Objective assessment of severity of cough and gastro-oesophageal reflux disease in patients with Idiopathic Pulmonary Fibrosis and efficacy of pulsed cyclophosphamide and methylprednisolone therapy in patients with progressive interstitial lung disease

Dr Prosenjit Dutta
Student Number: 130643737

Doctor of Medicine
Institute of Cellular Medicine, Newcastle University

November 2017

Abstract

Cough is a disabling symptom in patients with Idiopathic pulmonary fibrosis (IPF). Reflux disease is frequently associated with IPF and implicated in pathogenesis of both IPF and cough.

Therefore, acid suppression by omeprazole should ameliorate cough and improve quality of life in IPF patients, which is the hypothesis underlying the planned PPIPF study. My thesis is based on the baseline assessments of cough and reflux in IPF patients recruited for the study.

45 patients with mean age of 71.2 years; 35 (77.8%) male were recruited. 24-hour cough recording at baseline showed significantly raised cough frequency (mean 11.99/hour) with impaired quality of life (mean LCQ-total score 15.22).

Reflux related health questionnaires [RSI (mean score 15.6,) and GIQLI (mean score 105.56,)] suggested impaired quality of life, possibly due to reflux disease in our study cohort [but not DeMRQ (mean score 1.16)]. Consent for GI studies and bronchoscopy was low. GI studies demonstrated oesophageal dysmotility in 4 (44%) and acid reflux in 6 (67%) out of the 9 IPF patients, who completed the assessment. BAL samples showed leucocytosis in all 8 participants with bacterial growth on cultures in 2 participants.

Additionally, I undertook a case-series study to assess the outcome of pulsed cyclophosphamide and methyl-prednisolone therapy in patients with progressive interstitial lung disease (ILD) in our institute.

Medical records of 53 patients with mean age of 60 years; 29 (55%) male were reviewed. The median number of cyclophosphamide pulses received was 6. The average rate of change of lung function was significantly less after cyclophosphamide therapy both for FVC ($p=0.0004$) and TLco ($p=0.00015$). In our single centre, retrospective study pulsed cyclophosphamide and methyl-prednisolone was associated with stabilisation of lung function in a mixed cohort of patients with progressive ILD. Adverse events were common but transient and managed with dose reduction and/or delayed schedule.

Acknowledgements

I would like to acknowledge and thank everybody for his or her continued help and support. Without them the conduction of the study projects and my thesis would not have been possible.

I am indebted to my supervisors Professor John Simpson, Dr Ian Forrest, Dr Chris Ward, Institute of Cellular Medicine (ICM), Newcastle University for their advice, support and feedback with all my study projects. They designed the PPIPF study, obtained research funding and helped me with interpretation of results and reviewed my manuscripts. British Lung Foundation (BLF) funded the PPIPF study. Dr Ian Forrest performed bronchoscopy during the early phase of the study (to teach specific principles of research bronchoscopy). In addition, Prof Simpson, Dr Forrest helped me with designing the Cyclophosphamide study, interpreting and presenting results at academic meetings. My sincere thanks to Dr Wendy Funston, Clinical Research Fellow for help with continued recruitment and conduction of the PPIPF study (following my return to compulsory clinical training).

I am grateful to Vicky Ryan (senior trial statistician) and Helen Mossop (trial statistician) from Biostatistics Research Group, Institute of Health and Society, Newcastle University. Their help with setting up of online randomisation website, data management (via secure websites) for the PPIPF study have been invaluable for conduction of the study. They also helped in generating mandatory trial progress reports for the trial regulatory committees. My thesis is based on the baseline "raw" data they kindly provided (reproduced from the secure website where I have previously uploaded the data collected during study visits).

Further, I express my gratitude to Dr Bridget Griffiths, Consultant Rheumatologist in Freeman Hospital for her input in planning and carrying out the Cyclophosphamide project. I would also like to thank Ms Karen Walker, Rheumatology specialist nurse

for help with the list of names and prescription of the patients who received Cyclophosphamide therapy.

I am grateful to Dr Sylvia Worthy and Dr Anna Beattie (Consultant Thoracic Radiologist, Royal Victoria Infirmary) for their review of the chest CT scans of patients for both PPIPF and Cyclophosphamide studies.

I am grateful to Dr Peter Avery, Senior Lecturer, Institute of Mathematics and Statistics at Newcastle University for his help and support with the interpretation of results of the cyclophosphamide study. Without his advice poster publication of the cyclophosphamide study would not have been possible.

Chest OPD team in RVI: My sincere thanks to the Chest Clinic nursing staff and the receptionists for their help and support especially for their work in locating and collecting notes, arranging appointments for the research study. I have spent huge time in the clinic for screening, calling potential patients and actually conducting the study. Additionally, thanks to the lung function physiologists for their help in performing the lung function tests on the study patients.

Endoscopy Unit in RVI: I would like to thank Mr Rhys Jones (Surgical Registrar and Upper GI clinical research fellow), Rachel Colver (Specialist upper GI nurse) and Gillian (Health Care Assistant) for their support with the GI physiology study. I learnt to perform and report the test from Mr Rhys Jones. With help from Rachel and Gillian I have performed the GI physiology tests. Their continued support (specially with the mandatory administrative paperwork) with the GI procedures is much appreciated. Additionally I would like to thank the nurses in the bronchoscopy unit for their assistance with the research bronchoscopy procedures.

Sir William Leech Research Center in Freeman Hospital: I am grateful to Dr Gail Johnson and Kasim Jiwa, clinical scientists in the Sir William Leech Research Center

for their training in processing of BAL fluid samples. I can independently perform total and differential counts on BAL samples. I am thankful for Kasim's continued support with storage of the BAL samples.

NCTU: The NCTU monitored the trial. I would like to thank Ms Jessica Qian (Trial Manager), Dr Jennifer Wilkinson (Senior Trial Manager) for their continuous advice and guidance on conduction of the study. I am grateful to Mr Mark Palmer and Dr Leslie Hall who took over from Jessica (on maternity leave from March 2015) and Jennifer (seconded to other study projects) respectively for their support and assistance with my trial study. Additionally for the painstaking work with the trial master file and the investigator site file, I would like to thank Rebecca Forbes, Trial Manager (she took over from Mark) and Jared Thornton, Senior Trial Manager, (who took over from Leslie). They also organised the compulsory trial meetings for which I am obliged.

Secretaries: My sincere thanks to Ms Linda Ward (secretary to Prof Simpson), Ms Joanna McNichol, Ms Wendy Fairbairn (secretary to Dr Forrest and secretary for the ILD services) for their assistance with patient appointments, clinical notes and clinic/research letters.
I would also like to thank Ms Carole Fairless, secretary to Dr Griffiths for her help with regular collection of notes for the Cyclophosphamide audit.

Abbreviations

ACEi – Angiotensin converting enzyme inhibitors

AEC – Alveolar epithelial cell

ALP – Alkaline phosphatase

ALT – Alanine transaminase

ANA – Antinuclear antibody

ATS – American Thoracic Society

BAL – Bronchoalveolar lavage

BDNF – Brain derived neurotropic factor

BLF – British Lung Foundation

BMI – Body mass index

COPD – Chronic obstructive pulmonary disease

CRF – Case report forms

CTA – Clinical Trials Authorisation

CTD – Connective tissue disease

CTD-ILD – Connective tissue disease related-interstitial lung disease

CTIMP – Clinical Trial of an Investigational Medicinal Product

CTU – Clinical Trials Unit

DeMRQ – DeMeester Reflux associated Questionnaire

DMSC – Data and Safety Monitoring Committee

dsDNA – Double-stranded DeoxyriboNucleic acid antibody

ECG – Electrocardiogram

EMT – Epithelial-mesenchymal transition

FXa – Activated factor X

FEV1 – Forced expiratory volume in 1 second

FVC – Forced vital capacity

GGT – Gamma-glutamyl transferase

GI – Gastro-intestinal

GIQLI – Gastro-Intestinal Quality of Life index Questionnaire

GORD – Gastro-oesophageal reflux disease

GP – General practitioners

HRCT – High resolution computer tomography

IIP – Idiopathic interstitial pneumonia

ILD – Interstitial lung disease

ILD-MDT – Interstitial lung disease multidisciplinary team

IPF – Idiopathic Pulmonary Fibrosis

IV – Intravenous

Kco – Transfer factor coefficient of lung for carbon monoxide gas

LCQ – Leicester cough questionnaire

LOS – Lower oesophageal sphincter

MHRA – Medicine and Healthcare products Regulatory Agency

MID – Minimal important difference

NCTU – Newcastle Clinical Trials Unit

NGF – Nerve growth factor

NSIP – Non specific interstitial pneumonia

NUTH – The Newcastle upon Tyne Hospitals Foundation Trust

PAI – Plaminogen activator inhibitor

PARs – Proteinse-activated receptors

PDGF – platelet-derived growth factor

PIC – Participant Identification Center

PIS – Participant Information Sheet

PPI – Proton pump inhibitor

RCT – Randomised controlled trial

REC – Research Ethics Committee

RSI – Reflux Symptom Index Questionnaire

RVI – Royal Victoria Infirmary, Newcastle

SAP – Symptom-associated probability

SmPC – Summary of product characteristics

TERC – Telomerase RNA

TERT – Telomerase reverse transcriptase

TF – Tissue factor

TGF-β – Transforming growth factor-β

TLco – Transfer factor of lung for carbon monoxide gas

TLOSRs – Transient lower oesophageal sphincter relaxations

TMG – Trial Management Group

TNF-α – Tumour necrosis factor-α

TSG – Trial Steering Group

UIP – Usual interstitial pneumonia

VC – Vital capacity

ZES – Zollinger-Ellison syndrome

Table of Contents

List of tables

List of figures

Chapter 1. Background to the Thesis

My thesis is based on the findings from 2 projects common to the theme of interstitial lung disease (ILD).

The first relates to a single-centre randomised double-blinded placebo-controlled pilot trial of the proton pump inhibitor (PPI) omeprazole in idiopathic pulmonary fibrosis (IPF) (or the PPIPF study). The British Lung Foundation (BLF) funded the study. Professor John Simpson (Professor of Respiratory Medicine, Newcastle University) was the Chief Investigator; Dr Ian Forrest (Consultant Respiratory Physician, The Newcastle upon Tyne Hospitals NHS Foundation Trust) was the Principal Investigator; Dr Chris Ward (Senior Lecturer in Respiratory Medicine, Institute of Cellular Medicine, Newcastle University) and Professor Jacky Smith (Professor of Respiratory Medicine, University of Manchester) were Co-Investigators. I co-ordinated the trial in my role as a BLF Clinical Research Fellow.

The second project was a retrospective study to assess the efficacy of pulsed cyclophosphamide and methylprednisolone therapy in patients with progressive ILD.

The two projects were common to my interest in ILD and addressed the relative lack of effective therapies. Working on these two parallel projects provided the opportunity to broaden my research experience while improving my understanding of advanced management of patients with ILD.

Complying with the mandatory research regulations and initial teething problems of setting up a Clinical Trial of an Investigational Medicinal Product (CTIMP) meant delay in starting recruitment for the randomised controlled trial (RCT). Recruitment started in March 2014 (almost 6 months later than planned). In spite of several measures put in place to improve recruitment of participants to the PPIPF study this remained slower than expected (the initial plan was to recruit 60 participants over a period of 21 months). Therefore the decision was made to recruit for the study over an

extended period (until August 2016). In July 2015, I returned to my mandatory clinical training in Respiratory Medicine. Dr Wendy Funston, Clinical Research Fellow, kindly led further recruitment for the PPIPF study after I returned to clinical training. Being mindful of the MD programme timeline, my thesis is based on the baseline assessment of cough and reflux in patients with IPF, plus the cyclophosphamide study. In total 45 patients were recruited to PPIPF (last patient recruited in July 2016). The original intention was to recruit a minimum of 40 patients with complete data relating to the primary outcome measure, and this was achieved.

Additionally, in keeping with the principles of a double-blinded clinical trial, the trial statisticians and the trial managers of the Newcastle Clinical Trials Unit (NCTU) managed data collected during study period/visits. At the time of writing, all the participants have completed their study visits and data is being collated. Subsequent analysis can be performed only after further mandatory checks confirm satisfactory adherence to data collection regulations.

Chapter 2. Introduction: Idiopathic Pulmonary Fibrosis (IPF)

2.1 IPF: Background and pathogenesis

IPF is the most common type of idiopathic interstitial pneumonia (IIP) (Bradley *et al*., 2008; Raghu *et al*., 2011; Richeldi *et al*., 2017). In this condition, due to an as yet unknown aetiology, wide areas of healthy lungs are replaced by fibrotic tissue, which makes oxygen extraction difficult during respiration. Although the disease course is unpredictable, it tends to be chronically progressive with a median survival of 2-5 years from time of diagnosis (Richeldi *et al*., 2017). Patients predominantly suffer from breathlessness (on exertion and/or at rest) and cough, which can be debilitating. Till date there is currently no cure. Lung transplantation can improve survival but only a few patients are eligible given the complicated nature of the procedure and the subsequent follow up treatment regime. In recent years, two disease-modifying drug therapies (pirfenidone and nintedanib) have been approved for the treatment of mild to moderate IPF (Arai *et al*., 2013; King *et al*., 2014; Richeldi *et al*., 2014). Both drugs reduce the rate of decline in lung function in patients with IPF over a period of 1 year but have little impact on symptoms of cough and breathlessness, which can cause significant impairment of quality of life. Hence there is continued focus on symptom-based treatment in patients with IPF. Cough associated with IPF is extremely difficult to manage and new approaches are required (Chung and Pavord, 2008; Dicpinigaitis, 2008; Woodcock *et al*., 2010; Birring, 2011).

2.1.1 Pathogenesis of IPF

IPF is not a common medical problem. According to the British Thoracic Society ILD Registry programme's annual report of 2013/2014, it is estimated that there are 5000 new cases of IPF every year in the UK or an incidence rate of 7 – 9 per 100,000 (the true incidence is not known). Prevalence was estimated to be 15 – 25 per 100,000 but increases with age. About 5000 patients with IPF die per annum in the UK probably accounting for the low recorded prevalence rate.

