findings of the BLESS study, which investigated the use of erythromycin in non-CF bronchiectasis suggests that the reduction of *Haemophilus* in patients with particularly high loads of this bacteria leads to a significant reduction in exacerbation. However, no such benefit was detected in those with high loads of *Pseudomonas*. This directly contradicts the early hypothesis, as discussed previously, that intrinsic anti-*Pseudomonas* activity is responsible for the efficacy of long-term macrolides. Although not proven, the reduction in *Haemophilus* load may represent a partial explanation of exacerbation reduction across the spectrum of respiratory disease, as it is a well-recognised driver of exacerbations [232, 233].

There has been some evidence to suggest that prolonged treatment with azithromycin may also have antiviral properties. In a study of human bronchial epithelial cells pre-treated with azithromycin, *Gielen et al.* reported a significant increase in expression of Rhinovirus-induced interferons [234]. An interesting finding of this study was that this effect was not observed in erythromycin or telithromycin, suggesting this is a unique property of azithromycin. Similar findings were also reported by *Menzel et al.* who observed the in vitro effects of azithromycin in bronchial epithelial cells of both healthy subjects and COPD patients [235]. Interestingly, this study only showed a reduction in rhinovirus-induced interferons in COPD cells and not those of healthy volunteers. Moreover, unlike *Gielen et al.* this study showed a reduction of viral load with azithromycin treatment. Other studies have also shown that acute treatment with azithromycin or short-term prophylaxis has been associated with improved inflammatory profiles of murine models infected with Influenza A and parainfluenza type 1 [236, 237]. This antiviral effect has not, however, yielded positive results for the acute treatment of viral infections. This includes the failure of azithromycin to improve mortality of patients with COVID-19, regardless of whether they had respiratory disease [238]. Such findings leave the

clinical antiviral properties of azithromycin in doubt. Although in vitro and animal models suggest direct antiviral effects, it has been proposed that any protection that long-term azithromycin confers is likely due to its anti-inflammatory properties [239]. Despite this, further investigation is still required to explore the characteristics of viral infection in those with chronic respiratory disease who are being treated with long-term azithromycin. It is possible that the observed antiviral effects may result in a 'milder' viral illness in patients with airways diseases, thereby reducing the likelihood of needing acute treatments such as oral corticosteroids.

1.7.3 Immunomodulatory mechanisms of long-term azithromycin

The most common mechanism by which azithromycin is thought to work almost as a panacea in respiratory medicine is through its effects on both innate and adaptive immunity. One could write an entire thesis detailing the different mechanisms by which azithromycin has been thought to exert its immunomodulatory effects. Here we will provide a brief overview of a few select hypotheses for which convincing evidence has been presented.

1.7.3.1 Azithromycin and Neutrophils

One of the more studied cellular components of airway inflammation that has been described are neutrophil extracellular traps (NETs). These structures are formed when neutrophils produce a mesh of fibres composed of chromatin DNA, bactericidal proteins, and histones which exert a bacteriostatic effect on pathogens [240]. Although the process of NET formation has a role in the innate immune response, it has been shown that aberrant 'NETosis' is implicated in chronic airways disease inflammation, including COPD, asthma, and most convincingly in bronchiectasis [241-243]. However, the pathogenic role of excess NET formation may differ amongst respiratory diseases. One proposed theory is that persistent NET presence may results in pulmonary epithelial damage compromising the barrier function of epithelial cells. This may then exacerbate existing airway inflammation whilst simultaneously increasing the

likelihood of respiratory infection [244]. It has also been proposed that abnormal NET production is intrinsically involved to the pathogenesis of non-CF bronchiectasis. As NETs have been shown to be ineffective against killing common pathogens found in airways disease[245] it is believed that inappropriate NET production is only ineffective in removing the bacteria but then also exerts its damaging effects against the host. Moreover, this theory has been corroborated with the higher concentration of NETs found in patients with both *Pseudomonas*- and *Haemophilus*-dominant microbiomes [243].
Using sputum proteomics, it has been demonstrated that azithromycin can significantly reduce the sputum concentration of NETs in patients with asthma and non-CF bronchiectasis [243]. To date there is no agreed mechanism by which macrolides such as azithromycin modulate NET production in the airways. A murine emphysema model showed that erythromycin reduced NET levels by downregulating Th1 and Th17 cells [246]. Furthermore, it has been shown in healthy human neutrophils that azithromycin may reduce the production of reactive oxygen species and cell degranulation, therefore diminishing NET formation [247]. Despite such experimental evidence and convincing correlation between reduction in presence of NETs and improved disease control, the direct importance of NETs in chronic respiratory disease remains unproven. Work is required to elucidate the exact significance of NETs and NETosis which, if they are a vital component of pathogenesis, may be a useful therapeutic target.

1.7.3.2 Effect of azithromycin on leucocyte migration and activity

It is thought that long-term azithromycin may attenuate the infliltration of the airway with leucocytes. The exact mechanism by which this is believed to work is unclear, however, it is though that azithromycin modulates the production of adhesion molecules and chemokines. A study of healthy volunteers by *Culic et al.* showed that administration of azithromycin causes a reduction in serum IL-6, IL-8, and the cell adhesion molecule sV-CAM-1 [248]. Such findings were corroborated in a study by *Majanovic et al.* in steroid-naïve COPD patients. This study took sputum samples of such patients and treated them with multiple

macrolides including azithromycin, discovering in a reduction of a vast array of chemokines [249]. These laboratory findings were again reinforced by a clinical study of long-term azithromycin for patients with COPD who had a tracheostomy. This study by *Blasi et al.* revealed that IL-6 and TNF-α concentrations reduced whilst on treatment with azithromycin, whereas the standard care arm were found to have higher concentration of such cytokines. Moreover, this study also observed that concentrations of both IL-6 and TNF-α increased back to their baseline value upon cessation of azithromycin treatment [250]. However, it is unlikely that reduction of such mediators is the cardinal mechanism of azithromycin. This is evidenced by the fact that direct antagonism of these molecules does not lead to improved clinical outcomes in patients with respiratory disease [251, 252].

Azithromycin is also believed the regulate the activity of alveolar macrophages. The proportions of the two recognised phenotypes of macrophages, M1 (pro-inflammatory) and M2 (anti-inflammatory), are thought to be regulated by azithromycin therapy. *Murphy et al.* demonstrated a significant increase in the production of IL-10 and IL-12 which are known as the anti-inflammatory chemokines which are produced by M2-like macrophages [253]. Despite this evidence, there have been no studies examining the effect of azithromycin on macrophage phenotypes in patients with chronic respiratory disease.
Further studies have shown that azithromycin augments the phagocytic capacity of airway macrophages. *Hodge et al.* first demonstrated this in a study comparing the phagocytosis of macrophages in patients with COPD and healthy controls. They demonstrated that, although baseline phagocytosis was impaired in COPD patients, azithromycin greatly improved the phagocytosis of neutrophils and epithelial cells by alveolar macrophages [254]. A subsequent study from the same group reported similar findings in a clinical study of patients with COPD. Using bronchoalveolar lavage (BAL) samples, they again found that azithromycin greatly increased phagocytic ability of alveolar macrophages as well as showing an increase in mannose receptor expression in the serum [255]. Since this report, the importance of mannose receptor function and expression as well as

mannose-binding lectin (MBL) has been studied across the spectrum of respiratory disease. Variations in the genes that code for mannose receptors have been shown to increase the risk of developing asthma [256]. *Lin et al.* demonstrated that a genetic polymorphism leading to MBL deficiency increased the risk of recurrent exacerbation of COPD [257]. Moreover, reduced mannose-binding lectin expression is associated with poorer quality of life and worse radiological disease in bronchiectasis [258]. This protein plays a crucial role in the innate immune system by 'tagging' pathogens for phagocytosis by macrophages, therefore if this is upregulated by azithromycin it may improve the hosts ability to prevent infection.

1.7.3.3 Azithromycin and adaptive immunity

The evidence for azithromycin's effects on the adaptive immune system is less comprehensive, however, it has been shown to influence dendritic cell function. An in vitro study by *Iwamoto et al.* showed that azithromycin significantly reduced the expression of multiple components of the allogenic immune response in dendritic cells, including toll-like receptor 4, IL-12, and major histocompatibility complex (MHC) class II proteins [259]. This may reduce the allogenic inflammatory response which is believed to play a part in the development and progression of chronic respiratory disease. One clinical study by *Xuan et al.* has demonstrated that azithromycin treatment reduced the secretion of IL-10 and IL-12 in children with asthma. However, this study did not show any direct impact on cell surface molecules of dendritic cells such as CD83, CD80, and CD86 [260].

As stated above, none of the studies mentioned have provided a definitive mechanism for the immunomodulatory properties of azithromycin. The absence of clinical evidence demonstrating immunomodulation in patients with chronic respiratory disease in clinical studies casts doubt on whether the in vitro findings truly reflect what occurs in patients. Additionally, it is unclear whether the

changes in immune responses that we have observed in patients are just downstream effects of other systemic actions of azithromycin.

1.8 Conclusions

In this chapter I have discussed the current literature regarding the assessment of cough, the role and measurement of reflux in respiratory disease, as well as our current understanding of how azithromycin benefits patients with respiratory disease and what the mechanism behind this is. From this review I believe that our present knowledge incomplete.

I believe that the prevailing questions for which answers are elusive are as follows

1. How prevalent is non-acid reflux and oesophageal dysmotility in respiratory disease? Can we use HROM as a diagnostic tool to identify this issue in patients with refractory respiratory symptoms?
2. Although azithromycin reduces exacerbation frequency, what impact does it have on symptom burden in patients with chronic respiratory disease?
3. What is the effect of azithromycin on oeosphageal function in patients with respiratory disease? Does long-term azithromycin therapy concomitantly improve peristaltic function and cough in such patients?
4. Can we use more novel techniques of continuous cough monitoring and analysis to measure the effect of long-term azithromycin in patients with chronic respiratory disease?

The following experiments that are detailed in this thesis will set out to provide an answer to some of these questions. My intention is to generate evidence to support the hypothesis that azithromycin is a useful therapeutic for patients with refractory chronic cough and troublesome cough in a variety of respiratory disease. Moreover, I believe that azithromycin exerts this anti-tussive effect by improving oesophageal functioning in such patients.

Chapter 2

Investigating the Diagnostic Utility of High Resolution Oesophageal Manometry in Patients with Refractory Respiratory Symptoms

2.1 Introduction

We have established that persistent respiratory symptoms such as cough, wheeze, and breathlessness represent a huge burden on healthcare systems. Indeed, they are among the most common reasons for presentation to primary and secondary care in the United Kingdom, comprising up to 22% of all patient contacts.[261] Chronic respiratory diseases also account for more than 10% of productive life lost secondary to medical issues, and are known to cause great detriment to the psychological, physical, and socioeconomic wellbeing of patients.[262-264] Unfortunately, many patients who are treated with optimal medical therapy for respiratory diseases such as asthma, chronic cough (CC), and COPD may report daily symptoms [265, 266]. For patients and clinicians alike, the cause for these refractory symptoms is often frustratingly unclear and traditional investigations for pulmonary diseases may prove insufficient in elucidating the aetiology.

As discussed *in chapter 1* In recent years, the gut-lung axis has been suggested as a potential driver of symptom burden in those with respiratory disease [267-269]. Indeed, much of the current literature is focused on the link between gastroesophageal reflux disease (GORD) and conditions such as, COPD, asthma, CC and interstitial lung diseases (ILD).[30, 176, 270, 271] However, we have established that precious little work exists examining how the functioning of the oesophagus may play a role in respiratory pathophysiology.

In this study, I examined upper gastrointestinal tract function in people with chronic respiratory diseases and persistent symptoms despite optimal medical therapy. This chapter aims to examine the diagnostic utility of HROM in the cohort of patients where traditional avenues of investigation may be fruitless.

2.2 Methods

In this retrospective, single-centre observational study, data were collected for all patients that were investigated using HROM for refractory respiratory symptoms between January 1st 2011 and December 1st 2021. A list of all patients that had been referred for HROM by consultant physicians in respiratory medicine was collated. Referral details were then checked to ensure that the referral was made for investigation of oesophageal dysmotility and/or GORD in patients who were experiencing persistent symptoms despite optimal medical management. Approval for the data collection for this study was granted by the Hull University Teaching Hospitals NHS Trust clinical governance committee.

High Resolution Oesophageal Manometry

All HROM investigations were undertaken in the same GI physiology lab using similar testing equipment. Due to the length of the period that data were recorded, different versions of similar testing equipment were used. However, the same protocol for recording oesophageal manometry was used throughout. This protocol was as follows:

- All testing was carried out using a 36-channel solid-state unidirectional manometric catheter which was placed transnasally.
- Patients were asked to swallow 10 boluses of water at intervals of 20-30 seconds, followed by swallows of a solid bolus of a small piece of bread.
- Measurements were analysed and reported using Laborie Investigation and Diagnostic Software.
- The quality of each swallow was assessed and stratified into 6 categories of peristalsis: normal, ineffective, failed contraction, premature, hypercontractile, and fragmented.
- Data from the quality of the swallows as well as measurements of the integrated relaxation pressure (IRP), distal contractile integral (DCI),

distal latency (DL), and lower oesophageal sphincter resting pressure (LOSRP) were analysed and patients were given a manometric diagnosis using the contemporaneous Chicago Classification.[272] Examples of each of these diagnoses can be seen in figure 5.

24-hour pH and Impedance Monitoring

The 24-hour pH ambulatory and impedance study was carried out using Laborie Investigation and Diagnostic Software. A pH sensor was placed 5cm above the proximal border of the lower oesophageal sphincter, with 6 impedance-measuring electrodes positioned at intervals of 3cm, 5cm, 7cm, 9cm, 15cm and 17cm above the proximal border of the lower oesophageal sphincter. Patients were asked to carry out their normal activities of daily living for 24 hours before returning the equipment. Measurements were recorded to determine total duration of distal oesophageal acid exposure (Acid Exposure Time)[273], patient reported symptoms (using an event marker device), number of reflux events, and the character of the reflux event (whether it was gas/ liquid and acidic/ non-acidic). Data collected were analysed by Laborie Investigation and Diagnostic Software to determine the DeMeester score and identify potential reflux events. These were then manually analysed by a Specialist Clinical Scientist in GI Physiology.

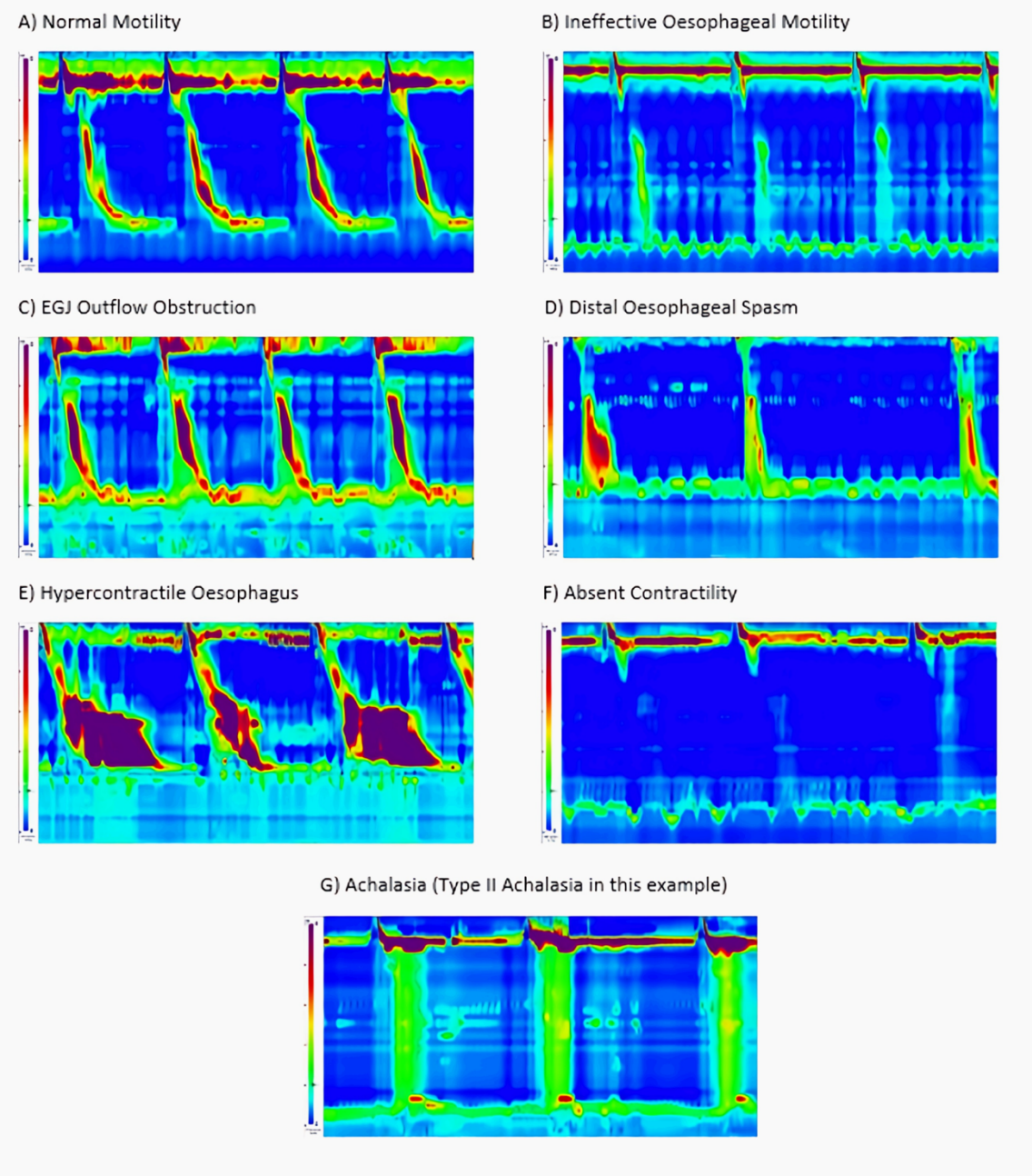


Figure 5. *Data acquired using HROM is visualised in the images above, converting manometric information and displaying the data as a topographic plot that represents both anatomy and physiology. Pressure is represented by colour; the lowest pressures are represented as blue, graduating to the higher pressures represented in purple. Sensor location is on the y-axis, and time is on the x-axis. The two horizontal bands of pressure correspond to the resting upper oesophageal sphincter (UOS) pressure (located at the top of the images), and the lower oesophageal sphincter (LOS) pressure (located at the bottom of the images). The pattern of colour in-between these two distinct bands of pressure represent the pressure profile within the oesophagus. Images A-G illustrate the manometric profile of the seven main categories of diagnosis from the Chicago Classification.*

Patient demographics

Demographic information including age, sex, smoking status, and primary respiratory diagnosis were collected for all patients through examination of electronic patient records. Previous clinical care event records were also examined to determine whether patients' symptoms have been assessed using the HARQ tool[113]. This questionnaire was selected above others as its primary purpose is to identify those patients with concomitant reflux and cough hypersensitivity.

Statistical Analysis

All demographic data are presented descriptively. Comparison of means was performed using independent t-tests, comparison of medians was performed using Mann-Whitney U testing, and comparison of proportions was performed using Chi-squared testing. Comparison of the relationship between any two variables was performed using simple linear regression analysis. All data were analysed using IBM SPSS Statistics 26 (IBM Corp., Armonk, NY, USA).

2.3 Results

Between the period January 1st 2011 to December 1st 2021, 441 patients with chronic respiratory disease were investigated with HROM. Of such patients, 64% were female and the mean age was 56.5 years (SD 13.9). The common primary diagnoses of the patients investigated included CC (77%, n=339), Asthma (10%, n=44), ILD (7%, n=29), Cystic Fibrosis (3%, n=12), and COPD (2%, n=10). Twenty-four percent of patients reported being a current or ex-smoker.

Manometry Results and Diagnoses

All patients had sufficient manometric assessment adequate to diagnose oesophageal disorders as per the contemporary Chicago Classification. Of all 441 patients, 34.5% (n=152) demonstrated normal motility on HROM, 54% (n=238) had Ineffective Oesophageal Motility (IOM), 7.3% (n=32) had Absent

Contractility, 3.2% (n=14) had Oesophageal-gastric Junction Outflow Obstruction (EGJOO), 0.5% (n=2) had Distal Oesophageal Spasm (DOS), 0.5% (n=2) had Achalasia, and a single patient met the criteria for Hypercontractile Oesophagus. The overall prevalence of disorders of oesophageal motility (i.e. any diagnosis other than normal motility) was 66% (n=290). There were no statistically significant differences between males and females in the prevalence of any of these manometric diagnoses. Furthermore, the proportion of patients with any disorder of oesophageal motility was not different between males and females (63% vs 67%, p=0.414). All data regarding the manometric findings of all patients can be found in Table 1. The prevalence of each manometric diagnosis stratified by respiratory diagnosis can be seen in Figure 6.

In addition to examination of oesophageal motility, patients were also assessed for the presence of a hiatus hernia. Of all patients, 41% (n=181) were found to have a hiatus hernia on HROM. The proportion of females who were found to have a hiatus hernia was significantly higher than that of males (45% vs 34%, p=0.029).

24-hour pH Testing

The results of 24-hour pH testing were available for 87% (n=383) of all patients. Of these patients, the median DeMeester score was 8.49 (range = 0.2-167.81) and 38% (n=144) of patients who underwent pH testing had a positive DeMeester score (defined as higher than 14.72), indicating peptic gastro-oesophageal reflux disease.

The median (range) number of reflux events in 24 hours in those who underwent testing was 64 (1 - 1050). We were able to further characterise each reflux event in 42% (n=161) of the patients who had pH testing into acid/non-acid and gaseous/liquid reflux. The median (range) proportion of reflux events that were characterised as acidic in all patients was 35% (0 – 87%) and the median proportion of reflux events characterised as gaseous was 41% (0 – 100%).

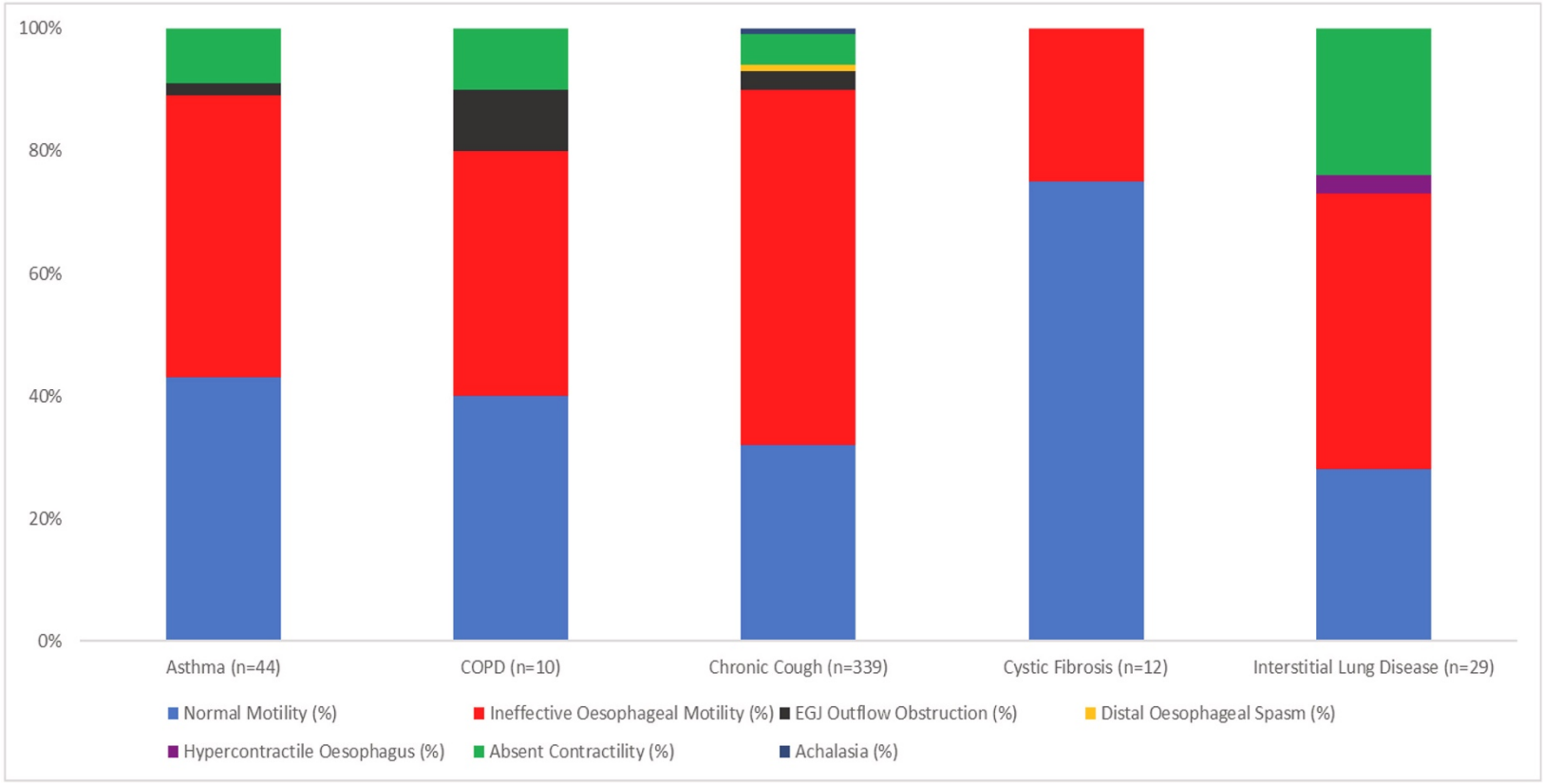


Figure 6. *Proportion of patients with each manometric diagnosis, stratified by the respiratory diagnoses frequently observed in this study.*

	All patients (n=441)	Males (n=158)	Females (n=283)	p-value*
Mean Age (SD)	56.5 (13.9)	55.0 (16.6)	56.4 (12.7)	0.328
Female (%)	283 (64)	-	-	
Mean HARQ score (SD)	38.8 (13.2)	38.0 (12.5)	39.3 (13.6)	0.313
Smoking History (%)	106 (24)	29 (18)	77 (27)	0.062
Diagnosis (%)				
Chronic Cough	339 (77)	113 (72)	226 (80)	-
Interstitial Lung Disease	29 (7)	14 (9)	15 (5)	-
Cystic Fibrosis	12 (3)	8 (5)	4 (1)	-
Asthma	44 (10)	16 (10)	28 (10)	-
COPD	10 (2)	4 (3)	6 (2)	-
Median Oesophageal Manometry Metrics (range)				
Integrated Relaxation Pressure (mmHg)	11.6 (-3.5-104)	10.35 (-2.3-104)	11.95 (-3.5-30.5)	0.003
Distal Contractile Integral (mmHg/sec/cm)	570 (0-5890)	553 (0 -3411)	572 (0-5890)	0.703
Distal Latency (sec)	6.7 (3.8-11.9)	6.6(3.8-10)	6.7 (4.6-11.9)	0.891
Lower Oesophageal Resting Pressure (mmHg)	26.2 (2.1-105)	24.5 (5.5-88.1)	25.9 (2.1-105)	0.310
Chicago Classification (%)				
Normal Motility	152 (34)	58 (37)	53 (39)	0.459
Ineffective Oesophageal Motility	238 (54)	84 (53)	154 (54)	0.800
EGJ Outflow Obstruction	14 (3)	2 (1)	12 (5)	0.088
Distal Oesophageal Spasm	2 (1)	2 (1)	0 (0)	-
Hypercontractile Oesophagus	1 (1)	1 (1)	0 (0)	-
Absent Contractility	32 (7)	10 (6)	22 (9)	0.575
Achalasia	2 (1)	1 (1)	1 (1)	0.675
Manometric Findings				
Any Oesophageal Dysmotility (%)	290 (66)	100 (63)	190 (67)	0.414
Hiatus Hernia (%)	181 (41)	54 (34)	127 (45)	0.029
Dysmotility or Hiatus Hernia (%)	360 (82)	119 (75)	241 (85)	0.010
Median 24-hour pH Study Findings (range)				
DeMeester Score	8.49 (0.2-167.81)	10.65 (0.2-167.81)	7.48 (0.2-144.14)	0.064
Number of Reflux Events	64 (1-1050)	74 (4-1050)	59.5 (1-332)	0.997
% of Reflux Events Acidic	35 (0-87)	38 (0-87)	31.5 (0-84)	0.483
% of Reflux Events Gaseous	41 (0-100)	43 (0-91)	40 (0-100)	0.969

Table 1. *Baseline demographics, HARQ scores, HROM and 24-hour pH study findings for patients included in this study.*

Correlation of Oesophageal Studies and Clinical Assessment with the HARQ

HARQ scores were available for 83% (n=366) of all patients, with the mean score being 38.8 (SD=13.2), the upper limit of normal in patients without airway reflux is a score of 14[36]. The mean HARQ score was significantly higher in those with any diagnosis of oesophageal dysmotility when compared to those with normal manometric studies (40.6 vs 35.3, $p<0.001$). Furthermore, linear regression analysis demonstrated a significant inverse correlation between a higher HARQ score and a lower DCI, when controlled for age. Our model predicted a 13.4mmHg/sec/cm reduction in DCI for every point increase on the HARQ (95% CI 19.9-6.8, $p<0.001$), with a correlation coefficient of -0.205. There were no significant relationships identified between the HARQ and IRP, DL, and LOSRP. Scatter plots examining the relationship between HARQ score and manometry readings can be seen in figure 7.

There were no significant relationships identified between the HARQ score and DeMeester scores or number of reflux events in 24 hours. There was no difference in the mean HARQ score between patients with ≥50 reflux events in 24 hours and those with <50 reflux events in 24 hours (39.2 vs 38.5, $p=0.318$). Furthermore, there was no difference in the mean HARQ score between those who met the diagnostic criteria for GORD (i.e. a positive DeMeester score) and those who did not (37.9 vs 39.2, $p=0.231$).

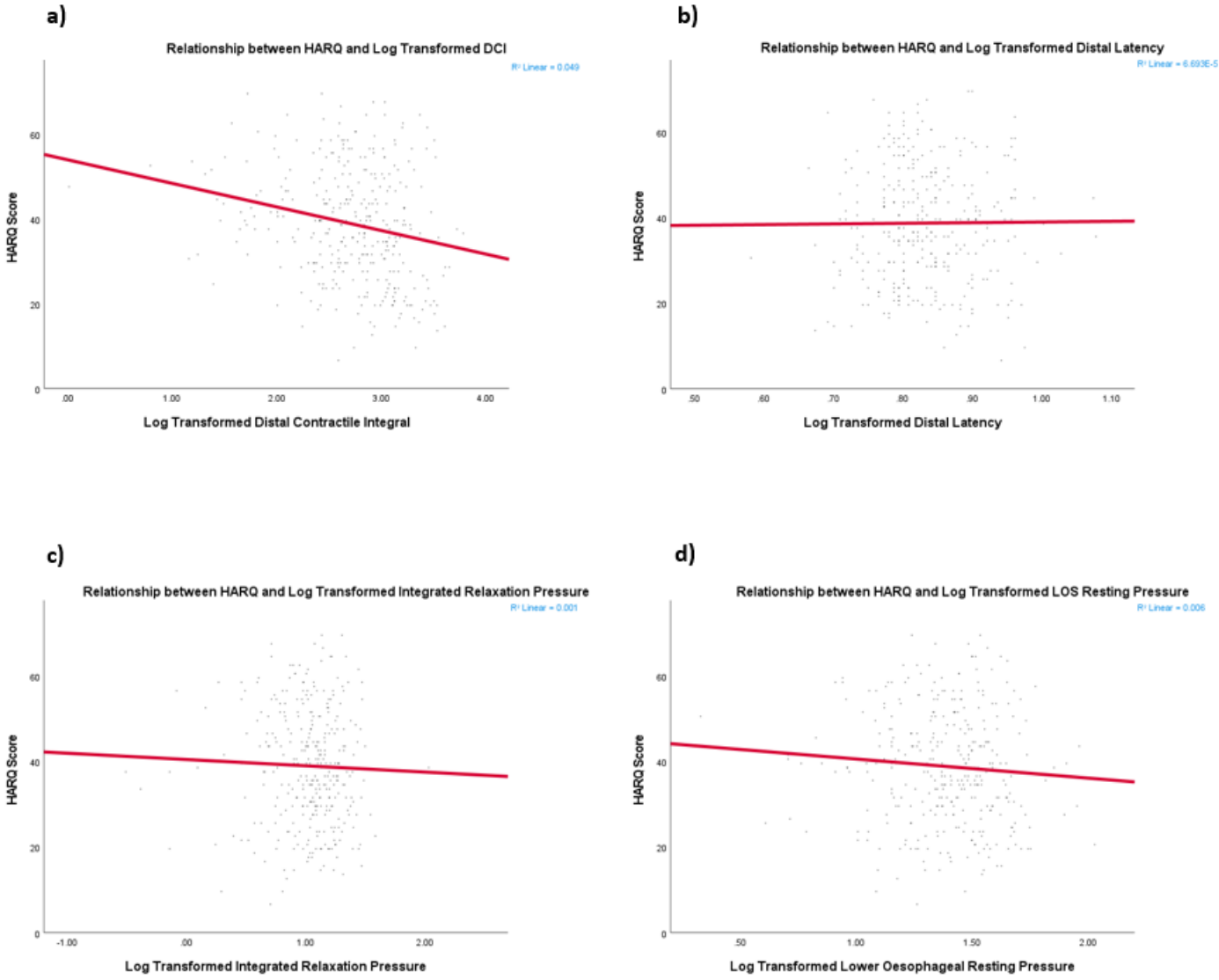


Figure 7. *Scatter diagrams with lines of best fit displaying the relationship between the HARQ scores and HROM metrics. a) Displaying a significant negative relationship between increasing HARQ score and Distal Contractile Integral. b) Displaying a non-significant relationship between HARQ score and Distal Latency. c) Displaying a non-significant negative relationship between the HARQ score and Integrated Relaxation Pressure. d) Displaying a non-significant negative relationship between the HARQ score and Lower Oesophageal Sphincter Resting Pressure*

2.4 Discussion

In this study, we report that 66% of patients with refractory respiratory symptoms demonstrate disorders of oesophageal motility. The proportion of patients with oesophageal dysmotility is consistently high over a range of respiratory diseases, including interstitial lung disease (72%), airways disease (57%), and CC (68%). The median DCI (the measure of the peristaltic vigour of the oesophagus) in our study was 570mmHg/sec/cm, less than half of the average DCI in a study of healthy, albeit slightly younger, individuals.[274] Subsequent 24-hour pH testing in our cohort of patients showed a lower prevalence of traditional GORD, with only 38% of patients exhibiting a positive DeMeester score. The (relatively) lower prevalence of traditional GORD may suggest that is not acidic reflux from the stomach which is implicated in respiratory disease, as has been historically suspected [176, 270, 275]. We hypothesise that impaired peristaltic activity of the oesophagus leading to aspiration of airway reflux may be the cardinal insult.

The concept of the gut-lung axis is one that has been previously recognised in systemic diseases that cause severely impaired oesophageal function such as systemic sclerosis. The latter has been closely linked with the development of ILD and airways disease.[276, 277] Indeed, one study showed that patients with absent contractility on HROM demonstrated a significantly lower forced vital capacity (FVC) and diffusion capacity for carbon monoxide (DLCO) than those with normal motility.[278] Such findings suggest a potential investigation and treatment strategy for patients with whom conventional approaches have failed. However, it is important to recognise that not all those with persistent respiratory symptoms will have oesophageal dysfunction, thus careful patient selection for an invasive procedure such as HROM is essential.

In all patients in this study, investigation with HROM was deemed appropriate as they reported reflux-related symptoms as evidenced by their HARQ score. This validated tool evaluates the likelihood that a patient's respiratory symptoms

could be attributed to airway reflux.[36] Our data appear to legitimise this method of selecting patients for investigation with HROM, as higher HARQ scores were significantly correlated with a lower DCI, even when adjusted for age. We believe the HARQ offers a useful screening tool for determining the appropriateness of oesophageal study in such patients.

As discussed in Chapter 1, the current therapeutic options to tackle the dysfunctional oesophagus in respiratory disease are limited. There is precedent of gastrointestinal intervention in diseases such as CC and ILD, as anti-reflux surgery has been utilised to improve symptoms in the former and prevent progression of disease in the latter.[279, 280] Surgical intervention, however, may be contraindicated for those with poor peristaltic activity of the oesophagus as peristalsis may be worsened by the correction of lower oesophageal sphincter anatomy.[281] Macrolide antibiotics such as azithromycin are commonly used in the management of a number of chronic respiratory diseases[282], have been shown to promote gastrointestinal motility through agonist activity at the motilin receptor. Indeed, macrolides reduce the rate of reflux events in patients with GORD and in patients who have undergone lung transplantation.[203, 283, 284] There is a requirement for studies aiming to examine the effect of macrolides and other prokinetic agents on oesophageal motility in patients with respiratory disease. In patients reporting a high burden of reflux symptoms, as measured by the HARQ, a potential 'treatable trait' may be identified, and therapeutic strategies aimed at improving oesophageal motility may be employed. Confirmation of dysmotility may be observed using HROM.

Our study has limitations, including the absence of lung function data for patients with airways disease and ILD to examine the relationship between oesophageal function and respiratory physiology. Furthermore, we cannot assume causation between oesophageal dysmotility and refractory respiratory symptoms. As this data was collected retrospectively, we were unable to collect any data on severity of symptoms using validated symptom questionnaires or visual analogue scales to examine the impact of oesophageal dysfunction on patient reported outcomes. We do, however, report a significant proportion of

patients with oesophageal dysmotility in a large cohort of patients with chronic respiratory disease.

2.5 Conclusions

In this study, we have observed oesophageal dysmotility in two-thirds of patients with refractory respiratory symptoms that were investigated with HROM. We also observe a low prevalence of traditional GORD in the same population. These findings suggest that motility disorders of the oesophagus, rather than acidic reflux, may contribute to persistent symptoms in chronic respiratory diseases. This study provides a rationale for further investigation of the use of prokinetic agents in patients with chronic respiratory disease.

Chapter 3
The effect of long-term macrolide antibiotic therapy on patient reported outcomes of cough and breathlessness in chronic respiratory disease: a systematic review and meta-analysis

3.1 Introduction

As discussed in previous chapters, the symptom of cough is extremely prevalent, accounts for a significant proportion of symptom burden, and is responsible for up to 10% of clinical contacts in primary care. [3, 285] In chronic respiratory diseases such as idiopathic IPF, COPD, and asthma, a higher burden of cough as measured by both objective and subjective assessment is associated with disease progression.[61, 286, 287] Despite this, the primary outcomes, and indeed secondary outcomes, of large-scale clinical trials are seldom related to objective or subjective measures of cough.

The use of long-term azithromycin has become commonplace in the field of respiratory medicine over the past 10 years. Although its exact mechanism remains debated, it has been shown in large randomised controlled trials to reduce exacerbation frequency in patients with COPD, asthma, and bronchiectasis.[221, 224, 226] In light of this, the British Thoracic Society (BTS) guideline for the use of long-term macrolides recommends their use in patients who experience frequent exacerbations in each of these diseases.[282] However, this guideline recommends against the use of long-term macrolides in unexplained chronic cough, moreover, the recent BTS clinical statement of chronic cough recommends only using long-term macrolides in those with chronic productive cough and recommends against its use in non-productive cough.[288]

As outlined previously in this thesis, the exact mechanism by which macrolides work is incompletely understood. However, the widely accepted theory is of their immunomodulatory properties.[289] The most convincing evidence is of

the positive effect of azithromycin on the ability of airway macrophages to phagocytose bacteria, which has been shown in COPD patients.[290] There have been many in-vitro studies investigating the effect of macrolides on other aspects of innate and adaptive immunity, with conflicting results.[247, 248, 291, 292]. A less studied mechanism of azithromycin is its effect on gastrointestinal motility, through its potent effect as an agonist of motilin receptors is well established.[202] In the previous chapter, we have shown that up to two-thirds of patients with chronic respiratory disease and high cough burden have an element of oesophageal dysmotility. This oesophageal dysmotility may well cause non-acidic gaseous refluxate to cause inflammation in upper and lower airways and cause sensitisation of vagal afferents engendering the development of cough hypersensitivity syndrome.[42, 157] However, the direct impact of azithromycin on objective oesophageal function and its correlation with objective and subjective assessments of cough severity is an area that will be explored in later chapters.

In this systematic review and meta-analysis of randomised controlled trials (RCTs) and non-randomised noncomparative studies, we synthesise the current evidence for the use of azithromycin in chronic respiratory disease. We aimed to assess the effects of azithromycin on subjective patient-reported outcomes of cough as well as objective 24-hour cough counts.

3.2 Methods

Protocol Registration

The review protocol was prospectively registered with PROSPERO (registration number CRD42023433530).

Eligibility Criteria

We searched for English language studies of adults (≥18 years old) with a diagnosis of chronic respiratory disease including Chronic Obstructive Pulmonary

Disease (COPD), Asthma, Chronic Cough, Interstitial Lung Disease (including Idiopathic Pulmonary Fibrosis), and non-cystic fibrosis Bronchiectasis who were being treated with long-term azithromycin. Both randomised controlled trials (RCTs) and non-randomised, noncomparative trials (NCT) of efficacy were eligible for inclusion. We excluded trials comparing azithromycin with other long-term macrolide therapy. All common azithromycin treatment regimens including once daily and three times weekly dosing were eligible for inclusion.

Search Strategy

We searched electronic literature databases MEDLINE, EMBASE, and Cochrane Central Register of Controlled Trials in June 2023 using the terms 1) azithromycin AND 2) cough AND 3) asthma; OR 4) COPD or chronic obstructive pulmonary disease; OR 5) ILD or interstitial lung disease; OR 6) IPF or idiopathic pulmonary fibrosis; OR 7) Chronic Cough or refractory chronic cough or idiopathic chronic cough; OR 8) Bronchiectasis. Reference lists of previous systematic reviews with similar endpoints were hand-searched for additional titles. The full search strategy, including the results of the searches for each database, can be found in the Appendix 1.

Study Screening and Selection

All titles, abstracts, and full-text articles were uploaded to the online review website Covidence (www.covidence.org). Title and abstract screening were performed by two reviewers independently (D L Sykes and N Rahunathan) with discrepancies resolved by a third reviewer (P Mason). Full-text articles of potentially eligible studies were again screened by two independent reviewers (D L Sykes and P Mason) with discrepancies resolved by consensus. Reasons for exclusion of full-text articles were recorded.

Outcomes of interest

To be eligible for inclusion in the analysis, studies had to include at least one of the following as either a primary or secondary outcome. The first main outcome of interest for this review was patient-reported outcome measures of cough including Leicester Cough Questionnaire, Hull Airway Reflux Questionnaire (HARQ), Cough Visual Analogue Scale (VAS), Cough Numerical Rating Scale (NRS), Cough Quality of Life Questionnaire. The second main outcome of interest for this review was objective cough-counting, studies were eligible if they used any method of cough-counting including (but not limited to): VitaloJAK^{TM}, Leicester Cough Monitor, and Hull Automatic Cough Counter.

Data Extraction

All pre-specified study data were extracted using the Covidence online data extraction tool. Two authors (D L Sykes and N Rahunathan) independently recorded data including:

- Study Data: First author, year of publication, geographical setting, source of funding

- Methods: Study design, study setting, duration of study, inclusion/exclusion criteria, azithromycin dosing regimen, type of cough recorder used, patient-reported outcome measure used

- Results: number of participants, mean age, % male, change in patient-reported outcome measures, change in objective cough count, adverse events, and mortality

Once all data were extracted by each author, discrepancies were resolved by consensus and the limitations of each study were discussed.

Risk of bias assessment

All studies were assessed for risk of bias by two independent reviewers (D L Sykes and N Rahunathan) using the Cochrane Risk of Bias Tool on the Covidence online review website. [293]This tool assesses each study for sequence generation, allocation concealment, blinding of participants, personnel and outcome assessors, incomplete outcome data, selective outcome reporting, and other sources of bias. Studies were also rated on their method of outcome measurement, analysis of groups, and statistical analysis.

NCT studies were assessed for risk of bias using the ROBINS-I risk of bias tool. Disagreements in assessment were resolved by consensus.

Statistical Analysis

Continuous data from patient-reported outcomes and objective cough counts were inputted as raw data in all meta-analyses in this review. The reported outcome of all meta-analyses was standardised mean difference (SMD). Meta-analysis was performed for all studies comparing the baseline mean (SD) and post-treatment follow-up mean (SD) of each outcome in the azithromycin treatment group.

Separate meta-analyses were performed for RCT data where the mean difference (SD) of each outcome between placebo and azithromycin groups were available. Where standard deviations were not available for a particular outcome they were imputed using a correlation coefficient from a different study in the review, as per the Cochrane Handbook.[294]

As there were only a small number of studies included in the final review, we decided not to use I^2 as a measure of heterogeneity as it is biased in small meta-analyses. Due to the differences in diseases studied and methods of assessment between the included studies, all meta-analyses were conducted using a random-effects model. All data analysis was performed using IBM SPSS Statistics 28 (IBM Corp., Armonk, NY, USA).

3.3 Results

Study Selection

The systematic search identified 1240 eligible studies, of which 389 were duplicates. A further 4 studies were included from citation searching, resulting in 855 studies eligible for screening by their title and/or abstract. From these titles, a full-text review was carried out on 33 studies, of which 6 studies met the criteria for inclusion in the final analyses. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram can be visualised in Figure 8.

Study Characteristics

A total of 6 studies (n=275) were included in the final analysis, including 4 RCTs [295-298] (n=224) and 2 NCTs[299, 300] (n=51); all 4 RCTs compared long-term azithromycin with placebo. Table 2 illustrates the characteristics of all included studies. Amongst the RCTs, the risk of bias was rated as low in all categories. As the same risk-of-bias checklist was applied to all studies, the risk-of-bias was significantly higher in the NCT trials as they were both open-label and did not have a comparator group. Figure 9 shows the risk-of-bias assessment for all studies.

The two NCT studies that were included in the review both were scored as having a 'serious' risk of bias using the ROBINS-I tool. The exact scoring for both *Fraser et al.* and *Martin et al.* can be seen in the Supplementary Materials.
Of the 4 RCTs included in the analysis, one trial each examined the use of azithromycin in COPD, asthma, chronic cough, and IPF. Of the 2 NCT trials, one trial was conducted in sarcoidosis and one in chronic cough.

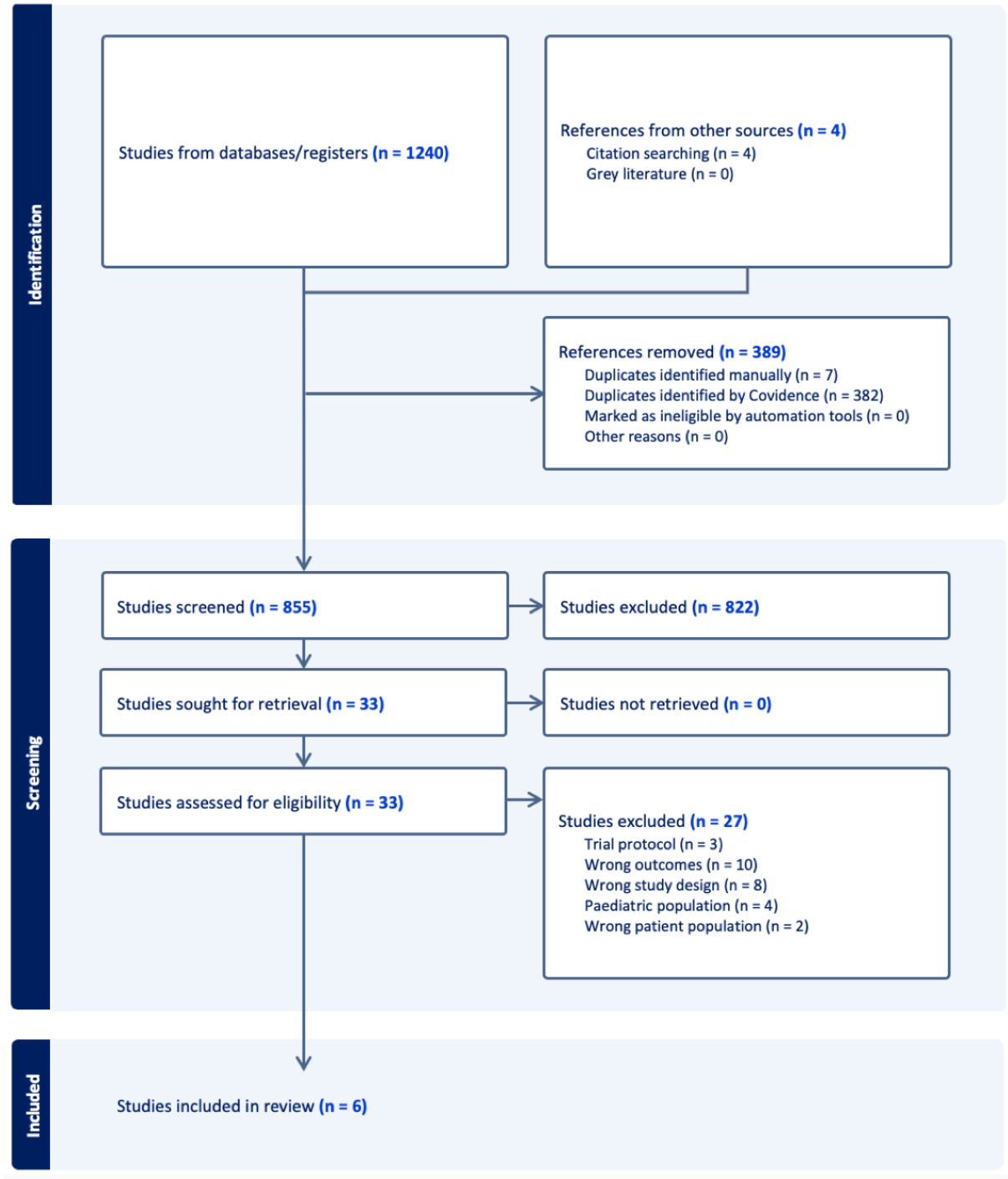
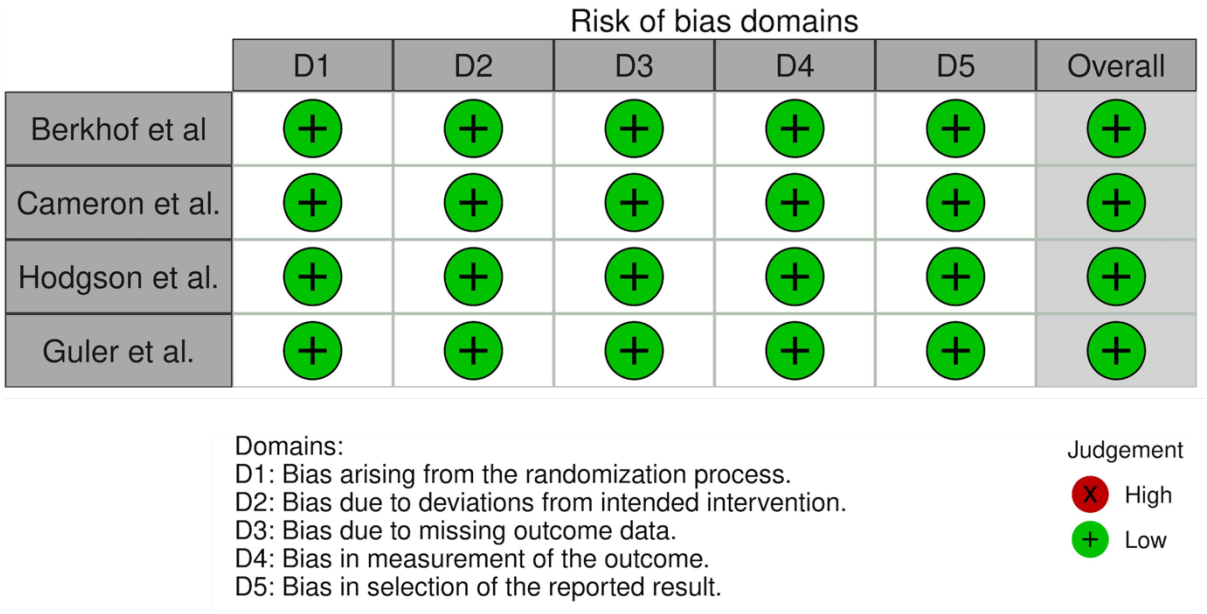


Figure 8. *PRISMA flow diagram showing quantities of studies excluded at each stage in the review.*

A



B

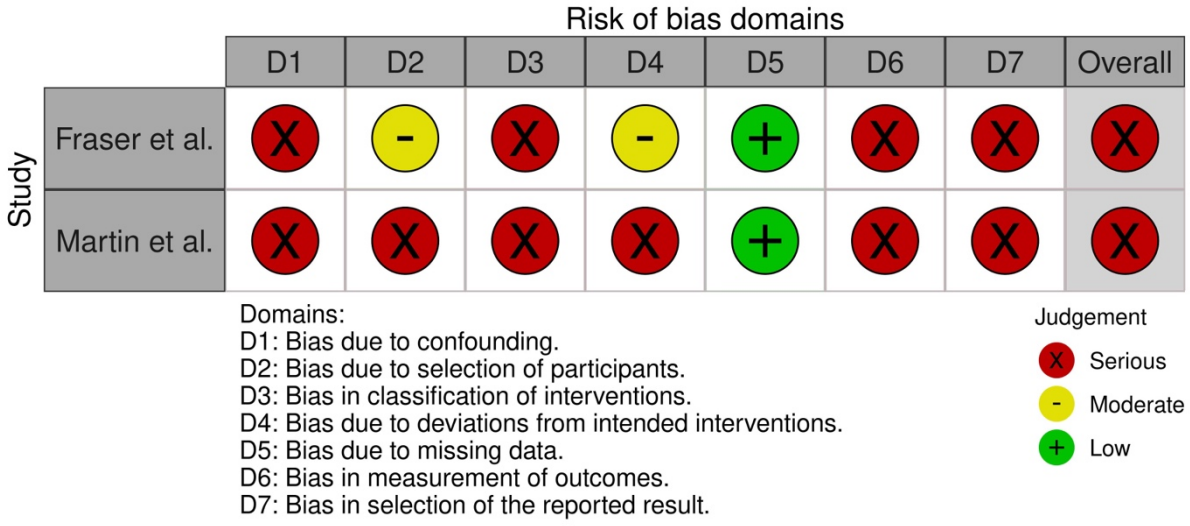


Figure 9. A) *Risk of bias assessment for all RCTs included in this review. B) Risk of bias assessment for all NCTs included in this review*

Study	*Fraser et al. 2020*	*Martin et al. 2019*	*Guler et al. 2021*	*Hodgson et al. 2016*	*Cameron et al. 2013*	*Berkhof et al. 2013*
Condition	*Sarcoidosis with associated cough*	*Chronic Cough of various aetiologies, including asthma, GORD, and early*	*Idiopathic Pulmonary Fibrosis*	*Chronic Cough*	*Asthma*	*COPD*
Study Design	*NCT pre-post clinical trial*	*NCT pre-post clinical trial*	*Randomised Placebo-Controlled Crossover Trial*	*Randomised Placebo-Controlled Trial*	*Randomised Placebo-Controlled Trial*	*Randomised Placebo-Controlled Trial*
Azithromycin Regimen	*250mg once daily*	*250mg three times per week*	*500mg three times per week*	*Azithromycin 500 mg daily for 3 days followed by 250 mg 3 times a week*	*250mg once daily*	*250mg three times per week*
Outcomes of interest	*LCQ, Cough Severity VAS, 24 hour cough counting*	*LCQ*	*LCQ, Cough Severity VAS, SGRQ, 24-hour cough counting*	*LCQ, Cough Severity VAS*	*LCQ*	*LCQ, SGRQ*
Follow-up	*12 weeks*	*12 weeks*	*12 weeks on both placebo and azithromycin*	*12 weeks*	*12 weeks*	*12-18 weeks*
Mean Baseline LCQ Score	*15.96*	*11.5*	*11.6*	*10.85*	*16.61*	*13.95*
Number of participants	*21*	*30*	*25*	*44*	*71*	*84*

Table 2. *Characteristics of studies included in the meta-analysis.*

Randomised Controlled Trials

Berkhof et al. was the largest study identified in the systematic review, examining the role of azithromycin in 84 COPD patients. It demonstrated a significantly greater mean increase in LCQ total score after 12 weeks in the azithromycin group compared with placebo (mean difference 1.3 [95% CI 0.3-2.3] p = 0.01), meeting the minimally clinical important difference for the LCQ. There was also a significant improvement in SGRQ total score over 12 weeks with azithromycin compared to placebo, mean difference -7.4 [95% CI -12.5; -2.5], p = 0.004).

Cameron et al. was conducted in a group of 71 smokers with asthma. No effect was seen on mean LCQ following 12 weeks of treatment with azithromycin. The mean difference was -1.06 [95% CI -2.16 to 0.05], p=0.06.

Hodgson et al. was a trial of 44 patients with chronic cough. There was statistically significant improvement in LCQ score in the azithromycin group from 10.2 to 12.6 (mean change, 2.4; 95% CI, 0.5 to 4.2; P = .01), which was not seen in the placebo group (mean change, 0.7; 95% CI, −0.6 to 1.9). However, the between-group difference was only observed at 4 weeks and not past this point (mean difference, 1.9 [95% CI, 0.1 to 3.8] P = .04). There was no significant difference between azithromycin and placebo in cough severity VAS scores (p=0.21).

Guler et al. observed the effect of azithromycin in 25 patients with IPF. This study found no difference in total LCQ score (mean difference 0.68 [95% CI -0.64-1.99], p=0.29). No difference between cough severity VAS scores (mean difference, 0.25 [95% CI -1.12-1.63], p=0.70). 24-hour cough recording demonstrated no difference in coughs per hour between placebo and azithromycin (mean difference, -3.9 [95% CI -10.2-2.3], p=0.19).

Noncomparative Trials

Martin et al. included 30 patients with chronic cough. This showed a significant improvement in LCQ at 12 weeks with a median improvement of 6.3 ($p<0.001$).

Fraser et al. recruited 21 patients with sarcoidosis and troublesome cough. This study showed a significant improvement in LCQ at 3 months (median change, 1.85 [-1.17-12.18], p=0.006). There was also a significant improvement in Cough VAS (median change, -9.0 [-93-20], p=0.009). A comparison of 24-hour cough counts showed a significant reduction from 228 (43-1950) at baseline to 81 (16-414) at 3 months (p=0.002).

Meta-Analyses

Leicester Cough Questionnaire

All 6 studies included in the analysis recorded LCQ scores at baseline and post-treatment follow-up. The meta-analysis found a significant improvement in LCQ scores with azithromycin treatment when compared to baseline scores (MD=2.24 [95% CI=0.28-4.20], p=0.02, I^2 =0.86). When the RCTs were analysed alone, with a comparison of azithromycin vs placebo, there was no significant improvement of LCQ scores (MD=1.0 [95% CI=-0.51-2.51], p=0.19, I^2 =0.68). Forest plots for the meta-analyses of all studies and for RCTs alone can be seen in Figure 10.

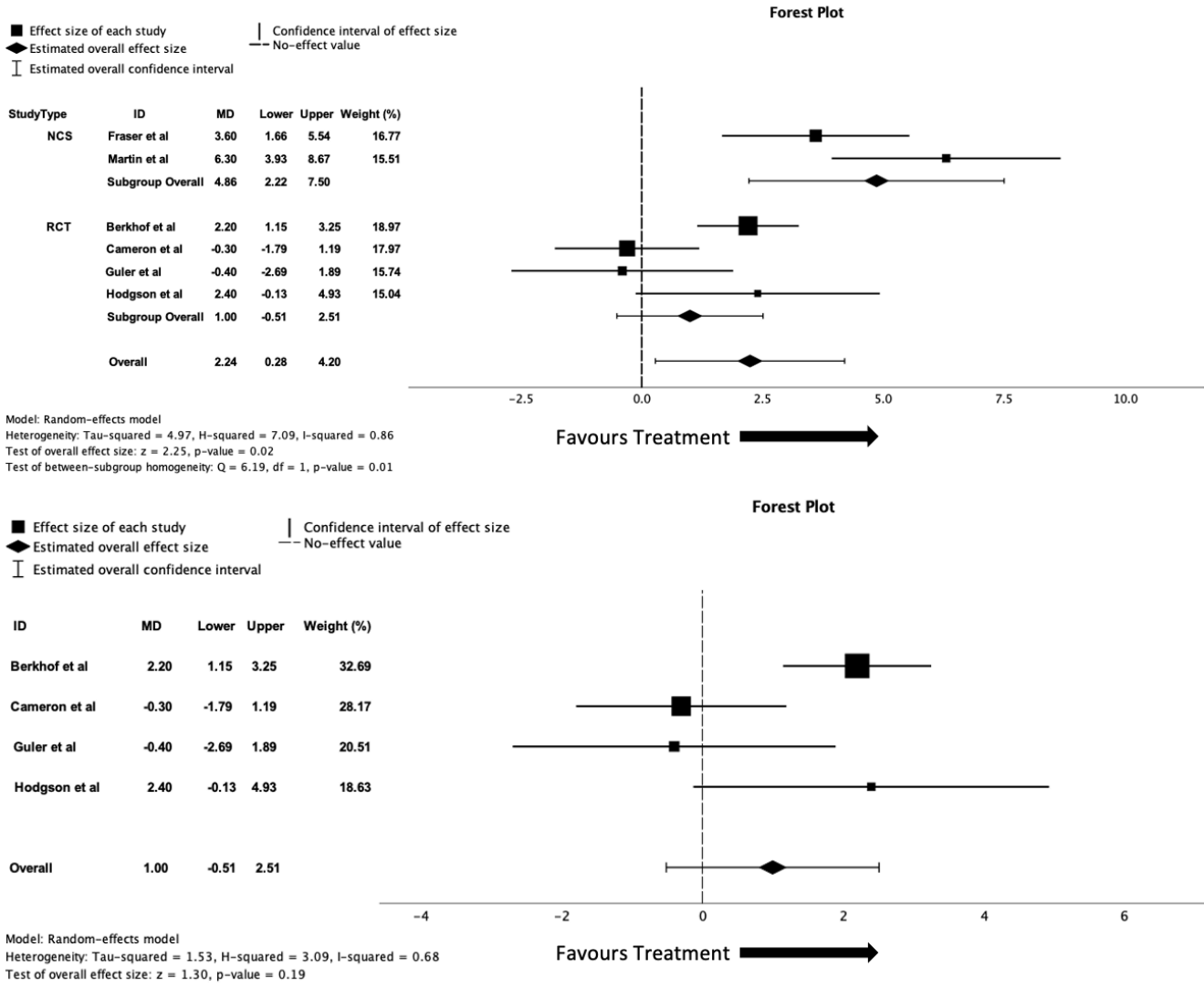


Figure 10. *A) Forest plot of all study data comparing baseline and follow-up LCQ scores with treatment of azithromycin. B) Forest plot of randomised controlled trials data comparing LCQ score means changes between both azithromycin with placebo groups.*

Cough Severity Visual Analogue Scale

Three studies (n=85) included in the analysis measured Cough Severity VAS as an outcome, 2 RCTs (n=64) and 1 (n=21) NCT study. When all studies were analysed together there was no effect for reduction of Cough Severity VAS score with azithromycin treatment compared with baseline score (SMD=-0.39 [95% CI -

0.92-0.14], p=0.15, I^2 = 0.54). When RCTs were analysed alone, comparing the effect on Cough Severity VAS mean difference in azithromycin and placebo groups, there was no significant difference between groups (SMD=-0.24 [95% CI -0.67-0.20], p=0.28, I^2=0.00). It is important to note that the Cough Severity VAS that was used in *Guler et al.* was actually a numerical rating scale (NRS), however, due to the similarity in the nature of assessment, it has been included in the meta-analysis. Forest plots for the meta-analyses of all studies and for RCTs alone can be seen in Figure 11.

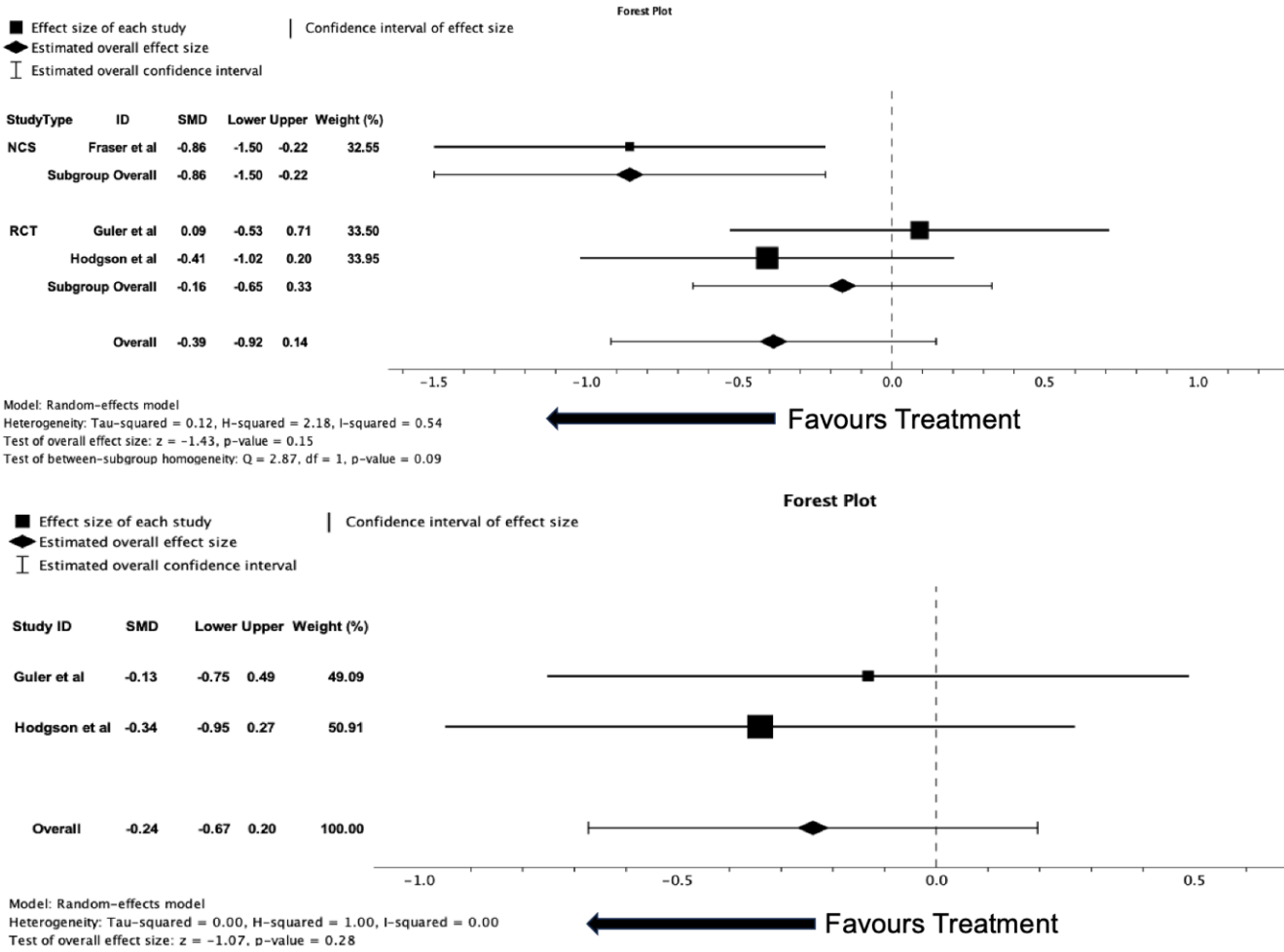


Figure 11. *A) Forest plot of all study data comparing baseline and follow-up Cough Severity VAS scores with treatment of azithromycin. B) Forest plot of randomised controlled trials*

data comparing Cough Severity VAS score means changes between both azithromycin with placebo groups.

St George's Respiratory Questionnaire

Two studies (n=104) included in the analysis measured the SGRQ as an outcome, both were RCTs. There was no significant reduction in SGRQ scores after a meta-analysis of the two studies (MD=-4.53 [95% -10.41-1.35], p=0.13, I^2=0.98). The forest plot for the meta-analysis of these two studies can be seen in Figure 12.

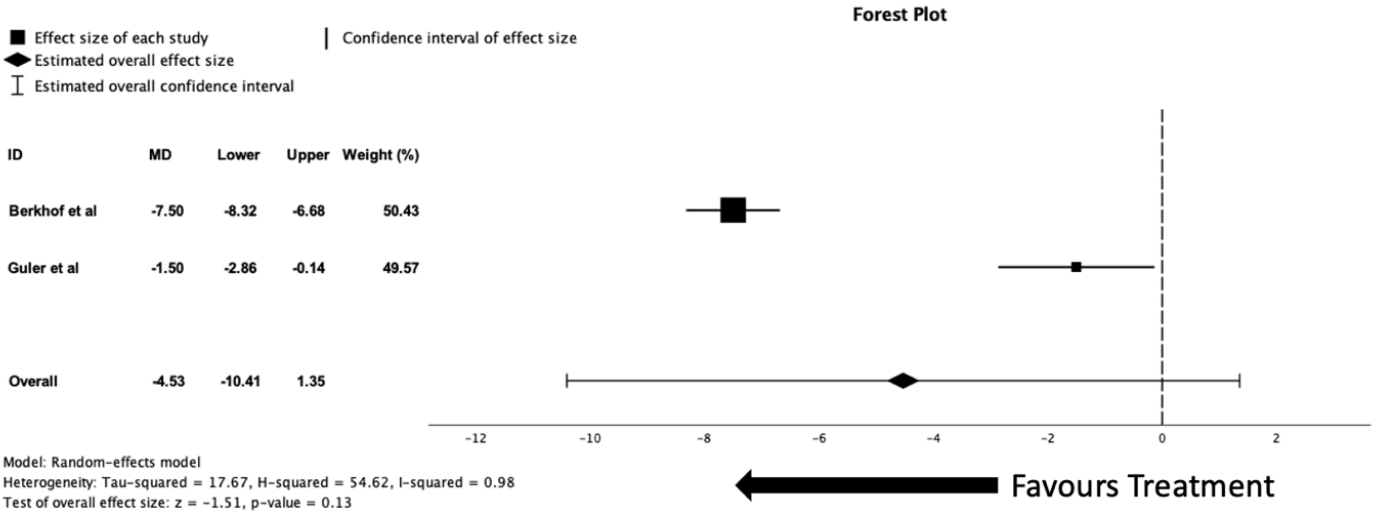


Figure 12. *Forest plot of randomised controlled trials data comparing SGRQ score means changes between both azithromycin with placebo groups.*

Objective Cough Counts

Two Studies (n=41) included in the analysis performed 24-hour cough recording at both baseline and follow-up after treatment with azithromycin. *Fraser et al.* utilised the Hull Automatic Cough Counter with the Leicester software algorithm for identifying coughs. *Guler et al.* recorded coughs with the NOX T3 device with Noxturnal software. There were differences in reporting the cough count, with *Fraser et al.* reporting 24-hour cough counts and *Guler et al.* reporting cough index (i.e. coughs per hour), for the meta-analysis, data were converted into cough index. There was no reduction in cough index in the meta-analysis (SMD= -0.41 [95% CI -1.04-0.32], p=0.09, I^2=0.00). The forest plot for this data can be seen in Figure 13.

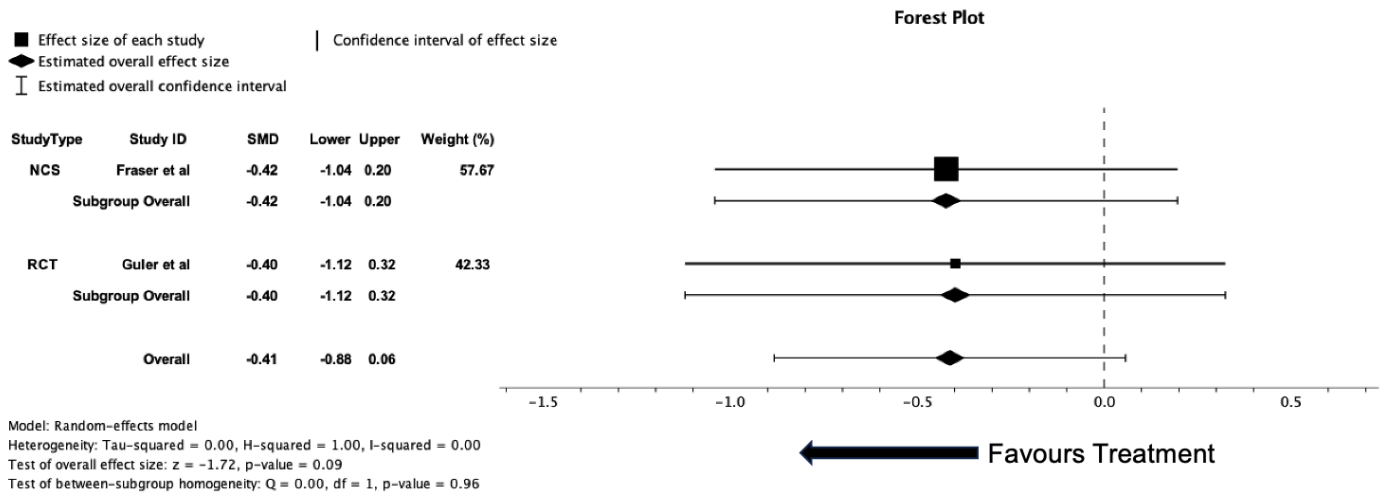


Figure 13. *Forest plot of studies with data comparing mean difference of coughs per hour on 24-hour cough monitoring at baseline and follow-up with azithromycin treatment*

3.4 Discussion

In this systematic review, we have pooled data from 4 RCTs and 2 noncomparative studies comprising 275 patients with various chronic respiratory diseases. We have shown a significant improvement in cough-related quality of life as measured by the LCQ when comparing pre-treatment baseline values with post-azithromycin treatment follow-up assessments (SMD=0.62 [95% CI=0.12-1.12], p=0.01). However, when placebo-controlled data from the 4 RCTs were pooled together, there was no statistically significant improvement with treatment over placebo.