IPF more commonly affects males and they present mostly between the ages of 50 – 70 years (the median age at diagnosis is 65 years) (Richeldi *et al*., 2017).
The diagnosis of IPF is made on the basis of a distinct radiological and histological pattern of "usual interstitial pneumonia" (UIP) in the absence of other identifiable causes (King *et al*., 2011; Chung *et al*., 2015). High resolution computer tomography (HRCT) scans show characteristic reticular changes with secondary bronchial dilatation predominantly in the basal, peripheral and sub-pleural regions of the lungs. In advanced cases, clusters of cystic airspaces (typically 3 – 10mm in diameters) in between the interlobular septae known as "honeycombing" are seen, mainly at the basal and sub-pleural regions. These characteristic features in HRCT scans, in association with typical signs and symptoms, have been accepted world wide as diagnostic of IPF in day-to-day clinical practice (Richeldi *et al*, 2017). Currently, routine surgical lung biopsy (previously regarded as the diagnostic gold standard) is not advocated except in the presence of non-diagnostic or atypical HRCT scan and/or clinical features.

The two HRCT scan images (Figure 1A and Figure 1B) below illustrate the radiological features of IPF:

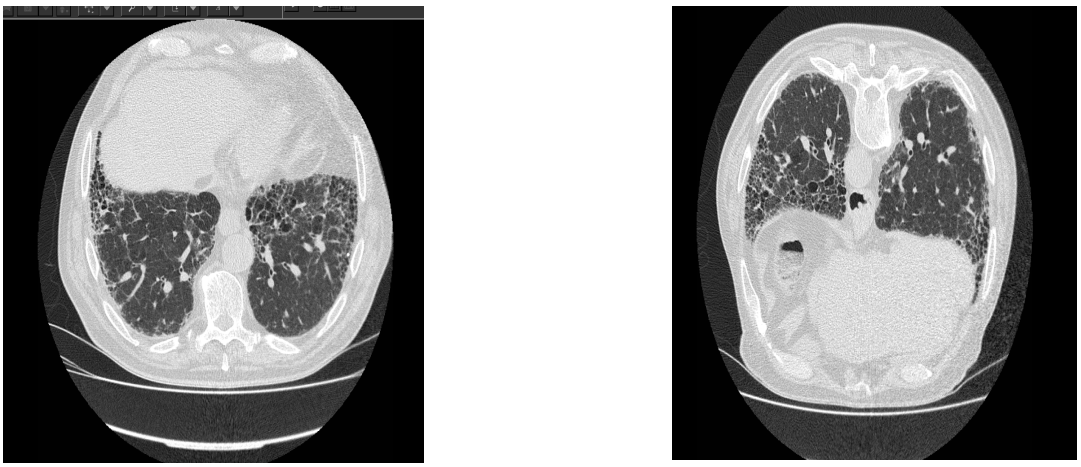


Figure 1: HRCT features of UIP
(A) supine, inspiratory scan of the lung bases. (B) prone, inspiratory scan of the lung bases.

Figure 1A is an HRCT image of a patient in the supine position. It shows changes of fibrosis bilaterally at the bases of the lungs in peripheral and sub-pleural areas associated with honeycombing changes, as is typically seen in IPF. Figure 1B is an HRCT image from the same patient in the prone position. It shows persistent changes with change of position (anonymised images obtained from an HRCT scan of a patient with IPF under hospital follow up).

The pathogenesis of fibrosis in IPF is not entirely understood but it involves a complex interaction between multiple inherent physiological and pathological processes. Previously, chronic inflammation of the lung leading to gradual fibrosis as a mechanism was proposed. However recent research suggests repeated alveolar epithelial injury in an aging lung (or in a genetically predisposed individual) in association with aberrant tissue repair leads to fibrosis in the lungs (King *et al*., 2011; Richeldi *et al*., 2017).

Two types of alveolar epithelial cells (AEC) line the lung alveoli. AEC type 1 are the primary lining cells and cover up to 90% of the alveolar surface (King *et al*., 2011). They help with gas exchange during respiration. Type 2 AECs secrete surfactants and also help in renewal of type 1 AECs during homoeostasis and after lung injury. Damage or injury to the type 2 AECs caused by repetitive or persistent environmental insult(s) play a prominent role in the pathogenesis of IPF. Abnormal AEC2s and loss of AEC1s lead to dysregulated tissue repair and excess collagen production leading to fibrosis in the lungs.

Repetitive lung injury causes damage and/or apoptosis to both AEC1 and AEC2 cells. Post injury AEC2 cells proliferate and migrate to attempt repair tissue damage. However, in patients with IPF, there is abnormal activation of AEC2 cells, which lead to increased secretion of a host of fibrosis-promoting growth factors and chemokines including transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), tumour necrosis factor-α (TNF-α) (King *et al*., 2011). These chemicals promote migration, proliferation of fibroblasts and differentiation to myofibroblasts

[derived from residential mesenchymal cells, bone marrow derived fibrocytes, lung interstitium pericytes, circulating fibrocytes, epithelial-mesenchymal transition (EMT) and endothelial-mesenchymal transition (Richeldi *et al*., 2017)]. In particular, activation of latent TGFβ1 promotes EMT and differentiation of fibroblasts to myofibroblasts (King *et al*., 2011). EMT is the process by which epithelial cells transform and are capable of migration and secretion of extracellular matrix (acquire properties similar to mesenchymal cells), thereby increasing the number of fibroblasts and myofibroblasts at the site of alveolar injury. Activated myofibroblasts synthesise and deposit excess amounts of extracellular matrix (mainly type 1 collagen) contributing to fibrosis.

In parallel, to abnormal extracellular matrix deposition, aberrant wound healing/repair leads to what is known as 'bronchiolisation of alveolar tissue'. At the site of epithelial injury, aberrant repair process (due to deregulation of developmental pathways) leads to abnormal re-epithelialisation resulting in bronchiolisation of the alveolar space (King *et al*., 2011).

Regeneration of AEC2 cells is impaired in IPF, leading to loss of AEC1 cells further preventing normal epithelialisation of damaged alveoli.

In addition, both the extrinsic and intrinsic coagulation pathways are also activated in IPF leading to activation of Factor X, which promotes fibrosis, by stimulation of fibroblasts. Coagulation and fibrosis play a crucial role in homeostasis in health. An imbalance between coagulation and fibrinolysis have been demonstrated within the alveolar space in experimental animal models of fibrosis (Crooks and Hart, 2015). The risk of IPF associated with at least one prothrombotic state is significantly high (OR=4.78) and is associated with increased mortality (Navaratnam *et al*., 2014). Tissue injury leads to increased release of tissue factor (TF) from AEC2 cells, alveolar macrophages (and endothelial cells), which on exposure to plasma forms a TF-factor VIIa complex. This complex, ultimately leads to activation of factor X (FXa), which in association with activated factor Va leads to activation of thrombin and formation of a clot by converting fibrinogen to fibrin. The initial TF-factor VIIa-FXa complex also activates factor IX, factor VIII, factor XI thereby triggering the

intrinsic coagulation cascade leading to persistent production of thrombin and sustaining coagulation. Increased levels of TF and Plasminogen activator inhibitor (PAI, an inhibitor of plasmin) have been demonstrated in the BAL fluid from patients with IPF, suggesting activation of coagulation and inhibition of fibrinolysis (Chambers and Scotton, 2012; Crooks and Hart, 2015).
The cellular response of activated coagulation pathway is chiefly mediated by proteinases in conjunction with a family of proteinase-activated receptors (PARs), comprising of four members, PAR1 to PAR4 (Chambers and Scotton, 2012). Thrombin and factorVIIa-FXa complex are potent activators of PAR1. Increased expression of PAR1 is seen on epithelial cells, alveolar macrophages and fibroblasts in patients with IPF. Activation of PAR1 leads to increased release of inflammatory and fibrotic mediators like TNF, TGFβ, PDGF and PAI. PAI inhibits fibrinolysis while TGFβ, PDGF promote proliferation and differentiation of myofibroblasts. PAR1 stimulation also promotes collagen production by lung fibroblasts and their differentiation to myofibroblasts (Chambers and Scotton, 2012).

Therefore, an abnormal epithelial cell (mainly AEC2) in association with abnormal extracellular matrix perpetuates dysregulated wound healing mechanism, which results in aberrant lung re-modelling. Re-epithelialisation of damaged alveolar epithelium is aberrant (in the absence of AEC1) and the rate of collagen deposition exceeds degradation, resulting in lung fibrosis, destruction of alveolar architecture and impairment of gas exchange during respiration.

The microscopic features support the proposed new theory of repetitive epithelial injury in pathogenesis of IPF. Histology shows “typical UIP pattern” with temporal and spatial heterogeneity within the lungs. This means areas of normal lung alternate with areas of interstitial fibrosis, lung destruction and honeycombing in varying severity. An aggregation of fibroblasts and myofibroblasts within the extracellular matrix called “fibroblastic foci” are found adjacent to the fibrotic tissues which represent areas of active fibrosis. Fibroblastic foci is a diagnostic feature for histopathological diagnosis of UIP fibrosis.

Figure 2 demonstrates the histological features of UIP pattern essential for diagnosis of IPF

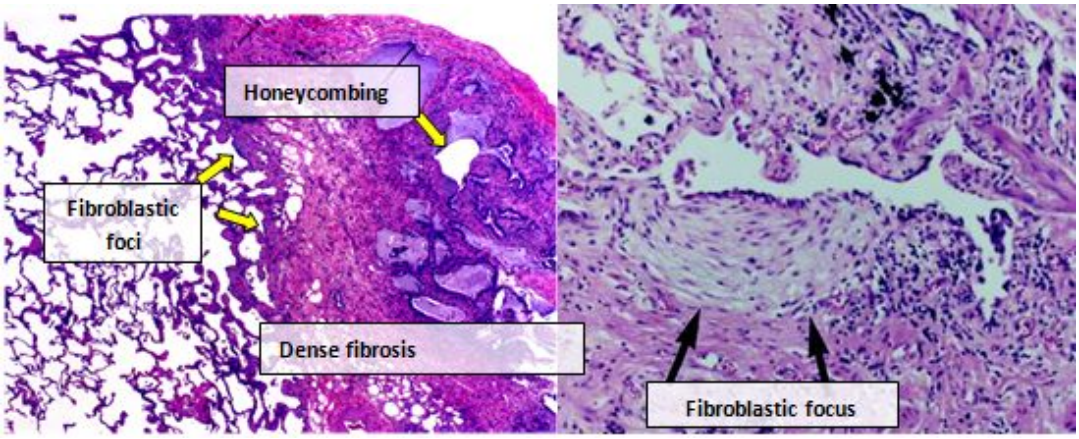


Figure 2: Histological features of UIP pattern fibrosis.
The picture on the left points to areas of fibrosis, honeycombing changes and fibroblastic foci. The image on the right demonstrates a fibroblastic focus at higher magnification. (Picture uploaded from Wikimedia.org after Internet search on google.co.uk)

Mutations in the surfactant protein C gene (SFTPC) and protein A2 gene (SFTPA2) have been reported in some cases of familial pulmonary fibrosis (where two or more members of the same family have been affected) (Garcia C. K., 2011). These proteins are solely expressed by AEC2 cells and mutations in the genes result in accumulation of abnormal proteins which can cause persistent epithelial injury.

Terminal bronchi express MUC5B gene, which encodes for mucin 5B, a precursor protein that helps in mucus production. A common variation in the MUC5B gene promoter region has been implicated as a genetic risk factor in the development of familial as well as sporadic cases of IPF. Overexpression of MUC5B lead to increased protein concentration causing impaired mucociliary clearance, retention of particles and repetitive epithelial injury. Alternatively, mucin play a vital role in innate immunity and immune dysregulation contributes to the development of lung fibrosis (Kaur *et al*., 2017).

Telomerase is an enzyme which promotes the addition of nucleotide to the terminal ends of the chromosomes. Telomerase reverse transcriptase (TERT) in association with telomerase RNA (TERC) preserve and maintain telomerase enzyme. Mutations

in the TERT and TERC genes have been demonstrated predominantly in familial pulmonary fibrosis (heterozygous mutation in TERT and/or TERC reported in approximately 15% of patients with familial pulmonary fibrosis). TERT mutation was the commonest genetic defect seen in familial pulmonary fibrosis (Garcia C. K., 2011). 40% of TERT mutation carriers reported some symptoms of pulmonary fibrosis by the mean age of 51 years and died at an early age (average age at death for males 57.7 years and females 66.6 years). Mutations in TERT/TERC genes were also associated with other symptoms like aplastic anaemia, cirrhosis and premature greying. The pathogenesis of fibrosis is proposed to be due to progressive shortening of telomere lengths (to successive cell division) and premature consumption of progenitor cells, thereby compromising epithelial repair after tissue injury (Garcia C. K., 2011; Kaur *et al*., 2017).

Interestingly, restricted telomere lengths have been demonstrated in a few patients with familial and sporadic IPF, in the absence of mutations in telomerase. Cronkhite et al. (2008) found short telomere lengths (less than 10 percentile when compared to healthy adults) in 24% and 23% of patients with familial and sporadic pulmonary fibrosis respectively, with no mutations in TERT or TERC genes. Radiological features consistent with IPF are often noted on CT scans of asymptomatic individuals, especially over the age of 75 years (King *et al*., 2011). Progressive shortening of telomeres has also been advocated as one of the possible mechanism in age-linked pulmonary fibrosis.

Although growing evidence suggests genetic predisposition increases the risk of development of IPF no genetic factors have shown direct cause-effect relationship, especially in cases of sporadic IPF.

Multiple environmental agents, namely cigarette smoking, exposure to organic/inorganic dust (metal and wood dusts, stone, silica) and viral infections (e.g. Epstein Barr Virus, Cytomegalovirus, Human Herpes Viruses 7 and 8) (Vannella *et. al.* 2008) have been implicated in the initiation and/or perpetuation of alveolar damage leading to pulmonary fibrosis. However no agent(s) have been consistently associated with development of IPF to establish a causal relation.

In recent years, the role of gastro-oesophageal reflux disease (GORD) and micro-aspiration in the development of lung fibrosis has generated interest (Fahim *et. al*., 2011; Raghu and Meyer, 2012; Lee *et al*., 2013). Recurrent aspiration of stomach and bile acids causing "repetitive alveolar cell injury" leading to progressive fibrosis has been proposed as a possible mechanism in the pathogenesis of IPF. This is discussed in further details in section 2.3.