The absence of improvement in LCQ scores and objective cough counts in the RCT-only meta-analysis compared to the improvements observed in the noncomparative studies are in keeping with the large placebo responses that have been observed in previous trials in chronic cough patients.[81] However, this response has not been seen in recent trials examining cough in IPF patients.[82, 301] When analysing the LCQ results from studies included in this review individually, there are 2 RCTs with improvement, 2 RCTs with no difference, and 2 noncomparative trials showing improvements. The heterogeneity in the findings observed may be attributable to patient selection; for example, *Cameron et al.* included asthmatic patients with a baseline LCQ of 16.6 in the treatment group and 16.9 in the placebo group. These values almost reach the cut-off for normality, which has previously been reported as 17.68.[302] Furthermore, the two RCTs that showed significant improvement in LCQ scores had patients with baseline LCQ scores of 10.2 and 14.5 (treatment groups), which may indicate that patients with more severe cough have a higher chance of responding to azithromycin. We have also observed variation in response based on the disease studied in each of the studies, as the positive RCTs were in groups of chronic cough and COPD patients, whereas the negative RCTs were in patients with asthma and IPF, and the two positive noncomparative studies were in patients with chronic cough and sarcoidosis.

Of the studies included in our final analysis, only two trials with a combined total of 41 patients utilised the technique of objective cough counting as one of the outcomes of their study. Both studies showed a reduction in cough counts with the use of azithromycin, and although our analysis did not show this to be statistically significant, we believe that this is due to a lack of statistical power from the small sample sizes in the individual studies and therefore in the meta-analysis. Both studies investigated azithromycin in interstitial lung disease, with Sarcoidosis in *Fraser et al.* and IPF in *Guler et al.* The data from both included studies represent a promising signal which warrants further investigation of the effect of long-term macrolides on objective cough in different respiratory diseases. Objective assessment of cough through 24-hour cough counting has been shown to correlate with more traditional outcome measures of respiratory disease control and has been shown to predict improvement in disease control in asthma[303], COPD[304], and IPF[305]. Traditionally, clinical trials of novel pharmacological interventions for idiopathic/ refractory chronic cough have used this metric as their primary outcome [81] and it has more recently been utilised as a primary outcome for trials of new therapeutics in IPF.[82] Despite its increasing usage, the optimal method of objective cough counting remains the centre of debate.[65, 306] Indeed, the two trials included in this review reported different metrics, with *Guler et al.* reporting cough index (coughs per hour) and *Fraser et al.* reporting 24-hour cough count. Other reported cough metrics include awake cough frequency, which is more applicable in idiopathic/refractory chronic cough as such patients tend not to cough as much overnight, but is less applicable to asthmatic patients who may well cough mostly throughout the night. More novel techniques of cough assessment are being explored, such as ambulatory and home continuous cough monitoring.[96, 307].

There are several limitations in this systematic review and meta-analysis. Firstly, there is a relative dearth of available studies that examine the impact of azithromycin on cough, furthermore, the included studies boast only small-moderate sample sizes. This review aimed to widen the number of participants

by including noncomparative studies as well as RCTs, which will of course alter the reliability of the results as such studies are not controlled or blinded which will introduce several biases that are not present in reviews of RCTs only. For this reason, we performed additional analysis on RCTs separately to gain further understanding of the effect of azithromycin compared with placebo on subjective measures of cough. Another potential limitation of this analysis is the heterogeneity of the patient population from which these studies were selected, as different respiratory diseases have distinct pathophysiology and differential cough burden which may affect the efficacy of azithromycin. Moreover, there was heterogeneity in certain aspects of the study protocols, such as the method of azithromycin dosing and objective cough measurement techniques. Despite these limitations, this systematic review was prospectively designed, registered, conducted, and reported in line with PRISMA guidelines.

3.5 Conclusions

In conclusion, we have demonstrated that long-term azithromycin therapy improves cough-related quality of life in various chronic respiratory diseases. However, the data from randomised controlled trials have not definitively proven this finding. We believe that to definitively establish the effect of azithromycin on cough and accurately identify patient sub-groups most likely to benefit, a large-scale clinical trial of patients with a broad spectrum of respiratory diseases and sufficiently severe cough, should be undertaken with pre-specified subgroup analyses.

Chapter 4

Investigating the effect of azithromycin on oesophageal motility in patients with chronic respiratory disease: a feasibility study

4.1 Introduction

The findings in chapter 3 demonstrate that the evidence for the efficacy of azithromycin on cough in chronic respiratory disease shows promise but remains unproven. Moreover, as was discussed in chapter 1, the mechanism by which azithromycin benefits patients with a broad range of respiratory disease remains an unsolved mystery. To understand the effect of azithromycin on oesophageal function and its impact on clinical outcomes in respiratory disease, a prospective clinical trial is necessary. As HROM is an invasive procedure, it was unclear whether patients would tolerate multiple procedures to better understand their disease. We therefore set out to perform a feasibility study to assess the acceptability of performing repeated HROM studies to measure oesophageal function in patients with a broad range of chronic respiratory diseases, who reported cough as a predominant symptom.

Feasibility studies have been increasingly used over the past 20 years as a tool to ascertain whether a research study can be performed[308]. The primary aims of any feasibility study are to assess whether an intervention and/or investigation is acceptable and appropriate to be tested in a larger clinical trial. Moreover, it is a reliable way to assess whether data collection, trial processes, and participant retention are feasible.[309] This allows for refinement of all aspects of a study protocol and informs researchers if any pre-conceived study procedures need to be adapted or potentially abandoned.

The feasibility study detailed in this chapter assessed the ability of the research team to successfully collect physiological data using HROM as well as clinical

data using continuous cough monitoring. This chapter will report the feasibility outcomes for this study in addition to HROM results. The clinical data collected for assessing the effect of azithromycin on objective and subjective measures of cough will be mentioned in this chapter, however, detailed analysis of this will be discussed in chapter 5.

4.2 Methods

To inform a larger randomised controlled trial to investigate the effect of azithromycin on oesophageal dysmotility and how this relates to respiratory symptoms, we undertook an open-label, single arm clinical trial. This study was prospectively registered on clinicaltrials.gov (trial identifier: NCT05469555) and ethical approval was granted by the regional ethics committee board (reference number: 22/NE/0194). The study was classified as a clinical trial of an investigational medicinal product (CTIMP) and was ratified by the Medicines and Healthcare products Regulatory Agency (MHRA).

Study Protocol

Patients with any chronic respiratory disease that were being considered for long term azithromycin therapy (defined as >6 months of 250mg daily or three times weekly) by their treating clinician were eligible to be recruited to this study. Patients were referred to the study team by clinicians working at Hull University Teaching Hospitals NHS Trust. Consenting patients were then contacted by the study team to undertake screening for the trial, prior to initiating their azithromycin treatment.

The trial comprised 3 study visits, one screening visit, one pre-azithromycin initiation visit, and one follow-up visit. The schedule of events for the study, including all investigations that patients undertook at each visit, can be seen in Table 3. At screening, provided that they met all of the pre-specified inclusion

criteria and did not meet any exclusion criteria, patients were consented to take part in the study. The full inclusion and exclusion criteria are displayed in table 4. The cardinal exclusion criteria were the inability to be treated with macrolide antibiotics, either due to allergy, sensitivity, or QTc prolongation (>450ms for males, >470ms for females) on electrocardiogram.

Visit	Screening	Baseline visit		Follow-up visit
Day	**-28 to -7**	**0**		**28 to 42**
Procedure/Assessment				
Written Informed Consent	X			
Inclusion/ Exclusion Criteria	X	X		
Invitation Experience survey	X			
Setup of the Hyfe Cough Tracker©	X			
Monitoring of cough frequency using the Hyfe Cough Tracker©	X	X		X
Vital signs		X		X
Weight and Height		X		X
Physical Examination		X		X
Electrocardiogram		X		X
Past Medical History		X		
Review of previous laboratory results		X		
AE Monitoring		X		X
MRC Dyspnoea grade		X		X
Hull Airway Reflux Questionnaire	X	X		X
Breathlessness, Cough, and Sputum Scale		X		X
St George's Respiratory Questionnaire		X		X
Leicester Cough Questionnaire		X*		X*
King's Brief Interstitial Lung Disease Questionnaire		X*		X*
COPD Assessment Test		X*		X*
Asthma Control Questionnaire		X*		X*
Visual Analogue Scales (Cough)		X		X
Numeric Rating Scale (Breathlessness)		X		X
Spirometry		X		X
High-Resolution Oesophageal Manometry		X		X
Health Care Utilisation				X
Evaluation of usual care received				X
Trial Experience Survey				X

Minimum 1 month on azithromycin therapy

Table 3. *Schedule of events for the study*

Inclusion Criteria	- Males and females aged ≥18 years. Have a diagnosis of chronic respiratory disease (COPD, asthma, interstitial lung disease, chronic cough, cystic fibrosis, and/or bronchiectasis) confirmed by a consultant respiratory physician. - Exhibit symptoms consistent with airway reflux, demonstrated by a score ≥14 on the Hull Airways Reflux Questionnaire - Are being initiated on azithromycin treatment as part of routine clinical care by their usual clinician. This will include all common treatment regimes, 250mg once daily, 250mg three times per week, and 500mg three time per week. - Are willing and able to consent to all trial procedures.
Exclusion Criteria	- Previous treatment with long-term macrolides in the past 3 months. - Unable to be investigated with HROM due to contraindications such as anatomical abnormalities or diseases of the oesophagus or unwilling/ unable to be investigated with HROM based on the clinical judgement of the investigators due to severity of lung disease. - Have another cardiorespiratory cause for their symptoms (such as heart failure or lung cancer). - Are unable or unwilling to consent to or complete the trial procedures. - Women of child bearing potential not using effective means of contraception. Effective methods of contraception include any oral contraceptive pill, any intrauterine device, any long term contraceptive implant, and any long-acting contraceptive injection - Have been involved in another medicinal trial (CTIMP) within past four weeks.

Table 4. *Pre-specified inclusion and exclusion criteria for participating in the study*

The nature of the patients' respiratory disease, with particular regard to their cough, was assessed using validated questionnaires at screening. Participants were then provided with a continuous cough monitor; the details of these assessments and their outcomes for the screening and subsequent visits will be discussed in the next chapter. Participants also completed an invitation experience questionnaire in which they were asked to describe their experience of being invited to participate in the trial.

Patients then reattended for their pre-azithromycin initiation visit approximately one week after screening where they undertook their baseline HROM study. Following this testing they were started on azithromycin 250mg once daily. Patients were advised to contact the trial team if they had common adverse effects of azithromycin such as gastrointestinal discomfort, nausea, diarrhoea, or vomiting. If the patients reported any of these symptoms, the study team advised the patient to reduce their dose to 250mg three times per week in the first instance and then discontinue altogether if the adverse effects were intolerable or if the patient did not wish to continue.

Patients re-attended for their follow-up visit approximately 1 month after their pre-azithromycin visit. During this visit they had a further HROM study performed, and all other clinical assessments were repeated. Patients then completed a study experience survey to assess the acceptability of the trial to the participants.

High-Resolution Oesophageal Manometry Protocol and Metrics

All participants were investigated using a standardised HROM technique. Participants were advised to fast 4 hours prior to investigation and were investigated with a 36-channel combined manometric and impedance monitoring catheter. This catheter was inserted trans-nasally to a length of 60cm from the nostril. Participants were then asked to lay supine for 5 minutes prior to measurement.

A standardised protocol for oesophageal function tests was followed, with further tests utilised if felt appropriate by the clinical scientist performing the test. The mandatory tests included:

- 10 separate swallows of 5mls of water with an interval of 15 seconds between each swallow.

- The participant was then asked to complete 5 rapid swallows, ingesting 2mls of water at each swallow.

- The final test consisted of the participant swallowing 5 small pieces of white bread in 5 separate swallows.

Each individual study was analysed manually by an expert gastrointestinal physiologist and a full analysis report was produced for each individual test in each participant. A list of the measured metrics of oesophageal function along with a brief explanation of their significance is given in table 5.

Metric	Explanation
Distal Contractile Integral	Calculated by multiplying the mean amplitude of contraction, the duration of contraction, and the length of the distal oesophageal segment. The average DCI from the 10 liquid swallows is collected and used to quantify the vigour of oesophageal contraction.
Integrated Relaxation Pressure	Calculated by analysing pressure data from sensors positioned along the oesophagus, specifically looking at the lowest pressure in the sphincter area during the relaxation phase following swallowing. The average IRP4sec is defined as the average minimum oesophagogastric junction pressure for 4 seconds of relaxation. This gives a quantification of the adequacy of relaxation at the oesophagogastric junction.
Lower Oesophageal Sphincter Resting Pressure	Basal and relaxation LOS pressure measurements is taken, and the LOS Resting Pressure is usually defined as the highest continuous pressure recorded in the sensor representing the lower oesophageal sphincter. This measurement informs us whether the oesophageal sphincter is excessively tight or if it loose, both of which may lead to reflux of ingested matter.
Chicago Classification	Using Version 4.0 of the Chicago Classification, patients are provided with a manometric diagnosis. These diagnoses include Normal Motility, Ineffective Oesophageal Motility, Absent Contractility, Achalasia, EGJ Outflow Obstruction, Hypercontractile Oesophagus, and Distal Oesophageal Spasm. Each diagnosis has criteria that is fulfilled using the measurements listed above.

Table 5. *HROM metrics with brief explanations regarding their measurement and significance.*

Feasibility Outcomes

To assess the feasibility of performing a larger randomised controlled trial we assessed the following feasibility outcomes:

- Recruitment: eligibility to consent ratio, recruitment rate (patients recruited to the trial per month), and participant retention to follow-up.
- Data quality: Completion of clinical outcomes at each visit and patterns of missing data for the trial measures. Completion of participant symptom questionnaires throughout the study. Completion of trial processes, principally HROM.
- Acceptability of assessment: This was primarily the willingness of participants to undertake both HROM tests. We also assessed this using the invitation and study experience questionnaires.

A 'traffic light' stop-go criteria was used to assess the feasibility of a larger study, based on the findings of the above outcomes. This will inform where the larger trial protocol and potential recruitment targets may have to be amended to ensure it can be realistically and successfully completed.

Exploratory Clinical Outcomes

The following physiological and clinical outcomes were assessed for a signal of change to inform primary and secondary endpoint selection and sample size for a larger randomised controlled trial:

- Oesophageal function: we evaluated the effect of azithromycin on the measures of oesophageal contraction and swallow coordination, as described in table 5. This was done by comparing the HROM studies for individual patients before and 1 month after initiation of azithromycin treatment.
- Subjective symptom burden: Symptoms were assessed before and 1 month after initiation of azithromycin. These were measured using validated questionnaires to assess severity of symptoms as well as

health-specific quality of life. As stated previously, the specific results of these will be displayed and discussed in Chapter 5.

- Relationship between oesophageal motility and symptoms: The change in oesophageal functioning and both objective and subjective measures of respiratory symptoms was examined in each patient. The relationship between these two parameters was examined using statistical analysis.
- Objective cough frequency: Effect of azithromycin therapy on continuous objective cough frequency as measured by the Hyfe Cough Tracker©. Findings from these assessments will be discussed in chapter 5.

Adverse events

Unwanted side effects and adverse events are, as previously described, relatively common with long-term azithromycin treatment [310, 311]. For the purposes of this trial, all adverse events, including severity and any actions taken to ameliorate them, were reported in congruence with the UK NHS Research Ethics Service Guidelines. All adverse events and their effect on the adherence to azithromycin are reported.

Statistical Analysis

Participant characteristics are summarised using descriptive statistics. Medians (range) will be reported for non-parametric data, mean (standard deviation) for continuous, normally distributed data, and raw count (number (%) are reported for nominal data. The primary feasibility outcomes are presented using raw numbers and percentages. Comparisons of baseline and follow-up HROM and questionnaire data were performed using paired t-tests for normally distributed data and Mann-Whitney U testing for skewed data. Correlations between symptoms and HROM metrics were performed using Pearson's correlation coefficient for normally distributed data and Spearman's rank correlation for non-normal data. All data were analysed using IBM SPSS Statistics 29 (IBM Corp., Armonk, NY, USA).

4.3 Results

Study Recruitment

The study team opened recruitment between July 2023 and May 2024 and 30 potential participants were assessed for eligibility with all patients who were screened being deemed eligible for inclusion, giving an eligibility rate of 100%. Of these 30 patients, all 30 provided informed consent to participate in the trial and completed the initial study visit, giving a consent rate of 100%.

Patient Characteristics

Of the 30 patients recruited to this study, 60% (n=18) had a diagnosis of chronic cough, 23% had a diagnosis of interstitial lung disease (n=7), and 17% had a diagnosis of obstructive airways disease (n=5). The cohort had a mean age of 65.2 (SD 11.3) and 57% (n=17) were female. All baseline characteristics are displayed in Table 6.

Feasibility Outcomes

The eligibility:consent ratio for the study was 1:1 as all participants who were deemed eligible for the study agreed to participate. The recruitment rate for the study was calculated by the total number of patients recruited per month for the duration of the study, this worked out as 3 patients per month (30 patients recruited over a 10-month period). Participant retention was 93% (n=28) at visit 2 and 87% (n=26) of all participants who were recruited were able to complete the study. The study therefore met all of its primary recruitment and retention feasibility outcomes, indicating that a larger randomised controlled trial is feasible. All feasibility outcomes and their respective ‘traffic light’ stop-go criteria are presented in table 7.

	All patients (n=30)
Age, mean (SD)	65.2 (11.3)
Female, % (n)	57 (17)
BMI, mean (SD)	28.8 (6.0)
Primary Respiratory Diagnosis, % (n)	
Chronic Cough	60 (18)
Interstitial Lung Disease	23 (7)
COPD	10 (3)
Asthma	7 (2)
Spirometry, mean (SD)	
FEV1 % predicted	81 (29)
FVC % predicted	92 (25)
FEV1/FVC ratio	68 (14)
Blood Eosinophil Count, median (range)	0.21 (0.01-0.95)
Baseline Cough VAS, mean (SD)	56.5 (19.6)
Baseline HARQ Score, mean (SD)	40.8 (10.6)

Table 6. *Baseline characteristics of patients included in the study*

Data Quality

For the initial visit all clinical assessments were carried out, with no missing data. The flow of patients throughout the clinical trial can be seen in figure 14.

Pre-azithromycin initation visit (visit 2)

For the 28 participants who attended visit 2, 97.6% of questionnaire data were collected, 96% (n=27) of patients had their initial HROM performed, and 97.4% of other clinical assessments, such as spirometry, ECG, and physical examination, were completed.

Follow-up visit (visit 3)

Of the 26 patients who remained in the trial until the final study visit, 96.8% of questionnaire data were able to be collected. 88.5% (n=23) of patients had both their initial and follow-up HROM performed (2 participants declined a second study and 1 participant was unable to have the procedure performed due to an anatomical issue at visit 2). 92.3% of the other clinical asssessements were completed for participants at visit 3.

Continuous asessement of cough

Participants were provided with a physcial paper cough diary which comprised of 30 days of consectuive cough VAS scoring. Of the 26 participants who completed the study, 69% (n=18) were able to return a completed diary. Of the patients who completed the study, 92% (n=24) had satisfactory levels (>12 hours per day for 90% of days monitored) of continuous cough recordings using the Hyfe Cough Tracker©.

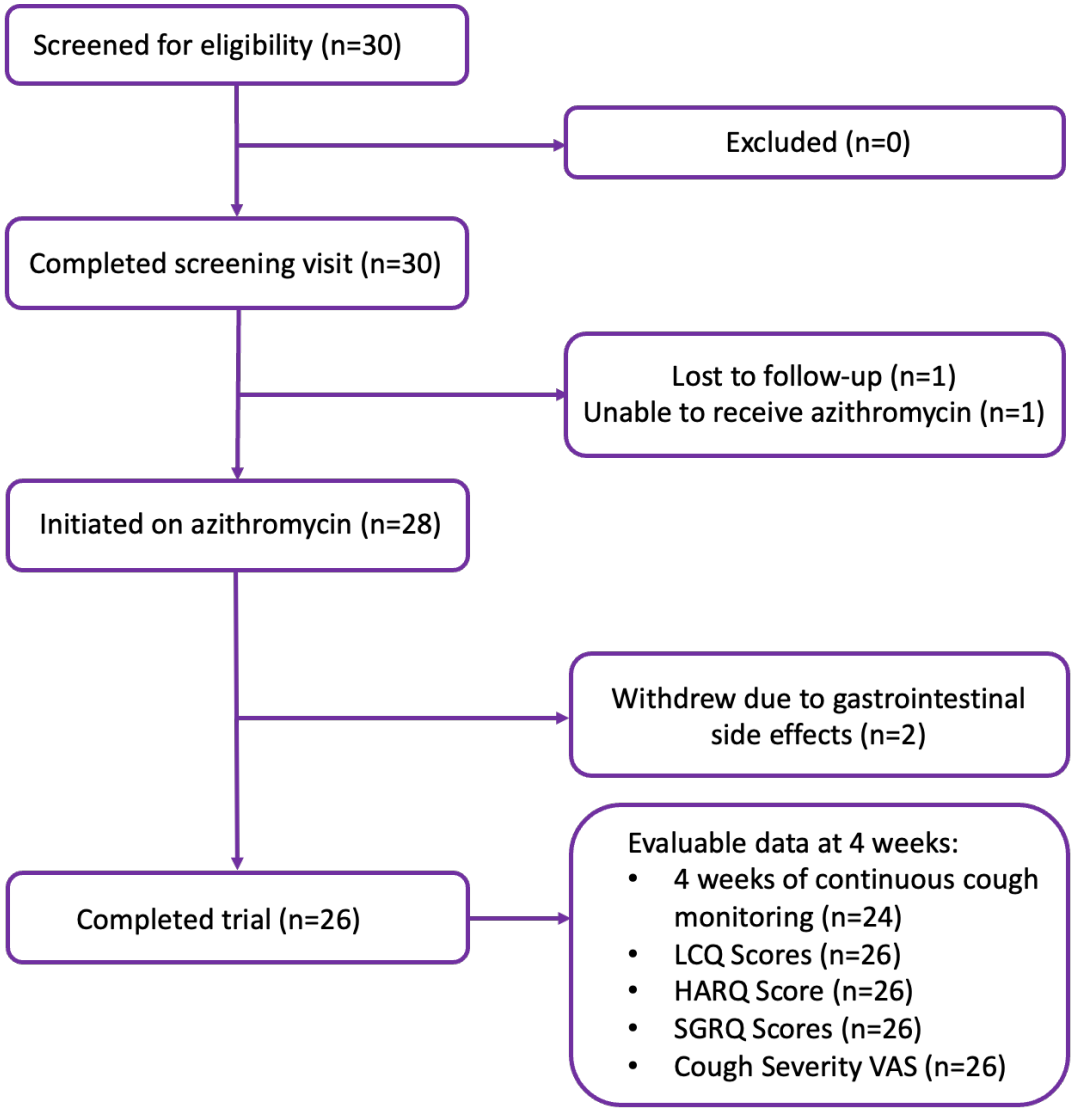


Figure 14. *CONSORT diagram of patient flow in the trial.*

Recruitment	Target	Result
Eligibility: Consent Ratio	Red >3:1 Amber 2:1 Green <1.5:1	1:1
Recruitment rate - participants per month	Red <2 Amber = 2 Green ≥3	3
Participant retention – Final Study Visit	Red <70% Amber 70-80% Green >80%	87%
Data Quality		
Completion rate of questionnaire data – Visit 2	Red <85% Amber 85-95% Green >95%	97.6%
Completion rate of other clinical assessments – Visit 2	Red <85% Amber 85-95% Green >95%	97.4%
Completion rate of HROM – Visit 2	Red <85% Amber 85-95% Green >95%	96%
Completion rate of questionnaire data – Visit 3	Red <75% Amber 75-85% Green >85%	96.8%
Completion rate of other clinical assessments – Visit 2	Red <75% Amber 75-85% Green >85%	92.3%
Completion rate of HROM – Visit 3	Red <75% Amber 75-85% Green >85%	88.5%

Table 7. *Results of the feasibility outcomes with their associated stop-go criteria to be successfully deemed feasible in a large scale randomised clinical trial.*

Invitation and Study Experience Surveys

Of the patients who attended the screening visit, 77% (n=23) completed the invitation experience survey. One of the core components of this survey was an 8 item Likert scale questionnaire, exploring the patient's understanding and thoughts around participating in the trial. The results from this questionnaire can be seen in figure 15. When asked what the patients' primary motivation for participating in the trial was 56% (n=13) stated it was to 'improve their cough', 48% (n=11) reported it was to help other people who may have a similar condition, 17% (n=4) stated they wanted to better understand why they cough, and 17% (n=4) wanted to help to find a new treatment for chronic cough (each participant could give more than one reason).

The proportion of patients who completed the study experience survey was 50% (n=13). When asked their overall experience of the trial 92% (n=12) stated it was 'good' and 8% (n=1) reported it to be 'fairly good'.

Effect of Azithromycin on Oesophageal Function – Exploratory Outcomes

The majority of patients included in this study had abnormal oesophageal function present on their baseline HROM study (52%, n=14). The Chicago Classification for all patients at baseline was either Normal (48%, n=13), Ineffective Oesophageal Motility (41%, n=11), or Absent Contractility (11%, n=3). The proportions of patients with these three classifications were not significantly different on repeat HROM testing at follow-up, as there were no instances of change of Chicago classification in any participant.

There was no significant difference between the DCI at baseline and follow-up (459 vs 493, p=0.362), although the median change in DCI in all patients was an increase of 12.8% (range -78.3% to 293.1%). The proportion of patients who had a >30% improvement in their HROM was 42% (n=10). Individual patient data for changes in DCI is displayed in figure 16.

There were no significant differences between baseline and follow-up measurements of IRP (10.75 [SD=5.5] vs 10.8 [SD=5.4], p=0.484) or LOSP (23.2 [SD=11.4] vs 24.1 [SD=13.4], p=0.371). All data for manometric findings can be found in table 8.

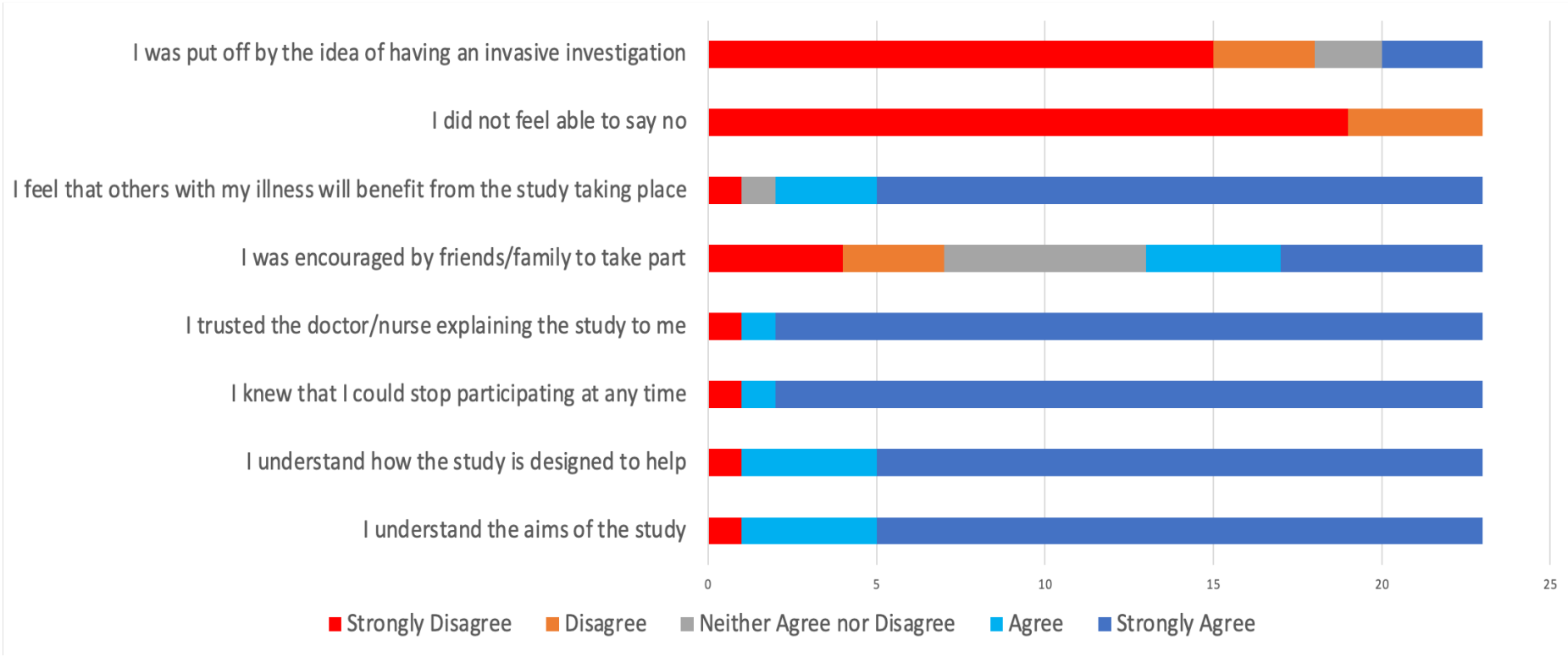


Figure 15. *Responses given by study participants to the trial invitation experience survey*

	Baseline (n=27)	Follow-up (n=23)	p-value
Distal contractile Integral, mmHg/cm/s, median (range)	459 (29-2732)	493 (70-2710)	0.362
Integrated Relaxation Pressure, mmHg, mean (SD)	10.75 (5.5)	10.8 (5.4)	0.484
Lower Oesophageal Sphincter Pressure, mmHg,mean (SD)	23.2 (11.4)	24.1 (13.4)	0.371
Distal Latency, ms, mean (SD)	6.7 (1.1)	6.6 (0.9)	0.464
Chicago Classification, % (n)			
Normal Motility	48 (13)	48 (11)	.
Ineffective Oesophageal Motility	41 (11)	39 (9)	.
Absent Contractility	11 (3)	13 (3)	.

Table 8. Results of manometric properties of all patients at baseline and follow-up visits.

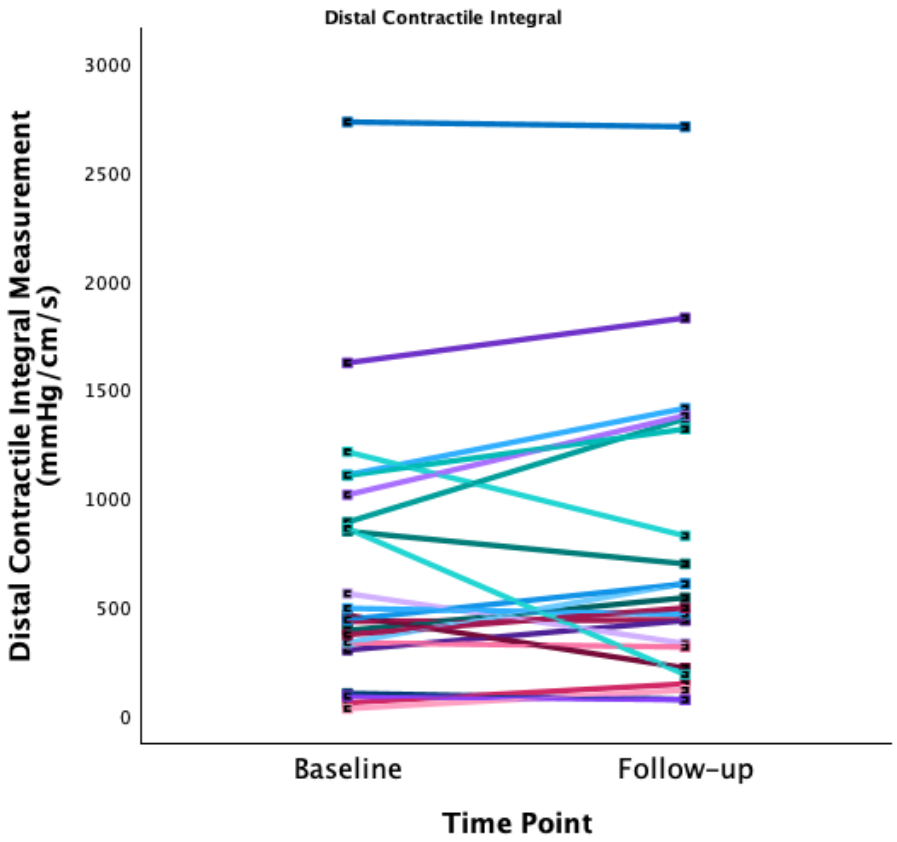


Figure 16. *Individual patient data for DCI measurements at baseline and follow-up for all participants.*

Relationship between HROM and Symptoms – Exploratory Outcomes

As previously stated, the effect of azithromycin on respiratory symptoms will be explored in the subsequent chapter, however, we performed exploratory analysis of the relationship between the change in objective and subjective measures of cough with the HROM findings.

There was no significant relationship between changes in DCI and changes in cough index (coughs per hour) in patients who had adequate cough monitoring performed, Pearson's correlation = 0.226 (p=0.325). There was also no significant correlation between the change in cough VAS scores or LCQ scores and change in DCI, Pearson's correlation =0.199 (p=0.362) and 0.174 (p=0.450), respectively. There was, however, a significant association between the change

in HARQ score and the change in DCI measurement with a Pearson's correlation of -0.448 (p=0.032). The scatter plots examining these relationships can be seen in figures 17-19.

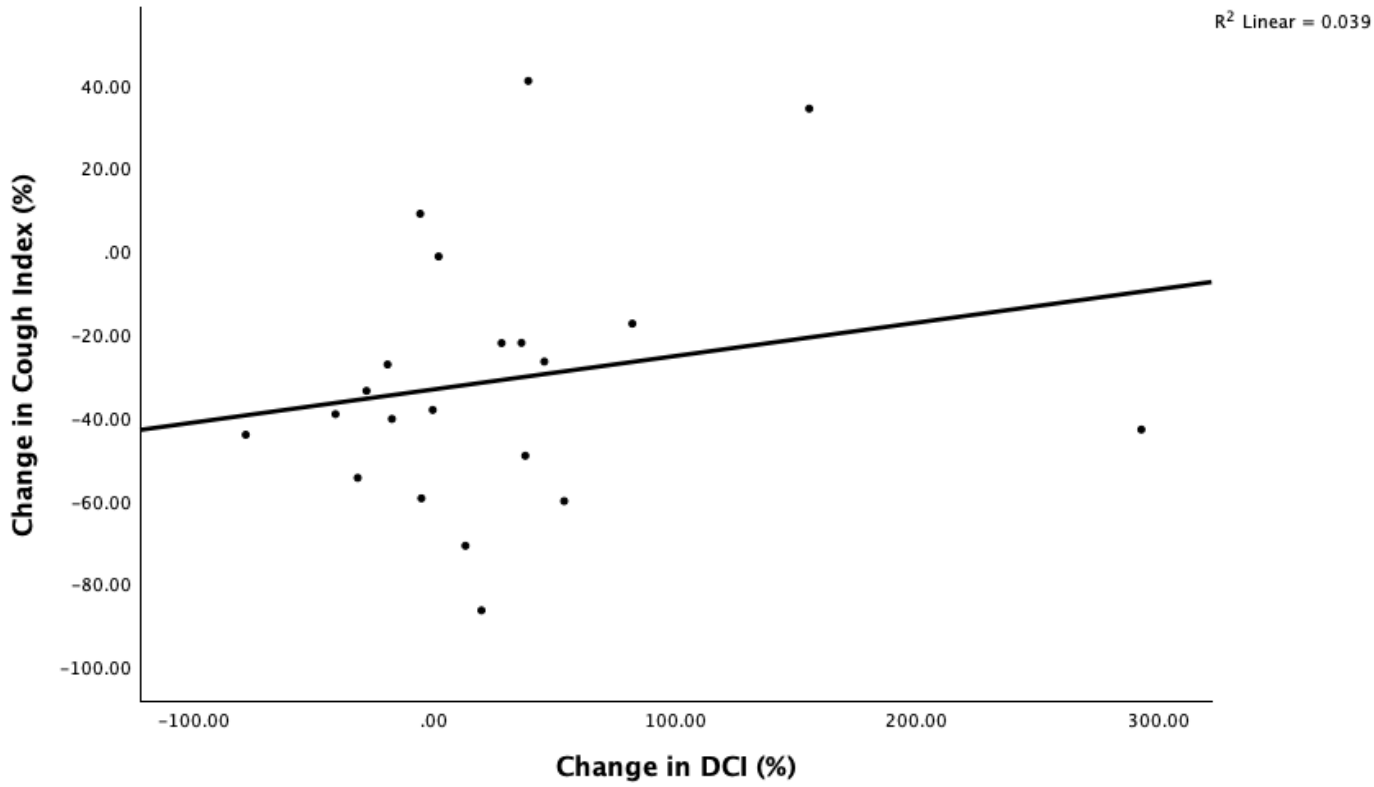


Figure 17. *Scatter plot examining the relationship between change in cough index (number of coughs per hour) and DCI between baseline and follow-up.*

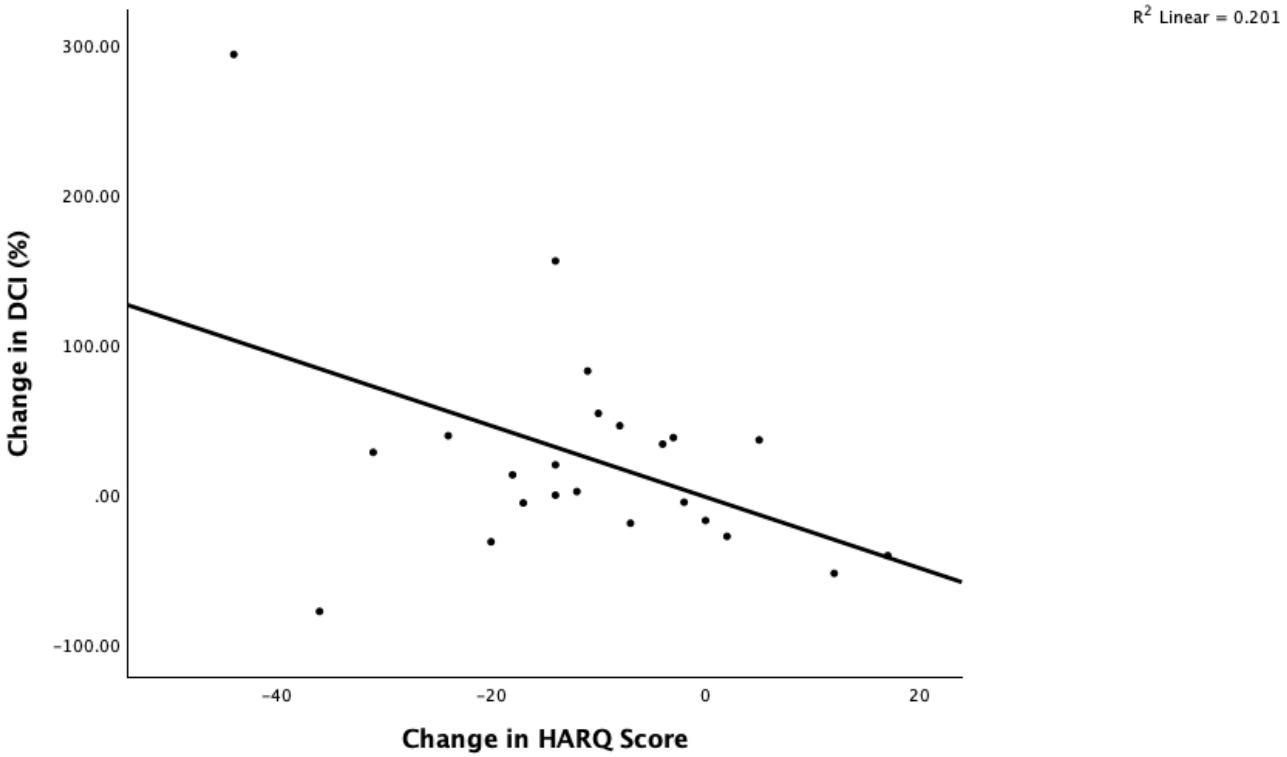


Figure 18. *Scatter plot examining the relationship between change in HARQ Scores and DCI between baseline and follow-up.*

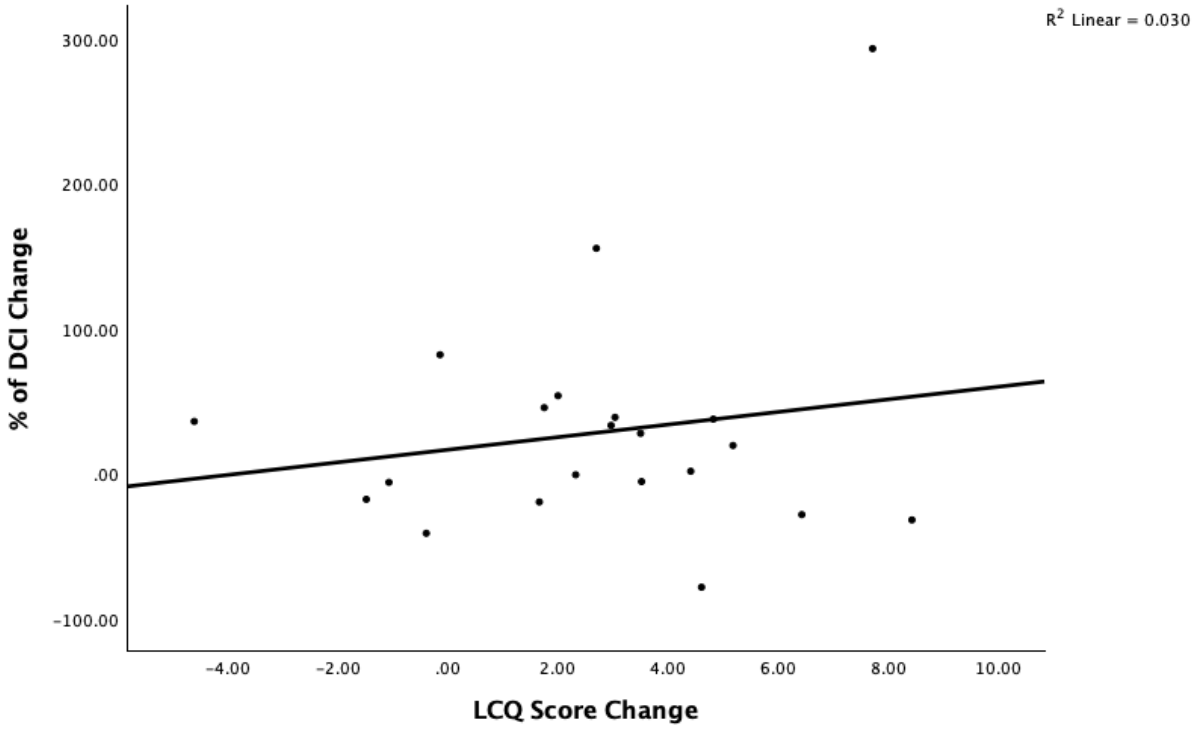


Figure 19. *Scatter plot examining the relationship between change in LCQ Scores and DCI between baseline and follow-up*

4.4 Discussion

The results of this trial show that a definitive large scale randomised controlled trial to assess the effectiveness of azithromycin on oesophageal function and symptom burden in patients with chronic respiratory disease is feasible and acceptable to patients. The findings of numerical improvements of DCI measurements in this group of patients after one month of azithromycin treatment suggests that investigating this as a primary endpoint in a large-scale trial is warranted. This is further evidenced by the fact that almost half of patients had an improvement of their DCI measurement $\geq$30% at follow-up. We believe that this signifies a clinically significant rise as previous data has shown an approximate 30% intra-individual variability between two separate HROM recordings[312].

In this study, all the pre-specified stop-go criteria for feasibility outcomes were achieved, including recruitment rate and participant retention. In addition, all pertinent data quality targets were met. The only exception to this was the completion rate of the paper cough VAS diary by participants, which we believe is likely attributable to the cumbersome nature of the document. In a large-scale trial, we believe that a digital cough VAS diary would be much more user-friendly and would lead to a lower rate of data attrition.

In the conception phase of the study, one of the chief concerns pertaining to the protocol was the willingness of participants to undergo two HROM investigations. Although it is not a painful procedure, it has risks such as bleeding and discomfort [143] and it is plausible that patients may not wish to undergo an investigation twice as part of a research study. However, the results from our invitation experience survey showed that 78% (n=18) of patients selected ‘strongly disagree’ or ‘disagree’ responses when asked whether the thought of having an invasive investigation discouraged them from taking part in the study. Furthermore, only 2 participants (that did not withdraw from the study) who were able to have their initial HROM study declined to have a further test at

follow-up, indicating that the study protocol for measuring oesophageal function was acceptable to patients and would be feasible in a larger study.

Of the candidate primary outcomes that were proposed, it appears that the change in DCI demonstrated the only numerical improvement in terms of oesophageal motility. The use of DCI is also concordant with the proposed mechanism by which azithromycin improves oesophageal motility. As discussed in chapters 1 and 2, azithromycin has been shown in vitro to be a potent motilin receptor agonist [202], such receptors are known to be expressed in the oesophagus. However, given that the primary effect of motilin agonism, specifically with macrolide antibiotics, in the oesophagus is a rise in lower oesophageal sphincter pressure[198, 313, 314], it may be surprising that our results did not show a signal of any change in this manometric parameter. However, 50% of the participants in our study were found to have a hiatus hernia, which has been shown to reduce the LOSP significantly due to anatomical displacement, impairing the sphincter's ability to maintain it's normal pressure[315]. This may preclude the measurement of LOSP being a meaningful clinical endpoint in a definitive trial.

Motilin agonism is not the only proposed mechanism for improved gastrointestinal motility with azithromycin therapy. There is evidence to suggest that deglycosylated azithromycin, a metabolite of azithromycin, may enhance intestinal smooth muscle function by binding to the structural protein transgelin[316]. This protein is predominantly distributed in smooth muscle tissue and exerts its effect via a c-terminal calponin-like molecule (CLIK23) and a positively charged amino acids which bind to actin filaments in the smooth muscle[317-319]. Transgelin promotes aggregation of G-actin to F-actin. This then in turn increases the length and strength of fibre bundles in intestinal smooth muscle cells, increasing their contractility strength[320]. In a study performed in healthy volunteers, the presence of deglycosylated azithromycin in the faeces was significantly associated with increased intestinal peristalsis[316]. However, this study did note that there was no significant association between deglycosylated azithromycin levels and contractility in the stomach.

Furthermore, they reported that deglycosylated azithromycin lacked any motilin-receptor agonist activity. Taken together, this suggests that differences in azithromycin metabolism between patients may result in differential effects on gastrointestinal motility. Some patients may experience lower gastrointestinal effects such as abdominal cramps, flatulence, and diarrhoea, whereas others may have increased gastric peristalsis, which may be beneficial for patients with reflux-associated chronic cough. Unfortunately, this study did not examine the effect of transgelin activation by deglycosated azithromycin in the oesophagus, which may be different to that of both the gastric and intestinal cells. Further work is required on the potential mechanistic effects of azithromycin on both motilin agonism and transgelin binding in patients with chronic refractory cough.

The primary limitation of this study is the lack of control group. As all patients had received azithromycin, we are unable to demonstrate any signal of improvement in manometric parameters that may be attributable to the azithromycin therapy. This also does not allow us to perform a formal sample size calculation for a larger randomised controlled trial. However, as previously stated, it would be logical to use the cut-off of at least 30% improvement in DCI as the minimum clinical important difference (MCID) between groups. Moreover, the lack of patient, clinician, and HROM operator blinding to the treatment and the phase of treatment, i.e. HROM before or after azithromycin treatment, leaves this study open to researcher bias. However, given that this was a feasibility study, where the primary endpoint was the acceptability of the trial processes to the patients and clinicians, the lack of blinding and randomisation is of lesser concern. It is also noteworthy that although we were able to conduct detailed study of oesophageal function in this trial, we did not assess gastric pH levels or impedance. As discussed previously in *Chapters 1 and 2,* there may be a significant proportion of patients with chronic respiratory disease that may be partially attributable to liquid acid reflux. With the protocol used in this study, we are not able to deduce the effect of azithromycin on stomach/ oeosphageal pH, number of reflux events, or nature of refluxate (i.e.

acidic, non-acidic, liquid, or gaseous). To investigate this aspect of the gut-lung axis, a definitive trial may warrant the inclusion of 24-hour pH and impedance monitoring.

4.5 Conclusions

A definitive randomised controlled trial to assess the efficacy of azithromycin on oesophageal function, as measured by repeated HROM testing, and its relationship to cough in chronic respiratory disease is feasible and acceptable to patients. The primary outcome of this trial should be the relationship of oesophageal contractility, measured by DCI, and objective cough counts measured by continuous cough monitoring and how these may change with long-term azithromycin treatment. Such future trials should utilise mixed methods to understand the effect of azithromycin on the patient's perspective, as well it's in/ ex vivo effect on motilin agonism and transgelin activity.

Chapter 5

Effect of azithromycin on continuous cough monitoring in chronic respiratory disease: an exploratory analysis

5.1 Introduction

It is established that long-term treatment with low-dose azithromycin is beneficial to the quality of life of patients with chronic respiratory diseases [222, 321]. It is believed that this improvement is driven by a reduction in exacerbations, however, some patients with respiratory diseases may not experience acute exacerbations. There are different phenotypes of airways diseases such as COPD and asthma, with some patients having high symptom burden despite having few exacerbations [322-324]. Moreover, patients with refractory chronic cough, that is not secondary to another respiratory ailment, may not have true exacerbations of their disease in the same way, but will experience disabling daily symptoms. Historically, symptoms have been somewhat neglected as a primary endpoint in clinical trials. Although useful tools, subjective measures of symptom burden and quality of life at discrete timepoints may be flawed. Measuring symptoms objectively is therefore vital to better understand the efficacy of medications, as relying on exacerbation frequency or subjective symptom measurement alone may not reflect the true efficacy of novel treatments.

Objective cough frequency has been shown to be a sensitive biomarker in many aspects of respiratory disease. In asthma patients, an increased cough frequency is associated with higher levels of type 2 inflammation and disease severity[325]. Moreover, a reduction in objective cough frequency has been shown to be an early predictor of response to biologic medications in patients with severe eosinophilic asthma[303]. A similar association between disease severity and increased objective cough counts has been observed in bronchiectasis[88]. Furthermore ,an increase cough reflex sensitivity has been found to increase risk

of exacerbation in COPD[326]. My systematic review (*Chapter 3)* highlights that only two clinical studies, one randomised controlled trial and one non-comparative, open-label trial, have assessed the effect of azithromycin on objective cough frequency. Both trials studied patients with ILD (1 IPF and 1 sarcoidosis) and had a combined total of 40 participants who completed all study visits. Thus, there is a dearth of high-quality data to answer this clinical question.

The current evidence examining objective cough in patients treated with azithromycin has been collected using traditional 24-hour cough counting methods at 2 or more time-points. As previously discussed in this thesis, novel, wearable, cough monitors have been developed which may more accurately reflect the nature of a patient's cough. Moreover, they may more sensitively detect changes in cough frequency as longer monitoring periods will reduce the impact of short-term variability of cough data.

In this clinical study we aimed to assess, for the first time, the effect of azithromycin on objective cough frequency using continuous cough monitoring over a prolonged period. To increase the generalisability of this study, we included patients with a broad range of respiratory diseases. We also aimed to assess the associations between objective and subjective measures of cough frequency using daily monitoring to highlight any day-to-day variability which may be unaccounted for using traditional, 24-hour cough counting.

5.2 Methods

As part of the feasibility trial described in *Chapter 4* we performed a pre-specified exploratory analysis of the effect of azithromycin on both objective and subjective measures of cough. Additionally, we assessed the relationship between continuous cough assessment using both objective and subjective measures. This analysis was performed in tandem with the feasibility study described in *Chapter 4,* with the same group of participants. Moreover, the ethical approval granted for study was given by the same regional ethics committee board (reference number: 22/NE/0194) and the study was registered under the same overall trial on clinicaltrials.gov (trial identifier: NCT05469555).

The recruitment, consent, and eligibility procedures have already been detailed in *Chapter 4.* This trial retained the same inclusion and exclusion criteria as the feasibility study, which can be seen in table 4. As with the feasibility study, the participants attended 3 study visits. The initial screening visit participants would be assessed for eligibility and, if they were willing and able to take part, would be supplied with the *Hyfe Cough Monitor®* and instructed on how to ensure adequate data capture. Participants then returned 1 week later, having worn the monitor in this time, to enable a 'baseline' objective cough pattern to be recorded.

At Visit 2, participants would undertake a comprehensive subjective assessment of their respiratory symptoms using validated questionnaires. Following this, if they did not have a prolonged QTc interval on ECG, participants would be provided with a prescription for azithromycin 250mg OD. The protocol for dose adjustments, based on whether the participants experienced gastrointestinal adverse effects, has been described in *Chapter 4*. In the 4-week period between Visit 2 and Visit 3, all participants were asked to complete a daily cough severity VAS diary. Participants retained the cough monitor for this period with the aim of completing continuous 24-hour a day monitoring for the full treatment

period.

After a minimum of 4 weeks' treatment, the participants returned for Visit 3 where they would repeat the holistic assessment of their respiratory symptoms with validated questionnaires. The participants returned their cough monitors and their involvement in the trial was complete. The schedule of events (table 3) outlines the structure of each study visit.

Hyfe Cough Monitor®

As discussed in *Chapter 1,* this wearable cough monitor enables participants' cough to be monitored continuously for the duration of the study. For the purpose of this study, we used 8 'Domino' Android™ smartwatches which had the software for the HyfeAI® cough detection algorithm pre-installed. Participants were instructed to wear the device on their wrist whilst they were awake, remove it for showering or bathing, and charge it by their bedside whilst sleeping. This strategy allowed for the participant to be monitored 24 hours a day whilst awake and asleep. A diagram of the cough watch can be seen in Figure 20.

Participants were also provided with a portable, plug-in modem which provided a wireless internet connection, this allowed the cough data stored on the watch to be uploaded to the *Hyfe* cloud. Data was uploaded when the watch was charging and in proximity to the plug-in modem. Each participant was given a unique user ID which would link to an online dashboard where live cough data uploaded to the cloud would be displayed. This dashboard was only available to the study team so that the participant was unaware of the trend of their cough counts. An example of this dashboard can be seen in Figure 21.

The digital dashboard provided data for the length of time that each participant was monitored, allowing for a more accurate assessment of cough frequency as days when participants were not wearing the watch for at least 12 hours were excluded from the final analyses.

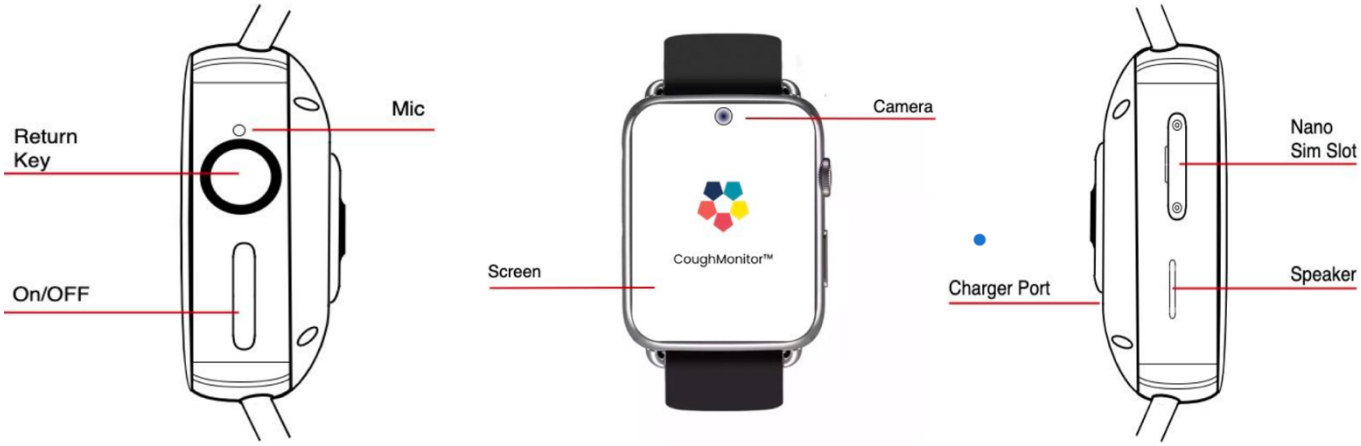


Figure 20. *Diagram of the 'Domino' Android™ smartwatch given to participants with the pre-installed HyfeAI® cough monitoring algorithm.*

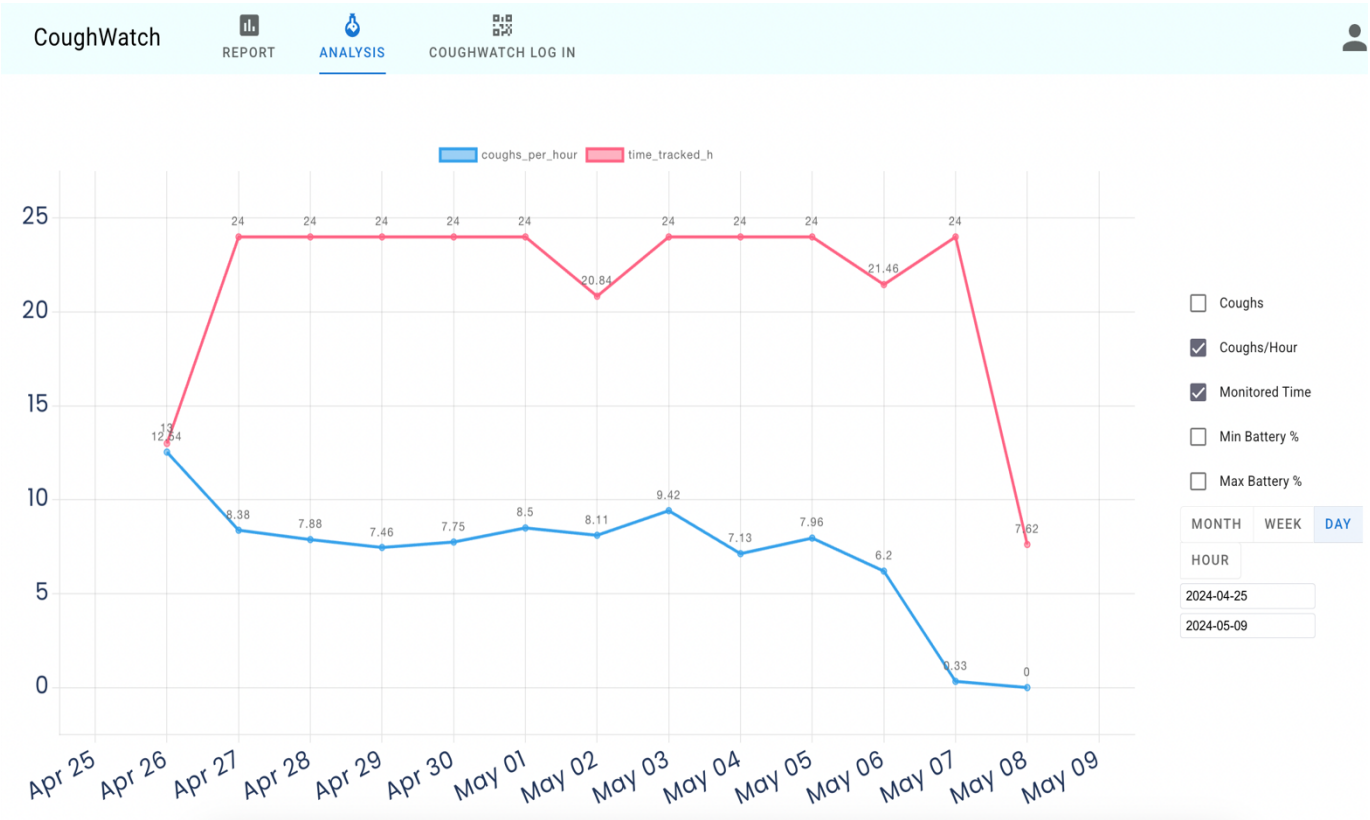


Figure 21. An example of the *Hyfe Cough Monitor® interactive dashboard which allows for live monitoring of participant's cough data, including the length of time that the participant was monitored for (displayed by the red line).*

Primary Outcome – Objective assessment of cough

The primary outcome of this exploratory analysis was the average daily cough count for participants at week 4 post-initiation of azithromycin compared with the 1 week of monitoring prior to the start of their treatment. As is customary for clinical trials measuring objective cough frequency, this will be reported as hours/hour. We will, however, also analyse total average daily cough counts.

Secondary Outcomes – Subjective Assessment of cough, other respiratory symptoms, and quality of life

The subjective assessment of cough severity in this trial was carried out with cough severity VAS at baseline and follow-up, as well as a 4-week daily VAS diary. Cough severity was also assessed using the HARQ and the breathlessness, cough, and sputum scale (BCSS) at baseline and follow-up. Subjective measure of breathlessness was measured using the medical research council (MRC) dyspnoea scale.
Cough-specific quality of life was measured using the LCQ. Data collected from the LCQ was also broken down into the physical, psychological, and social domains. Respiratory-disease specific quality of life was measured using the SGR with total scores calculated along with scores for symptoms, activity, and impact domains.

Data was collected for disease-specific questionnaires including the COPD Assessment test (CAT), Asthma Control Questionnaire 6 (ACQ6), King's Brief ILD Questionnaire (K-BILD), as well as the numerical rating scale (NRS) for breathlessness. However, due to inconsistencies in the administration and recording of these questionnaires, data on these will not be presented.

Secondary Outcomes – Novel cough metrics

1.) *Relief of cough*

To investigate whether the time that participants spent not coughing or not in 'bouts' of coughing was correlated with improvements in

subjective assessments of cough we used a novel method of analysis termed 'relief of cough'. Previously published data from patients attending a specialist chronic cough centre demonstrated that the most commonly reported time elapsed before patients felt as though their cough bout had ended was 15 minutes.[327] This time period was used as a rolling bracket within the analysis to determine the duration of time with no coughs recorded i.e. the patient was relieved of their cough. For example, if we take one hour's cough counting: Patient A coughs repeatedly and every cough is within 15 minutes of the one that preceded it; they would have no relief of cough. However, Patient B may record the same total number of coughs but with all coughs occurring within a discrete 20-minute period and not coughing again; they would have had 40 minutes of relief of cough. The proportion of time that a participant was relieved of cough during each 24-hour monitored period was then calculated. This was performed by subtracting the total time of 'relief of cough' from the total time that the participant was actively monitored in that 24 hours. For example, if a participant had a total relief of cough time of 18 hours and were able to be monitored for 24 hours in a single day, their proportion of time relieved of cough would be 0.75 or 75%.

2.) *Cough Density*

Using another novel metric that has previously been developed by *Morice et al.,* we aimed to assess the number of times that patients coughed in each bout. By subtracting the total relief of cough time from the 24-hour observation period we derive the total time spent coughing. The number of coughs in that period comprise the cough density. Using the same scenario as above, we can derive that Patient A has a lower cough density as they spent more time coughing. Patient B, in contrast, has a higher cough density because of a greater time relieved of their cough.

Secondary Outcomes – Cough variability and correlation with subjective assessment

We aimed to validate a previously performed method of analysing the variability of objective cough frequency, reported by *Morice et al.[328],* and to examine whether the variability of cough frequency is consistent in different groups of patients. This analysis was performed by calculating the average absolute deviation (AAD) and the average relative AD (AAD/mean) of the daily cough frequency for each patient.

To assess the relationship between objective and subjective measures of cough we performed linear regression between the daily cough VAS scores from the participants' diaries and the daily objective cough frequency, this is reported as Pearson's correlation coefficient.

Statistical Analysis

Participant baseline characteristics are summarised using descriptive statistics. Medians (range) are reported for non-parametric data, mean (standard deviation) for continuous, normally distributed data and raw count (number (%)) are reported for nominal data. Comparisons of baseline and follow-up objective cough frequencies and patient reported outcome measures (PROMs) were performed using paired t-tests for normally distributed data and Mann-Whitney U testing for skewed data. Correlations between daily cough VAS and cough frequency were performed using Pearson's correlation coefficient for normally distributed data and Spearman's rank correlation for non-normal data. For analysis of repeated measures, for example mean cough frequency per week of treatment, one-way ANOVA analysis was used for normally distributed data and Friedman's ANOVA was used for non-parametric data. All data were analysed using IBM SPSS Statistics 29 (IBM Corp., Armonk, NY, USA).

5.3 Results

Of the 30 patients recruited to this study 60% (n=18) had a diagnosis of chronic cough, 23% had a diagnosis of interstitial lung disease (n=7), and 17% had a diagnosis of airways disease (n=5). The cohort had a mean age of 65.2 (SD=11.3) and 57% (n=17) were female. All baseline characteristics are displayed in Table 8.

At 4-weeks follow-up, 80% (n=24) of patients met the pre-determined criteria for satisfactory usage of the cough monitor over the duration of the clinical trial. Azithromycin was discontinued in 2 participants due to adverse effects (both were attributed to gastrointestinal upset). One patient was ineligible to initiate treatment due to a prolonged corrected QT interval (QTc) on their ECG. One patient was lost to follow-up after the initial screening visit. The CONSORT diagram demonstrating patient flow through the study can be seen in Figure 14.

	All patients (n=30)
Age, mean (SD)	65.2 (11.3)
Female, n (%)	17 (57)
BMI, mean (SD)	28.8 (6.0)
Primary Respiratory Diagnosis, n (%)	
Chronic Cough	18 (60)
Interstitial Lung Disease	7 (23)
COPD	3 (10)
Asthma	2 (7)
Spirometry, mean (SD)	
FEV1 % predicted	81 (29)
FVC % predicted	92 (25)
FEV1/FVC ratio	68 (14)
Blood Eosinophil Count, median (range)	0.21 (0.01-0.95)
Baseline Cough VAS, mean (SD)	56.5 (19.6)
Baseline HARQ Score, mean (SD)	40.8 (10.6)

Table 9. *Baseline characteristics for the participants included in this exploratory open-label study*

Effect of azithromycin on cough frequency

There was a significant reduction in the median coughs per hour from the pre-treatment baseline monitoring week to follow-up at week 4 (8.9 vs 5.5, p=0.002). There was a significant reduction in median coughs per hour seen within 1 week of treatment (8.9 vs 7.5, p=0.003) and this improvement was sustained at week 2 (9.79 vs 6.8, p=0.003) and week 3 (9.79 vs 6.8, p=0.003). When using Friedman's ANOVA there was a significant reduction in coughs per hour when all time points were compared (p<0.001). The sustained reduction in cough frequency can be visualised in figure 22. There was also a significant reduction in median average total daily cough count at follow-up when compared to baseline (175.5 [range 17-948] vs 110.5 [range 17-550], p<0.001).

The median change in frequency of coughs per hour in all patients who completed 5 weeks of continuous cough monitoring was -32.3% (Range = -78.4% - 37.87%). Of the 24 patients who had full monitoring, 29% (n=7) had a >50% reduction in their average median coughs per hour between baseline and week 4. The average coughs per hour decreased numerically in 87.5% (n=21) of participants with complete cough data. The logarithmically transformed cough counts for all patients at baseline and week 4 can be seen in Figure 23.

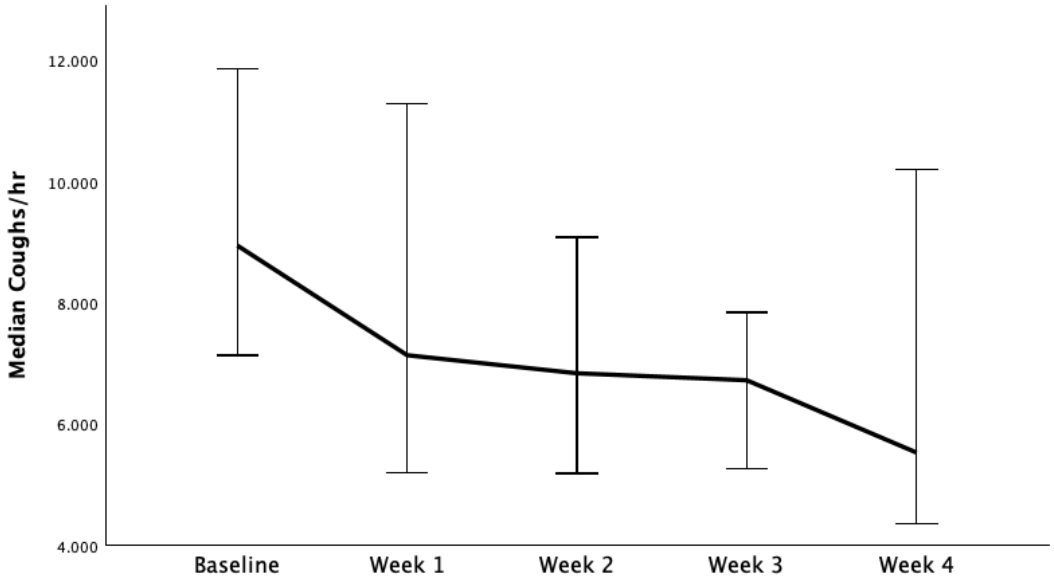


Figure 22. *Displaying the change in median coughs per hour from pre-treatment baseline per week in follow-up.*

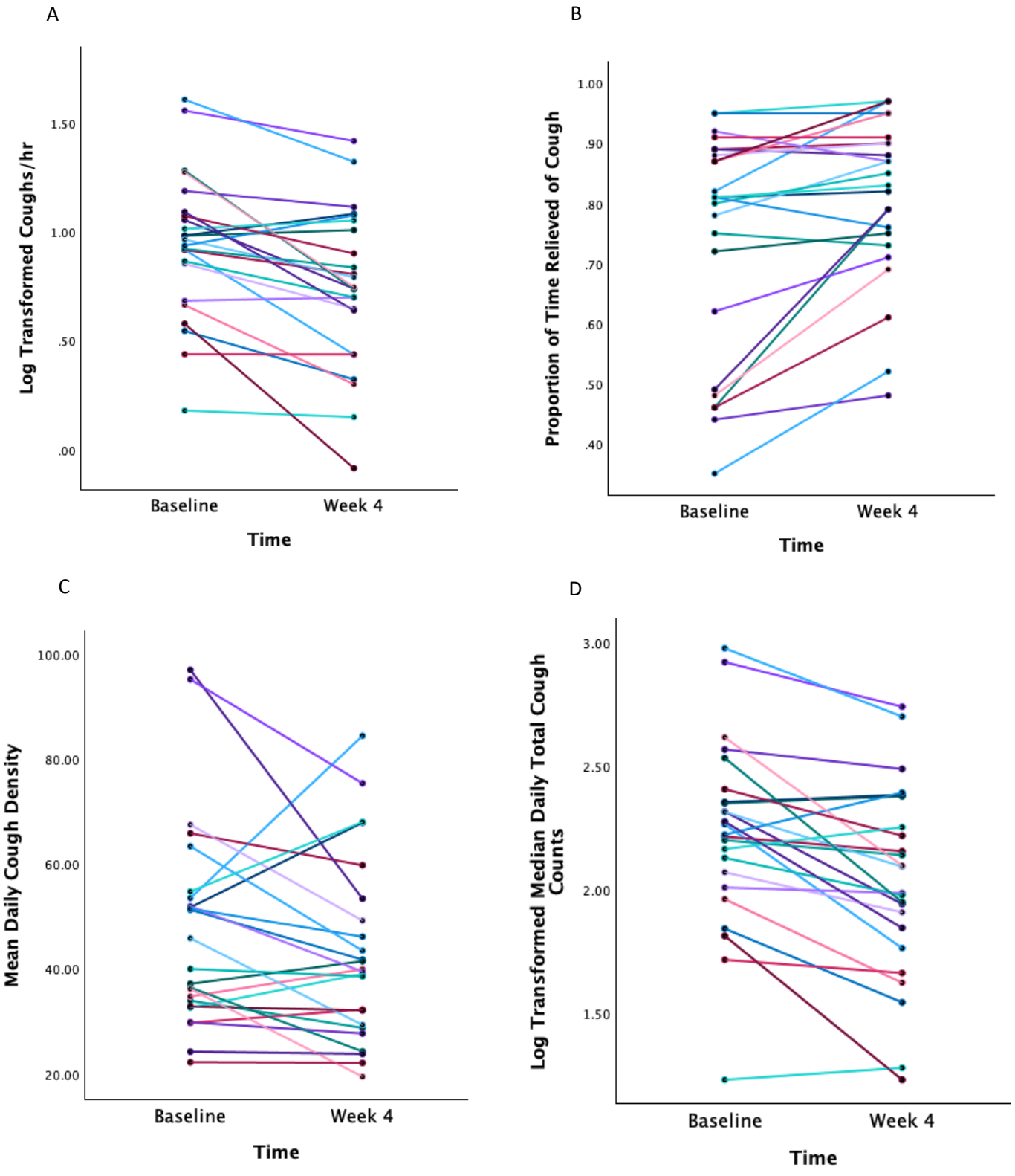


Figure 23. *Spaghetti plots showing individual patient data at baseline and 4-week follow-up for objective cough metrics: A) Logarithmically transformed*

coughs/hr B) Mean proportion of time relieved of cough C) Mean daily cough density D) Logarithmically transformed median daily cough counts

Effect of azithromycin on novel cough metrics

Our analysis found a significant improvement in the proportion of time that participants were relieved of their cough, improving from a baseline mean of 74% of recording time spent relieved of cough to a mean of 81% of time by week 4 ($p<0.001$). There was not, however, any significant improvement in the cough density metric between baseline and follow-up, despite a numeric improvement (47.5 [SD=19.7] vs 42.8 [SD=17.5], p=0.069).