2.2 IPF and cough

73-86% of patients with IPF suffer from cough (Crystal *et al*., 1976; Turner-Warwick *et al*. 1980; Key *et al*., 2010). It can be a presenting and a complicating feature of IPF. Cough is often non-productive and persistent with significant impact on quality of life (both social and physical). Key et al. (2010) objectively measured cough rates in patients with IPF and investigated the association between the objective and subjective measures of cough. Their study showed significantly higher cough counts in IPF patients (median cough rate of 9.4 per hour) compared to asthmatics and healthy volunteers (cough rate of less than 1 per hour). The study confirmed that cough is a major disabling symptom, with a strong correlation between objective cough assessment and cough-related quality of health measures in patients with IPF.

Physiologically, cough is a protective reflex, which helps clear mucus, secretions and noxious substances from the airways (McGarvey *et al*., 2007). It is mediated by the afferent vagus nerve, which mainly innervate the central and proximal airways (Kilduff *et al*.,2014). Studies have shown heightened cough reflex sensitivity (measured by the concentration of inhaled capsaicin needed to induce 2 – 5 coughs) in patients with IPF (Doherty *et al*., 2000). This suggests up-regulation of sensory c fibres in the airways, which are sensitive to chemical stimulation. Similar increase in sensitivity of the cough reflex has also been reported in patients without IPF who have chronic cough and reflux disease.

Patients with IPF express increased levels of neurotrophins in their lungs (Harrison *et al*., 2013). Induced sputum from IPF patients contains higher concentrations of nerve

growth factor (NGF) and brain-derived neurotropic factor (BDNF) compared to normal controls (Hope-Gill *et al*., 2003). Bronchoalveolar lavage (BAL) fluid from IPF patients contains higher levels of NGF than healthy controls (Jones R. M., 2012; Harrison *et al*., 2013). A single immunological study found enhanced expression of NGF and TrKA (neurotrophic tyrosine kinase receptor type 1) in the lungs of patient with IPF compared to other interstitial lung diseases (Ricci *et al*., 2007). The fibroblastic foci in particular showed immune staining for BDNF and TrKB (neurotrophic tyrosine kinase receptor type 2). Data show NGF can enhance both cough and airway obstruction via a mechanism that involves the activation of the TrKA receptor and TRPV1 (Transient Receptor Potential Vanilloid – 1) (Caterina *et al*., 1997; Caterina and Julius, 1999; Groneberg *et al*., 2004).

Treatment of cough in IPF remains difficult. Current practice involving opiate-based anti-tussives (like codeine or low dose slow release morphine) has limited therapeutic benefits (Pavord and Chung, 2008). In a small uncontrolled, open label study, high dose steroids for a month were shown to reduce cough sensitivity to capsaicin and cough symptom score suggesting that cough in IPF should be amenable to pharmacological therapy. In another open-labelled trial of oral interferon-α, five of 20 patients reported improvement in cough symptoms (Lutherer *et al*., 2011). However, these are small open label studies and it is well known that cough as a symptom is highly influenced by placebo effect. Gabapentin has shown promise in refractory chronic cough, improving cough-specific quality of life compared to placebo after 8 weeks of therapy (Ryan *et al*., 2012). However, patients in the study were chronic coughers with no active respiratory disease (i.e. IPF) or infection. Also, side effects occurred in 31% of patients, including nausea, fatigue and dizziness.

A recently concluded double-blinded, two-treatment, two-period cross over trial comparing thalidomide with placebo showed beneficial effects on cough and quality of life as determined by questionnaires and visual analogue scale in IPF (Horton *et al*, 2012). However study participants represented a small number of self-referred patients with mild IPF, who had other possible causes of cough (70% had reflux

disease and 30% were on angiotensin converting enzyme inhibitors (ACEi) for hypertension). In addition the side effect profile of thalidomide included constipation, dizziness, drowsiness, increased risk of infection and peripheral neuropathy, and hence thalidomide cannot be regarded as an ideal therapeutic option.

Thus there is a need for better treatments for cough, which can only be developed through better understanding of the pathogenesis of cough in IPF (interestingly IPF, despite being a disease in the peripheries of the lung, causes disabling cough, a reflex typically mediated by nerves which are predominantly centrally located).

2.3 IPF and Gastro-oesophageal reflux disease (GORD)

Recurrent aspirations secondary to gastro-oesophageal reflux have been postulated in the pathogenesis of IPF by perpetuating epithelial injury. As stated previously, the cough receptors are predominantly located proximally and the pathological changes in the lungs in IPF are predominantly peripheral. This raises a strong possibility of a link between gastro-oesophageal reflux disease, cough and IPF.

2.3.1 Gastro-oesophageal reflux disease

Reflux of gastric contents into the oesophagus can be a physiological event (Bredenoord *et al*, 2013). In healthy adults reflux episodes can occur through out the day but are mainly postprandial.

GORD disease is defined as reflux that causes distressing symptoms (typically heartburn and regurgitation) with or without mucosal injury to the oesophagus (Bredenoord *et al.,* 2013). Patients with GORD often show oesophageal erosion, ulceration or intestinal metaplasia at endoscopy. However, by definition oesophageal lesions are not essential for a diagnosis of GORD and this subgroup of patients are generally said to suffer from non-erosive reflux disease.

"The anti-reflux barrier" prevents reflux. It consists of three major components:

1. The crural diaphragm
2. The lower oesophageal sphincter (LOS)

3. The “anatomical flap valve”

The right crux of the diaphragm forms a sling that surrounds the distal oesophagus, creating a teardrop shaped hiatal canal. This structure serves as an extrinsic sphincter and reinforces the high-pressure zone of the lower oesophageal sphincter.

The LOS is a 3-4 cm segment of tonically contracted circular smooth muscle at the distal end of the oesophagus (manometry reveals this to be a high pressure area). It is also known as the “intrinsic sphincter”. In healthy individuals the resting tone of the LOS may vary from 10 – 35 mm Hg relative to intragastric pressure. The most common mechanism for reflux is transient lower oesophageal sphincter relaxations (TLOSRs). These are mediated by the vagus nerve in response to gastric distension to enable gas venting from the stomach. These are independent of swallows. On average, a TLOSR lasts for about 20 sec., which is significantly longer than the typical swallow-induced relaxation.

In healthy individuals, the oesophagus enters the stomach at an acute angle called the angle of His. The “anatomical flap valve” is present at the oesophago-gastric junction and its primary functions are to maintain the angle of His and to retain the terminal part of the oesophagus in the abdomen.

Both the intrinsic and extrinsic sphincters are weakened by disruption of the flap valve and migration of the LOS above the crural canal, contributing to reflux. Severe reflux disease occurs when the LOS is permanently displaced proximally above the diaphragm and swallow-associated reflux from the hiatal sac impairs oesophageal clearance. Endoscopically, the flap valve can be inspected and graded with the Hill classification (as shown in Figure 3).

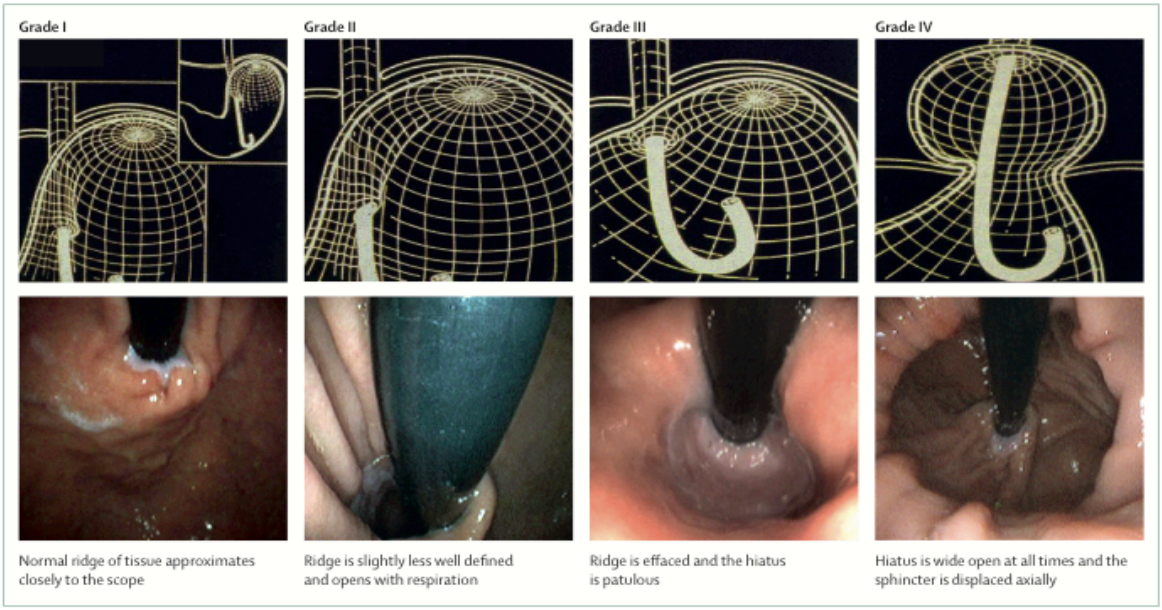


Figure 3. Progressive anatomical disruption of the gastro-oesophageal junction as it relates to the flap valve anti-reflux barrier.
Upper panels: 3-D endoscopic anatomy with endoscope retroflexed. Lower panels: endoscopic manifestations of the flap valve grade (Figure adapted from Bredenoord et al. Lancet 2013; 381:1934).

Peristaltic dysfunction of the oesophageal body can predispose to reflux disease. Failed peristalsis and hypotonic peristaltic contractions can both result in incomplete emptying and prolonged mucosal exposure to refluxate. An extended period of acid exposure is known to cause oesophagitis and Barrett's oesophagus.

The "acid pocket" refers to a layer of unbuffered gastric acid that sits on top of the meal in the postprandial period. It is close to the gastric cardia and is facilitated by the absence of peristaltic contraction in the proximal stomach (and hence the potential to reflux easily). In patients with reflux disease, the acid pocket is located more proximally and could even extend beyond the manometrically located LOS.

Physiological or pathological conditions that chronically increase intra-abdominal pressure augment the risk of reflux disease. The gastro-oesophageal pressure gradient is amplified in pregnancy, cough and obesity and plays a pivotal role in associated reflux symptoms.

Thus signs and symptoms of reflux can occur secondary to distal oesophageal acid exposure when frequency and volume of reflux is excessive or the oesophageal mucosa is hypersensitive and/or injured.

2.3.2 IPF and gastro-oesophageal reflux disease

GORD is detected in 10-20% of healthy western population (Fahim *et al.*, 2011). GORD is a known cause of persistent cough (McGarvey *et al.*, 2007; Chung and Pavord, 2008). A diagnosis is usually made after resolution of the symptoms following treatment with high dose anti-acid therapy, usually by a proton pump inhibitor (PPI) like omeprazole or lansoprazole.

GORD has been associated with a number of chronic respiratory conditions. 62% of patients with severe chronic obstructive pulmonary disease (COPD) demonstrated pathological reflux disease in comparison to 19% of healthy controls (Casanova *et al*., 2004). In a study by Terada et al., (2008), approximately 27% of COPD patients reported reflux symptoms compared to 12% of healthy volunteers and presence of reflux symptoms were significantly associated with exacerbation of COPD ($p<0.01$). Subsequently, Benson et al., (2015) also demonstrated a high prevalence of reflux disease in a large cohort of COPD patients (n=2135) and reflux symptoms with or without antacid therapy was associated with increased risk of exacerbation of COPD and hospitalisation (HR=1.58, 95% CI=1.35-1.86).

Similarly, both symptomatic and silent reflux disease has been demonstrated in patients with non cystic fibrosis bronchiectasis (prevalence rate 26-75%) and cystic fibrosis patients (prevalence 35-81%) and seem to be associated with a more severe disease pattern (Lee *et al.,* 2011; Robinson and DiMango, 2014).

GORD is significantly more frequent in patients with IPF than in age- and sex-matched controls. As early as the 1970s, Pearson reported 6 cases of pulmonary fibrosis with hiatus hernia (Pearson *et al*, 1971). Mays et al (1976) later demonstrated fluoroscopic evidence of gastro-oesophageal reflux in patients with IPF compared to age-matched controls.

In 1998, Tobin et al, demonstrated abnormal distal acid exposure in 16 out of 17 patients with IPF compared to four out of eight controls, using ambulatory pH monitoring. Interestingly, only 4 of the IPF patients with reflux disease reported typical reflux symptoms. The study also showed that proximal supine reflux was common. Although this study had a small sample population, subsequent studies have reported similar findings.

In a study by Raghu et al (2006), sixty-five consecutive patients with well-defined IPF were subjected to 24-hour pH monitoring and oesophageal manometry. A total of 133 consecutive patients with intractable asthma and symptoms of GORD were used for comparison. The prevalence of abnormal acid GORD in IPF patients was 87%, with 76% and 63% demonstrating abnormal distal and proximal oesophageal acid exposures, respectively. Abnormal acid GORD was significantly more common in IPF patients than in asthma patients. Only 47% of IPF patients experienced classic GORD-related symptoms. There was no correlation between IPF severity and acid GORD severity. In conclusion, abnormal acid gastro-oesophageal reflux is highly prevalent, but often clinically occult in patients with idiopathic pulmonary fibrosis.

A recent study by Kilduff et al. (2014), on 18 subjects with IPF recorded a high proportion of proximal reflux events (mean 74.7%) and supine reflux events in the study cohort as a whole. Acid reflux events were noted to be within normal limits by the study but the size of the sample cohort was limited.

Raghu et al in 2006 presented a retrospective review of the clinical outcomes of four patients with newly diagnosed IPF and increased acid reflux, who chose to be treated solely with anti-acid reflux therapy. Pulmonary function test (PFT) results in all four patients stabilized and/or improved while their conditions were maintained with adequate treatment for acid reflux.

Studies have suggested that surgical correction of reflux in IPF reduces disease progression. Linden et al. (2006), compared post-operative lung function tests in 14

IPF patients on a transplant waiting list with reflux disease who underwent Nissen fundoplication, to 31 IPF patients who did not undergo anti-reflux surgery. Over an average period of 15 months follow up, patients who had undergone surgery maintained stable lung function and stabilisation in oxygen requirement was also noticed.

In a retrospective study of 204 patients with IPF by Lee et al. (2011), therapy for reflux disease was an independent predictor of longer survival. In addition use of reflux medication was associated with lower radiologic fibrosis score.