A comparison of the average daily total number of episodes that participants were relieved of their cough between baseline and follow-up also did not show any significant difference (17.3 [SD=4.8] vs 17.9 [SD=4.6], p=0.243). The trends of the change in these cough metrics per week can be seen in figures 24-26.

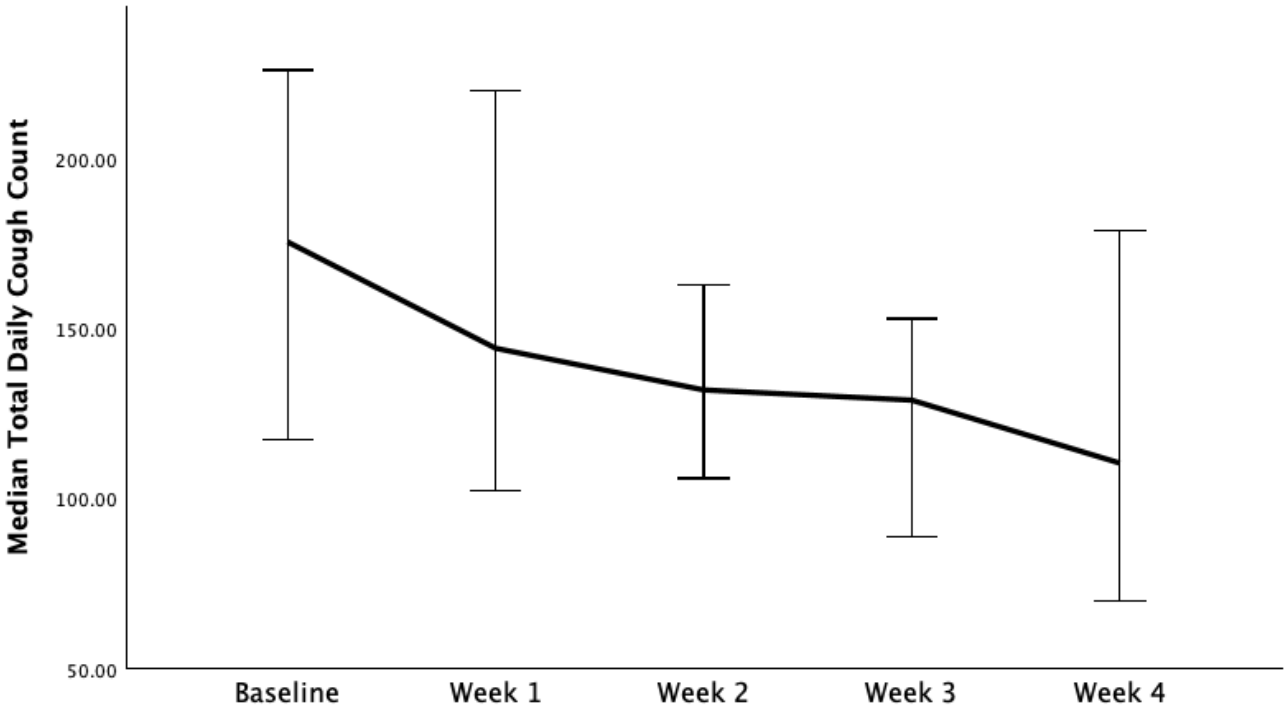


Figure 24. *Displaying the change in median total daily cough counts from pre-treatment baseline per week in follow-up.*

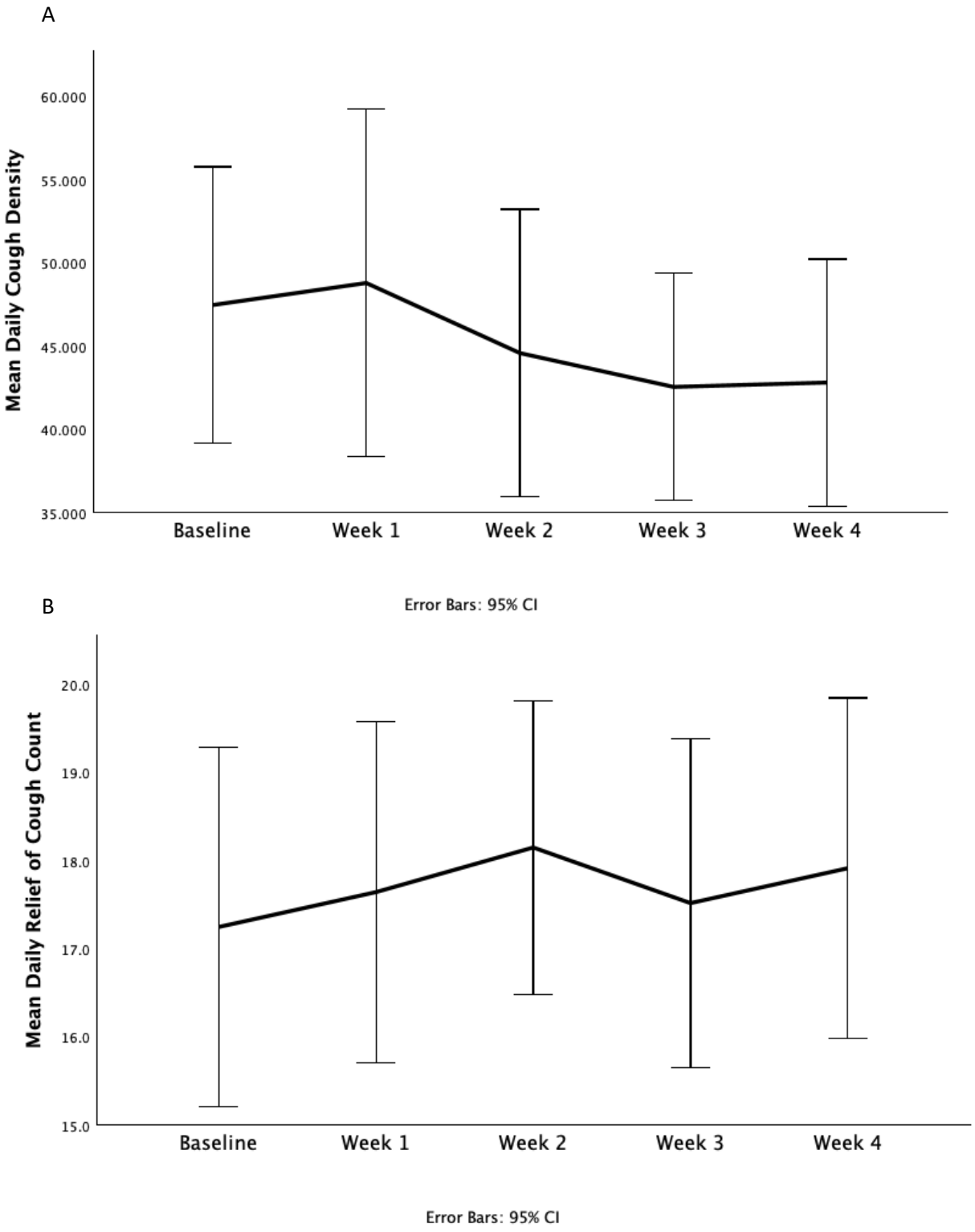


Figure 25. *A) Displaying the change in median daily cough density from pre-treatment baseline per week in follow-up. B) Change in median daily relief of cough count from pre-treatment baseline per week in follow-up.*

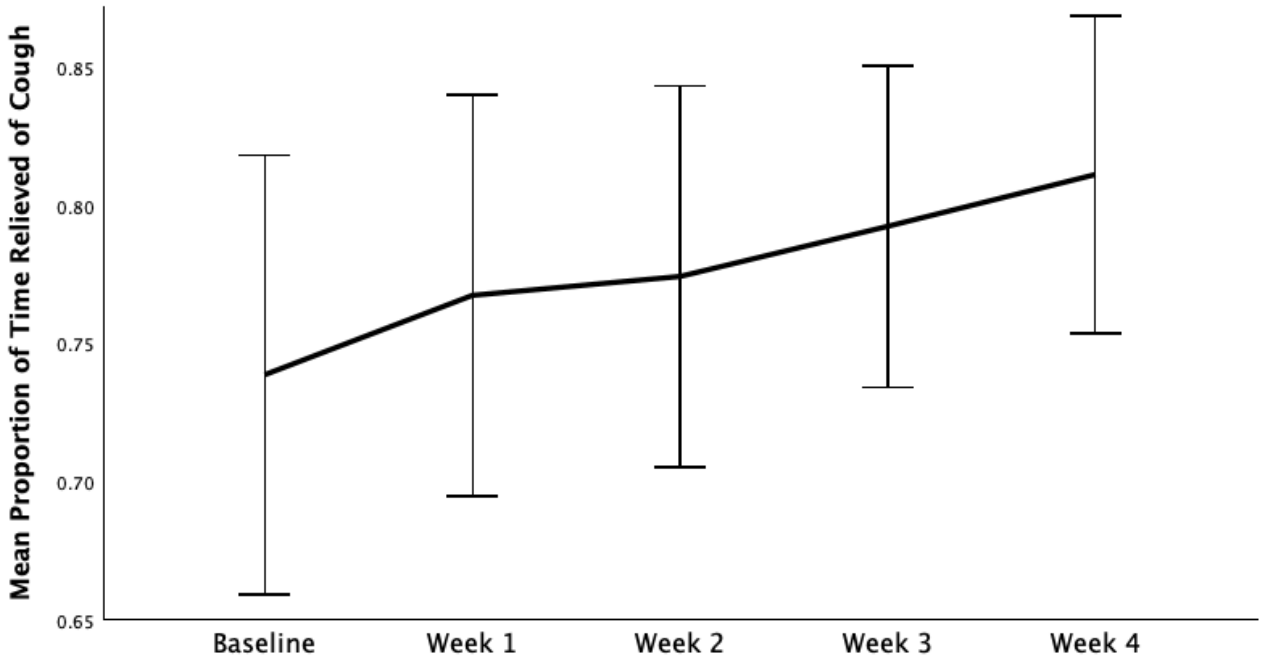


Figure 26. *Displaying change in the mean proportion of monitoring time that participants were relieved of cough (0.8= 80% of monitored time relieved of cough).*

Cough Frequency Metric	Baseline	Follow-up	P-value
Daily Total Cough Counts, median (range)	175.5 (17-948)	110.5 (17-550)	<0.001
Daily Cough Index (Coughs/hr), median (range)	8.9 (1.5-40.6)	5.5 (0.8-26.2)	0.002
Daily Cough Density, mean (SD)	47.5 (19.7)	42.8 (17.5)	0.069
Daily Total Relief of Cough count, mean (SD)	17.3 (4.82)	17.9 (4.6)	0.243
Daily Proportion of Time Relieved of Cough, mean (SD)	0.74 (0.19)	0.81 (0.14)	<0.001

Table 10. Objective cough metrics at pre-treatment baseline and 4-week follow-up.

Effect of azithromycin on patient reported outcome measures

Of the 30 patients recruited to the study, 86.7% (n=26) had complete data for cough-related patient reported outcome measures (PROMs). There was a significant improvement in mean cough severity visual analogue score (VAS) at 4-week follow-up when compared to baseline (48.4 [SD=23.1] vs 38.6 [SD=28.1], p=0.01). Of the 26 participants who completed both the baseline and follow-up VAS assessments, only 19% (n=5) exceeded the minimal clinically important difference (MCID) of 30mm improvement. There was also significant improvement in the mean Hull Airways Reflux Questionnaire (HARQ) score before and after treatment (41.3 [SD=10.4] vs 29.9 [SD=13.7], p<0.01). The MCID for improvement, defined as a reduction in 16 points, was met in 35% (n=9) of the participants for the HARQ.

Regarding cough-specific quality of life there was a significant improvement in the mean Leicester Cough Questionnaire (LCQ) score between baseline and follow-up (11.1 [SD=3.7] vs 13.6 [SD=4.1], p=0.01). Such improvements were observed in all 3 of the physical, psychological, and social domains of the LCQ. The MCID for improvement in the LCQ was defined as an increase in score of at least 1.7 for the total score and 0.9 in individual domain scores[329]. This was met in 65% (n=17) of participants for the overall score, 62% (n=16) for the physical domain, 58% (n=15) for the psychological domain, and 65% (n=17) of participants for the social domain.

There was also significant improvement in overall health-related quality of life, as measured by the St George's Respiratory Questionnaire (SGRQ), at follow-up (SGRQ total mean 50.2 [SD=25.3] vs 44.8 [SD=24.6], p=0.008). A lower score in the SGRQ signifies a better quality of life. Significant improvements were also seen in the symptom domain (mean score = 68.4 [SD=21.6] vs 62.7 [SD=19.2], p=0.011) and the impacts domain of the SGRQ (mean score = 48.5 [SD=28.0] vs 38 [SD=24.3], p<0.001). There was an observed worsening of quality of life in activity domain of the SGRQ, but this was only of borderline significance (mean score = 42.2 [SD=29.7] vs 46.9 [SD=34.2], p=0.048). The MCID for improvement

in the total SGRQ and all of its domains was defined as a reduction of 4 points [330]. This score was met in 35% (n=9) of participants for the total score, 50% (n=13) for the symptoms domain, 19% (n=5) for the activity domain, and 46% (n=12) of the impact domain.

The only PROM that did not demonstrate a significant improvement was the Breathlessness, Cough, and Sputum Scale (BCSS) as there was no significant difference between mean baseline and follow-up scores (5.6 [SD=2.9] vs 4.2 [SD=2.7], p=0.13). When analysed by specific domain of the BCSS there was no significant change in the breathlessness domain, however, there were significant reductions in both the cough (2.42 [SD=0.9] vs 1.92 [SD=1.1], p=0.015) and sputum (1.83 [SD=1.2] vs 1.0 [SD=1.1], p=0.003) domains. The MCID for the BCSS was defined a reduction of 1 point for the total score[331], as the literature is scarce for the MCID of individual domains, the 1 point reduction was also applied to the breathlessness, cough, and sputum domains. This was met in 62% (n=16) of participants for the total score, in 31% (n=8) for the breathlessness domain, in 46% (n=12) for the cough domain, and 54% (n=14) for the sputum domain.

All PROM data can be seen in table 10 and all individual patient data comparing baseline and follow-up data for PROMs is illustrated in figures 27 and 28.

Questionnaire	Baseline	Follow-up	p-value
Cough VAS, mean (SD)	48.4 (23.1)	38.6 (28.1)	0.01
Hull Airways Reflux Questionnaire, mean (SD)	41.3 (10.4)	29.9 (13.7)	<0.001
Leicester Cough Questionnaire Total, mean (SD)	11.1 (3.7)	13.6 (4.1)	0.01
LCQ Physical Domain, mean (SD)	3.9 (1.5)	4.7 (1.5)	0.02
LCQ Psychological Domain, mean (SD)	3.7 (1.4)	4.7 (1.6)	0.02
LCQ Social Domain, mean (SD)	3.5 (1.3)	4.3 (1.4)	0.05
St George's Respiratory Questionnaire Total, mean (SD)	50.2 (25.3)	44.8 (24.6)	0.008
SGRQ Symptoms Domain, mean (SD)	68.4 (21.6)	62.7 (19.2)	0.011
SGRQ Activity Domain, mean (SD)	42.2 (29.7)	46.9 (34.2)	0.048
SGRQ Impact Domain, mean (SD)	48.5 (28.0)	38.0 (24.3)	<0.001
Breathlessness, Cough, Sputum Scale, mean, SD	5.6 (2.9)	4.2 (2.7)	0.13
BCSS Breathlessness Domain, mean (SD)	1.33 (1.2)	1.25 (1.2)	0.392
BCSS Cough Domain, mean (SD)	2.42 (0.9)	1.92 (1.1)	0.015
BCSS Sputum Domain, mean (SD)	1.83 (1.2)	1.0 (1.1)	0.003

Table 11. Patient reported outcomes at pre-treatment baseline and 4-week follow-up. VAS: visual analogue scale.

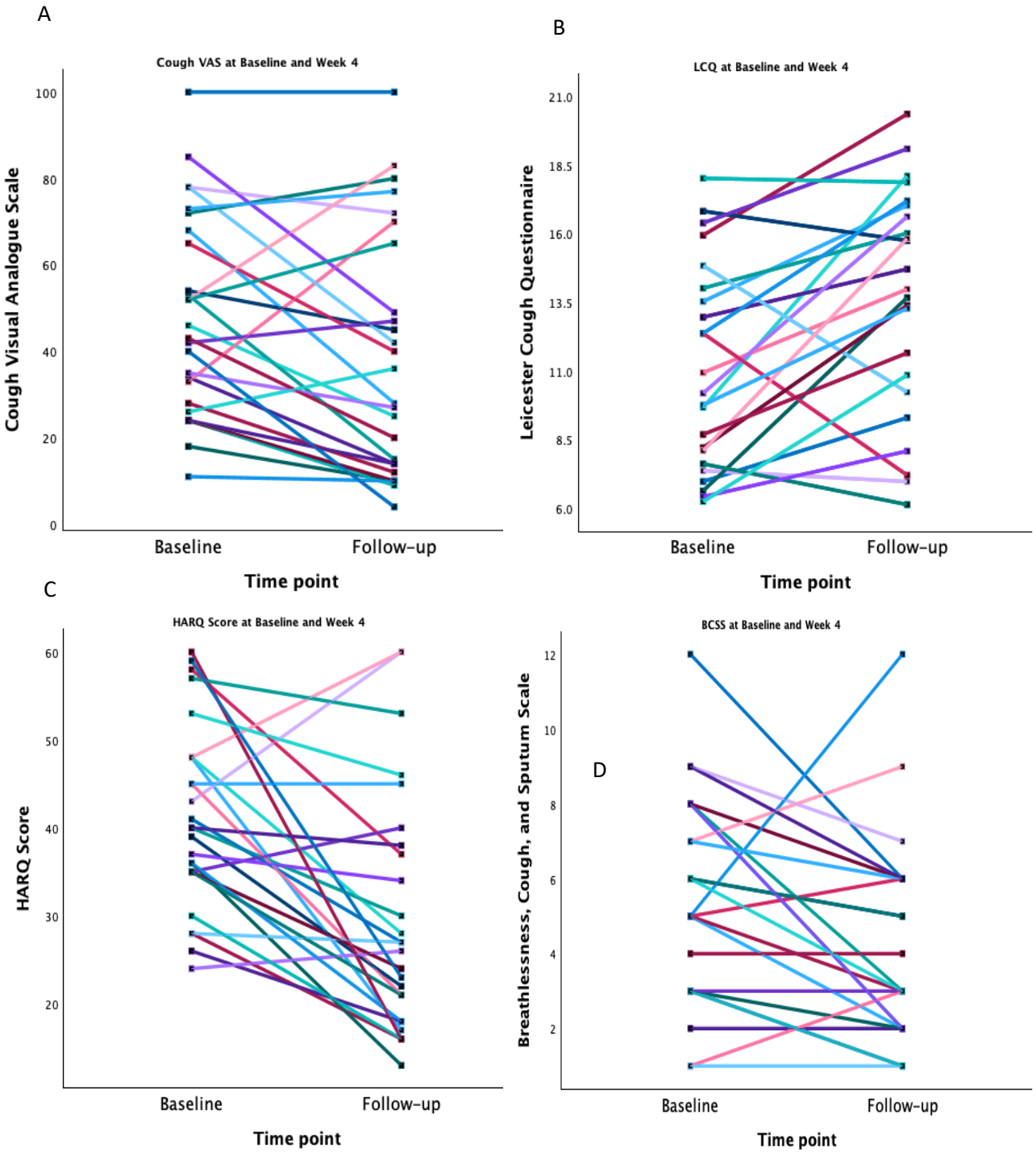


Figure 27. *Scores for each patient reported outcome measure at baseline and follow-up for all patients. Each line represents individual patient data.*

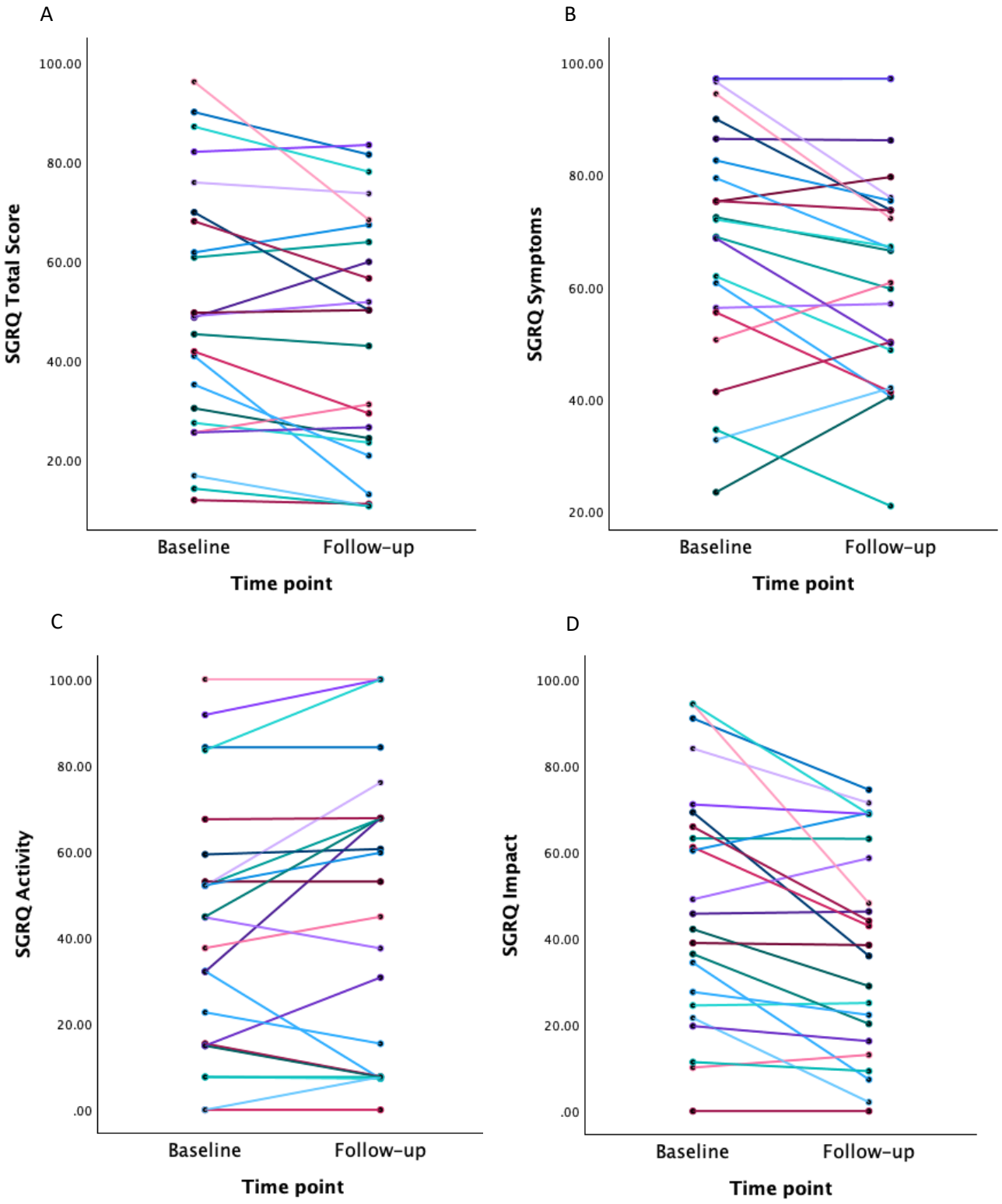


Figure 28. *Scores for the SGRQ including A) Total score, B) Symptom domain, C) Activity domain, and D) Impact domain at baseline and follow-up for all patients. Each line represents individual patient data.*

Cough Diary Data

As stated in *Chapter 4*, 69% (n=18) of participants who completed all study visits returned a physical cough diary in which they recorded a daily cough severity VAS score. There was a significant reduction in mean daily VAS scores from week 1 to week 4 of diary entry (47.5 [SD=18.5] vs 36.7 [SD=26.1], p=0.003). There was, however, no significant difference between all 4 weeks mean daily VAS scores using ANOVA analysis, p=0.490. The mean cough VAS per week can be seen in Figure 29. There was significant day-today variability in VAS scores, although no formal statistics were performed to analyse this variability, one can see the overall trend in scores in Figure 30.

In order to understand the relationship between daily objective and subjective daily measures of cough we performed an analysis to measure the correlation between daily total cough count and daily cough VAS. There was no significant correlation between the two variables and the mean and median correlations were 0.153 and 0.213, respectively. The average cough counts, cough VAS scores, and the corresponding correlations can be seen in Table 11. Such correlations are also displayed graphically in Figure 31.

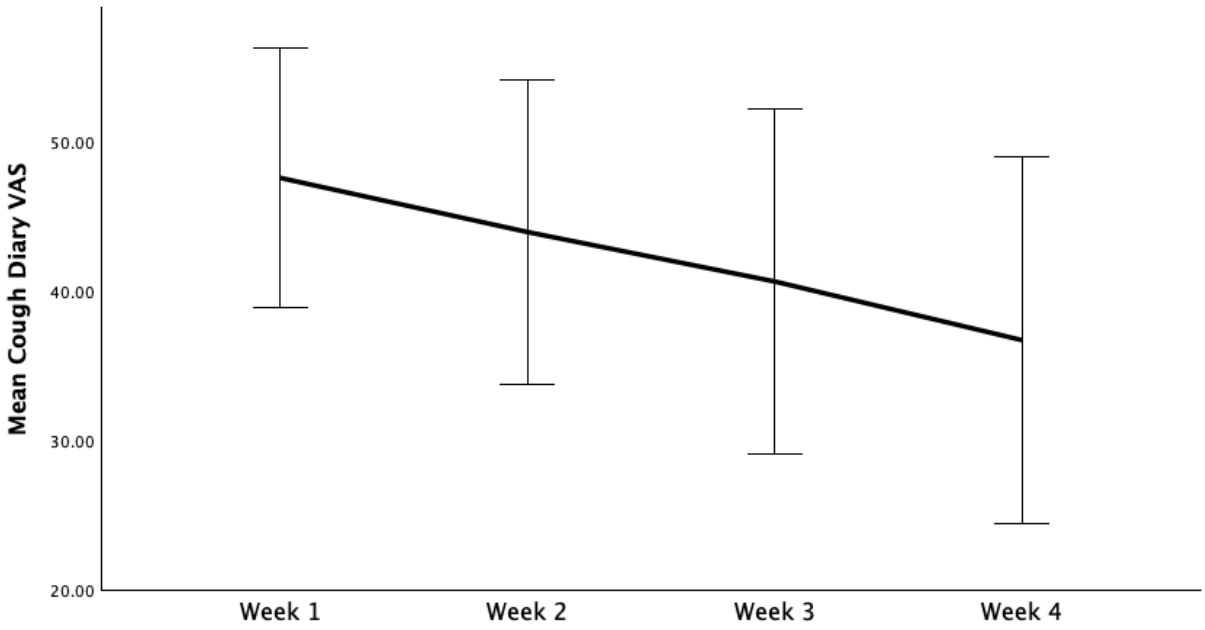


Figure 29. *Change in weekly mean cough severity VAS scores calculated from completed cough diaries*

Participant number	Daily Cough Count, mean (SD)	Daily Cough VAS, mean (SD)	Correlation coefficient
Participant 22	152.5 (74.2)	13.9 (8.1)	0.727
Participant 28	165.4 (46.3)	39.7 (12.7)	0.456
Participant 13	102.6 (46.9)	54.9 (7.9)	0.453
Participant 30	119.4 (58.9)	43.4 (12.3)	0.392
Participant 6	145.0 (32.3)	30.9 (20.1)	0.380
Participant 27	624.4 (204.3)	40.6 (7.5)	0.292
Participant 18	128.4 (47.6)	52.3 (18.3)	0.253
Participant 19	90.3 (55.4)	29.9 (15.6)	0.250
Participant 15	42.9 (17.9)	37.6 (16.9)	0.213
Participant 7	56.1 (38.0)	13.8 (22.7)	0.158
Participant 2	124.4 (101.3)	15.8 (18.5)	0.058
Participant 4	20.8 (9.9)	10.8 (6.2)	0.036
Participant 3	143.6 (29.8)	52.8 (13.2)	-0.064
Participant 29	169.6 (71.3)	49.2 (14.9)	-0.129
Participant 5	105.3 (51.5)	28.9 (13.6)	-0.248
Participant 17	318.5 (91.4)	61.4 (10.9)	-0.284
Participant 11	51.4 (41.3)	49.5 (4.1)	-0.344
Participant 8	80.5 (25.7)	100 (0)	N/A

Table 12. *Summarising the daily cough count and VAS scores, with the corresponding correlation co-efficient for all individual patients that completed the cough diary. Note that subjects are ranked by the strength of the correlation between cough count and VAS score*

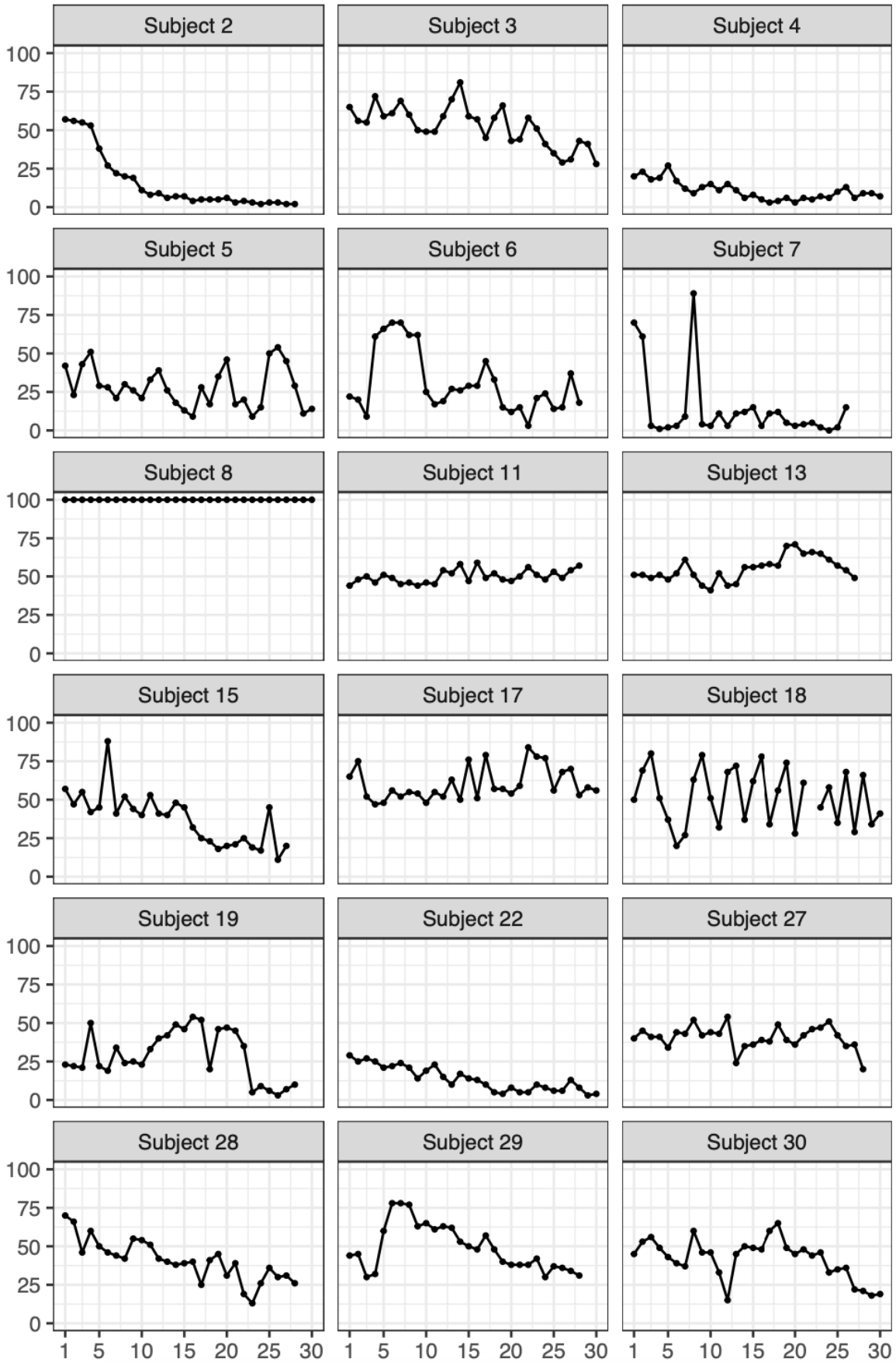


Figure 30. *Daily VAS scores for each participant that completed the 4-week diary, with the day on the x-axis and corresponding VAS score on the y-axis*

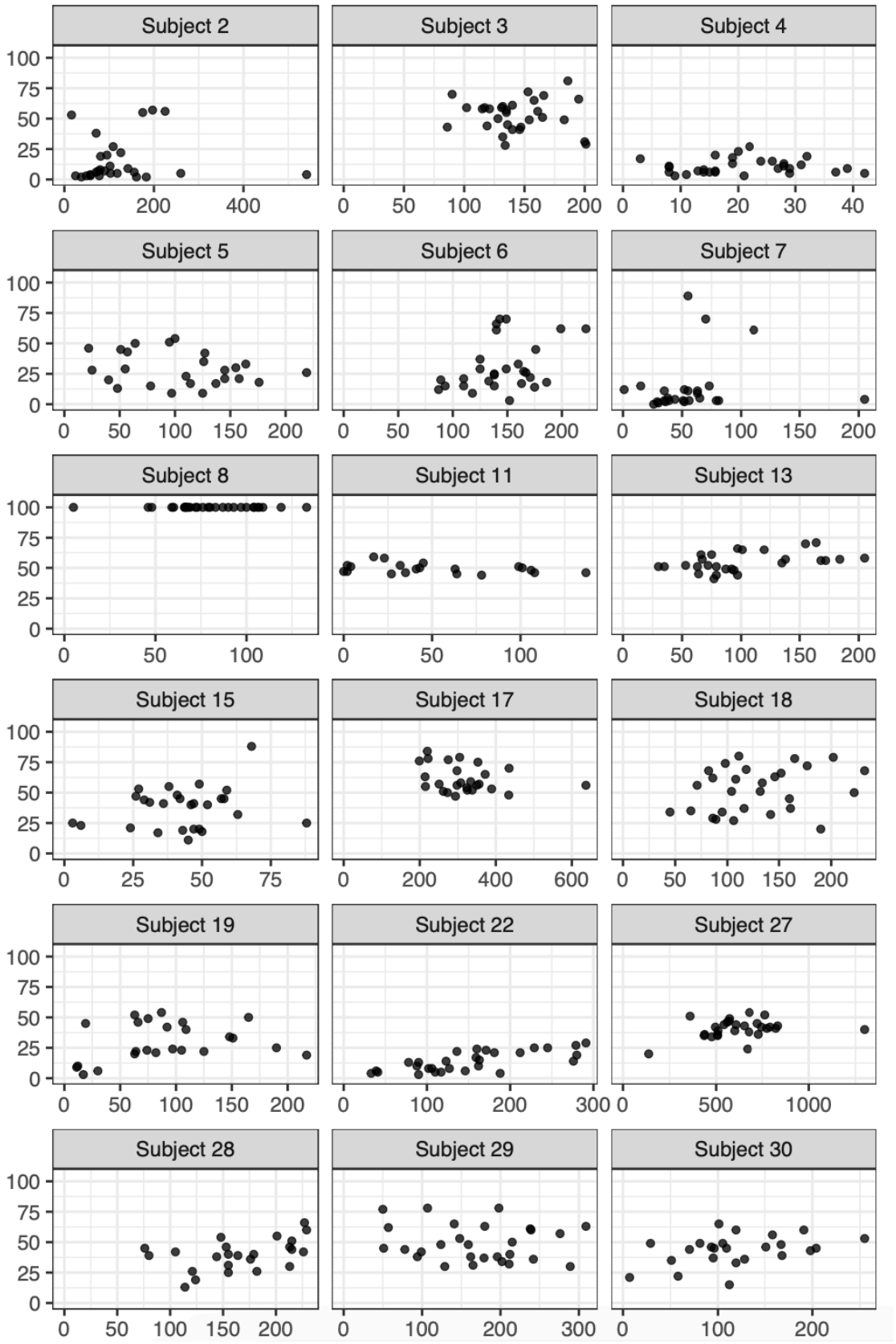


Figure 31. *Scatter plot matrix for all participants that completed the 4-week cough diary, with daily total cough counts displayed on the x-axis and daily cough VAS scores displayed on the y-axis.*

Correlation of change in Patient Reported Outcome Measures with Cough Metrics

Change in Total Cough Count

There was not a significant correlation between change in total cough count and change in HARQ (Pearson's = 0.373, p=0.073), LCQ total (Pearsons =-.314, p=0.155), LCQ Physical (Pearsons=-0.370, p=0.090), LCQ Psychological (Pearson's=-0.385, p=0.077), LCQ Social (Pearsons =-0.075, p=0.741), SGRQ Total (Pearsons =.339, p=0.133), or SGRQ Symptom Domain (Pearson's = 0.346, p=0.125).