2.3.3 Reflux, Aspiration and IPF

Studies have demonstrated biomarkers of aspiration in the BAL fluid samples of patients with IPF. Savarino et al. (2013), reported that 40 consecutive IPF patients had significantly higher ($p<0.01$) oesophageal acid exposure, and number of weakly acidic and proximal reflux events, compared to 40 non-IPF ILD patients and 50 healthy volunteers. Patients with IPF had more bile acids and pepsin ($p<0.03$) in BAL fluid (62% and 67%, respectively) and saliva (61% and 68%, respectively) than non-IPF patients (25% and 25% in BAL fluid, and 33% and 36%, respectively, in saliva) and controls (0% and 0% in BAL fluid and saliva, respectively).

In 2012, Lee et al. measured gastric pepsin levels in BAL fluid from 24 patients with acute exacerbation of IPF and compared this to 30 patients with stable disease. They detected measurable pepsin levels in most patients with stable IPF. On average the pepsin concentration in BAL fluid was higher in patients with acute exacerbation compared to stable controls (median level of 46.8 ng/ml in acute exacerbation versus 35.4 nanogram/ml in controls). The study demonstrated pepsin level was an indicator of acute exacerbation status ($p=0.04$) in IPF. This was mainly driven by a subgroup of 8 patients (in the acute exacerbation group) with markedly high pepsin concentration in their BAL fluid (greater than or equal to 70 ng/ml).

Therefore, findings from the above studies demonstrate a stronger association of GORD with IPF and suggest aspiration secondary to GORD may play an important role in the natural progression of IPF.

The definitive treatment of reflux disease is surgery in the form of fundoplication. This is done under general anaesthesia and mechanical ventilation. The majority of patients with IPF also suffer from additional cardiovascular co-morbidities (like hypertension, diabetes mellitus, stroke, ischaemic heart disease) putting them at higher risk for adverse events relating to general anaesthetics. This means only a few IPF patients are suitable for surgery. In this context effective medical therapy may be more useful. International consensus recognises the potential relationship between reflux and IPF and agrees further research is necessary to improve care in IPF.

Chapter 3. Methods: PPIPF study

Based on the literature, both acid and non-acid reflux is common in patients with IPF.

3.1 PPIPF study hypothesis

Therefore the hypotheses underlying the planned study were:
1) Acid reflux causes cough in patients with IPF
2) Acid suppression therapy will significantly ameliorate cough with immediate improvement in quality of life
3) Acid suppression therapy by itself might be sufficient to reduce the rate of decline in IPF.

3.2 Usefulness of the PPIPF study

The study aimed to
1) Assess severity of cough and its impact on quality of life in patients with IPF
2) Objectively measure both acid and non-acid reflux in patients with IPF.

Anti-acid therapy blocks or neutralises gastric acid in the stomach but does not actually prevent reflux. The role of non-acid reflux in pathogenesis of cough and fibrosis is not clearly understood. Fibrosis of lung parenchyma can trigger up-regulation of central cough receptors by mechanical distortion of the proximal airways thereby providing an alternative explanation for cough in IPF. Additionally, lung fibrosis can disrupt the anti-reflux barrier by altering the anatomical relation between LOS and crural diaphragm. Fibrosis can also impair oesophageal motility, which in turn will slow bolus clearance, increasing exposure time to reflux, thereby precipitating reflux disease frequently found in IPF patients. Hence there is a theoretical possibility that acid suppression therapy might not improve cough in patients with IPF.

Even if anti-acid therapy in the trial failed to improve cough in IPF patients, the study is expected to provide further insight into role of non-acid therapy in IPF and inform

management strategies. If the study showed significant improvement in cough with acid suppression therapy then it would provide robust scientific evidence for an inexpensive therapeutic option for a distressing symptom in IPF. This will help design further multi-centre definitive trials prior to routine clinical practice. Long term antacid therapy is potentially associated with increased risk of infection (like pneumonia) due to alteration of the normal gut organism. Our study was planned to assess adverse events associated with antacid therapy. Either way the study was expected to generate new knowledge to guide future treatment for IPF patients.

3.3 Study drug: Omeprazole - drug class and licensed indication

Omeprazole is a widely used acid suppressant medication belonging to the class of drugs commonly referred to as proton pump inhibitors (PPI). It is a selective inhibitor of the hydrogen/potassium-adenotriphosphatase (H^+/K^+-ATPase) enzyme system found on the surface of gastric parietal cells, which is responsible for secretion of hydrogen ions or protons in the gastric lumen (the acid/proton pump). Omeprazole therefore inhibits both basal and stimulated acid secretion by reversibly inhibiting the final step of gastric acid production (Robinson, M., 2004).

Omeprazole is available over the counter and prescribed in [Vanderhoff and Tahboub, 2002; Summary of product characteristics (SmPC): omeprazole, version 4, 2013, Bristol Laboratories Ltd]:

1. Treatment and prevention of relapse of gastric ulcers
2. Treatment and prevention of relapse of duodenal ulcers
3. Treatment and/or eradication of *Helicobactor pylori*-induced peptic ulcer disease (in combination with antibiotics)
4. Treatment and prevention of non-steroidal anti-inflammatory drug (NSAID)-induced peptic ulcer disease
5. Treatment of reflux oesophagitis.

Long-term omeprazole therapy is employed in the management of:

1. Zollinger-Ellison syndrome
2. Symptomatic gastro-oesophageal reflux disease
3. Healed reflux oesophagitis.

3.3.1 Dose and duration

Omeprazole 20 mg daily is usually prescribed for the management of peptic ulcer disease and symptomatic reflux disease. In most patients with duodenal ulcers healing occurs in 2 weeks whereas gastric ulcers may take up-to 4 weeks to heal. Patients with inadequate response or incomplete healing are often prescribed 40mg omeprazole (higher dose) daily for an extended period (SmPC: omeprazole, version 4, 2013, Bristol Laboratories Ltd).

Omeprazole is acid-labile and hence is administered orally as enteric-coated capsules or tablets. Absorption of omeprazole is rapid via the small intestine and achieves peak plasma concentration in 1-2 hours post-ingestion (Vanderhoff and Tahboub, 2002). In patients with duodenal ulcer once a day 20 mg omeprazole will maintain an intra-gastric pH >/= 3 over a mean time of 17 hours in a 24 hour period (Miner Jr *et al*., 2003; SmPC: omeprazole, version 4, 2013, Bristol Laboratories Ltd).

For these reasons a twice daily dosing schedule of omeprazole 20 mg was chosen for the study.

3.3.2 Clinical pharmacology

Omeprazole is a weak base. It is concentrated and converted to active form in the gastric parietal cells. It is completely metabolised by the hepatic cytochrome P450 enzyme system. It is mainly excreted via the urine (80%) while the rest is excreted via the faeces (Welage and Berardi, 2000;Vanderhoff and Tahboub, 2002).

Omeprazole is a competitive inhibitor of CYP2C19 and CYP3A4 enzymes (Arnold, R., 1994;Reilly, J.P., 1996;Welage and Berardi, 2000). This raises the risk of

interaction with other medications that are metabolised by these hepatic enzymes. In addition, the half-life of omeprazole is prolonged in patients with hepatic disease due to delayed metabolism. For these reasons patients with documented hepatic cirrhosis (or advanced hepatic disease) and patients with current drug therapy with warfarin, phenytoin, diazepam or azoles (anti-fungal medications) were excluded from the study (Reilly, J. P., 1996;Vanderhoff and Tahboub, 2002).
In addition, recent evidence suggests warfarin is associated with more adverse events in patients with IPF hence patients on warfarin therapy were excluded from the study (Noth *et al*., 2012).
Patients on anti-fungal therapy could be included in the study on completion of therapy.

3.3.3 Adverse effects

Omeprazole is a commonly prescribed anti-acid therapy. The overall incidence of side effects is reported to be less than 5% (Arnold, R., 1994;Reilly, J.P., 1996; Welage and Berardi, 2000). Commonly reported side effects are headache, abdominal pain, constipation, diarrhoea, flatulence, nausea and vomiting (occurring in between 1-10% of patients). Prolonged periods (greater than equal to 3 months) of omeprazole therapy have been associated with hypomagnesaemia and increased risk of fractures (hips, wrist, spine). Rare (frequency > 1/10,000 to < 1/1,000) documented side effects associated with omeprazole therapy include hypersensitivity reactions, hyponatraemia, leukopenia, thrombocytopenia, skin rashes and interstitial nephritis.

3.3.4 Administration schedule

Participants were prescribed omeprazole 20 mg twice a day for 3 months.

3.3.5 Manufacture and supply

Omeprazole and matched placebo were supplied by Victoria Pharmaceuticals, Royal Hospitals, Belfast BT12 6BA.

3.4 PPIPF study design

The study was a prospective, randomised double-blinded, placebo-controlled single-centre, pilot trial of omeprazole in patients with IPF. The study was designated as a "pilot trial" as it sought to provide proof of concept of reduction in cough with anti-acid therapy in patients with IPF. Therefore major focuses of the study were rate of eligibility, participant recruitment and retention, and yield and quality of data, especially with regards to the proposed secondary outcomes (Johnson *et al*., 2005). This will help design further multi-centre definitive studies in future.

3.4.1 Patient population/Recruitment

Patients were recruited from the specialist ILD clinic at the Royal Victoria Infirmary (RVI) in Newcastle. RVI is the regional tertiary specialist centre for ILD. Eligible patients from across the region are routinely referred to the RVI clinic for initiation of anti-fibrotic therapy (i.e. pirfenidone and/or nintedenib) in IPF.

I regularly attended the Tuesday afternoon clinic to recruit participants for the study. I screened patients with a diagnosis of IPF aged between 40 and 85 years. Cases with an incident or prevalent diagnosis of IPF were recruited.

3.4.2 Sample size

This was a pilot trial. The analysis of data was expected to be mainly descriptive, hence no formal sample size was calculated (Hertzog 2008). Good practice guidelines recommend 20-30 participants per treatment arm should provide sufficient information to adequately assess feasibility and distribution of data, and to estimate the standard deviation of major study parameters (Lancaster *et al*., 2002; Schelling 2003). Therefore the aim of the study was to recruit 60 IPF patients in total (approximately 30 patients in the omeprazole arm and 30 patients in the placebo arm). This was expected to ensure at least 20 patients per treatment arm completing study follow up, allowing for an attrition rate of up to 33%.

3.4.3 Inclusion criteria

Patients who fulfilled all of the following criteria were recruited for the study:

- The ILD multidisciplinary team (ILD-MDT) at Newcastle considered IPF as the most likely diagnosis
- The patient had a history of cough, with or without exertional breathlessness
- The presence of predominantly basal, sub-pleural honeycombing changes on high resolution computed tomography (HRCT) scan of the chest
- The presence of bilateral basal crepitation on auscultation of chest
- Lung function tests consistent with a restrictive ventilatory pattern, with vital capacity (VC) <90% predicted and/or transfer factor capacity of the lung for carbon monoxide (TLco) <90% predicted
- Age between 40 – 85 years.

Patients with evidence of emphysema on their scan were also eligible for the study as long as IPF was the predominant feature and all of the above criteria were met.

In the event of no clear consensus as to the diagnosis in the ILD-MDT, the plan was to include patients in the study only if 2 external experts (from outside the region) in ILD ratified the diagnosis of IPF as being mostly likely.

Patients already on anti-acid therapy (i.e. PPI) at screening were potentially eligible for recruitment to the study. In those patients, the indication for PPI therapy was reviewed. Patients who had been on PPI for a short period (e.g. 2 months) were eligible after a month of cessation of anti-acid therapy. Patients on PPI therapy for prolonged periods were also eligible after discontinuation of PPI unless they had a clear indication for prolonged therapy. Apart from Zollinger-Ellison Syndrome (ZES), there are only a few medical conditions that require prolonged therapy with PPI. Hence, I reviewed each patient's history and case notes and/or contacted his/her general practitioner (GP). If there was no known diagnosis of ZES or history of

significant gastro-intestinal bleed or dyspepsia, I requested patients to consider a trial of supervised discontinuation of PPI therapy.

I contacted the respective GPs of patients who expressed a wish to take part in the study but were on long-term PPI therapy. If they were in agreement, the patient signed a consent form to agree to discontinue PPI therapy for a period of 2 weeks. If patients developed any symptoms during the supervised 2-week period then they re-started therapy and were excluded from the study. If however they remained asymptomatic (without their usual PPI therapy) for the 2-week period, they were recruited for the study. Similarly, patients on antacids like raft alginates and/or pro-kinetics were also potentially eligible for the study if they remained well off the treatment for a period of at least 2 weeks.

3.4.4 Exclusion criteria

Patients were excluded from the study if they fulfilled any of the following criteria:

- Documented allergy to omeprazole or any other PPI
- Concurrent therapy with warfarin, diazepam, phenytoin or azole therapy (like ketoconazole)
- Requirement for regular therapy with antacids such as PPI, raft alginates and/or prokinetics like metoclopramide (especially during the trial period)
- History of exacerbation of IPF and/or history of upper or lower respiratory tract infection within the prior 4 weeks of starting the study drug
- Treatment started specifically for IPF (e.g. prednisolone, pirfenidone, nintedanib, N-acetylcysteine) in the 4 weeks before starting the study drug
- Documented history of hepatic cirrhosis or advanced liver disease
- Pregnancy and/or breast feeding
- ILD not considered to be IPF by the regional ILD-MDT (i.e. patient's ILD was considered to be related to other conditions like rheumatoid lung disease, systemic sclerosis, sarcoidosis, asbestosis)
- Simultaneous participation in another CTIMP for IPF.

3.4.5 Intervention

Patients were randomised 1:1 either to receive omeprazole 20mg twice daily or matching placebo. Medication was taken orally before food for 3 months.

3.4.6 Primary outcomes

Primary efficacy outcome

The frequency of objectively measured cough from baseline i.e. from beginning of the study to the end of treatment (or within the last 2 weeks of completion of treatment) between the omeprazole and the placebo group.

Primary feasibility outcomes

- Assess eligibility rate, recruitment, randomisation and study completion rate
- Assess feasibility and acceptability of study-related procedures.

3.4.7 Secondary outcomes

The key focus behind the proposed secondary outcomes was analysis of data collection and data quality. The plan was to assess the following efficacy outcomes:

- Change in subjective assessment of cough at the end of treatment period as measured by a validated cough questionnaire
- Change in subjective assessment of reflux symptoms at the end of treatment as measured by validated reflux questionnaires
- Change in acid and non-acid reflux at the end of treatment
- Change in VC and TLco at the end of treatment
- Change in 6 minute walk test at the end of treatment
- Concentrations of cytokines (such as interleukin-8 and transforming growth factor beta) and bile salts/acids in BAL fluid at the end of treatment as evidence of on-going lung inflammation
- Infection in BAL fluid at the end of treatment
- Adverse events as reported by patients.

3.5 PPIPF study procedures

3.5.1 Screening

IPF patients attending the dedicated ILD clinic at RVI were screened and recruited for the study if they fulfilled the inclusion criteria and consented. I attended the clinic regularly every Tuesday. I reviewed the medical records of the patients attending or due to attend the clinic. I then provided information relating to the study either in person (when they attended the clinic appointment), or by letter to the eligible patients. I also made it clear to the patients that they may take as long as they liked to consider the information provided, and could contact if they had any further queries. Patients who were on an antacid therapy were also considered as outlined in sub-section 3.4.3.

I then followed up the potential patients with a phone call or at their next clinic review (whichever was earliest). I answered any questions and clarified any doubts or concerns with regards to the study or related procedures.

3.5.2 Consent

I was responsible for requesting consent from each participant. Participants were randomised and entered into the trial only after they had received information relating to the study and signed a written consent form. Once patients expressed interest in the study project, and had read the patient information, they were requested to sign the study consent form. I requested written consent from the participants in the ILD clinic and retained a copy of the form in the case report form. A copy of the consent form was filed in the patient's clinical records, and the patient kept another copy. A letter was sent out to the GP informing him/her of the patient's participation in the study. Copies of the GP letter were filed in each patient's medical notes and in the case report form.

Patients were aware that they could withdraw from the study at any time without any prejudice. Data recorded up to the point of withdrawal were included in the study analysis, unless patients withdrew consent to use the collected data.

3.5.3 Randomisation

Participants were randomised via a secure password-protected web site administered by the NCTU. It was available 24 hours a day. The web address of the randomisation site was https://apps.ncl.ac.uk/random/.

Participants were either randomised to the omeprazole arm or the placebo arm in a 1:1 ratio using random permuted blocks of size twelve. To ensure concealment of allocation, a statistician who had no other involvement with the study generated the randomisation schedule. Randomisation generated a unique 3 digit "Study ID number" for each participant. This unique number was entered on to the prescription, which was then delivered to the Clinical Trials Pharmacy at RVI. Randomisation also generated a unique "Trial patient number (pack number)" for the medication pack held at Pharmacy. The Study ID number matched the Trial Patient number, hence allocation to either omeprazole or the placebo group was double-blinded (i.e. both the participant and the research team were blinded to the allocation).

3.5.4 Drug termination criteria

The study drug (omeprazole or placebo) was stopped if any one of the following conditions were met at any time during the study period (criteria were agreed pre-trial):

- Completed the stipulated 90 days of treatment
- The patient suffered an adverse event deemed related to the study drug
- The patient needed regular treatment with an antacid on clinical grounds (as decided by the patient's usual clinical team or GP)
- The patient declined to continue and/or complete the study
- Death or a clinical decision to discontinue routine medical treatment.

3.5.5 Process of unblinding

An agreed protocol (between the trial team and the pharmacy) was put in place prior to beginning the trial for emergency unblinding, if such a situation arose. If it was deemed essential to know the treatment allocation of a participant, I could be contacted via the RVI switchboard or via a 24-hour mobile number (both numbers were provided to the participants and were also printed in the PIS). I would then contact the clinical trials pharmacist at RVI (during working hours) or the on-call pharmacist for The Newcastle upon Tyne Hospitals NHS Foundation Trust (if out of working hours) for emergency unblinding. I would then record (at the earliest opportunity) the date, time and reason for unblinding in the case report form.

3.6 PPIPF study assessments

Participants who consented for the study had the following information collected and underwent the following study procedures.

3.6.1 Demographics

I recorded patients' age, gender, smoking history, medical co-morbidities and current list of medication. In addition, baseline observations including height, weight, body mass index (BMI), pulse rate, blood pressure, oxygen saturations and respiratory rate were recorded. This information helped in assessing patients for suitability of study procedures and later in stratification during analysis of study results.

3.6.2 Questionnaires

I used the following 4 questionnaires to assess the impact of cough and reflux on health and daily activities of study participants. Questionnaires were administered via the chest clinic at RVI during study visit day 1 (pre–treatment) and study visit day 4 (post-treatment).

Leicester Cough Questionnaire (LCQ)

This is a fully validated 19-item cough-related questionnaire (Birring *et al*., 2003). It is divided into 3 domains (physical, social and psychological) to assess the overall impact of cough in day-to-day life and also to assess any improvement after intervention. It has a 7-point Likert response scale and the total score is calculated by adding the domain scores (ranges from 3-21). Each domain score is derived by dividing the total score from items within the domain by the number of items in the domain (ranges from 1-7). A higher score implies better quality of life.

LCQ is easy to administer, reproducible and responsive to change [questionnaire was developed with help from patients with chronic cough (Birring *et al*., 2003)]. Birring *et al*. (2003) indicated that a change of 2.56 in the total LCQ score is likely to be clinically significant. Yousef *et al*. (2011) in their study of assessment of quality of life in patients with acute cough demonstrated LCQ-acute and it domains were highly responsive and the minimal important difference (MID) for total LCQ was 2.5. However Raj *et al*. (2009) demonstrated a LCQ MID of 1.3 for patients with chronic cough.

Although LCQ was not specifically designed for patients with IPF, it is a well established cough-related quality of life questionnaire. It has been frequently used in longitudinal studies objectively assessing severity of cough and studies assessing efficacy of anti-tussives.

Reflux Symptom Index (RSI)

This is a fully validated 9-item questionnaire to assess the possibility of laryngo-pharyngeal reflux (Belafsky *et al*., 2002). Each item is rated on a scale of 0 – 5 (0 = no problems, 5 = severe problems). Possible scores range from 0 – 45. A composite score of 10 or below is taken as normal (Cohen *et al*., 2005), while scores above 13 suggest significant reflux disease (Belafsky *et al*., 2002). Lower scores imply better

health. RSI questionnaire is highly reproducible and widely used in clinical practice for diagnosis and management of laryngo-pharyngeal reflux disease.

Gastro-Intestinal Quality of Life Index Questionnaire (GIQLI) and De-Meester Reflux Questionnaire (DeMRQ)

These two questionnaires are designed to assess quality of life in patients with gastro-intestinal disease in clinical practice and in clinical studies.

GIQLI is a 36-item questionnaire relating to symptoms attributable to gastro-intestinal disease (Eypasche *et al*., 1995). There are 5 possible options or responses to each question with 4 points allocated to the "most desirable option" and 0 points allocated to the "least desirable option". Possible score range from 0 – 144 with higher score indicating better quality of life. Healthy individuals have a mean score of 122.6 +/- 8.5 (Yano *et al*., 2009). This is an exhaustive questionnaire comprising of a set of core questions applicable to patients with any gastro-intestinal disease and several organ specific questions. Therefore it is not a specific questionnaire for gastro-intestinal reflux disease. However it is validated, reproducible and is a comprehensive measure of patient's perception of impact of disease (and related treatment) on his/her life.

The DeMRQ questionnaire has been previously used in our research group. This is a short 3-item questionnaire to assess health-related quality of life in patients with reflux disease (DeMeester *et al*., 1976). The 3 items/questions relate to symptoms usually associated with reflux disease (i.e. heartburn, regurgitation, dysphagia). Each item is rated on a scale of 0 – 3 depending upon frequency and severity of occurrence (0 = no/none, 3 = severe). Possible scores range from 0 – 9 with low scores indicating better quality of life. This questionnaire has not been extensively used in clinical trials or research previously. However it is easy to administer and included in the study due to earlier experience in our research group.

3.6.3 Lung function tests

Lung function tests are often performed in patients with IPF as part of their routine clinical assessment. Hence IPF patients are familiar with the procedure. It is well known that early changes in vital capacity (VC) are associated with progression in IPF. Also, exploring various secondary end-points is one of the main reasons for conducting a pilot trial (Johnson *et al*., 2005). Therefore spirometry [Forced expiratory volume in 1 second (FEV1) in litres and Forced vital capacity (FVC) in litres] and gas transfer factors [Transfer factor of lung for carbon monoxide gas (TLco) in mmol/min/Kpa) and Transfer factor coefficient of lung for carbon monoxide gas (Kco) in mmol/min/Kpa/litres] were assessed for the purpose of this trial. Lung function tests were performed by the specialist physiologists at the Chest Clinic in RVI, using an Nspire body plethysmograph as per standard international guidelines (Miller *et al*., 2005;Macintyre *et al*., 2005).

3.6.4 Six minute walk test (6MWT)

6MWT measures the distance that a patient can walk on a flat, hard surface in 6 minutes. It is an useful measure of exercise capacity in various cardio-pulmonary diseases. Short term reproducibility of 6MWT is excellent when performed by the same person or technician [American Thoracic Society (ATS) statement: guidelines for the six minute walk test, March 2002]. The median distance walked by healthy men and women in 6 minutes was 576 m and 494 m respectively (Enright *et al*., 1998). Reidelmeier et al., (1997), reported that a minimal difference of 54 m in 6MWT distance was associated with subjective change in exercise capacity in stable, severe COPD patients.

6MWT is commonly undertaken in patients with IPF to assess overall functional capacity. In a study of 123 IPF patients by Swigris et. al., (2010), 6MWT distance did not change significantly from baseline over a period of 12 months and the MID for 6 minutes walk distance was estimated to be approximately 28 m. However the study included only a small number of moderately severe IPF patients and data from

patients who died or who were unable to perform 6MWT were not included in the analysis. Du Bois et. al., (2010), assessed validity, reliability and responsiveness of 6MWT in a large group of IPF patients (n=826). They determined an MID of 24-45 m. They also demonstrated that a decline of more than 50 m in 6 minutes walk distance over 24-weeks was associated with 4 fold increased risk in mortality in 1 year ($p<0.001$). Therefore, both the studies concluded 6MWT is a valid and reliable measure of disease condition in IPF. The later study suggested 6MWT is an important prognostic marker and can be used as a key endpoint for clinical trials in patients with IPF.

I performed the 6MWT at the Chest Clinic in RVI as per standard international guidelines. Two points (distance between which is known or measured prior to the test) are marked on a level surface and the patient walks between the points for a period of 6 minutes. The patient can stop and/or rest at any point if they are too breathless to continue or have other symptoms such as joint pains, chest pain etc. The total distance managed is calculated at the end of 6 minutes and any change in symptoms or reason for early discontinuation of test is recorded.

3.6.5 Twenty-four hour cough recording

I am grateful to Prof Jacky Smith (Co-Investigator, University of Manchester) and her team for their continued help and support with the 24-hour ambulatory cough recording. I have visited Manchester (Cough Research Centre) on multiple occasions to learn setting up of cough monitors, securing electronic transfer of cough recording (to Manchester), and formatting of the recording data cards. They provided the cough recorders and the cough sensors for the study.

The cough recorder objectively measures cough frequency over a period of 24 hours (McGuiness *et al*., 2012). It involves participants wearing a CE marked lightweight digital sound recording device Vitalojak (Vitalograph Ltd, Birmingham, UK) for 24 hours while performing their day-to-day activities. There are two microphones attached to the recorder – the first is an Air Microphone, which can be clipped to a

lapel or collar (of the shirt or jacket of the participant) and records all sounds. The second is a Chest Sensor, which is attached to the skin over the top of the sternum and records sounds from the chest wall. The device has an internal 4GB data card on which the sound is recorded. The device is inserted in a small bag, which is then secured around the waistline with help of an adjustable belt with self-locking clips (as shown in Figure 4 and Figure 5).

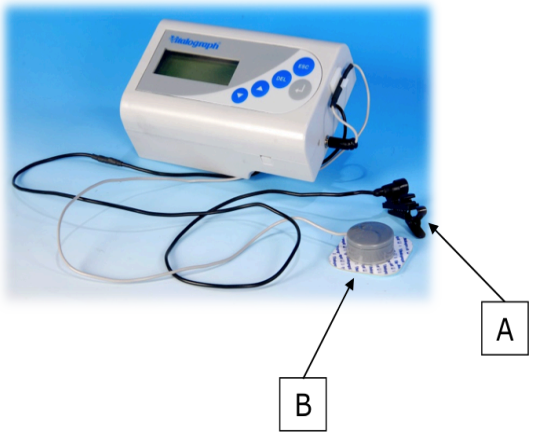


Figure 4: Vitalojak cough recording device with the 2 microphones.
A: Air Microphone; B: Chest Sensor

Figure 5: Cough recording device carried around waistline in a bag (adapted from "cough count training" power point presentation provided by the cough research team at Manchester).

The procedure is well tolerated with no major discomfort reported by previous research participants (McGuiness *et al*., 2012). On completion of recording, anonymised sound recording is transferred electronically via a File Zilla application to a secure server at the University of Manchester. The actual cough count is calculated from the 24-hour recording with help of a specially developed, fully validated software programme (CoolEdit 2000, Syntrillium Software Corp., USA). Cough is counted manually, hence the 24-hour recording is cut down and compressed by the custom-made software by removing background noises, silent periods and the majority of speech, with the aim of retaining only the coughs. This generates 24 tracks or files each representing 1 hour of real time. An expert cough counter in Manchester then listens to all the files and electronically tags all the cough sounds thereby

generating a report of cough counts per hour (including daytime and night time cough rates). The reports were then returned to us.

The act of coughing involves the intake of a large volume of air followed by rapid expulsion associated with an explosive sound. A typical cough consists of an "explosive phase", an "intermediate phase" and a "voiced phase".

Figure 6 demonstrates a series of coughs with the explosive and the voiced phases marked (adapted from "cough count training" power point presentation provided by the cough research team at Manchester).