Change in Coughs per Hour

There was not a significant correlation between change in coughs/hr and change in SGRQ Symptom domain (Pearsons = 0.311, p=0.170), SGRQ total (Pearsons =0.341, p=0.131), HARQ (Pearson's = 0.368, p=0.077), LCQ total (Pearson's = -0.357, p=0.102), LCQ Psychological domain (Pearsons = -0.421, p=0.051), LCQ Social domain (Pearson's = -0.102, p=0.652). There was a minimally significant correlation with LCQ Physical domain (Pearson's = -0.425, p=0.048).

Change in Cough Density

There was not a significant correlation between the change in cough density and change in SGRQ total score (Pearson's =-0.048, p=0.835), SGRQ Symptom domain (Pearsons = -0.198, p=0.390), LCQ total score (Pearson's = -0.119, p=0.597), LCQ Physical domain (Pearson's = -0.007, p=0.976), LCQ Psychological domain (Pearson's = -0.120, p=0.595), LCQ Social domain (Pearson's = -0.177, p=0.431), or HARQ (Pearson's = 0.058, p=0.786).

Change in Proportion of Time Relieved of Cough

There was not a significant correlation between change in total cough count and change in HARQ (Pearson's = -0.142, p=0.508), SGRQ total score (Pearson's =-0.228, p=0.320), SGRQ Symptom domain (Pearsons = -0.138, p=0.549), LCQ total

score (Pearson's = 0.284, p=0.200), LCQ Physical domain (Pearson's = 0.412, p=0.056), LCQ Psychological domain (Pearson's = 0.275, p=0.216), LCQ Social domain (Pearson's = 0.082, p=0.718).

Variability in daily cough counts using continuous cough monitoring

We analysed the daily variability of cough counts for the 18 participants who had complete cough monitoring data, i.e. had been continuously monitored for 24 hours per day for the duration of the study. This was done by calculating the average of all daily cough counts for an individual and then subtracting each day's cough count from the average. The average of all of the differences is the average absolute deviation.

The calculated mean of the average absolute deviation between each day's cough frequency and the overall average daily frequency for all participants was 2 coughs/hour (SD = 1.3). The mean of the average relative AAD (AAD/mean) for all participants was 35.1% (SD = 13.2). Assuming the average cough frequency best estimates the cough burden of a patient during the measurement period, the AAD shows that any given single-day measurement is liable of an average error of 2 coughs/hour or 35.1%. Average AD and average relative AD for all participants in the study can be seen in Table 12.

Participant number	Average AD	Average Relative AD (%)
Participant 27	6.094	23.4
Participant 22	3.116	41.4
Participant 2	3.044	57.2
Participant 17	2.719	20.7
Participant 29	2.305	30.5
Participant 28	2.145	27.4
Participant 5	2.114	42.2
Participant 19	1.958	41.3
Participant 13	1.929	42.5
Participant 18	1.690	31.9
Participant 11	1.590	65.6
Participant 6	1.261	20.2
Participant 3	1.083	18.4
Participant 7	1.040	43.1
Participant 8	0.916	27.9
Participant 15	0.456	24.3
Participant 14	0.332	38.1

Table 13. *Average absolute deviation and average relative absolute deviation (AAD/mean) of cough counts for each participant.*

5.4 Discussion

We have shown in this open-label, single-arm exploratory study that treatment with azithromycin 250mg once daily leads to a significant reduction in a broad range of both objective and subjective measures of cough. This study included patients with a variety of chronic respiratory diseases, including asthma, COPD, chronic cough, and interstitial lung disease. As azithromycin has previously been shown to reduce exacerbation frequency across the spectrum of respiratory disease, we believe that it's use in treating respiratory symptoms, especially chronic cough, may also be beneficial for a wide range of patients with chronic respiratory conditions.

The studies performed in *Chapter 4 and Chapter 5* serve as initial proof-of-concept data that there is a signal of effect of azithromycin in patients with chronic respiratory disease and oesophageal dysmotility. This warrants further investigation with a large phase 3 randomised-controlled trial. In this cohort of patients, selected because of their chronic cough, over 50% demonstrated oesophageal dysmotility on HROM testing which reinforces the data shown in *Chapter 2* that this is a common co-existing phenomenon. Although most patients showed improvements in objective cough counts and a numerical increase in mean oesophageal contractility that was not statistically significant, this study was not sufficiently powered to establish a relationship between improvement in gastrointestinal motility and reduction in cough counts. There was, as reported in *Chapter 4*, a significant relationship found between oesophageal motility and symptoms of airway reflux. Evidenced by the significant Pearson's correlation between the mean change in DCI and mean change in HARQ scores in patients (figure 18), this study shows that azithromycin may at least improve the symptoms of reflux in patients with chronic respiratory disease. This is reinforced by the analysis of the retrospective data collected in *Chapter 2*, which demonstrated those with oesophageal dysmotility returned a significantly higher mean HARQ score and there was a significant Pearson's correlation between DCI values and HARQ scores (figure 7). Taken together, it is reasonable to suggest that

the HARQ is a sensitive tool for detecting reflux-related respiratory symptoms and that azithromycin may be a viable treatment option for such patients. We believe, however, that a definitive randomised controlled trial assessing the effect of azithromycin on both cough and oesophageal motility, and the relationship between the two, is needed.

There is little doubt that the cohort of patients in this study possessed elements of cough hypersensitivity. This is evidenced by the fact that all participants had a HARQ score above the upper limit of normal (i.e. >14). Moreover, with a mean baseline HARQ score of 41.3 this population possessed a similar average score to the global COUGH-1 and COUGH-2 trials, consisting of over 2000 patients with UCC or RCC, which reported a mean HARQ of 39.7[114]. Previous data have shown that raised HARQ scores are associated with increased cough reflex sensitivity in patients with both asthma and chronic cough [332]. Further evidence to support the HARQ as a surrogate marker for cough hypersensitivity is the significant improvements in HARQ scores that have been demonstrated with neuromodulating medications such as gabapentin, pregabalin, and baclofen[333, 334]. The results of this study indicate that azithromycin may also reduce cough reflex hypersensitivity as there was a significant reduction in mean HARQ score at follow-up when compared to pre-treatment baseline (41.3 vs 29.9, $p<0.001$), moreover there were numerical reductions in HARQ scores in 85% (n=22) of patients. It is worth noting that to more rigorously test the hypothesis of azithromycin being a treatment for airway reflux-induced CHS, a definitive trial would have to include mechanistic sub-studies. An assessment of cough reflex sensitivity would be the gold standard of assessing the physiological impact of azithromycin in CHS.

Inhalation challenge testing, using capsaicin, citric acid, and ATP are widely used in small proof-of-concept mechanistic studies to demonstrate the physiological mechanism of novel therapeutics for chronic cough[335]. Studies of actively sensitised guinea pigs demonstrated a significant reduction in cough reflex

sensitivity and number of coughs elicited by an inhaled capsaicin challenge test, when treated with both erythromycin and azithromycin[336]. However, it has been debated whether such experiments testing cough reflex sensitivity and respiratory neuropathology in animal models translate to clinical studies in human subjects[337]. To investigate cough reflex sensitivity in a potential definitive trial, a select subgroup of consenting patients (in both the treatment and placebo groups) would undertake a tussive inhalation challenge test at pre-treatment baseline and post-treatment follow-up. The traditional, and most reported, endpoint of this experiment would be the concentration of inhaled capsaicin required to elicit 2 coughs and/or 5 coughs, usually expressed as C_2 and C_5, respectively[338]. More recently, such endpoints have been called into question, with some suggesting that they are less likely to be able to discriminate between health and disease compared to recording the total number of coughs elicited by a single concentration of capsaicin (or any other tussive stimuli)[339, 340]. Regardless of the specific endpoint used, a measurement of cough reflex sensitivity using tussive challenges is required in a future trial to corroborate the findings of the HARQ scores in this study.

Treatment with azithromycin significantly improved scores on all PROMs, with the exception of the BCSS, in this trial. The findings of the meta-analyses performed in *Chapter 3* demonstrate the inconsistent data on the effect of azithromycin on subjective assessments of cough severity and cough-specific quality of life. Figure 10a shows that when both RCTs and non-comparative trials were collated, there was a significant improvement in LCQ scores. Around two-thirds of patients in this study met the MCID for improvement in both the total LCQ score and the LCQ physical domain. Furthermore, our data showed a correlation between improvement in LCQ scores and reduction in objective cough frequency. Despite this finding being of borderline significance it is consistent with previous studies investigating the relationship between these two measures [71, 116]. Despite improvements being observed across the board of PROMs, it is imperative that a future definitive trial, as with all clinical trials of anti-tussive therapeutics, select

primary and secondary endpoints that are representative of the patient experience.

Findings from *Nguyen et al.* demonstrate a significant association between improvements in cough severity VAS and objective cough frequency, measured at only two discrete time points[107]. Our study clearly shows that, when measured daily, cough severity VAS does not significantly correlate with daily cough frequency. If we assume a critical Pearson correlation co-efficient for n=18 would be 0.444 at the 0.05 significance level, then only 3 participants demonstrated a significant moderate correlation between objective daily cough frequency and daily VAS scores. Moreover, despite the fact there were significant reductions in total cough frequency and mean cough VAS scores in the total cohort of this study, there was no significant correlation found between the change in cough VAS and change in cough frequency at the individual participant level. This, combined with the cough severity VAS modest test-retest reliability, calls into question the validity of cough severity VAS measurement as a tool to sensitively detect improvement in cough. Despite it being a simple and easy-to-use PROM, it may lack sufficient depth of assessment to sensitively detect and understand the change in disease experience in response to pharmacological therapy. Despite the ability of the HARQ to assess cough reflex sensitivity and the LCQ to detect the overall impact of a patient's cough on their quality of life, there have been limited tools to assess cough severity. In recent times, indeed published after the completion of this trial, there has been an attempt to address this unmet need for a sufficiently detailed cough severity PROM. *Kum et al.* developed the McMaster Cough Severity Questionnaire (MCSQ), an 8-item, one-week recall questionnaire that consists of 7-point Likert scale responses addressing the two domains of frequency and intensity[111]. Whilst it has not yet been studied concomitantly with objective cough frequency, it may better correlate with changes in cough counts compared with QoL questionnaires, because it focuses more on the characteristics of a patient's cough rather than it's impacts. In a large randomised controlled trial of azithromycin for patients with chronic cough, it would be important to include the MCSQ as a formal assessment. By also observing its

correlation with objective cough counts and traditional PROMs, we could gain a more comprehensive understanding of treatment response.

For many years, 24-hour cough frequency, expressed as total coughs or coughs/hour, at distinct time points within a trial has been the 'gold standard' for objectively assessing cough in patients with respiratory disease. This method has been used in the majority large-scale clinical trials of novel antitussive medications.[81, 341] However, with the development of continuous cough monitors, such as the *Hyfe Cough Monitor* used in this study, we now have the ability to measure cough frequency for prolonged periods.[95, 101] We believe that this study was the first to use a wearable continuous cough monitor to measure response to a pharmaceutical treatment over an extended period. One previous study used smartphone-based applications to monitor treatment responses to anti-tuberculosis therapy over a 2-week period[342]. Although this study reported a median daily recording time of 23.9 hours, the absence of a wearable design for the recording device raises doubts about the accuracy of the cough recording data. Since participants in our study could easily wear the cough monitor on their wrist, minimising the time spent outside the device's recording range, the cough counts are more likely to be representative of the true cough frequency.

Continuous cough monitors have demonstrated that there is significant intra-subject day-to-day and within-day variability of cough frequency.[328, 343] Although it was not a prespecified endpoint in our trial, we have confirmed, as others have previously shown, a wide day-to-day variability of cough frequency as revealed by continuous cough monitoring. Using a cohort of patients with a variety of chronic respiratory disease, a different device and methodology of cough monitoring, we have demonstrated a strikingly similar result of day-to-day variation in cough counts. Our findings of a 35% daily variability in average cough frequency are comparable to the 29% seen in *Morice et al*, for which the same method of calculating the average absolute deviation in cough frequency for each patient was used. Such variability calls into question the utility of single-day recordings as a reliable indicator of true cough frequency. The consequences of

this are that a study using this method to measure response to antitussive therapy is inherently subject to type 2 statistical error. There have been several promising investigational medicinal products that have 'failed' in phase 2 and 3 studies since their primary endpoint was 24-hour or awake cough frequency. Thus, we suggest that continuous cough monitoring over a prolonged period of time should be the gold standard tool for clinical trials. The situation is analogous to the development of anti-hypertensive medications where single recordings of blood pressure require large numbers of patients to demonstrate effect, whereas in clinical practice, repeated measurements are recommended in diagnosis of hypertension and monitoring of treatment.

In this study we performed novel analysis of cough counts, including measuring the 'cough density' and the proportion of time that participants had 'relief-of-cough'. Both such methods of analysis were developed with a patient-led approach, as many patients often report their cough as an episodic phenomenon. Such episodes of coughs have been given a litany of different names, including fits, paroxysms, attacks, and epochs[344]. However, the current literature has settled on 'bouts' as the accepted nomenclature[345, 346]. Previous studies have suggested very short periods of time to describe the duration of a bout. In a trial examining the effect of lesogaberan on chronic cough, *Badri et al.* defined a 'bout' as continuous coughing without a 2 second pause, after which one bout would end and a cough occurring more than 2 seconds afterwards would belong to a distinct bout[74]. Such a definition has also been used in a study of acute cough in healthy patients with an upper respiratory tract infection[85]. More recent analysis of cough bouts has suggested that the cough-free period should be extended to 3 seconds. In an analysis of 47 patients with chronic cough, *Dockry et al.* found that defining a 'bout' as continuous coughing with no more than 3 second pause between sequential coughs had the strongest correlation with cough VAS scores[347]. The findings of this study have two fundamental flaws, firstly they do not present data for bouts with a window of longer than 3 seconds between coughs. Secondly, the authors defined accuracy of the definition of a bout by performing retrospective correlation between the time window (i.e. 1

second, 2 seconds etc.) and cough severity VAS scores. As has been discussed extensively in this thesis, cough severity does not adequately reflect patient experience in chronic cough.

A similar method of analysis was performed by *Holt et al.,* where number of cough bouts, cough-free time, and cough bout length were all investigated through correlations with cough severity VAS measurements[345]. The authors found that counting the number of cough bouts in 24 hours, using inter-cough periods of less than 3 seconds, correlated strongly with cough severity VAS. They also found that removal of single-cough bouts resulted in stronger correlations with the VAS scores. This method of measuring cough bouts as short-lived phenomena may be applicable to some patients, others may have a different experience. The method of analysis developed by *Morice et al.*, and the one utilised in this study, approaches the definition of bouts differently. The relief of cough metric takes into account that many patients will have prolonged, intense bouts of coughing where they will cough many times in a period of 10-15 minutes[348]. These bouts are likely to be much more troubling to patients than a bout of 2-3 coughs which would, using the definition suggested by *Holt et al.*, constitute a bout.

Using 24-hour cough count data from the CANAL phase 2a trial investigating the effect of the kappa-opioid receptor nalbuphine on cough in IPF, both discussed methods of cough bout analysis have been applied to the same population. using short inter-cough interval windows of 1 second to 10 seconds, *Smith et al.* demonstrated a significant reduction in cough bouts with nalbuphine when compared to placebo, regardless of the time interval used[349]. Similar results were presented when using relief of cough analysis as *Morice et al.* demonstrated a significant improvement in relief-of-cough time and cough intensity (the same metric in our study was named as cough density)[350]. The relief of cough metric has also been used to reanalyse previously negative data from the VOLCANO-2 trial of orvepitant in chronic cough[351]. The original analysis of this trial found no significant reduction in awake cough frequency; however, the re-analysis found a significant reduction in cough density and increase in relief-of-cough

time[348]. Such results suggest that both methodologies of quantifying cough bouts have merit and require further investigation. In a future, definitive, large-scale clinical trial we would look to utilise both methods of analysis and correlate them with existing PROMs as well as the new MCSQ, to see if either more accurately reflects patients' experiences. It would also be paramount that an accompanying qualitative sub-study be performed to gather a more granular outlook on how changes in cough bouts affect patients.

There are, of course, limitations to this study. The lack of randomisation, blinding, or a control group in this open-label, single-arm study does not allow us to definitively report the efficacy of azithromycin for cough, as the reduction in cough frequency could be regression to the mean or a placebo response, which is known to be significant in studies of chronic cough[352]. The small sample size of participants in this study also impedes our ability to reliably correlate objective and subjective measures of cough. Although patients were blinded from their live cough count data, researchers had access to the *Hyfe cough monitor* dashboard which may introduce researcher bias if they had intentionally or unintentionally informed patients of their cough patterns. There was also variation in the adherence to cough monitoring as technological issues, such as local data signal and watch battery life, stifled our ability to continuously monitor all participants for the entirety of the 5 weeks that they participated in the trial.

5.5 Conclusions

In this non-controlled study, we have demonstrated a significant reduction in objective and subjective cough, using both established and novel metrics of cough analysis, with daily azithromycin treatment in patients with chronic respiratory disease. We believe this is the first study to utilise continuous cough monitoring with a wearable device to assess the changes in cough frequency with a pharmacological agent. A future large-scale randomised controlled trial is necessary to definitively test the efficacy of azithromycin as an antitussive agent in this patient population.

Concluding Remarks

In this thesis I have comprehensively assessed the role of oesophageal dysmotility as a treatable trait for cough in chronic respiratory disease. I have elucidated the gut-lung axis as a potential aetiological contributor to refractory chronic cough and presented evidence to justify further investigating azithromycin. This cheap, safe, and effective medication may work as a prokinetic agent to improve symptom burden in such patients. Through the robust methodology of systematic review and meta-analysis we have established the benefit of azithromycin for cough-related quality of life across various respiratory conditions. This study identified a relative paucity of data on the effect of azithromycin on objective cough frequency, with only two small-scale studies having investigated this. To address this chasm in the literature, we performed the first clinical trial to utilise wearable continuous cough monitoring to evaluate changes in objective cough frequency following pharmacological intervention. Additionally, using the established methodology of a feasibility study, we demonstrated the potential for conducting a definitive clinical trial examining the relationship between oesophageal function and chronic cough, and the influence of azithromycin on both. Our findings suggest that investigating patients with mildly invasive procedures such as HROM, in addition to continuous cough monitoring, was feasible and acceptable to patients.

A future large-scale clinical trial to assess the hypothesis presented in this thesis would be ideally powered to detect a clinically meaningful difference in both reduction in cough count and improvement in oesophageal function with long-term azithromycin therapy. I believe that this would best performed using a parallel-arm, placebo-controlled randomised control trial. This study may benefit from 3-month course azithromycin treatment in order to assess the whether its effects are sustained over a prolonged period of time. The primary endpoint of such a trial would likely remain reduction in cough frequency, however, the secondary endpoints would be vital in ascertaining the effect of oesophageal

function as well as the effect of azithromycin on the novel cough metrics described in this thesis.

Despite the work in this furthering our understanding, there are many unanswered questions surrounding the gut-lung axis in chronic respiratory disease. We have discussed the role of acidic GORD in the development and progression of diseases such as IPF, but it remains unclear whether it is the acid or the non-acidic gaseous refluxate responsible. Furthermore, we still do not have definitive evidence of the mechanism of injury that contributes to respiratory pathology. For example, it may be direct contact with stomach acid that leads to epithelial injury in the large and small airways, engendering eosinophilic and/or neutrophilic inflammation. An alternative hypothesis is that of airway reflux, where it has been suggested that prolonged exposure to gaseous, non-acid reflux may cause localised neuronal injury to the vagal afferents in the upper and lower airways. This damage results in hypersensitivity of the vagal afferents, reducing the stimulus required to invoke the cough reflex. Airway reflux-induced cough hypersensitivity has previously only been referred to as a cause of the refractory or unexplained chronic cough. However, increased cough reflex sensitivity, caused by airway reflux, in those with underlying respiratory diseases such as asthma, COPD, or ILD may exacerbate or perpetuate their condition. Thus, in further work it is crucial to perform mechanistic sub-studies to better understand the effects of both acid and non-acid refluxate on pulmonary epithelial and neuronal cells. A deeper understanding of this pathophysiology is imperative for clinical practice, as it may allow us to develop relevant clinical and biochemical biomarkers. Such biomarkers could enable us to better select patients who may benefit from therapies such as azithromycin.

The field of objective cough measurement has progressed rapidly in the time since the conception of this thesis. The clinical trial conducted in this thesis ventured into the uncharted territory of continuous cough monitoring. Although not the primary aim of this work, we have demonstrated not only that day-to-day cough frequency has significant variability but also that there is very little correlation between daily objective counts and subjective cough assessment. As the world of

chronic cough still yearns for the perfect patient-centred clinical endpoint, we have conducted novel analysis of cough counts, including relief of cough and cough density. Such metrics undoubtedly require further development and investigation. Nonetheless, these metrics, which are designed using the patient's perspective, are vital if we are to prove the efficacy of novel antitussive agents.

In summary, this thesis has employed a broad range of methodologies to demonstrate the prevalence, mechanisms, and clinical features of oesophageal dysmotility in patients with chronic respiratory disease and has identified azithromycin as a potential treatment for this patient cohort. The feasibility and preliminary clinical findings from the clinical trial conducted as part of this thesis provide a mandate to perform a large-scale randomised controlled trial investigating the effects of azithromycin on cough and oesophageal function in such patients.

References

1. Statistics OFN. Monthly mortality analysis, England and Wales: February 2023 2023 [Available from: https://www.ons.gov.uk/peoplepopulationandcommunity/birthsdeathsandmarriages/deaths/bulletins/monthlymortalityanalysisenglandandwales/february2023#leading-causes-of-death.

2. Johnson MJ, Currow DC, Booth S. Prevalence and assessment of breathlessness in the clinical setting. Expert review of respiratory medicine. 2014;8(2):151-61.

3. Bergmann M, Haasenritter J, Beidatsch D, Schwarm S, Hörner K, Bösner S, et al. Prevalence, aetiologies and prognosis of the symptom cough in primary care: a systematic review and meta-analysis. BMC Family Practice. 2021;22:1-19.

4. Naser AY, Mansour MM, Alanazi AF, Sabha O, Alwafi H, Jalal Z, et al. Hospital admission trends due to respiratory diseases in England and Wales between 1999 and 2019: an ecologic study. BMC Pulmonary Medicine. 2021;21:1-10.

5. Hubbard R. The burden of lung disease. BMJ Publishing Group Ltd; 2006. p. 557-8.

6. Bausewein C, Booth S, Gysels M, Kühnbach R, Haberland B, Higginson IJ. Understanding breathlessness: cross-sectional comparison of symptom burden and palliative care needs in chronic obstructive pulmonary disease and cancer. Journal of palliative medicine. 2010;13(9):1109-18.

7. Dicpinigaitis PV. Prevalence of stress urinary incontinence in women presenting for evaluation of chronic cough. ERJ Open Research. 2021;7(1).

8. Ruhl CE, Everhart JE. Risk factors for inguinal hernia among adults in the US population. American journal of epidemiology. 2007;165(10):1154-61.

9. Irwin RS, Dudiki N, French CL, Altman KW, Azoulay E, Barker AF, et al. Life-threatening and non-life-threatening complications associated with coughing: a scoping review. Chest. 2020;158(5):2058-73.

10. Bone AE, Hepgul N, Kon S, Maddocks M. Sarcopenia and frailty in chronic respiratory disease: Lessons from gerontology. Chronic respiratory disease. 2017;14(1):85-99.

11. Jørgensen NR, Schwarz P, Holme I, Henriksen B, Petersen L, Backer V. The prevalence of osteoporosis in patients with chronic obstructive pulmonary disease—a cross sectional study. Respiratory medicine. 2007;101(1):177-85.

12. de Theije C, Costes F, Langen RC, Pison C, Gosker HR. Hypoxia and muscle maintenance regulation: implications for chronic respiratory disease. Current Opinion in Clinical Nutrition & Metabolic Care. 2011;14(6):548-53.

13. Marengoni A, Vetrano DL, Manes-Gravina E, Bernabei R, Onder G, Palmer K. The relationship between COPD and frailty: a systematic review and meta-analysis of observational studies. Chest. 2018;154(1):21-40.

14. Milne KM, Kwan JM, Guler S, Winstone TA, Le A, Khalil N, et al. Frailty is common and strongly associated with dyspnoea severity in fibrotic interstitial lung disease. Respirology. 2017;22(4):728-34.

15. Sheth JS, Xia M, Murray S, Martinez CH, Meldrum CA, Belloli EA, et al. Frailty and geriatric conditions in older patients with idiopathic pulmonary fibrosis. Respiratory medicine. 2019;148:6-12.

16. McGarvey L, Carton C, Gamble LA, Heaney LG, Shepherd R, Ennis M, et al. Prevalence of psychomorbidity among patients with chronic cough. Cough. 2006;2(1):1-6.

17. Ho SF, O'Mahony MS, Steward JA, Breay P, Buchalter M, Burr ML. Dyspnoea and quality of life in older people at home. Age and ageing. 2001;30(2):155-9.

18. Young EC, Smith JA. Quality of life in patients with chronic cough. Therapeutic advances in respiratory disease. 2010;4(1):49-55.

19. Hutchinson A, Barclay-Klingle N, Galvin K, Johnson MJ. Living with breathlessness: a systematic literature review and qualitative synthesis. European Respiratory Journal. 2018;51(2).

20. Omachi TA, Katz PP, Yelin EH, Gregorich SE, Iribarren C, Blanc PD, et al. Depression and health-related quality of life in chronic obstructive pulmonary disease. The American journal of medicine. 2009;122(8):778. e9-. e15.

21. Cox IA, Arriagada NB, de Graaff B, Corte TJ, Glaspole I, Lartey S, et al. Health-related quality of life of patients with idiopathic pulmonary fibrosis: a systematic review and meta-analysis. European Respiratory Review. 2020;29(158).

22. Stanescu S, Kirby SE, Thomas M, Yardley L, Ainsworth B. A systematic review of psychological, physical health factors, and quality of life in adult asthma. NPJ primary care respiratory medicine. 2019;29(1):37.

23. Ortega HG, Liu MC, Pavord ID, Brusselle GG, FitzGerald JM, Chetta A, et al. Mepolizumab treatment in patients with severe eosinophilic asthma. New England Journal of Medicine. 2014;371(13):1198-207.

24. Serisier DJ, Martin ML, McGuckin MA, Lourie R, Chen AC, Brain B, et al. Effect of Long-term, Low-Dose Erythromycin on Pulmonary Exacerbations Among Patients With Non-Cystic Fibrosis Bronchiectasis The BLESS Randomized Controlled Trial. Jama-Journal of the American Medical Association. 2013;309(12):1260-7.

25. Lipson DA, Barnhart F, Brealey N, Brooks J, Criner GJ, Day NC, et al. Once-daily single-inhaler triple versus dual therapy in patients with COPD. New England Journal of Medicine. 2018;378(18):1671-80.

26. Richeldi L, Du Bois RM, Raghu G, Azuma A, Brown KK, Costabel U, et al. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. New England Journal of Medicine. 2014;370(22):2071-82.

27. Morice A, Fontana G, Belvisi M, Birring S, Chung K, Dicpinigaitis PV, et al. ERS guidelines on the assessment of cough. European respiratory journal. 2007;29(6):1256-76.

28. Faruqi S, Thompson R, Wright C, Sheedy W, Morice AH. Quantifying chronic cough: objective versus subjective measurements. Respirology. 2011;16(2):314-20.

29. Qiu Z, Yu L, Xu S, Liu B, Zhao T, Lue H, et al. Cough reflex sensitivity and airway inflammation in patients with chronic cough due to non‐acid gastro‐oesophageal reflux. Respirology. 2011;16(4):645-52.

30. Havemann BD, Henderson CA, El-Serag HB. The association between gastro-oesophageal reflux disease and asthma: a systematic review. Gut. 2007;56(12):1654-64.

31. Raghu G, Pellegrini CA, Yow E, Flaherty KR, Meyer K, Noth I, et al. Laparoscopic anti-reflux surgery for the treatment of idiopathic pulmonary fibrosis (WRAP-IPF): a multicentre, randomised, controlled phase 2 trial. The Lancet Respiratory Medicine. 2018;6(9):707-14.

32. Faruqi S, Molyneux ID, Fathi H, Wright C, Thompson R, Morice AH. Chronic cough and esomeprazole: A double‐blind placebo‐controlled parallel study. Respirology. 2011;16(7):1150-6.

33. Chan WW, Chiou E, Obstein KL, Tignor AS, Whitlock TL. The efficacy of proton pump inhibitors for the treatment of asthma in adults: a meta-analysis. Archives of Internal Medicine. 2011;171(7):620-9.

34. Abrahamsson T, Jakobsson H, Andersson AF, Björkstén B, Engstrand L, Jenmalm M. Low gut microbiota diversity in early infancy precedes asthma at school age. Clinical & Experimental Allergy. 2014;44(6):842-50.

35. Russell SL, Gold MJ, Hartmann M, Willing BP, Thorson L, Wlodarska M, et al. Early life antibiotic‐driven changes in microbiota enhance susceptibility to allergic asthma. EMBO reports. 2012;13(5):440-7.

36. Morice AH. Airway reflux as a cause of respiratory disease. Breathe. 2013;9(4):256-66.

37. Bloom CI, Saglani S, Feary J, Jarvis D, Quint JK. Changing prevalence of current asthma and inhaled corticosteroid treatment in the UK: population-based cohort 2006–2016. European Respiratory Journal. 2019;53(4).

38. Louis R, Satia I, Ojanguren I, Schleich F, Bonini M, Tonia T, et al. European Respiratory Society guidelines for the diagnosis of asthma in adults. European Respiratory Journal. 2022;60(3).

39. Excellence NIfHaC. Asthma: diagnosis, monitoring and chronic asthma management (BTS, NICE, SIGN) 2024 [Available from: https://www.nice.org.uk/guidance/ng245.

40. Agustí A, Celli BR, Criner GJ, Halpin D, Anzueto A, Barnes P, et al. Global Initiative for Chronic Obstructive Lung Disease 2023 Report: GOLD Executive Summary. American Journal of Respiratory and Critical Care Medicine. 2023;207(7):819-37.

41. Morice AH, Millqvist E, Bieksiene K, Birring SS, Dicpinigaitis P, Ribas CD, et al. ERS guidelines on the diagnosis and treatment of chronic cough in adults and children. European Respiratory Journal. 2020;55(1).

42. Song W-J, Morice AH. Cough hypersensitivity syndrome: a few more steps forward. Allergy, asthma & immunology research. 2017;9(5):394-402.

43. Chung KF, McGarvey L, Mazzone S. Chronic cough and cough hypersensitivity syndrome. The Lancet Respiratory Medicine. 2016;4(12):934-5.

44. Wijsenbeek M, Cottin V. Spectrum of fibrotic lung diseases. New England Journal of Medicine. 2020;383(10):958-68.

45. Wijsenbeek M, Suzuki A, Maher TM. Interstitial lung diseases. The Lancet. 2022;400(10354):769-86.

46. Costabel U, Miyazaki Y, Pardo A, Koschel D, Bonella F, Spagnolo P, et al. Hypersensitivity pneumonitis. Nature Reviews Disease Primers. 2020;6(1):65.

47. Minagawa S, Araya J, Numata T, Nojiri S, Hara H, Yumino Y, et al. Accelerated epithelial cell senescence in IPF and the inhibitory role of SIRT6 in TGF-β-induced senescence of human bronchial epithelial cells. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2011;300(3):L391-L401.

48. Strongman H, Kausar I, Maher TM. Incidence, prevalence, and survival of patients with idiopathic pulmonary fibrosis in the UK. Advances in therapy. 2018;35:724-36.

49. Chung JH, Chawla A, Peljto AL, Cool CD, Groshong SD, Talbert JL, et al. CT scan findings of probable usual interstitial pneumonitis have a high predictive value for histologic usual interstitial pneumonitis. Chest. 2015;147(2):450-9.

50. Zemp E, Elsasser S, Schindler C, Kunzli N, Perruchoud AP, Domenighetti G, et al. Long-term ambient air pollution and respiratory symptoms in adults (SAPALDIA study). American journal of respiratory and critical care medicine. 1999;159(4):1257-66.

51. Janson C, Chinn S, Jarvis D, Burney P. Determinants of cough in young adults participating in the European Community Respiratory Health Survey. European Respiratory Journal. 2001;18(4):647-54.

52. Arinze JT, de Roos EW, Karimi L, Verhamme KM, Stricker BH, Brusselle GG. Prevalence and incidence of, and risk factors for chronic cough in the adult population: the Rotterdam Study. ERJ open research. 2020;6(2).

53. Zhang Q, Qiu M, Lai K, Zhong N. Cough and environmental air pollution in China. Pulmonary pharmacology & therapeutics. 2015;35:132-6.

54. Habre R, Moshier E, Castro W, Nath A, Grunin A, Rohr A, et al. The effects of PM2. 5 and its components from indoor and outdoor sources on cough and wheeze symptoms in asthmatic children. Journal of exposure science & environmental epidemiology. 2014;24(4):380-7.

55. Bentayeb M, Norback D, Bednarek M, Bernard A, Cai G, Cerrai S, et al. Indoor air quality, ventilation and respiratory health in elderly residents living in nursing homes in Europe. European Respiratory Journal. 2015;45(5):1228-38.

56. Sun Y, Hou J, Cheng R, Sheng Y, Zhang X, Sundell J. Indoor air quality, ventilation and their associations with sick building syndrome in Chinese homes. Energy and Buildings. 2019;197:112-9.

57. Koren HS, Graham DE, Devlin RB. Exposure of humans to a volatile organic mixture. III. Inflammatory response. Archives of Environmental Health: An International Journal. 1992;47(1):39-44.

58. Osman LM, McKenzie L, Cairns J, Friend J, Godden DJ, Legge J, et al. Patient weighting of importance of asthma symptoms. Thorax. 2001;56(2):138-42.

59. Lee HY, Lee Y, Lee J-H, Lee S-E, Sim DW, Kang N, et al. Association of Cough Severity with Asthma Control and Quality of Life in Patients with Severe Asthma. Lung. 2024:1-10.

60. Hughes R, Rapsomaniki E, Janson C, Keen C, Make BJ, Burgel P-R, et al. Frequent productive cough: symptom burden and future exacerbation risk among patients with asthma and/or COPD in the NOVELTY study. Respiratory medicine. 2022;200:106921.

61. Burgel P-R, Nesme-Meyer P, Chanez P, Caillaud D, Carré P, Perez T, et al. Cough and sputum production are associated with frequent exacerbations and hospitalizations in COPD subjects. Chest. 2009;135(4):975-82.

62. Landt E, Çolak Y, Lange P, Laursen LC, Nordestgaard BG, Dahl M. Chronic Cough in Individuals With COPD: A Population-Based Cohort Study. Chest. 2020;157(6):1446-54.

63. Lindberg A, Sawalha S, Hedman L, Larsson L-G, Lundbäck B, Rönmark E. Subjects with COPD and productive cough have an increased risk for exacerbations and death. Respiratory Medicine. 2015;109(1):88-95.

64. Hall JI, Lozano M, Estrada-Petrocelli L, Birring S, Turner R. The present and future of cough counting tools. Journal of thoracic disease. 2020;12(9):5207.

65. Zhang M, Sykes DL, Brindle K, Sadofsky LR, Morice AH. Chronic cough—the limitation and advances in assessment techniques. Journal of Thoracic Disease. 2022;14(12):5097.

66. Wang Z, Wang M, Wen S, Yu L, Xu X. Types and applications of cough-related questionnaires. Journal of Thoracic Disease. 2019;11(10):4379.

67. Mai Y, Fang L, Zhong S, de Silva SDSH, Chen R, Lai K. Methods for assessing cough sensitivity. Journal of Thoracic Disease. 2020;12(9):5224.

68. Fontana GA, Widdicombe J. What is cough and what should be measured? Pulmonary pharmacology & therapeutics. 2007;20(4):307-12.

69. Kelsall A, Decalmer S, Webster D, Brown N, McGuinness K, Woodcock A, et al. How to quantify coughing: correlations with quality of life in chronic cough. European Respiratory Journal. 2008;32(1):175-9.

70. Urban C, Kiefer A, Conradt R, Kabesch M, Lex C, Zacharasiewicz A, et al. Validation of the LEOSound® monitor for standardized detection of wheezing and cough in children. Pediatric pulmonology. 2022;57(2):551-9.

71. Decalmer SC, Webster D, Kelsall AA, McGuinness K, Woodcock AA, Smith JA. Chronic cough: how do cough reflex sensitivity and subjective assessments correlate with objective cough counts during ambulatory monitoring? Thorax. 2007;62(4):329-34.

72. Lee GO, Comina G, Hernandez-Cordova G, Naik N, Gayoso O, Ticona E, et al. Cough dynamics in adults receiving tuberculosis treatment. PloS one. 2020;15(6):e0231167.

73. Radine A, Werner C, Raidt J, Dougherty GW, Kerschke L, Omran H, et al. Comparison of nocturnal cough analysis in healthy subjects and in patients with cystic fibrosis and primary ciliary dyskinesia: a prospective observational study. Respiration. 2018;97(1):60-9.

74. Badri H, Gibbard C, Denton D, Satia I, Al-Sheklly B, Dockry RJ, et al. A double-blind randomised placebo-controlled trial investigating the effects of lesogaberan on the objective cough frequency and capsaicin-evoked coughs in patients with refractory chronic cough. ERJ Open Research. 2022;8(1).

75. Turner RD, Bothamley GH. How to count coughs? Counting by ear, the effect of visual data and the evaluation of an automated cough monitor. Respiratory medicine. 2014;108(12):1808-15.

76. Smith JA, Earis JE, Woodcock AA. Establishing a gold standard for manual cough counting: video versus digital audio recordings. Cough. 2006;2:1-6.

77. Pavesi L, Subburaj S, Porter-Shaw K. Application and validation of a computerized cough acquisition system for objective monitoring of acute cough: a meta-analysis. Chest. 2001;120(4):1121-8.

78. Smith JA, Holt K, Dockry R, Sen S, Sheppard K, Turner P, et al. Performance of a digital signal processing algorithm for the accurate quantification of cough frequency. European Respiratory Journal. 2021;58(2).

79. Barton A, Gaydecki P, Holt K, Smith JA. Data reduction for cough studies using distribution of audio frequency content. Cough. 2012;8:1-7.

80. McGuinness K, Holt K, Dockry R, Smith J. P159 Validation of the VitaloJAK™ 24 hour ambulatory cough monitor. Thorax. 2012;67(Suppl 2):A131-A.

81. McGarvey LP, Birring SS, Morice AH, Dicpinigaitis PV, Pavord ID, Schelfhout J, et al. Efficacy and safety of gefapixant, a P2X3 receptor antagonist, in refractory chronic cough and unexplained chronic cough (COUGH-1 and COUGH-2): results from two double-blind, randomised, parallel-group, placebo-controlled, phase 3 trials. The Lancet. 2022;399(10328):909-23.

82. Maher TM, Avram C, Bortey E, Hart SP, Hirani N, Molyneux PL, et al. Nalbuphine Tablets for Cough in Patients with Idiopathic Pulmonary Fibrosis. NEJM Evidence. 2023:EVIDoa2300083.

83. Birring S, Fleming T, Matos S, Raj A, Evans D, Pavord I. The Leicester Cough Monitor: preliminary validation of an automated cough detection system in chronic cough. European Respiratory Journal. 2008;31(5):1013-8.

84. Birring SS, Fleming T, Matos S, Raj AA, Evans DH, Pavord ID. Validation of the Leicester Cough Monitor (LCM): preliminary findings using an automated cough detection system in patients with chronic cough. European Respiratory Journal. 2008.

85. Lee KK, Matos S, Evans DH, White P, Pavord ID, Birring SS. A longitudinal assessment of acute cough. American journal of respiratory and critical care medicine. 2013;187(9):991-7.

86. Vertigan AE, Kapela SL, Gibson PG. Chronic cough in vocal cord dysfunction: description of a clinical entity. Respiratory medicine. 2020;168:105990.

87. Jackson AR, Hull JH, Hopker JG, Fletcher H, Gowers W, Birring SS, et al. The impact of a heat and moisture exchange mask on respiratory symptoms and airway response to exercise in asthma. ERJ Open Research. 2020;6(2).

88. Spinou A, Lee KK, Sinha A, Elston C, Loebinger MR, Wilson R, et al. The objective assessment of cough frequency in bronchiectasis. Lung. 2017;195:575-85.

89. Ryan NM, Birring SS, Gibson PG. Gabapentin for refractory chronic cough: a randomised, double-blind, placebo-controlled trial. The Lancet. 2012;380(9853):1583-9.

90. Vertigan AE, Kapela SL, Ryan NM, Birring SS, McElduff P, Gibson PG. Pregabalin and speech pathology combination therapy for refractory chronic cough: a randomized controlled trial. Chest. 2016;149(3):639-48.

91. Vertigan AE, Kapela SL, Birring SS, Gibson PG. Feasibility and clinical utility of ambulatory cough monitoring in an outpatient clinical setting: a real-world retrospective evaluation. ERJ Open Research. 2021;7(4).

92. Barry SJ, Dane AD, Morice AH, Walmsley AD. The automatic recognition and counting of cough. Cough. 2006;2:1-9.

93. Crooks MG, Hayman Y, Innes A, Williamson J, Wright CE, Morice AH. Objective measurement of cough frequency during COPD exacerbation convalescence. Lung. 2016;194:117-20.

94. Zhang M, Brindle K, Robinson M, Ingram D, Cavany T, Morice A. Chronic cough in cystic fibrosis: the effect of modulator therapy on objective 24-h cough monitoring. ERJ Open Research. 2022;8(2).

95. Chaccour C S-OI, Siegel S, Megson G, Winthrop KL, Berto-Botella J, de-Torres JP, Jover L, Brew J, Galvosas M, Rudd M, Small P. Validation of the Hyfe cough monitoring system: a multicenter clinical study. 2024.

96. Lee S-E, Rudd M, Kim T-H, Oh J-Y, Lee J-H, Jover L, et al. Feasibility and Utility of a Smartphone Application-Based Longitudinal Cough Monitoring in Chronic Cough Patients in a Real-World Setting. Lung. 2023;201(6):555-64.

97. Raberahona M, Zimmer A, Rakotoarivelo RA, Randrianarisoa PA, Rambeloson G, Rakotomijoro E, et al. Continuous digital cough monitoring during 6-month pulmonary tuberculosis treatment. ERJ Open Research. 2024.

98. Griffith D, Levin A, Rudd M, Small P, Daley C. Using Continuous Cough Monitoring to Assess Bronchiectasis Therapy. D25 EMERGING RESEARCH IN COUGH, CYSTIC FIBROSIS AND BRONCHIECTASIS. 2024:A7084-A.

99. Altshuler E, Tannir B, Jolicoeur G, Rudd M, Saleem C, Cherabuddi K, et al. Digital cough monitoring–A potential predictive acoustic biomarker of clinical outcomes in hospitalized COVID-19 patients. Journal of Biomedical Informatics. 2023;138:104283.

100. Abubakar-Waziri H, Edwards DA, Bhatta DB, Hull JH, Rudd M, Small P, et al. Inhaled alkaline hypertonic divalent salts reduce refractory chronic cough frequency. ERJ Open Research. 2024;10(5).

101. Kuhn M, Nalbant E, Kohlbrenner D, Alge M, Kuett L, Arvaji A, et al. Validation of a small cough detector. ERJ Open Research. 2023;9(1).

102. Boulet L-P, Coeytaux RR, McCrory DC, French CT, Chang AB, Birring SS, et al. Tools for assessing outcomes in studies of chronic cough: CHEST guideline and expert panel report. Chest. 2015;147(3):804-14.

103. Smith JA, Owen EC, Jones AM, Dodd ME, Webb AK, Woodcock A. Objective measurement of cough during pulmonary exacerbations in adults with cystic fibrosis. Thorax. 2006;61(5):425-9.

104. Yates H, Adamali HI, Maskell N, Barratt S, Sharp C. Visual analogue scales for interstitial lung disease: a prospective validation study. QJM: An International Journal of Medicine. 2018;111(8):531-9.

105. Judson MA, Chopra A, Conuel E, Koutroumpakis E, Schafer C, Austin A, et al. The assessment of cough in a sarcoidosis clinic using a validated instrument and a visual analog scale. Lung. 2017;195:587-94.

106. Brightling C, Monterio W, Green R, Parker D, Morgan M, Wardlaw A, et al. Induced sputum and other outcome measures in chronic obstructive pulmonary disease: safety and repeatability. Respiratory medicine. 2001;95(12):999-1002.

107. Martin Nguyen A, Bacci ED, Vernon M, Birring SS, Rosa CL, Muccino D, et al. Validation of a visual analog scale for assessing cough severity in patients with chronic cough. Therapeutic advances in respiratory disease. 2021;15:17534666211049743.

108. Baddini-Martinez J, Chinarelli T, Orlandini CB, de Oliveira MR, Bricoleri I, Cardamoni V, et al. The Multidimensional Cough Index: A Practical Scale to Measure Cough and Sputum. The American Journal of the Medical Sciences. 2021;362(4):396-402.

109. Hsu J, Stone R, Logan-Sinclair R, Worsdell M, Busst C, Chung K. Coughing frequency in patients with persistent cough: assessment using a 24 hour ambulatory recorder. European Respiratory Journal. 1994;7(7):1246-53.

110. Kwon J-W, Moon J-Y, Kim S-H, Song W-J, Kim M-H, Kang M-G, et al. Korean version of the Cough Symptom Score: clinical utility and validity for chronic cough. The Korean Journal of Internal Medicine. 2017;32(5):910.

111. Kum E, Guyatt GH, Abdulqawi R, Dicpinigaitis P, Dupont L, Field SK, et al. The McMaster Cough Severity Questionnaire (MCSQ): a cough severity instrument for patients with refractory chronic cough. European Respiratory Journal. 2024.

112. La-Crette J, Lee K, Chamberlain S, Saito J, Hull J, Chung K, et al. P150 The Development of a Cough Hypersensitivity Questionnaire (CHQ). Thorax. 2012;67(Suppl 2):A127-A.

113. Morice A, Faruqi S, Wright C, Thompson R, Bland J. Cough hypersensitivity syndrome: a distinct clinical entity. Lung. 2011;189(1):73-9.

114. Morice A, Birring S, Dicpinigaitis P, McGarvey L, Pavord I, Smith J, et al. Cough Triggers and Symptoms Among Patients with Refractory or Unexplained Chronic Cough in Two Phase 3 Trials of the P2X3 Receptor Antagonist Gefapixant (COUGH-1 and COUGH-2). Journal of Allergy and Clinical Immunology. 2021;147(2):AB61.

115. Birring SS, Prudon B, Carr AJ, Singh SJ, Morgan MDL, Pavord ID. Development of a symptom specific health status measure for patients with chronic cough: Leicester Cough Questionnaire (LCQ). Thorax. 2003;58(4):339-43.

116. Birring SS, Matos S, Patel RB, Prudon B, Evans DH, Pavord ID. Cough frequency, cough sensitivity and health status in patients with chronic cough. Respiratory medicine. 2006;100(6):1105-9.

117. Raj AA, Pavord DI, Birring SS. Clinical Cough IV:What is the Minimal Important Difference for the Leicester Cough Questionnaire? In: Chung KF, Widdicombe J, editors. Pharmacology and Therapeutics of Cough. Berlin, Heidelberg: Springer Berlin Heidelberg; 2009. p. 311-20.

118. Berkhof FF, Boom LN, ten Hertog NE, Uil SM, Kerstjens HAM, van den Berg JWK. The validity and precision of the Leicester Cough Questionnaire in COPD patients with chronic cough. Health and quality of life outcomes. 2012;10:4.

119. Fathi H, Moon T, Donaldson J, Jackson W, Sedman P, Morice AH. Cough in adult cystic fibrosis: diagnosis and response to fundoplication. Cough. 2009;5(1):1.

120. Fukuhara A, Saito J, Birring SS, Sato S, Uematsu M, Suzuki Y, et al. Clinical Characteristics of Cough Frequency Patterns in Patients with and without Asthma. The Journal of Allergy and Clinical Immunology: In Practice. 2020;8(2):654-61.

121. Fletcher KE, French CT, Irwin RS, Corapi KM, Norman GR. A prospective global measure, the Punum Ladder, provides more valid assessments of quality of life than a retrospective transition measure. Journal of Clinical Epidemiology. 2010;63(10):1123-31.

122. French CT, Irwin RS, Fletcher KE, Adams TM. Evaluation of a Cough-Specific Quality-of-Life Questionnaire. Chest. 2002;121(4):1123-31.

123. Lechtzin N, Hilliard ME, Horton MR. Validation of the Cough Quality-of-Life Questionnaire in Patients With Idiopathic Pulmonary Fibrosis. Chest. 2013;143(6):1745-9.

124. Smith J, Owen E, Earis J, Woodcock A. Cough in COPD: Correlation of Objective Monitoring With Cough Challenge and Subjective Assessments. Chest. 2006;130(2):379-85.

125. Bausewein C, Farquhar M, Booth S, Gysels M, Higginson IJ. Measurement of breathlessness in advanced disease: A systematic review. Respiratory Medicine. 2007;101(3):399-410.

126. Santos LM, Pedro PI, Dias A, Forte CB, Raposo P, Rodrigues MF. Relationship between dyspnea/oxygen saturation and leg discomfort/6-minute walking distance in patients with COPD participating in pulmonary rehabilitation. Pulmonology. 2019;25(6):357-60.

127. Laviolette L, Laveneziana P. Dyspnoea: a multidimensional and multidisciplinary approach. European Respiratory Journal. 2014;43(6):1750-62.

128. Malamood M, Shahsavari D, Parkman HP. Modern evaluation of esophageal function in the gastrointestinal motility laboratory: a narrative review. Annals of Esophagus. 2023;6.

129. Hatlebakk JG. Endoscopy in gastro-oesophageal reflux disease. Best practice & research Clinical gastroenterology. 2010;24(6):775-86.

130. Pannala R, Krishnan K, Watson RR, Vela MF, Dayyeh BKA, Bhatt A, et al. Devices for esophageal function testing. VideoGIE. 2022;7(1):1-20.

131. Savarino E, Bredenoord AJ, Fox M, Pandolfino JE, Roman S, Gyawali CP, et al. Advances in the physiological assessment and diagnosis of GERD. Nature reviews Gastroenterology & hepatology. 2017;14(11):665-76.

132. Silny J. Intraluminal multiple electric impedance procedure for measurement of gastrointestinal motility. Neurogastroenterology & Motility. 1991;3(3):151-62.

133. Zentilin P, Dulbecco P, Savarino E, Giannini E, Savarino V. Combined multichannel intraluminal impedance and pH-metry: a novel technique to improve detection of gastro-oesophageal reflux: literature review. Digestive and Liver Disease. 2004;36(9):565-9.

134. Boluda S, Mokhtari K, Megarbane B, Annane D, Mathon B, Cao A, et al. Golgi localization of SARS-CoV-2 spike protein and interaction with furin in cerebral COVID-19 microangiopathy: a clue to the central nervous system involvement? Free Neuropathology. 2023;4:1.

135. Piper D, Fenton BH. pH stability and activity curves of pepsin with special reference to their clinical importance. Gut. 1965;6(5):506.

136. Stef JJ, Dodds WJ, Hogan WJ, Linehan JH, Stewart ET. Intraluminal esophageal manometry: an analysis of variables affecting recording fidelity of peristaltic pressures. Gastroenterology. 1974;67(2):221-30.

137. Murray J, Clouse R, Conklin J. Components of the standard oesophageal manometry. Neurogastroenterology & Motility. 2003;15(6):591-606.

138. Gyawali C. High resolution manometry: the Ray Clouse legacy. Neurogastroenterology & Motility. 2012;24.

139. Gyawali CP, Kahrilas PJ. A short history of high-resolution esophageal manometry. Dysphagia. 2023;38(2):586-95.

140. Clouse RE, Staiano A, Alrakawi A. Development of a topographic analysis system for manometric studies in the gastrointestinal tract. Gastrointestinal endoscopy. 1998;48(4):395-401.

141. Salvador R, Dubecz A, Polomsky M, Gellerson O, Jones CE, Raymond DP, et al. A new era in esophageal diagnostics: the image-based paradigm of high-resolution manometry. Journal of the American College of Surgeons. 2009;208(6):1035-44.

142. Sifrim D, Holloway R. Transient lower esophageal sphincter relaxations: how many or how harmful? Official journal of the American College of Gastroenterology| ACG. 2001;96(9):2529-32.

143. Trudgill NJ, Sifrim D, Sweis R, Fullard M, Basu K, McCord M, et al. British Society of Gastroenterology guidelines for oesophageal manometry and oesophageal reflux monitoring. Gut. 2019;68(10):1731-50.

144. Rogers BD, Gyawali CP. Enhancing High-Resolution Esophageal Manometry: Use of Ancillary Techniques and Maneuvers. Gastroenterology Clinics of North America. 2020;49(3):411-26.

145. Kahrilas PJ, Ghosh SK, Pandolfino JE. Esophageal motility disorders in terms of pressure topography: the Chicago Classification. Journal of clinical gastroenterology. 2008;42(5):627-35.

146. Herregods T, Roman S, Kahrilas P, Smout A, Bredenoord A. Normative values in esophageal high‐resolution manometry. Neurogastroenterology & Motility. 2015;27(2):175-87.

147. Hill C. Dr. Thomas Sydenham (1624–1689): his Life and Original Writingsby Kenneh Dewhurst, London, Wellcome Historical Medical Library, 1966, pp. viii, 180, illus., 35s. Medical History. 1967;11(2):206-7.

148. Irwin RS, Zawacki JK, Curley FJ, French CL, Hoffman PJ. Chronic cough as the sole presenting manifestation of gastroesophageal reflux. American Review of Respiratory Disease. 1989;140(5):1294-300.

149. Decalmer S, Stovold R, Houghton LA, Pearson J, Ward C, Kelsall A, et al. Chronic cough: relationship between microaspiration, gastroesophageal reflux, and cough frequency. Chest. 2012;142(4):958-64.

150. Smith JA, Houghton LA. The oesophagus and cough: laryngo-pharyngeal reflux, microaspiration and vagal reflexes. Cough. 2013;9:1-9.

151. Chen Z, Chen H, Chen F, Gu D, Sun L, Zhang W, et al. Vagotomy decreases the neuronal activities of medulla oblongata and alleviates neurogenic inflammation of

airways induced by repeated intra-esophageal instillation of HCl in guinea pigs. Physiological Research. 2017;66(6):1021-8.

152. Chen Z, Gu D, Fan L, Zhang W, Sun L, Chen H, et al. Neuronal activity of the medulla oblongata revealed by manganese-enhanced magnetic resonance imaging in a rat model of gastroesophageal reflux-related cough. Physiological Research. 2019;68(1):119-27.

153. Javorkova N, Varechova S, Pecova R, Tatar M, Balaz D, Demeter M, et al. Acidification of the oesophagus acutely increases the cough sensitivity in patients with gastro‐oesophageal reflux and chronic cough. Neurogastroenterology & Motility. 2008;20(2):119-24.

154. Kahrilas PJ, Howden CW, Hughes N, Molloy-Bland M. Response of chronic cough to acid-suppressive therapy in patients with gastroesophageal reflux disease. Chest. 2013;143(3):605-12.

155. Chang AB, Lasserson TJ, Gaffney J, Connor FL, Garske LA. Gastro‐oesophageal reflux treatment for prolonged non‐specific cough in children and adults. Cochrane Database of Systematic Reviews. 2011(1).

156. Zerbib F, Bruley des Varannes S, Roman S, Pouderoux P, Artigue F, Chaput U, et al. Normal values and day‐to‐day variability of 24‐h ambulatory oesophageal impedance‐pH monitoring in a Belgian–French cohort of healthy subjects. Alimentary pharmacology & therapeutics. 2005;22(10):1011-21.

157. Morice AH. Airway reflux as a cause of respiratory disease. Breathe. 2013;9(4):257-66.

158. Vardar R, Sweis R, Anggiansah A, Wong T, Fox M. Upper esophageal sphincter and esophageal motility in patients with chronic cough and reflux: assessment by high‐resolution manometry. Diseases of the Esophagus. 2013;26(3):219-25.

159. Almansa C, Smith JA, Morris J, Crowell MD, Valdramidou D, Lee A, et al. Weak peristalsis with large breaks in chronic cough: association with poor esophageal clearance. Neurogastroenterology & Motility. 2015;27(3):431-42.

160. Burton L, Falk GL, Beattie J, Novakovic D, Simpson S, Van der Wall H. Findings from a novel scintigraphic gastroesophageal reflux study in asymptomatic volunteers. American Journal of Nuclear Medicine and Molecular Imaging. 2020;10(6):342.

161. Burton L, Falk GL, Parsons S, Cusi M, Van Der Wall H. Benchmarking of a Simple Scintigraphic Test for Gastro-oesophageal Reflux Disease That Assesses Oesophageal Disease and Its Pulmonary Complications. Mol Imaging Radionucl Ther. 2018;27(3):113-20.

162. Ghisa M, Marinelli C, Savarino V, Savarino E. Idiopathic pulmonary fibrosis and GERD: links and risks. Therapeutics and clinical risk management. 2019:1081-93.

163. Raghu G, Freudenberger T, Yang S, Curtis J, Spada C, Hayes J, et al. High prevalence of abnormal acid gastro-oesophageal reflux in idiopathic pulmonary fibrosis. European Respiratory Journal. 2006;27(1):136-42.

164. Desai S, Mackintosh J, Chua F, Renzoni E, Kouranos V, Margaritopoulos G, et al. P157 Hiatus hernia is highly prevalent in idiopathic pulmonary fibrosis and impacts disease progression. BMJ Publishing Group Ltd; 2018.

165. Savarino E, Carbone R, Marabotto E, Furnari M, Sconfienza L, Ghio M, et al. Gastro-oesophageal reflux and gastric aspiration in idiopathic pulmonary fibrosis patients. European Respiratory Journal. 2013;42(5):1322-31.

166. Prednisone, Azathioprine, and N-Acetylcysteine for Pulmonary Fibrosis. New England Journal of Medicine. 2012;366(21):1968-77.

167. Bois MC, Hu X, Ryu JH, Yi ES. Could prominent airway-centered fibroblast foci in lung biopsies predict underlying chronic microaspiration in idiopathic pulmonary fibrosis patients? Human Pathology. 2016;53:1-7.

168. Fidler L, Sitzer N, Shapera S, Shah PS. Treatment of gastroesophageal reflux in patients with idiopathic pulmonary fibrosis: a systematic review and meta-analysis. Chest. 2018;153(6):1405-15.

169. Tran T, Assayag D, Ernst P, Suissa S. Effectiveness of Proton Pump Inhibitors in Idiopathic Pulmonary Fibrosis: A Population-Based Cohort Study. Chest. 2021;159(2):673-82.

170. Campo S, Morini S, Re MA, Monno D, Lorenzetti R, Moscatelli B, et al. Esophageal dysmotility and gastroesophageal reflux in intrinsic asthma. Digestive diseases and sciences. 1997;42:1184-8.

171. Amarasiri DL, Pathmeswaran A, Dassanayake AS, de Silva AP, Ranasinha CD, de Silva HJ. Esophageal motility, vagal function and gastroesophageal reflux in a cohort of adult asthmatics. BMC gastroenterology. 2012;12:1-8.

172. Jack CI, Calverley PM, Donnelly RJ, Tran J, Russell G, Hind CR, et al. Simultaneous tracheal and oesophageal pH measurements in asthmatic patients with gastro-oesophageal reflux. Thorax. 1995;50(2):201-4.

173. Sharma B, Sharma M, Daga MK, Sachdev GK, Bondi E. Effect of omeprazole and domperidone on adult asthmatics with gastroesophageal reflux. World J Gastroenterol. 2007;13(11):1706-10.

174. Gadel AA, Mostafa M, Younis A, Haleem M. Esophageal motility pattern and gastro-esophageal reflux in chronic obstructive pulmonary disease. Hepato-gastroenterology. 2012;59(120):2498-502.

175. Iliaz S, Iliaz R, Onur ST, Arici S, Akyuz U, Karaca C, et al. Does gastroesophageal reflux increase chronic obstructive pulmonary disease exacerbations? Respiratory Medicine. 2016;115:20-5.

176. Benson VS, Müllerová H, Vestbo J, Wedzicha JA, Patel A, Hurst JR, et al. Associations between gastro-oesophageal reflux, its management and exacerbations of chronic obstructive pulmonary disease. Respiratory medicine. 2015;109(9):1147-54.

177. Dettmar PW, Lenham RK, Parkinson AJ, Woodcock AD. Pepsin detection as a diagnostic test for reflux disease. Reflux Aspiration and Lung Disease. 2018:91-104.

178. Strugala V, Woodcock AD, Dettmar PW, Faruqi S, Morice AH. Detection of pepsin in sputum: a rapid and objective measure of airways reflux. European Respiratory Journal. 2015;47(1):339-41.

179. Xu X, Chen Q, Liang S, Lv H, Qiu Z. Comparison of gastroesophageal reflux disease questionnaire and multichannel intraluminal impedance pH monitoring in identifying patients with chronic cough responsive to antireflux therapy. Chest. 2014;145(6):1264-70.

180. Xu X, Yang Z, Chen Q, Yu L, Liang S, Lü H, et al. Comparison of clinical characteristics of chronic cough due to non‐acid and acid gastroesophageal reflux. The clinical respiratory journal. 2015;9(2):196-202.

181. Page A, O'Donnell T, Blackshaw L. Inhibition of mechanosensitivity in visceral primary afferents by GABAB receptors involves calcium and potassium channels. Neuroscience. 2006;137(2):627-36.

182. Lidums I, Lehmann A, Checklin H, Dent J, Holloway RH. Control of transient lower esophageal sphincter relaxations and reflux by the GABAB agonist baclofen in normal subjects. Gastroenterology. 2000;118(1):7-13.

183. Zhang Q, Lehmann A, Rigda R, Dent J, Holloway R. Control of transient lower oesophageal sphincter relaxations and reflux by the GABAB agonist baclofen in patients with gastro-oesophageal reflux disease. Gut. 2002;50(1):19-24.

184. Wei W, Liu R, ZhangTong Y, Qiu Z. The efficacy of specific neuromodulators on human refractory chronic cough: a systematic review and meta-analysis. Journal of Thoracic Disease. 2016;8(10):2942.

185. Xie S, Xie M, Shen Y, Cheng D. Gabapentin for chronic refractory cough: A system review and meta-analysis. Heliyon. 2023;9(5).

186. Dong R, Xu X, Yu L, Ding H, Pan J, Yu Y, et al. Randomised clinical trial: gabapentin vs baclofen in the treatment of suspected refractory gastro‐oesophageal reflux‐induced chronic cough. Alimentary Pharmacology & Therapeutics. 2019;49(6):714-22.

187. Lehmann A, Blackshaw LA, Elebring T, Jensen J, Mattsson JP, Nilsson KA, et al. W1789 The New Reflux Inhibitor Azd3355 Has a Low Propensity for Inducing Central Side Effects Due to Its Affinity for the GABA Carrier. Gastroenterology. 2008;4(134):A-715-A-6.

188. Badri H, LGibbard C, Denton D, Satia I, Dockry RJ, Holt K, et al. Effect of centrally and peripherally acting GABAB agonism on the healthy human cough reflex. Pulmonary pharmacology & therapeutics. 2021;71:102079.

189. Dicpinigaitis P. Effect of the GABA-agonist baclofen on bronchial responsiveness in asthmatics. Pulmonary Pharmacology & Therapeutics. 1999;12(4):257-60.

190. Itoh Z, Aizawa I, Nakamura T. Effect of dopamine and its antagonists on contractile activity of the lower esophageal sphincter and the stomach (author's transl). Nihon Heikatsukin Gakkai Zasshi. 1980;16(2):99-107.

191. Mikami H, Ishimura N, Fukazawa K, Okada M, Izumi D, Shimura S, et al. Effects of metoclopramide on esophageal motor activity and esophagogastric junction compliance in healthy volunteers. Journal of neurogastroenterology and motility. 2016;22(1):112.

192. Anda LA, Araiza-Casillas R, Avendaño-Reyes M. Metoclopramide response in patients with early diffuse systemic sclerosis. Effects on esophageal motility abnormalities. Clinical and experimental rheumatology. 2005;23:685-8.

193. Bron B, Massih L. Domperidone: a drug with powerful action on the lower esophageal sphincter pressure. Digestion. 1980;20(6):375-8.

194. Al-Biltagi M, Bediwy AS, Deraz S, Amer HG, Saeed NK. Esomeprazole, versus esomeprazole and domperidone in treatment of gastroesophageal reflux in children with difficult-to-treat asthma. Eur Respiratory Soc; 2013.

195. Warusevitane A, Karunatilake D, Sim J, Lally F, Roffe C. Safety and effect of metoclopramide to prevent pneumonia in patients with stroke fed via nasogastric tubes trial. Stroke. 2015;46(2):454-60.

196. Liu JieSheng LJ, Xie ZongLin XZ. Effect of lansoprazole combined with domperidone on airway inflammatory cells and mediators in patients with gastro-esophageal reflux related cough. 2014.

197. Tomomasa T, Kuroume T, Arai H, Wakabayashi K, Itoh Z. Erythromycin induces migrating motor complex in human gastrointestinal tract. Digestive diseases and sciences. 1986;31:157-61.

198. Pennathur A, Tran A, Cioppi M, Fayad J, Sieren GL, Little AG. Erythromycin strengthens the defective lower esophageal sphincter in patients with gastroesophageal reflux disease. The American journal of surgery. 1994;167(1):169-73.

199. Kitazawa T, Kaiya H. Motilin comparative study: structure, distribution, receptors, and gastrointestinal motility. Frontiers in Endocrinology. 2021;12:700884.

200. Korimilli A, Parkman HP. Effect of atilmotin, a motilin receptor agonist, on esophageal, lower esophageal sphincter, and gastric pressures. Digestive diseases and sciences. 2010;55:300-6.

201. Peeters T, Matthijs G, Depoortere I, Cachet T, Hoogmartens J, Vantrappen G. Erythromycin is a motilin receptor agonist. American Journal of Physiology-Gastrointestinal and Liver Physiology. 1989;257(3):G470-G4.

202. Broad J, Sanger GJ. The antibiotic azithromycin is a motilin receptor agonist in human stomach: comparison with erythromycin. British journal of pharmacology. 2013;168(8):1859-67.

203. Rohof W, Bennink R, De Ruigh A, Hirsch D, Zwinderman A, Boeckxstaens G. Effect of azithromycin on acid reflux, hiatus hernia and proximal acid pocket in the postprandial period. Gut. 2012;61(12):1670-7.

204. Mertens V, Blondeau K, Pauwels A, Farre R, Vanaudenaerde B, Vos R, et al. Azithromycin reduces gastroesophageal reflux and aspiration in lung transplant recipients. Digestive diseases and sciences. 2009;54:972-9.

205. Corris PA, Ryan VA, Small T, Lordan J, Fisher AJ, Meachery G, et al. A randomised controlled trial of azithromycin therapy in bronchiolitis obliterans syndrome (BOS) post lung transplantation. Thorax. 2015;70(5):442-50.

206. Banić Tomišić Z. The story of azithromycin. Kemija u industriji: Časopis kemičara i kemijskih inženjera Hrvatske. 2011;60(12):603-17.

207. Hoepelman IM, Schneider MME. Azithromycin: the first of the tissue-selective azalides. International Journal of Antimicrobial Agents. 1995;5(3):145-67.

208. Foulds G, Shepard R, Johnson R. The pharmacokinetics of azithromycin in human serum and tissues. Journal of Antimicrobial Chemotherapy. 1990;25(suppl_A):73-82.

209. Girard AE, Girard D, English AR, Gootz TD, Cimochowski CR, Faiella JA, et al. Pharmacokinetic and in vivo studies with azithromycin (CP-62,993), a new macrolide with an extended half-life and excellent tissue distribution. Antimicrobial agents and chemotherapy. 1987;31(12):1948-54.

210. Gaynor M, Mankin AS. Macrolide antibiotics: binding site, mechanism of action, resistance. Current topics in medicinal chemistry. 2003;3(9):949-60.

211. Williams J. Spectrum of activity of azithromycin. European Journal of Clinical Microbiology and Infectious Diseases. 1991;10:813-20.

212. Cazzola M, Salzillo A, De Giglio C, Piccolo A, Califano C, Noschese P. Treatment of acute exacerbation of severe-to-very severe COPD with azithromycin in patients vaccinated against Streptococcus pneumoniae. Respiratory Medicine. 2005;99(6):663-9.

213. Baalbaki N, Giuliano C, Hartner CL, Kale-Pradhan P, Johnson L. Azithromycin versus beta-lactams in hospitalized patients with acute exacerbations of COPD. Journal of General Internal Medicine. 2022;37(16):4183-8.

214. Parnham MJ, Haber VE, Giamarellos-Bourboulis EJ, Perletti G, Verleden GM, Vos R. Azithromycin: mechanisms of action and their relevance for clinical applications. Pharmacology & therapeutics. 2014;143(2):225-45.

215. Organization WH. WHO guideline on mass drug administration of azithromycin to children under five years of age to promote child survival: World Health Organization; 2020.

216. Equi A, Balfour-Lynn I, Bush A, Rosenthal M. Long term azithromycin in children with cystic fibrosis: a randomised, placebo-controlled crossover trial. The Lancet. 2002;360(9338):978-84.

217. Wolter J, Seeney S, Bell S, Bowler S, Masel P, McCormack J. Effect of long term treatment with azithromycin on disease parameters in cystic fibrosis: a randomised trial. Thorax. 2002;57(3):212-6.

218. Kobayashi H. Biofilm disease: its clinical manifestation and therapeutic possibilities of macrolides. The American journal of medicine. 1995;99(6):26s-30s.

219. Wagner T, Soong G, Sokol S, Saiman L, Prince A. Effects of azithromycin on clinical isolates of Pseudomonas aeruginosa from cystic fibrosis patients. Chest. 2005;128(2):912-9.

220. Saiman L, Anstead M, Mayer-Hamblett N, Lands LC, Kloster M, Hocevar-Trnka J, et al. Effect of azithromycin on pulmonary function in patients with cystic fibrosis uninfected with Pseudomonas aeruginosa: a randomized controlled trial. JAMA. 2010;303(17):1707‐15.

221. Albert RK, Connett J, Bailey WC, Casaburi R, Cooper Jr JAD, Criner GJ, et al. Azithromycin for prevention of exacerbations of COPD. New England Journal of Medicine. 2011;365(8):689-98.

222. Uzun S, Djamin RS, Kluytmans JA, Mulder PG, van't Veer NE, Ermens AA, et al. Azithromycin maintenance treatment in patients with frequent exacerbations of chronic obstructive pulmonary disease (COLUMBUS): a randomised, double-blind, placebo-controlled trial. The Lancet Respiratory Medicine. 2014;2(5):361-8.

223. Wong C, Jayaram L, Karalus N, Eaton T, Tong C, Hockey H, et al. Azithromycin for prevention of exacerbations in non-cystic fibrosis bronchiectasis (EMBRACE): a randomised, double-blind, placebo-controlled trial. The Lancet. 2012;380(9842):660-7.

224. Altenburg J, de Graaff CS, Stienstra Y, Sloos JH, van Haren EH, Koppers RJ, et al. Effect of azithromycin maintenance treatment on infectious exacerbations among patients with non–cystic fibrosis bronchiectasis: the BAT randomized controlled trial. Jama. 2013;309(12):1251-9.

225. Brusselle GG, VanderStichele C, Jordens P, Deman R, Slabbynck H, Ringoet V, et al. Azithromycin for prevention of exacerbations in severe asthma (AZISAST): a multicentre randomised double-blind placebo-controlled trial. Thorax. 2013;68(4):322-9.

226. Gibson PG, Yang IA, Upham JW, Reynolds PN, Hodge S, James AL, et al. Effect of azithromycin on asthma exacerbations and quality of life in adults with persistent uncontrolled asthma (AMAZES): a randomised, double-blind, placebo-controlled trial. The Lancet. 2017;390(10095):659-68.

227. Tong X, Guo T, Liu S, Peng S, Yan Z, Yang X, et al. Macrolide antibiotics for treatment of asthma in adults: a meta-analysis of 18 randomized controlled clinical studies. Pulmonary pharmacology & therapeutics. 2015;31:99-108.

228. Taylor SL, Leong LE, Mobegi FM, Choo JM, Wesselingh S, Yang IA, et al. Long-term azithromycin reduces Haemophilus influenzae and increases antibiotic resistance in severe asthma. American journal of respiratory and critical care medicine. 2019;200(3):309-17.

229. Rogers GB, Bruce KD, Martin ML, Burr LD, Serisier DJ. The effect of long-term macrolide treatment on respiratory microbiota composition in non-cystic fibrosis bronchiectasis: an analysis from the randomised, double-blind, placebo-controlled BLESS trial. The lancet Respiratory medicine. 2014;2(12):988-96.