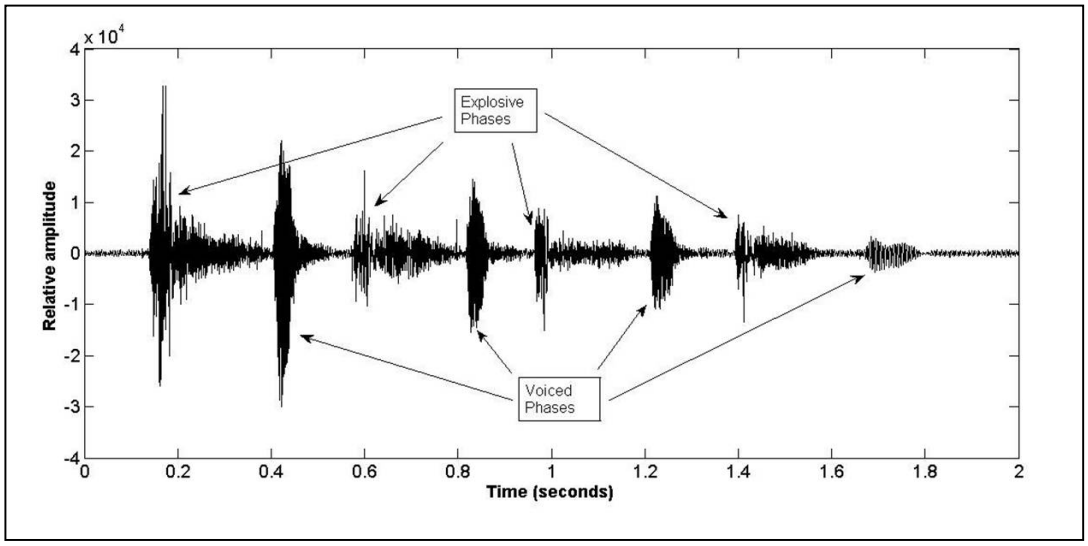


Figure 6: Series or "spasms" of coughs. Arrows point to the explosives and voiced phases of each cough (adapted from "cough count training" presentation provided by the cough research team at Manchester)

During manual cough counting, every "explosive cough sound" heard is tagged. Sounds from throat clearing or sneezing can be mistakenly marked. The microphone and chest sensor are synchronised during compression of the files and are reviewed together – this helps to confirm that the cough tagged has come from the participant being studied (cough from another person nearby is not recorded or registered in the chest sensor).

Figure 7 shows an example where each explosive phase of cough is tagged with a dotted line thereby representing 4 coughs (adapted from "cough count training" power point presentation provided by the cough research team at Manchester):

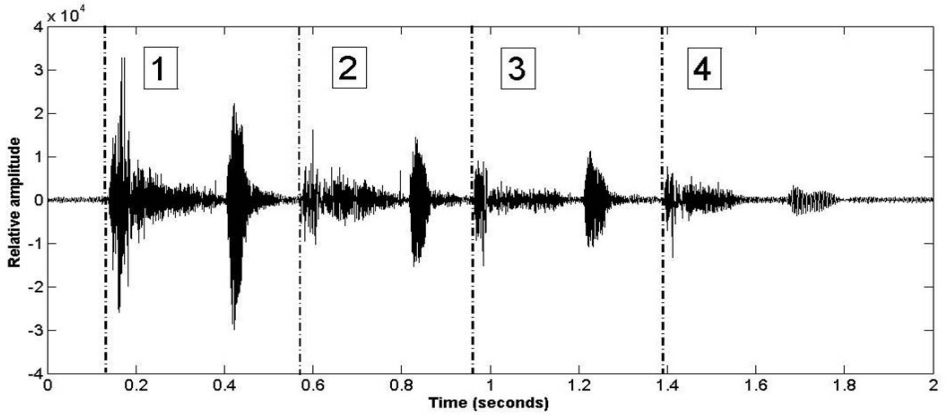


Figure 7: Example of 4 coughs where each of the explosive phases have been marked with a dotted line (adapted from "cough count training" presentation provided by the cough research team at Manchester).

For this study, Prof Smith and her team (which consists of 3 experts in cough counting) performed all the cough counting. The same cough counter performed the majority of the counts (and all the recordings from any particular individual), to reduce the likelihood of inter-operator variability. In addition 10% of the cough recordings were randomly selected for re-count by a second counter. Differences between the two cough counters were deemed acceptable as long as the difference was within 95% limits of agreement based on previously collected data analysed by the most experienced cough counters.

3.6.6 Gastro-intestinal physiology study

I am most grateful to Mr Rhys Jones (Clinical Research Fellow, Upper GI Surgery Department, RVI) from whom I learnt to perform and interpret GI physiology studies. I am also grateful to Rachel Colver (Specialist GI Nurse), with whom I have worked closely at the Northern Oesophago–Gastric Unit, for her help and support in performing the GI studies.

I also attended a 3-day course in the Netherlands to consolidate my experience in the performance and interpretation of GI studies. I performed and analysed the majority of the GI studies undertaken by the participants. The tests were performed in the Endoscopy Unit, RVI.

The GI physiology element was designed to detect and assess the severity of reflux disease. It consisted of two parts:

1. High Resolution Manometry – oesophageal study
2. 24-hour ambulatory pH - impedance study

High Resolution Manometry – oesophagus (HRM)

The peristaltic function of the oesophagus and the integrity of the LOS are assessed by the HRM study. It was performed with a 20-channel oesophageal catheter using an MMS Solar Gastric water-perfused high-resolution manometer. The 20 channels are actually pressure sensors, which assess the function of the oesophageal body and relaxation of the LOS with the help of 10 "wet" swallows (10 x 5 ml of water). The catheter is inserted via the nose and secured in place once the LOS is localised (with help of pressure tracing on the monitor). Patients are then instructed to swallow water (at least 10 times). This generates pressure waves (as the liquid bolus propagates through the oesophagus towards the stomach), which are recorded as graphs. With the development of a colour topographic technique, different colours could be assigned to different pressures generated across the oesophagus. Therefore the huge volume of recorded pressure traces (during the 10 swallows) can be easily represented as coloured plots. Any variation from the typical or normal swallow pattern can be easily identified, thereby facilitating detection of abnormal oesophageal and/or LOS function (Figure 8).

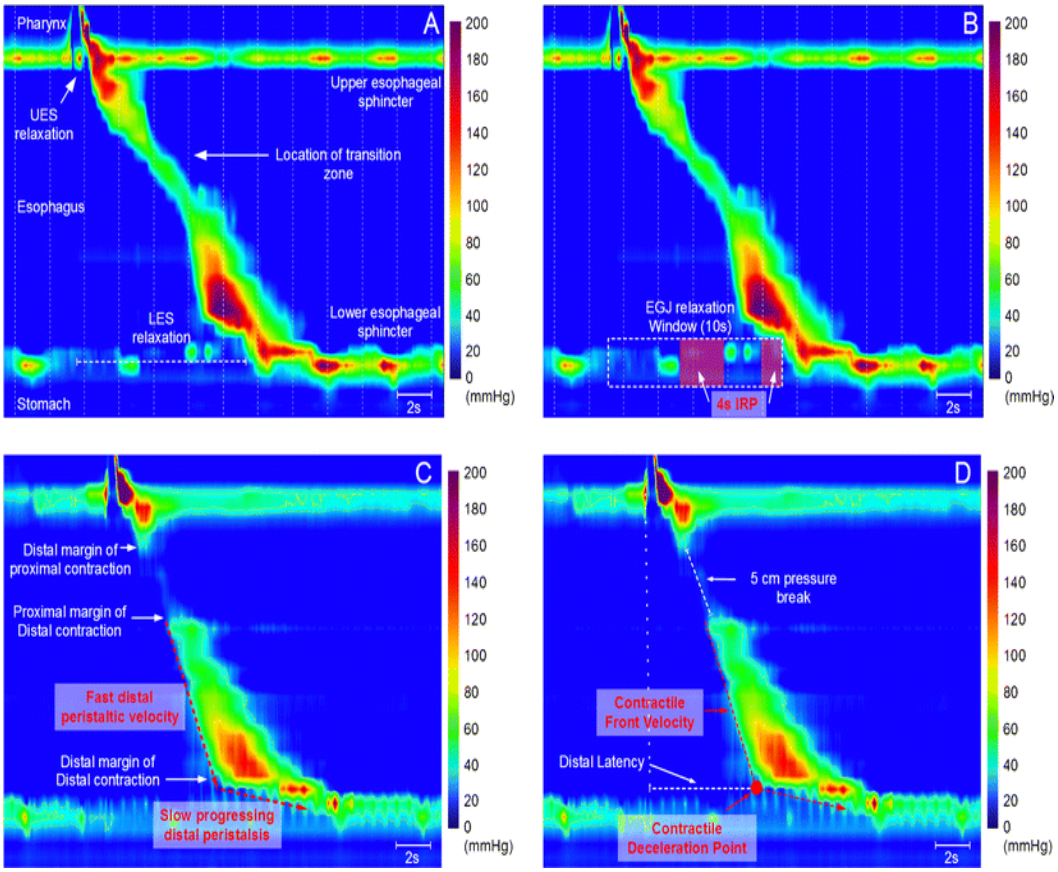


Figure 8: HRM plots of oesophagus, illustrating a normal swallow.
A: anatomical landmarks identified with HRM; B: LOS relaxation assessment; C: identification of peristaltic landmarks using isobaric contours lines; D: assessment of peristaltic function (adapted from the open access journal "clinical application of oesophageal impedance monitoring and high resolution-manometry" Current Gastroenterology Reports, June 2002, volume 14, issue 3, 197-205).

Figure 9 below shows a HRM plot of an abnormal peristalsis during swallow. It shows a "large break" in peristalsis in the oesophageal body (marked by the large rectangle). The marked smaller rectangle depicts LOS relaxation (adapted from power point presentation provided at the HRM training course in Netherlands, April 2014)

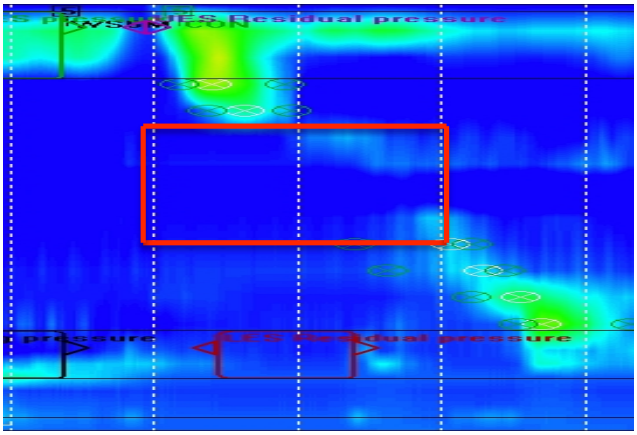


Figure 9: HRM plot of an abnormal peristalsis.
A "large break" (marked by the large rectangle) in the oesophageal body is illustrated by the large rectangle (adapted from power point presentation provided at the HRM training course in Netherlands, April 2014).

HRM techniques can assess both the oesophageal body and LOS at the same time (as opposed to conventional manometry methods) and is better at localising the LOS and detecting peristalsis abnormality, hence HRM was used for our study. Analysis was performed according to the Chicago classification system (Brendenoord *et al*., 2012).

HRM study was always performed prior to the 24-hour pH-impedance study for the following 3 reasons:

1. The study localises the LOS and guides subsequent accurate placement of the pH-impedance catheter
2. The study assesses the peristaltic function of the oesophagus, which influences subsequent decisions relating to anti-reflux surgery
3. The study helps exclude additional oesophageal motility disorders (such as non-obstructive dysphagia).

Twenty-hour hour pH-impedance study

This test was performed to objectively quantify reflux disease. The test was performed using a multichannel (8) intraluminal combined pH-impedance catheter by MMS Ohmega Systems.

The catheter was inserted transnasally and placed 5 cm above the LOS (localised during the preceding HRM study) and connected to a data box. Regurgitation of gastric contents into the oesophagus is recorded over a period of 24 hours while the patient carries on his/her daily activities as far as possible. The patient is requested to record symptom episodes by pressing a button on the data recorder (data box) so that symptom-event correlation can be assessed.

The 8 metal rings in the catheter allow measurement of electric resistance or "impedance" across 6 oesophageal segments. The attached pH catheter records the pH of the refluxate. The baseline resting impedance is that of the oesophageal mucosa. Resistance (or impedance) increases with the passage of air and decreases with the passage of food/liquid through oesophagus. Hence, depending on timing, change of measured impedance, direction and extent, any reflux events can be detected. The nature of reflux can also be characterised as acidic (pH < 4), weakly acidic (pH 4-7) or non acidic (pH >7).

Over a period of 24 hours the number, duration and proximal extent of reflux is recorded and results are compared with values recorded from studies of healthy volunteers (Zerbib *et al*., 2005).

Figure 10 shows a typical acid reflux event recorded during 24-hour pH-impedance study (an episode is marked by vertical lines with small squares on top). Impedance decreases initially at the distal channels (starts at channel 6) followed by the proximal channels. Impedance returns to baseline first at the proximal channels (channel 1) and then at the distal channels. A drop in impedance is associated with drop in pH to below 4 (as shown by the pH catheter tracing) thereby signifying an acid reflux event with proximal extension (adapted from pH-impedance study of Trial patient 003).