230. Valery PC, Morris PS, Byrnes CA, Grimwood K, Torzillo PJ, Bauert PA, et al. Long-term azithromycin for Indigenous children with non-cystic-fibrosis bronchiectasis or chronic suppurative lung disease (Bronchiectasis Intervention Study): a multicentre, double-blind, randomised controlled trial. The lancet Respiratory medicine. 2013;1(8):610-20.

231. Cuevas E, Huertas D, Montón C, Marin A, Carrera-Salinas A, Pomares X, et al. Systemic and functional effects of continuous azithromycin treatment in patients with severe chronic obstructive pulmonary disease and frequent exacerbations. Frontiers in Medicine. 2023;10:1229463.

232. Wilkinson TM, Aris E, Bourne S, Clarke SC, Peeters M, Pascal TG, et al. A prospective, observational cohort study of the seasonal dynamics of airway pathogens in the aetiology of exacerbations in COPD. Thorax. 2017;72(10):919-27.

233. Ackland J, Barber C, Heinson A, Azim A, Cleary DW, Christodoulides M, et al. Nontypeable Haemophilus influenzae infection of pulmonary macrophages drives neutrophilic inflammation in severe asthma. Allergy. 2022;77(10):2961-73.

234. Gielen V, Johnston SL, Edwards MR. Azithromycin induces anti-viral responses in bronchial epithelial cells. Eur Respir J. 2010;36(3):646-54.

235. Menzel M, Akbarshahi H, Bjermer L, Uller L. Azithromycin induces anti-viral effects in cultured bronchial epithelial cells from COPD patients. Scientific Reports. 2016;6(1):28698.

236. Beigelman A, Mikols CL, Gunsten SP, Cannon CL, Brody SL, Walter MJ. Azithromycin attenuates airway inflammation in a mouse model of viral bronchiolitis. Respiratory research. 2010;11:1-11.

237. Sato K, Suga M, Akaike T, Fujii S, Muranaka H, Doi T, et al. Therapeutic effect of erythromycin on influenza virus-induced lung injury in mice. American Journal of Respiratory and Critical Care Medicine. 1998;157(3):853-7.

238. Group RC. Azithromycin in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. Lancet (London, England). 2021;397(10274):605.

239. Khoshnood S, Shirani M, Dalir A, Moradi M, Haddadi MH, Sadeghifard N, et al. Antiviral effects of azithromycin: A narrative review. Biomedicine & Pharmacotherapy. 2022;147:112682.

240. Papayannopoulos V, Staab D, Zychlinsky A. Neutrophil elastase enhances sputum solubilization in cystic fibrosis patients receiving DNase therapy. PloS one. 2011;6(12):e28526.

241. Grabcanovic-Musija F, Obermayer A, Stoiber W, Krautgartner W-D, Steinbacher P, Winterberg N, et al. Neutrophil extracellular trap (NET) formation characterises stable and exacerbated COPD and correlates with airflow limitation. Respiratory research. 2015;16:1-12.

242. Wright TK, Gibson PG, Simpson JL, McDonald VM, Wood LG, Baines KJ. Neutrophil extracellular traps are associated with inflammation in chronic airway disease. Respirology. 2016;21(3):467-75.

243. Keir HR, Shoemark A, Dicker AJ, Perea L, Pollock J, Giam YH, et al. Neutrophil extracellular traps, disease severity, and antibiotic response in bronchiectasis: an international, observational, multicohort study. The lancet Respiratory medicine. 2021;9(8):873-84.

244. Saffarzadeh M, Juenemann C, Queisser MA, Lochnit G, Barreto G, Galuska SP, et al. Neutrophil extracellular traps directly induce epithelial and endothelial cell death: a predominant role of histones. PloS one. 2012;7(2):e32366.

245. Storisteanu DM, Pocock JM, Cowburn AS, Juss JK, Nadesalingam A, Nizet V, et al. Evasion of neutrophil extracellular traps by respiratory pathogens. American journal of respiratory cell and molecular biology. 2017;56(4):423-31.

246. Zhang H, Qiu S-L, Tang Q-Y, Zhou X, Zhang J-Q, He Z-Y, et al. Erythromycin suppresses neutrophil extracellular traps in smoking-related chronic pulmonary inflammation. Cell death & disease. 2019;10(9):678.

247. Bystrzycka W, Manda-Handzlik A, Sieczkowska S, Moskalik A, Demkow U, Ciepiela O. Azithromycin and chloramphenicol diminish neutrophil extracellular traps (NETs) release. International journal of molecular sciences. 2017;18(12):2666.

248. Čulić O, Eraković V, Čepelak I, Barišić K, Brajša K, Ferenčić Ž, et al. Azithromycin modulates neutrophil function and circulating inflammatory mediators in healthy human subjects. European journal of pharmacology. 2002;450(3):277-89.

249. Marjanović N, Bosnar M, Michielin F, Willé DR, Anić-Milić T, Čulić O, et al. Macrolide antibiotics broadly and distinctively inhibit cytokine and chemokine production by COPD sputum cells in vitro. Pharmacological research. 2011;63(5):389-97.

250. Blasi F, Bonardi D, Aliberti S, Tarsia P, Confalonieri M, Amir O, et al. Long-term azithromycin use in patients with chronic obstructive pulmonary disease and tracheostomy. Pulmonary pharmacology & therapeutics. 2010;23(3):200-7.

251. Malaviya R, Laskin JD, Laskin DL. Anti-TNFα therapy in inflammatory lung diseases. Pharmacology & therapeutics. 2017;180:90-8.

252. Berry M, Brightling C, Pavord I, Wardlaw AJ. TNF-α in asthma. Current opinion in pharmacology. 2007;7(3):279-82.

253. Murphy BS, Sundareshan V, Cory TJ, Hayes Jr D, Anstead MI, Feola DJ. Azithromycin alters macrophage phenotype. Journal of Antimicrobial Chemotherapy. 2008;61(3):554-60.

254. Hodge S, Hodge G, Brozyna S, Jersmann H, Holmes M, Reynolds PN. Azithromycin increases phagocytosis of apoptotic bronchial epithelial cells by alveolar macrophages. European Respiratory Journal.28(3):486-95.

255. Hodge S, Hodge G, Jersmann H, Matthews G, Ahern J, Holmes M, et al. Azithromycin improves macrophage phagocytic function and expression of mannose receptor in chronic obstructive pulmonary disease. American journal of respiratory and critical care medicine. 2008;178(2):139-48.

256. Hattori T, Konno S, Hizawa N, Isada A, Takahashi A, Shimizu K, et al. Genetic variants in the mannose receptor gene (MRC1) are associated with asthma in two independent populations. Immunogenetics. 2009;61:731-8.

257. Lin C-L, Siu L-K, Lin J-C, Liu C-Y, Chian C-F, Lee C-N, et al. Mannose-binding lectin gene polymorphism contributes to recurrence of infective exacerbation in patients with COPD. Chest. 2011;139(1):43-51.

258. Chalmers JD, McHugh BJ, Doherty C, Smith MP, Govan JR, Kilpatrick DC, et al. Mannose-binding lectin deficiency and disease severity in non-cystic fibrosis bronchiectasis: a prospective study. The Lancet Respiratory Medicine. 2013;1(3):224-32.

259. Iwamoto S, Kumamoto T, Azuma E, Hirayama M, Ito M, Amano K, et al. The effect of azithromycin on the maturation and function of murine bone marrow-derived dendritic cells. Clin Exp Immunol. 2011;166(3):385-92.

260. Xuan W, Xi-rong Z, Gang L. Effect of azithromycin on function of peripheral blood dendritic cells in children with bronchial asthma. Journal of International Translational Medicine. 2017;5(1):23-8.

261. Morrison KE, Colón-González FJ, Morbey RA, Hunter PR, Rutter J, Stuttard G, et al. Demographic and socioeconomic patterns in healthcare-seeking behaviour for respiratory symptoms in England: a comparison with non-respiratory symptoms and between three healthcare services. BMJ open. 2020;10(11):e038356.

262. Kassebaum NJ, Arora M, Barber RM, Bhutta ZA, Brown J, Carter A, et al. Global, regional, and national disability-adjusted life-years (DALYs) for 315 diseases and injuries and healthy life expectancy (HALE), 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. The Lancet. 2016;388(10053):1603-58.

263. Renwick DS, Connolly MJ. Impact of obstructive airways disease on quality of life in older adults. Thorax. 1996;51(5):520-5.

264. Brignall K, Jayaraman B, Birring SS. Quality of life and psychosocial aspects of cough. Lung. 2008;186(1):55-8.

265. Walke LM, Byers AL, Tinetti ME, Dubin JA, McCorkle R, Fried TR. Range and severity of symptoms over time among older adults with chronic obstructive pulmonary disease and heart failure. Archives of internal medicine. 2007;167(22):2503-8.

266. Chamberlain SA, Garrod R, Douiri A, Masefield S, Powell P, Bücher C, et al. The impact of chronic cough: a cross-sectional European survey. Lung. 2015;193(3):401-8.

267. Róka R, Rosztóczy A, Izbéki F, Taybani Z, Kiss I, Lonovics J, et al. Prevalence of respiratory symptoms and diseases associated with gastroesophageal reflux disease. Digestion. 2005;71(2):92-6.

268. Lee AL, Goldstein RS. Gastroesophageal reflux disease in COPD: links and risks. International journal of chronic obstructive pulmonary disease. 2015;10:1935.

269. Nazemiyeh M, Nouri-Vaskeh M, Somi MH, Saeedi E, Sharifi A. Lung function parameters in patients with gastroesophageal reflux without respiratory symptoms: a case-control study. Gastroenterology and hepatology from bed to bench. 2019;12(4):287.

270. Irwin RS. Chronic cough due to gastroesophageal reflux disease: ACCP evidence-based clinical practice guidelines. Chest. 2006;129(1):80S-94S.

271. Salvioli B, Belmonte G, Stanghellini V, Baldi E, Fasano L, Pacilli A, et al. Gastro-oesophageal reflux and interstitial lung disease. Digestive and Liver Disease. 2006;38(12):879-84.

272. Yadlapati R, Kahrilas PJ, Fox MR, Bredenoord AJ, Prakash Gyawali C, Roman S, et al. Esophageal motility disorders on high‐resolution manometry: Chicago classification version 4.0©. Neurogastroenterology & Motility. 2021;33(1):e14058.

273. Gyawali CP, Kahrilas PJ, Savarino E, Zerbib F, Mion F, Smout AJ, et al. Modern diagnosis of GERD: the Lyon Consensus. Gut. 2018;67(7):1351-62.

274. Bogte A, Bredenoord A, Oors J, Siersema P, Smout A. Normal values for esophageal high‐resolution manometry. Neurogastroenterology & Motility. 2013;25(9):762-e579.

275. Allaix ME, Fisichella PM, Noth I, Herbella FA, Segura BB, Patti MG. Idiopathic pulmonary fibrosis and gastroesophageal reflux. Implications for treatment. Journal of Gastrointestinal Surgery. 2014;18(1):100-5.

276. Solomon JJ, Olson AL, Fischer A, Bull T, Brown KK, Raghu G. Scleroderma lung disease. European Respiratory Review. 2013;22(127):6-19.

277. Christmann RB, Wells AU, Capelozzi VL, Silver RM, editors. Gastroesophageal reflux incites interstitial lung disease in systemic sclerosis: clinical, radiologic, histopathologic, and treatment evidence. Seminars in arthritis and rheumatism; 2010: Elsevier.

278. Kimmel JN, Carlson DA, Hinchcliff M, Carns MA, Aren KA, Lee J, et al. The association between systemic sclerosis disease manifestations and esophageal high‐resolution manometry parameters. Neurogastroenterology & Motility. 2016;28(8):1157-65.

279. Faruqi S, Sedman P, Jackson W, Molyneux I, Morice AH. Fundoplication in chronic intractable cough. Cough. 2012;8(1):1-7.

280. Linden PA, Gilbert RJ, Yeap BY, Boyle K, Deykin A, Jaklitsch MT, et al. Laparoscopic fundoplication in patients with end-stage lung disease awaiting transplantation. The Journal of Thoracic and Cardiovascular Surgery. 2006;131(2):438-46.

281. Rai Z, Morice AH, Sedman P. Anti Reflux Surgery. Reflux Aspiration and Lung Disease: Springer; 2018. p. 357-63.

282. Smith D, Du Rand IA, Addy C, Collyns T, Hart S, Mitchelmore P, et al. British Thoracic Society guideline for the use of long-term macrolides in adults with respiratory disease. BMJ Open Respiratory Research. 2020;7(1):e000489.

283. Chini P, Toskes PP, Waseem S, Hou W, McDonald R, Moshiree B. Effect of azithromycin on small bowel motility in patients with gastrointestinal dysmotility. Scandinavian journal of gastroenterology. 2012;47(4):422-7.

284. Mertens V, Blondeau K, Pauwels A, Farre R, Vanaudenaerde B, Vos R, et al. Azithromycin reduces gastroesophageal reflux and aspiration in lung transplant recipients. Digestive diseases and sciences. 2009;54(5):972-9.

285. Hirons B, Rhatigan K, Kesavan H, Turner RD, Birring SS, Cho PS. Cough in chronic lung disease: a state of the art review. Journal of Thoracic Disease. 2023;15(10).

286. Çolak Y, Afzal S, Lange P, Laursen LC, Nordestgaard BG, Dahl M. Role and impact of chronic cough in individuals with asthma from the general population. The Journal of Allergy and Clinical Immunology: In Practice. 2019;7(6):1783-92. e8.

287. Ryerson CJ, Abbritti M, Ley B, Elicker BM, Jones KD, Collard HR. Cough predicts prognosis in idiopathic pulmonary fibrosis. Respirology. 2011;16(6):969-75.

288. Parker SM, Smith JA, Birring SS, Chamberlain-Mitchell S, Gruffydd-Jones K, Haines J, et al. British Thoracic Society Clinical Statement on chronic cough in adults. Thorax. 2023;78(Suppl 6):s3-s19.

289. Pollock J, Chalmers JD. The immunomodulatory effects of macrolide antibiotics in respiratory disease. Pulmonary Pharmacology & Therapeutics. 2021;71:102095.

290. Hodge S, Reynolds PN. Low‐dose azithromycin improves phagocytosis of bacteria by both alveolar and monocyte‐derived macrophagesin chronic obstructive pulmonary disease subjects. Respirology. 2012;17(5):802-7.

291. Ishida Y, Abe Y, Harabuchi Y. Effects of macrolides on antigen presentation and cytokine production by dendritic cells and T lymphocytes. International Journal of Pediatric Otorhinolaryngology. 2007;71(2):297-305.

292. Ratzinger F, Haslacher H, Poeppl W, Hoermann G, Kovarik J, Jutz S, et al. Azithromycin suppresses CD4+ T-cell activation by direct modulation of mTOR activity. Scientific reports. 2014;4(1):7438.

293. Higgins J, Savović J, Page M, Elbers R, Sterne A. Chapter 8: assessing risk of bias in a randomized trial. Cochrane Handbook for Systematic Reviews of Interventions version 6.1 (updated September 2020). Cochrane 2020. 2022. 2022.

294. Higgins JP, Li T, Deeks JJ. Choosing effect measures and computing estimates of effect. Cochrane handbook for systematic reviews of interventions. 2019:143-76.

295. Berkhof FF, Hertog NED, Uil SM, Kerstjens HAM, van den Berg JWK. Azithromycin and cough-specific health status in patients with chronic obstructive pulmonary disease and chronic cough: A randomised controlled trial. Respiratory Research. 2013;14(1):125.

296. Hodgson D, Anderson J, Reynolds C, Oborne J, Meakin G, Bailey H, et al. The effects of azithromycin in treatment-resistant cough: A randomized, double-blind, placebo-controlled trial. Chest. 2016;149(4):1052-60.

297. Guler SA, Clarenbach C, Brutsche M, Hostettler K, Brill AK, Schertel A, et al. Azithromycin for the treatment of chronic cough in idiopathic pulmonary fibrosis a randomized controlled crossover trial. Annals of the American Thoracic Society. 2021;18(12):2018-26.

298. Cameron EJ, Chaudhuri R, Mair F, McSharry C, Greenlaw N, Weir CJ, et al. Randomised controlled trial of azithromycin in smokers with asthma. European Respiratory Journal. 2013;42(5):1412-5.

299. Fraser SD, Thackray-Nocera S, Shepherd M, Flockton R, Wright C, Sheedy W, et al. Azithromycin for sarcoidosis cough: An open-label exploratory clinical trial. ERJ Open Research. 2020;6(4):1-7.

300. Martin MJ, Lee H, Clayton C, Pointon K, Soomro I, Shaw DE, et al. Idiopathic chronic productive cough and response to open-label macrolide therapy: An observational study. Respirology. 2019;24(6):558-65.

301. Wu Z, Spencer LG, Banya W, Westoby J, Tudor VA, Rivera-Ortega P, et al. Morphine for treatment of cough in idiopathic pulmonary fibrosis (PACIFY COUGH): a prospective, multicentre, randomised, double-blind, placebo-controlled, two-way crossover trial. The Lancet Respiratory Medicine. 2024.

302. Reynolds JE, Jetté ME, Wright ML, Sundar KM, Gillespie AI, Slovarp LJ. Normative values for the leicester cough questionnaire in healthy individuals. Annals of Otology, Rhinology & Laryngology. 2023;132(6):705-8.

303. Faruqi S, Sykes DL, Crooks MG, Brindle K, Thompson J, Morice AH. Objective assessment of cough: an early marker of response to biological therapies in asthma? Lung. 2020;198:767-70.

304. Calverley P, Pauwels R, Löfdahl C-G, Svensson K, Higenbottam T, Carlsson L, et al. Relationship between respiratory symptoms and medical treatment in exacerbations of COPD. European Respiratory Journal. 2005;26(3):406-13.

305. van Manen MJ, Birring SS, Vancheri C, Vindigni V, Renzoni E, Russell A-M, et al. Effect of pirfenidone on cough in patients with idiopathic pulmonary fibrosis. European Respiratory Journal. 2017;50(4).

306. Cho PS, Birring SS, Fletcher HV, Turner RD. Methods of cough assessment. The Journal of Allergy and Clinical Immunology: In Practice. 2019;7(6):1715-23.

307. Do W, Russell R, Wheeler C, Javed H, Dogan C, Cunningham G, et al. Performance of cough monitoring by Albus Home, a contactless and automated system for nocturnal respiratory monitoring at home. ERJ Open Research. 2022;8(4).

308. Orsmond GI, Cohn ES. The distinctive features of a feasibility study: objectives and guiding questions. OTJR: occupation, participation and health. 2015;35(3):169-77.

309. Bowen DJ, Kreuter M, Spring B, Cofta-Woerpel L, Linnan L, Weiner D, et al. How we design feasibility studies. American journal of preventive medicine. 2009;36(5):452-7.

310. Li H, Liu D-H, Chen L-L, Zhao Q, Yu Y-Z, Ding J-J, et al. Meta-analysis of the adverse effects of long-term azithromycin use in patients with chronic lung diseases. Antimicrobial agents and chemotherapy. 2014;58(1):511-7.

311. Pomares X, Montón C, Bullich M, Cuevas O, Oliva JC, Gallego M, et al. Clinical and safety outcomes of long-term azithromycin therapy in severe COPD beyond the first year of treatment. Chest. 2018;153(5):1125-33.

312. Bogte A, Bredenoord A, Oors J, Siersema P, Smout A. Reproducibility of esophageal high‐resolution manometry. Neurogastroenterology & Motility. 2011;23(7):e271-e6.

313. Deloose E, Verbeure W, Depoortere I, Tack J. Motilin: from gastric motility stimulation to hunger signalling. Nature Reviews Endocrinology. 2019;15(4):238-50.

314. Pennathur A, Cioppi M, Fayad J, Little A. Erythromycin, motilin, and the esophagus. Surgery. 1993;114(2):295-8; discussion 8.

315. Kahrilas P, Lin S, Chen J, Manka M. The effect of hiatus hernia on gastro-oesophageal junction pressure. Gut. 1999;44(4):476-82.

316. Zhong W, Sun B, Ruan H, Yang G, Qian B, Cao H, et al. Deglycosylated azithromycin targets transgelin to enhance intestinal smooth muscle function. Iscience. 2020;23(9).

317. Elsafadi M, Manikandan M, Dawud RA, Alajez N, Hamam R, Alfayez M, et al. Transgelin is a TGFβ-inducible gene that regulates osteoblastic and adipogenic differentiation of human skeletal stem cells through actin cytoskeleston organization. Cell death & disease. 2016;7(8):e2321-e.

318. Lee E-K, Han G-Y, Park HW, Song Y-J, Kim C-W. Transgelin promotes migration and invasion of cancer stem cells. Journal of proteome research. 2010;9(10):5108-17.

319. Thompson O, Moghraby JS, Ayscough KR, Winder SJ. Depletion of the actin bundling protein SM22/transgelin increases actin dynamics and enhances the tumourigenic phenotypes of cells. BMC cell biology. 2012;13:1-10.

320. Hsieh T-B, Jin J-P. Evolution and function of calponin and transgelin. Frontiers in Cell and Developmental Biology. 2023;11:1206147.

321. Wang X, Luo J, Wang D, Liu B, Liu C. The efficacy and safety of long-term add-on treatment of azithromycin in asthma: a systematic review and meta-analysis. Medicine. 2019;98(38):e17190.

322. Wu J-j, Xu H-r, Zhang Y-x, Li Y-x, Yu H-y, Jiang L-d, et al. The characteristics of the frequent exacerbator with chronic bronchitis phenotype and non-exacerbator phenotype in patients with chronic obstructive pulmonary disease: a meta-analysis and system review. BMC Pulmonary Medicine. 2020;20:1-10.

323. Calle Rubio M, Casamor R, Miravitlles M. Identification and distribution of COPD phenotypes in clinical practice according to Spanish COPD Guidelines: the FENEPOC study. International Journal of Chronic Obstructive Pulmonary Disease. 2017:2373-83.

324. McDowell PJ, Heaney LG. Different endotypes and phenotypes drive the heterogeneity in severe asthma. Allergy. 2020;75(2):302-10.

325. Holmes J, McGarvey LP, Birring SS, Fletcher H, Heaney LG. An observational study to determine the relationship between cough frequency and markers of inflammation in severe asthma. European Respiratory Journal. 2022;60(6).

326. Cho PSP, Fletcher HV, Turner RD, Patel IS, Jolley CJ, Birring SS. The Relationship Between Cough Reflex Sensitivity and Exacerbation Frequency in Chronic Obstructive Pulmonary Disease. Lung. 2020;198(4):617-28.

327. Morice A, Brindle K, Pawsey S, Kazmi S. Electronic cough recording: How to better reflect the patient experience? European Respiratory Journal.64(suppl 68):PA2144.

328. Morice A, Alge M, Kuett L, Hart S, Rigby A, Elkayam D. Cough frequency has a high daily variation in patients with chronic cough. ERJ Open Research. 2024.

329. Birring S, Muccino D, Bacci ED, Vernon MK, Nguyen AM. Defining Minimal Clinically Important Differences (MCID) on the Leicester Cough Questionnaire (LCQ): Analyses of a Phase 2 Randomized Controlled Trial in Chronic Cough. Journal of Allergy and Clinical Immunology. 2019;143(2):AB52.

330. Jones PW. St. George's respiratory questionnaire: MCID. COPD: Journal of Chronic Obstructive Pulmonary Disease. 2005;2(1):75-9.

331. Leidy NK, Rennard SI, Schmier J, Jones MK, Goldman M. The breathlessness, cough, and sputum scale: the development of empirically based guidelines for interpretation. Chest. 2003;124(6):2182-91.

332. Kurokawa R, Kanemitsu Y, Fukumitsu K, Takeda N, Tajiri T, Nishiyama H, et al. Reflux-related symptoms reflect poor asthma control and the presence of airway neuronal dysfunction. Allergology International. 2022;71(3):318-24.

333. Zhang M, Chen Q, Dong R, Yu L, Ai Z, Xu X, et al. Prediction of therapeutic efficacy of gabapentin by Hull Airway Reflux Questionnaire in chronic refractory cough. Therapeutic Advances in Chronic Disease. 2020;11:2040622320982463.

334. Zhang M, Morice AH, Si F, Zhang L, Chen Q, Wang S, et al. Antitussive efficacy of the current treatment protocol for refractory chronic cough: our real-world experience in a retrospective cohort study. Therapeutic Advances in Respiratory Disease. 2023;17:17534666231167716.

335. Morice AH, Kitt MM, Ford AP, Tershakovec AM, Wu W-C, Brindle K, et al. The effect of gefapixant, a P2X3 antagonist, on cough reflex sensitivity: a randomised placebo-controlled study. European Respiratory Journal. 2019;54(1).

336. Tokuda A, Ohkura N, Fujimura M, Furusho S, Abo M, Katayama N. Effects of macrolides on antigen-induced increases in cough reflex sensitivity in guinea pigs. Pulmonary Pharmacology and Therapeutics. 2010;23(1):55-61.

337. Adner M, Canning BJ, Meurs H, Ford W, Ramos Ramírez P, van den Berg MP, et al. Back to the future: re-establishing guinea pig in vivo asthma models. Clinical Science. 2020;134(11):1219-42.

338. Holmes J, Heaney LG, McGarvey LP. Objective and subjective measurement of cough in asthma: a systematic review of the literature. Lung. 2022;200(2):169-78.

339. Holt KJ, Belcher J, Smith JA. Novel capsaicin cough endpoints effectively discriminate between healthy controls and patients with refractory chronic cough. Respiratory Medicine. 2023;208:107142.

340. Hilton EC, Baverel PG, Woodcock A, Van Der Graaf PH, Smith JA. Pharmacodynamic modeling of cough responses to capsaicin inhalation calls into question the utility of the C5 end point. Journal of allergy and clinical immunology. 2013;132(4):847-55. e5.

341. McGarvey L, Smith JA, Morice A, Birring SS, Chung KF, Dicpinigaitis PV, et al. A randomized, double-blind, placebo-controlled, parallel-group phase 2b trial of P2X3 receptor antagonist sivopixant for refractory or unexplained chronic cough. Lung. 2023;201(1):25-35.

342. Huddart S, Asege L, Jaganath D, Golla M, Dang H, Lovelina L, et al. Continuous cough monitoring: a novel digital biomarker for TB diagnosis and treatment response

monitoring. The International Journal of Tuberculosis and Lung Disease. 2023;27(3):221-2.

343. Chung KF, Chaccour C, Jover L, Galvosas M, Song W-j, Rudd M, et al. Longitudinal cough frequency monitoring in persistent coughers: Daily Variability and Predictability. Lung. 2024;202(5):561-8.

344. Sanchez-Olivieri I, Rudd M, Gabaldon-Figueira JC, Carmona-Torre F, Del Pozo JL, Moorsmith R, et al. Performance evaluation of human cough annotators: optimal metrics and sex differences. BMJ Open Respiratory Research. 2023;10(1):e001942.

345. Holt KJ, Dockry RJ, McGuinness K, Barrett E, Smith JA. An exploration of clinically meaningful definitions of cough bouts. ERJ Open Research. 2024;10(6).

346. Kum E, Guyatt GH, Munoz C, Beaudin S, Li S-A, Abdulqawi R, et al. Assessing cough symptom severity in refractory or unexplained chronic cough: findings from patient focus groups and an international expert panel. ERJ Open Research. 2022;8(1).

347. Dockry R, Holt K, Smith J, McGuinness K. S17 A relevant definition of cough bouts. BMJ Publishing Group Ltd; 2022.

348. Morice A, Brindle K, Pawsey S, Kazmi S. Electronic cough recording: How to better reflect the patient experience? : European Respiratory Society.

349. Smith J, Carroll K, Clark D, Molyneaux P. Nalbuphine Extended-release Reduces Cough Bouts in Patients With Idiopathic Pulmonary Fibrosis. A104 ADVANCES IN THE DIAGNOSIS AND TREATMENT OF ILD: American Thoracic Society; 2024. p. A2869-A.

350. MORICE A, CARROLL KJ, CLARK D. ANALYSIS OF RELIEF-OF-COUGH IN PATIENTS WITH IDIOPATHIC PULMONARY FIBROSIS TREATED WITH ORAL NALBUPHINE EXTENDED RELEASE. CHEST. 2024;166(4):A3495-A6.

351. Smith J, Ballantyne E, Kerr M, Mcgarvey L, Morice A, Sher M. The neurokinin-1 receptor antagonist orvepitant improves chronic cough symptoms: results from a Phase 2b trial. Eur Respir J. 2019;54(Suppl. 63):PA600.

352. Zhang M, Zhang B, Morice AH. Decoding the impact of the placebo response in clinical trials for chronic cough. ERJ Open Research. 2024;10(5).

Definitions

ACQ6: Asthma Control Questionnaire 6

AAD: Average Absolute Deviation

BAL: Bronchoalveolar Lavage

BCSS: Breathlessness, Cough, and Sputum Scale

CC: Chronic Cough

COPD: Chronic Obstructive Pulmonary Disease

CT: Computerised Tomography

CAT: COPD Assessment Test

CHS: Cough Hypersensitivity Syndrome

CI: Confidence Interval

DCI: Distal Contractile Integral

DL: Distal Latency

DLCO: Diffusion Capacity for Carbon Monoxide

ECG: Electrocardiogram

EGJOO: Esophageal-Gastric Junction Outflow Obstruction

ERs: Expiration Reflexes

FEV1: Forced Expiratory Volume in 1 Second

FEV1/FVC: Ratio of FEV1 to FVC

FVC: Forced Vital Capacity

GABA: Gamma-Aminobutyric Acid

GOLD: Global Initiative for Chronic Obstructive Lung Disease

GORD: Gastro-Oesophageal Reflux Disease

HARQ: Hull Airways Reflux Questionnaire

HACC: Hull Automatic Cough Counter

HROM: High Resolution Oesophageal Manometry

HRCT: High Resolution Computed Tomography

ICC: Intraclass Correlation Coefficient

ILD: Interstitial Lung Disease

IPF: Idiopathic Pulmonary Fibrosis

LCQ: Leicester Cough Questionnaire

LOS: Lower Oesophageal Sphincter

LOSP: Lower Oesophageal Sphincter Pressure

LHQ: Laryngeal Hypersensitivity Questionnaire

mMRC: Modified Medical Research Council

MCI: Multidimensional Cough Index

MIC: Minimal Inhibitory Concentration

MD: Mean Difference

MII-pH: Multichannel Impedance-pH

NRS: Numerical Rating Scale

NCT: Noncomparative Trials

NICE: National Institute for Health and Care Excellence

NHS: National Health Service

OGD: Oesophagogastroduodenoscopy

PPI: Protein-Pump Inhibitors

PROs: Patient-Reported Outcomes

PF-ILD: Progressive Fibrosing Interstitial Lung Diseases

RCTs: Randomised Controlled Trials

RCC: Reflux Cough

RCC: Reflux Chronic Cough

SABA: Short-Acting Beta2-Agonists

SGRQ: St George's Respiratory Questionnaire

SD: Standard Deviation

SMD: Standardised Mean Difference

TLOSR: Transient Lower Oesophageal Sphincter Relaxations

TB: Tuberculosis

UOS: Upper Oesophageal Sphincter

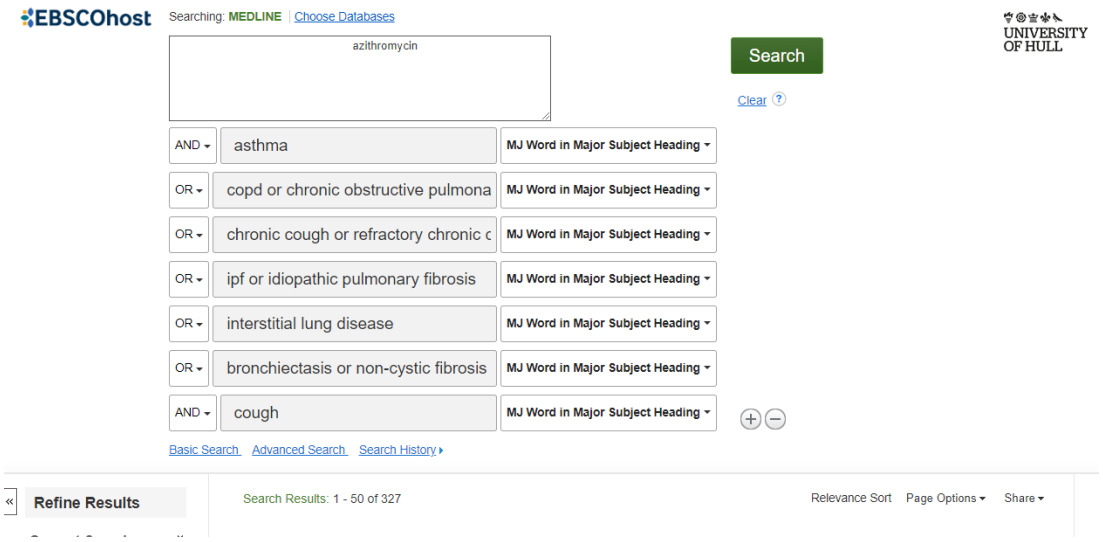
UIP: Usual Interstitial Pneumonia

VAS: Visual Analogue Scale

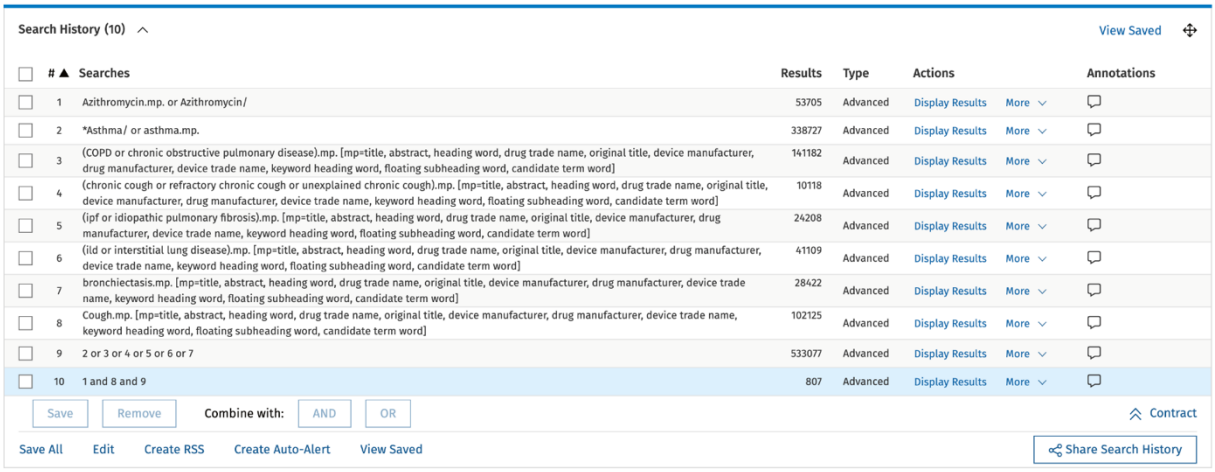
WHO: World Health Organization

WPLBs: Weak Oesophageal Peristalsis with Large Breaks

Appendices



Appendix Figure 1 – Search strategy and results for MEDLINE database



Appendix Figure 2 – Search strategy and results for EMBASE database

Appendix Figure 3 – Search strategy and results for CENTRAL database

1	azithromycin	
2	AND Asthma	
3	OR COPD OR Chronic Obstructive Pulmonary Disease	
4	OR Chronic Cough OR Refractory Chronic Cough	
5	OR IPF OR Idiopathic Pulmonary Fibrosis	
6	OR Interstitial Lung Disease	
7	Or Bronchiectasis OR Non-cystic fibrosis bronchiectasis	
8	AND Cough	327

Appendix Table 1 – Search strategy and results for MEDLINE database

1	azithromycin	53705
2	Asthma	338727
3	(COPD OR Chronic Obstructive Pulmonary Disease) .mp	141182
4	(Chronic Cough OR Refractory Chronic Cough Or Unexplained Chronic Cough) .mp	10118
5	(IPF OR Idiopathic Pulmonary Fibrosis) .mp	24208
6	(Interstitial Lung Disease OR ILD) .mp	41109
7	Bronchiectasis .mp	28422
8	Cough	102125
9	2 OR 3 OR 4 OR 5 OR 6 OR 7	533077
10	1 AND 8 AND 9	807

Appendix Table 2 – Search strategy and results for EMBASE database

1	azithromycin	
2	AND Asthma	
3	OR COPD OR Chronic Obstructive Pulmonary Disease	
4	OR Chronic Cough OR Refractory Chronic Cough	
5	OR IPF OR Idiopathic Pulmonary Fibrosis	
6	OR Interstitial Lung Disease OR ILD	
7	Or Bronchiectasis OR Non-cystic fibrosis bronchiectasis	
8	AND Cough	96

Appendix Table 3 – Search strategy and results for CENTRAL database