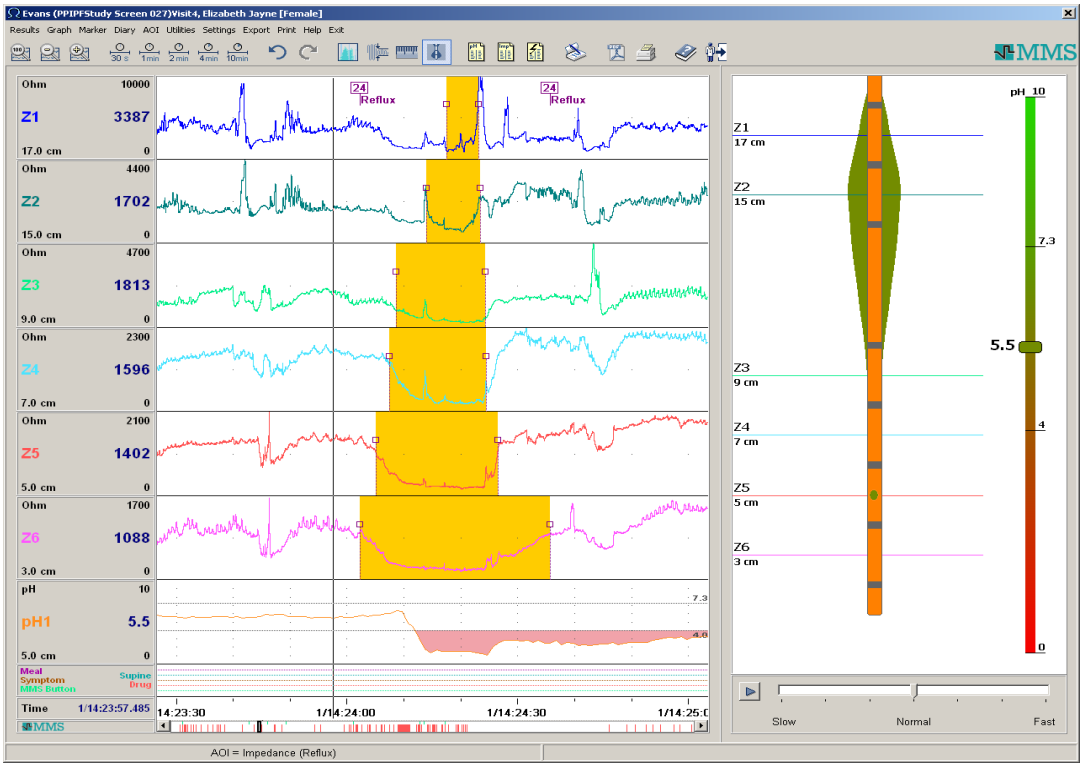


Figure 10: Typical acid reflux event.
Impedance decreases in a distal to proximal direction and then returns to baseline from proximal to distal direction. This is associated with a drop in pH below 4 (adapted from pH-impedance study of Trial Patient 003).

I have attached an illustration (Table 1) of the main results available from the 24-hour pH-impedance study (particularly those we planned to record for the purpose of the research study). This report format was initially designed by Mr Rhys Jones and reproduced with his kind permission.

Table 1: Proposed layout of data/results to be recorded from the 24-hour pH-impedance study for the research study.

Trial patient: XXX

	Normal range		**Pre – study drug**	**Post – study drug**
pH assessment				
% time pH<4	<4.2			
Number of pH drops to <4	<50			
Number of long reflux episodes > 5	<4.0			
Longest reflux episode	2.2			
DeMeester score	<14.72			
Impedance assessment				
Total number of reflux episodes	25-58			
Bolus clearance time (s)	8-13			
Symptom-event correlation				
Symptom-associated probability (%)	<95			

The DeMeester score refers to a commonly used composite score with which to quantify acid reflux based on pH monitoring. It takes account of the four pH measurements also listed in Table 1.

Bolus clearance time is the mean duration of oesophageal bolus exposure. Bolus exposure is defined as the interval between an impedance drop to <50% of the baseline and recovery of the impedance level to 50% of the baseline value for ≥5 seconds.

The symptom-associated probability is a statistical analysis of the correlation between reflux events (as recorded by pH or impedance monitoring) and patient-reported symptom episodes. In symptomatic individuals, such symptom analyses are useful in deciding whether a patient's symptoms are related to reflux.

3.6.7 Bronchoscopy and BAL assessment

I am competent in performing bronchoscopy and BAL (these are mandatory clinical skills for a respiratory trainee). Dr Ian Forrest performed bronchoscopy during the early phase of the study (to teach specific principles of research bronchoscopy), after which I performed the procedures.

I am thankful to Dr Gail Johnson and Kasim Jiwa at the Sir William Leech Respiratory Research Centre for my training in processing BAL samples. I have processed lavage samples obtained for separate on-going research studies under their supervision and have counted archived cytospin samples. I have processed BAL samples at the William Leech Research Laboratory, and centrifuged these for retention of supernatant at -80°C for future analysis of various cytokine concentrations in batched samples in Prof Simpson's laboratory at Newcastle University.

Bronchoscopy

Bronchoscopy was performed in the Endoscopy Unit at RVI according to standard practice and international guidelines. The procedure was performed usually under sedation (with intravenous midazolam) with topical anaesthesia provided by 2% lignocaine throat spray. Patients received supplemental oxygen via nasal prongs. Electrocardiogram (ECG) tracing and oxygen saturations were continuously monitored throughout the procedure. Previous HRCT scans were reviewed and where possible BAL samples were taken from a segment with changes of IPF but without advanced honeycombing, in order to minimise the small theoretical risk of pneumothorax. Three aliquots of saline, each of 60 ml, were instilled and aspirated as BAL sample. The patient was monitored for at least 2 hours after the procedure. Patients who had sedation were advised not to drive, work, drink alcohol, operate moving machinery (including drills etc. at home), or sign legal documents for the remainder of the day, and had to return home accompanied by a responsible adult.

BAL assessment

The quantity of BAL fluid aspirated was recorded. An aliquot of BAL fluid (at least 5 ml) was sent to the NHS Microbiology Laboratory (Freeman Hospital) for culture (and sensitivity if positive) of commonly known respiratory pathogens.

The rest of the sample was transferred to the research laboratory on ice. The BAL sample was processed as per established standard operating procedures (Standard Operating Procedures; BAL processing SOP Index S 01.version 3, Sir William Leech Centre, Freeman Hospital).

The sample was centrifuged at 1250 rpm (183g) for 6 minutes at 4^{o}C. The supernatant was decanted in centrifuge tubes. The cell pellet was reconstituted in 1-50 millilitres of Dulbecco's Phosphate-Buffered Saline solution (to generate an opaque suspension), and total cell count was estimated using an Improved Neubauer counting chamber. Up to 12 cytospins (using Shandon cytospin) were prepared on glass slides and air-dried. One cytospin was stained with Giemsa stain to perform a differential cell count. The rest of the cytospins were stored at -20^{o}C for future examination and/or research.

The decanted supernatant was centrifuged at 2500 rpm (734g) for 6 minutes at 4^{o}C. The supernatant was then divided into 600 microlitre aliquots and stored at – 80^{o}C.
After completion of preparation of cytospin, the cell suspension was re-centrifuged at 1250 rpm (183g) for 6 minutes at 4^{o}C. The supernatant was discarded and the cells were re-suspended in Dulbecco's Phosphate-Buffered Saline to achieve a concentration of 2 -3 million cells/ml. A maximum of 6 x 1 ml aliquots was retained and centrifuged at 3000 rpm (325g) for 4 minutes. The supernatant was discarded and cell pellets were stored at – 80^{o}C for future research studies.

The stored supernatant could be assessed in future for markers of inflammation and aspiration including (but not limited to) IL-8, transforming growth factor beta, pepsin (Stovold *et al*. 2007), and bile salts.

3.6.8 Patient visits for study assessments

Patients visited hospital and/or clinic for 6 days in total as summarised in Table 2 below. Patients attended for 3 consecutive days on 2 occasions.

On the first day (Visit 1) patients were consented (once eligibility confirmed) and baseline demographics recorded. Health related quality of life questionnaires were administered. They underwent lung function tests, 6MWT and then 24-hour ambulatory cough recording were commenced. Patients returned the cough recorder the next day (Visit 2) and 24-hour ambulatory pH-impedance study were commenced. On the third day (Visit 3) patients were randomised and issued with trial drug (omeprazole or placebo) and patient diary card (after completion of 24-hour pH-impedance study).

Patients were instructed to take study medication for 90 days and further study visits were planned for day 88-day 90.

Visit 4 was on day 88 for similar assessments as in Visit 1 (except consent and demographics). GI physiology study was repeated on day 89 (Visit 5).
Patients underwent bronchoscopy on day 90 (visit 6).

Patients took the last dose of medication on the evening of Day 90 and returned the empty IMP bottle the next day (or within 2 weeks).

Table 2: PPIPF study visits

	Days -7 to 0 (ie any 3 days in the lead up to starting study medication			Days 1-87	Days 88-90 §§		
Confirm eligibility	*						
Consent	*						
Demographics	*						
VC and TLco	*				*		
6 minute walk test	*				*		
Cough questionnaire	*				*		
Reflux questionnaire	*				*		
Commence 24h cough monitoring	*				*		
Commence 24h oesophageal physiology tests		*				*	
Randomization			*				
Issue omeprazole or placebo			*				
Issue adverse event diary	*						
Take study medication				*	*	*	*
Bronchoscopy and BAL							*

§§ - the tests scheduled for "days 88-90": some participants had tests performed within the 2 weeks prior to completion of omeprazole/placebo (this was pre- agreed in the study protocol). (Adapted from the PPIPF study protocol, version 4.0, 3.7.14).

3.7 PPIPF study pharmacovigilance/safety monitoring

During the trial period safety of participants was prioritised. The trial complied with all the mandatory regulations and principles set out in the Research Governance Framework and The Medicines for Human Use (Clinical Trials) Regulations 2004 and subsequent amendments. A favourable ethical opinion from a NHS Research Ethics Committee [NRES Committee Yorkshire & The Humber - Leeds West granted approval for the study on 20.09.2013 (Ref 13/YH/0284)] and a Clinical Trial Authorisation (CTA) from the MHRA were obtained prior to initiation of the trial (granted on 13.09.2013). In addition, the sponsor, The Newcastle upon Tyne Hospitals NHS Foundation Trust (NuTH) requires all clinical trials of investigational medicinal products (CTIMPs) to be managed through a registered Clinical Trials Unit, hence Newcastle Clinical Trials Unit (NCTU) monitored this trial. I completed Good Clinical Practice (GCP) training.

3.7.1 Data Monitoring and Safety Committee (DMSC)/ Trial Steering Group (TSG) appointment

A 3-member, independent DMSC was established for the trial. The committee consisted of 2 clinicians and 1 independent statistician. The panel members were Dr A. M. Wilson (chair), Senior Clinical Lecturer in Respiratory Health, University of East Anglia, Norwich; Dr Owen J. Dempsey, consultant chest physician, Aberdeen Royal Infirmary, Aberdeen; and Evie Gardner, Senior Biostatistician, Northern Ireland Clinical Trials Unit, The Royal Hospital, Belfast. I drafted the trial-specific DMSC charter (based on guidelines set by NCTU), which was approved and accepted by all members. The first DMSC meeting was held on 23/6/14. Subsequently, the DMSC convened roughly every 3 months during the trial period (DAMOCLES study group, 2005).

A 3-member independent TSG was also established for the trial. The members consisted of 2 clinicians and 1 lay patient member (not a study participant). The members of the steering group were Dr Michael Gibbons, consultant chest physician, Royal Devon and Exeter Hospital; Dr Helen Palfrey, consultant chest physician,

Papworth NHS Foundation Trust and Mr Ian Perry (lay patient member). Dr Gibbons was the chair. I drafted the trial-specific TSG charter, which was approved by the chair. The first TSG meting was on 31/10/14 and subsequent meetings were held every 6 months.

In addition, I designed the trial-specific Serious Adverse Event Form based on a template from NCTU. Also the SOHO66 system (a secure password-protected web based system) was set up for reporting of serious adverse events in accordance with NCTU guidance.

3.7.2 Patient diary card

I designed a "patient diary card" for the study participants (attached Appendix 5). This was a symptom diary card that was issued to participants so that they could record any symptoms or illness while participating in the study. This helped in assessment of drug compliance and safety of the investigational medicinal product (IMP) during final analysis.

3.7.3 Data collection

In accordance with suggestions by NCTU, research data were collated via an electronic Case Report Form (CRF). Data were uploaded via a password-protected secure website: www.macro.inferomed.com/NewcastleCTU/

I designed the paper CRF on which the electronic CRF was based. My study involved multiple hospital visits for study participants. Study procedures were performed at different departments – the Chest Clinic, Endoscopy Unit, and the laboratory – hence access to the electronic CRF (via internet) was difficult at times. Hence, being pragmatic I collected data on paper CRFs and then uploaded the data at the earliest possible opportunity.

3.7.4 Regulations, Ethics, Governance

Ethics: NRES Committee Yorkshire & The Humber - Leeds West granted approval for the study on 20.09.2013 (Ref 13/YH/0284).
MHRA approval for the study was granted on 13.09.2013.
Trust R&D approval: The Newcastle upon Tyne NHS Hospitals Foundation Trust granted final R&D approval on 11.02.2014 (Ref 6754).

There was a considerable interval between initial REC, MHRA approval and final R&D approval. The reason is outlined in details in Section 13. In summary, our initial submission (to REC and MHRA) was reviewed by NCTU, who suggested an amendment to the protocol (no change to proposed study outcomes were suggested). Hence a request for substantial amendments was filed for approval to REC and MHRA. R&D approval was delayed pending favourable opinion (after re-submission) from both REC and MHRA.

The Ethics Committee approved amendments on 10.01.2014.
The MHRA approved amendments on 15.01.2014.
Hence the study started recruitment in March 2014.
The first patient was randomized on 28.03.2014.

My research project was accepted as an NIHR portfolio study.
The study is registered with clinicaltrials.gov (NCT02085018) and ISRCTN (ISRCTN07139948).

Chapter 4. Results

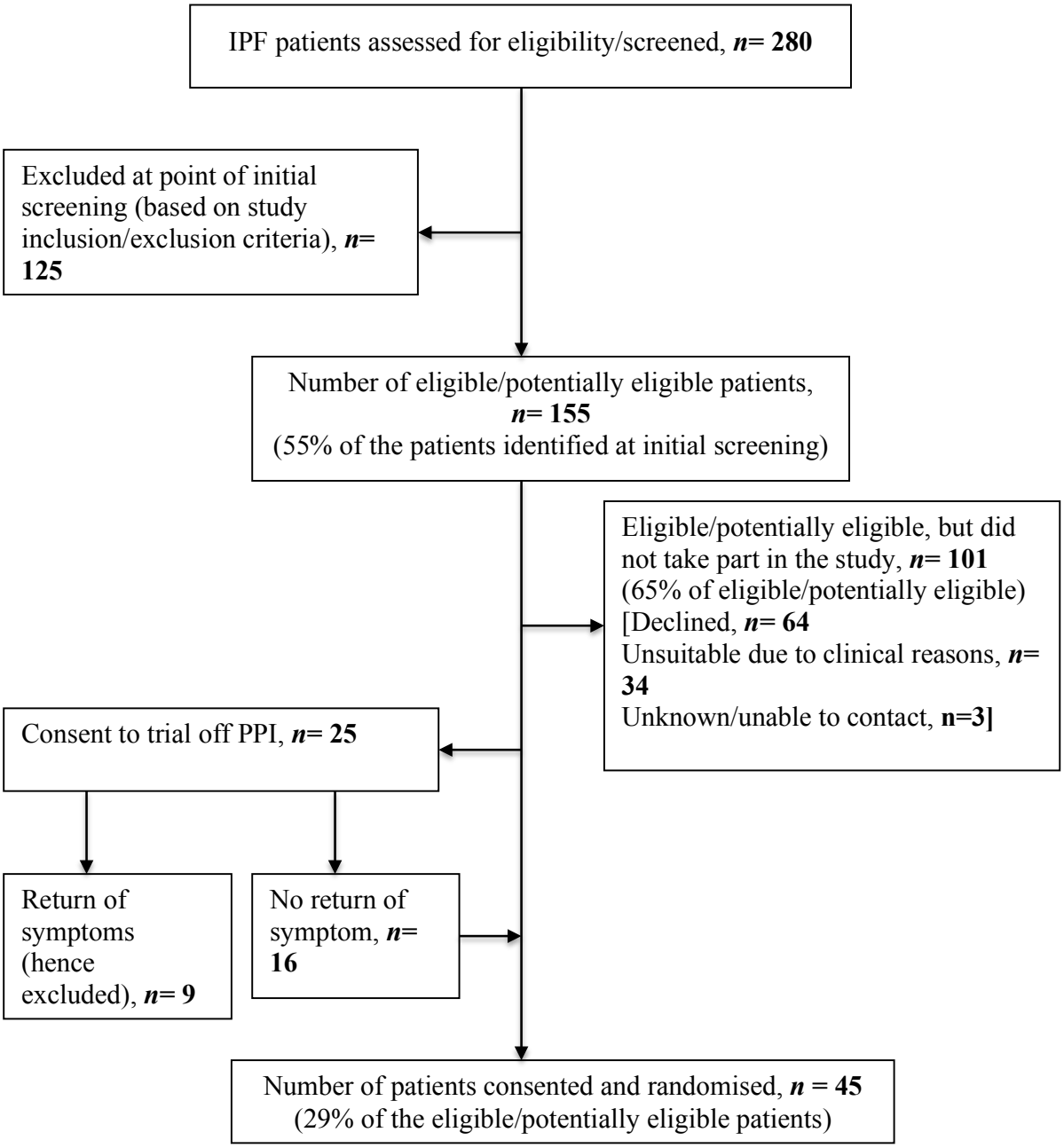
Prior to returning to clinical training (July 2015), I had recruited 33 participants for the PPIPF study. I am grateful to Dr Wendy Funston who further recruited and randomised 12 participants. In total 45 participants were enrolled for the study. Study closed to recruitment on 19/11/2016.

I am grateful to the NCTU, Vicky Ryan (senior trial statistician) and Helen Mossop (trial statistician) for their help with the collected data which was securely stored via the electronic website. Vicky and Helen kindly provided the "raw data" from the secure website. The results presented for the thesis are based on the baseline assessments (i.e. pre drug treatment) of cough and reflux of the participants. They provide an account of recruitment, completed study procedures and baseline characteristics of the participants of PPIPF study. Some of the results have already been presented at the mandatory DMSC and/or the TSG open progress reports for the PPIPF study (which I have helped Vicky and her team to prepare).

4.1 Screening and recruitment

The following figure (Figure 11) is a CONSORT diagram outlining screening, eligibility and recruitment of IPF patients for the study:

Figure 11: Consort diagram of patient screening and recruitment to study



In summary, **280** IPF patients were identified and screened for the study. Of the total patients screened, 125 (45%) were excluded based on the study inclusion and

exclusion criteria. I have outlined the reasons why identified IPF patients at initial screening were excluded from the study in Table 3 below.

155 patients were eligible or potentially eligible (based on current use of PPI and/or antacid, prokinetics). Of them 34 patients were excluded on clinical grounds i.e. history of GI bleed, gastrectomy surgery for cancer, fundoplication surgery, recent diagnosis of malignancy or patient deemed a candidate for palliative care in view of end stage pulmonary fibrosis (I have outlined the reasons and the number of potential participants excluded on clinical grounds in Table 3 and discussed in further details later in this section).

Therefore **118** IPF patients were invited to take part in the study (we were unable to contact and/or receive communication from 3 IPF patients identified at screening).

Table 3: Why identified IPF patients were excluded from the study based on inclusion/exclusion criteria, clinical grounds and/or other reasons:

Reasons		**Number of patients**	**Percent [1] (Rounded)**
Not fulfilling inclusion criteria, n= 59	ILD-MDT did not considered IPF as the likely diagnosis	9	3.2
	Patient has no cough	24	8.6
	No honeycombing on CT scan	5	1.8
	No bilateral basal fibrosis on CT scan	1	0.4
	No restrictive defect on lung function	2	0.7
	Age > 85 years	18	6.4
Fulfilling exclusion criteria, n= 66	Unable to stop concurrent PPI/antacid therapy	34	12.1
	Previous known allergy or intolerance to omeprazole or other PPI	3	1.1
	Concomitant use of interacting drugs [2]	23	8.2
	History of liver disease (cirrhosis)	4	1.4
	Concomitant enrolment in other trials	2	0.7
Clinically unsuitable, n= 34	Clinical co-morbidities	22	7.9
	Patient for palliative care only	7	2.5
	Died (during screening for example due to lung cancer)	5	1.8
Declined (after invitation to participate), n= 64	Travel/work commitments/study procedure	17	6.1
	Unwell/frail	6	2.1
	Other reasons	6	2.1
	Reason unknown	35	12.5
Unable to contact, n= 3		3	1.1
Return of symptoms off PPI, n= 9		9	3.2
	Total	235	83.9

(1) Percentage calculated with total number of IPF patients screened (n=280) as the denominator; (2) Concomitant drugs included warfarin (n=20); diazepam (n=1); phenytoin (n=2).

64 eligible IPF patients declined to participate (17 cited travel commitments with study procedures related reasons, 6 felt too unwell/frail for the study, 6 patients were not keen to discontinue their long term antacid therapy and/or were concerned about placebo, while no particular reason known for the other 35 patients).

25 patients who were on long term PPI consented for 2-week period of supervised discontinuation of PPI therapy. 16 patients successfully completed the "period of supervised discontinuation" and were randomised for the study. 9 patients had symptoms off PPI hence excluded.

In total **45** (29 % of the eligible or potentially eligible) patients were consented and randomised for the study (achieving a mean recruitment rate of 1.5 participant per month).

4.2 Patient characteristics at baseline or randomisation

During screening baseline demographics, co-existing medical illness and current drug therapy were recorded.

Table 4 below shows patient's demographics at baseline (prior to initiation of drug therapy).

Table 4: Baseline patient demographics (n=45)

Baseline Characteristic Number (%)	Cohort N= 45
Gender	
Female	10 (22.2)
Male	35 (77.8)
Ethnicity	
Caucasian	45 (100)
Smoking History	
Never smoked	10 (22.2)
Ex smoker	34 (75.6)
Current smoker	1 (2.2)
Continuous Baseline Characteristic	Cohort N= 45 Mean (SD) [Range]
Age (years)	71.2 (6.9) [56.0-85.0]
Physical examination	
BMI (kg/m^2)	29.2 (5.0) [22.7-50.2]
Blood Pressure (mmHg)	
Systolic	123.3 (14.5) [90-153]
Diastolic	71.8 (11.5) [50-107]
Heart rate (beats per minute)	76.0 (13.8) [51-107]
Respiratory rate (per minute)	22.0 (3.6) [14-28]
Oxygen saturation (%)	95.0 (2.1) [89-99]
Lung function tests	
FEV1 (litres)	2.04 (0.55) [0.67-3.14]
FEV1 (% predicted)	77.67 (16.79) [46-122]
FVC (litres)	2.54 (0.71) [1.55-4.32]

FVC (% predicted)	75.49 (17.34) [44-122]
TLCO (mmol/min/kPa) (n=44)[1]	3.99 (1.46) [1.53-7.16]
TLCO (% predicted)	49.00 (15.67) [19-87]
KCO (mmol/min/kPa/litre) (n=44)[1]	1.11 (0.27) [0.56-1.66]
KCO (% predicted)	85.25 (21.33) [45-128]
6 minute walk test	
Distance walked (m) (n=44)[2]	373.26 (115.53) [50.0[3]-550.0]

(1) Participant ID 014 – gas transfer factors were not possible to measure due to sub-optimal technique; (2) Participant ID 027 – had above knee amputation hence 6MWT was not performed; (3) Participant attended in wheelchair, 6MWT was difficult to perform.

Participants recruited for the study were predominantly male IPF patients between the age group of 65-84 years (with 5 patients between 40-64 years and 2 patients who were 85 years old). Mean smoking history of approximately 20 pack years (data available from 31 participants only). On an average each participant suffered from 3 or more additional medical illness and were prescribed 7 or more medications (for regular and/or "as needed" use).

Table 5 below shows the extent of co-existing medical illness among the participants at screening. A table of pre-specified comorbidities was listed in the CRF to be recorded routinely. I have also documented the other medical conditions that were most commonly reported in addition to the listed conditions.

Table 5: List of co-existing medical illness among participants (n=45):

Medical comorbidities	Number of patients	Percent (%)
Hypertension	19	42.2
Diabetes Mellitus	10	22.2
Hyperlipidaemia	1	2.2
Stroke	3	6.7
Heart failure	5	11.1
Atrial Fibrillation	2	4.4
Ischaemic heart disease	13	28.9
Osteoarthritis	7	15.6
Osteoporosis	3	6.7
Peripheral vascular disease	1	2.2
Malignancy - Prostate	3	6.7
Gout	6	13.3
Hypothyroidism	4	8.9
Chronic kidney disease	3	6.7
Asthma	2	4.4
Emphysema	2	4.4
Increased BMI	6	13.3

Table 6 below is a list of concomitant medications that the participants were taking at screening. The list is not exhaustive; I have reported the most frequently prescribed medications among the participants.

Table 6: List of concomitant medication at screening (n=45):

Drug	Number of patients	Percent (%)
Aspirin	20	44.4
Pirfenidone	18	40.0
Simvastatin	13	28.9
Atorvastatin	10	22.2
Paracetamol	10	22.2
Lansoprazole	9	20.0
Levothyroxine	9	20.0
Losartan	9	20.0
Amlodipine	8	17.8
Carbocisteine	8	17.8
Omeprazole	7	15.6
Codeine	6	13.3
Glyceryl trinitrate spray	6	13.3
Alendronic acid	5	11.1
Allopurinol	5	11.1
Bendroflumethiazide	5	11.1
Bisoprolol	5	11.1
Colecalciferol and calcium carbonate	5	11.1
Isosorbide mononitrate	5	11.1
Metformin	5	11.1
Prednisolone	5	11.1
Salbutamol inhaler	9	20.0
Furosemide	4	8.9
Oxygen	4	8.9
Ramipril	4	8.9

As outlined previously, 34 potentially eligible IPF patients were not included in the study due to “clinical reasons”. These patients were eligible for the study (based on study inclusion and/or exclusion criteria) but were not recruited due to clinical and/or technical considerations as explained below.

2 patients had already undergone gastric surgery (1 fundoplication for reflux disease and 1 gastrectomy surgery for cancer) hence it would not be possible to perform GI physiology study with them. Patients with previous history of significant GI bleed were deemed unsafe to discontinue antacid (PPI) therapy (considered risky for trial of “ 2 week period of supervised discontinuation of PPI therapy”). 5 patients with IPF

died (for example, due to lung cancer, acute exacerbation of fibrosis) during screening period. 7 patients with advanced disease (who were unsuitable for specific anti-fibrotic therapy) preferred palliative care on a domiciliary basis precluding them from participation in the study (which entailed multiple hospital visits for study related procedures). Therefore, often it has been a challenge to recruit potential IPF patients with end stage disease for the trial due to the inherent nature of the study.

4.3 Subjective assessment of cough and gastro-oesophageal reflux disease in patients with IPF

The impact of cough on daily life of the participants was assessed by the LCQ. LCQ was primarily developed for use in patients with chronic cough (hence "normal score" for healthy individuals is not known). Subsequently it has been validated to assess impact of cough severity in various respiratory conditions like bronchiectasis (Murray *et al*., 2009) and COPD (Berkhof *et al*., 2012).
Participants filled the questionnaire on the first day of study visit prior to initiation of drug therapy. Table 7 below summarises impact of cough on quality of life as reported by patients at baseline (assessed by the LCQ).

Table 7: Impact of cough on quality of life at baseline (n=45)

Cough related quality of life questionnaire at baseline	Cohort N=45 Mean (SD) [Range]
Leicester Cough Questionnaire [possible range: 3-21; higher score indicate better quality of life]	
LCQ – Total	15.22 (3.18) [7.92-20.63]
LCQ – physical domain	05.13 (1.01) [2.63-6.75]
LCQ – psychological domain	4.93 (1.27) [2.29-7.00]
LCQ – social domain	5.16 (1.17) [1.75-7.00]

The above results suggested impairment of quality of life in patients with IPF due to cough across all domains (physical, social, psychological).

The impact of reflux symptoms on health and day-to-day life of the participants was assessed by the RSI, GIQLI and demesster reflux questionnaires (DeMRQ). In addition to the LCQ, participants were requested to fill the reflux related quality of life questionnaires on the first day of study visit prior to initiation of drug therapy. Table 8 below summaries impact of reflux on quality of life as reported by patients at baseline as assessed by the above mentioned questionnaires.

Table 8: Impact of reflux on quality of life at baseline (n=45)

Reflux related quality of life questionnaires at baseline	Cohort N=45 Mean (SD) [Range]
Reflux Symptom Index Questionnaire [possible range: 0-45; lower score indicate better quality of life]	15.69 (9.30) [0-33]
Gastro-Intestinal Quality of Life Index Questionnaire [possible range: 0-144; higher score indicate better quality of life]	105.56 (17.66) [62-135]
De-Meester Reflux Associated Questionnaire [possible range: 0-9; lower score indicate better quality of life]	1.16 (1.15) [0-5]
Score = 0	16 (35.6%)
Score = 1	13 (28.9%)
Score = 2	11 (24.4%)
Score = 3	4 (8.89%)
Score = 5	1 (2.22%)

Overall high RSI score (mean 15.69) would suggest increased incidence of gastro-oesophageal reflux disease in our study population. Accordingly the GIQLI score

(mean 105.56) also suggest some impairment of quality of life possibly due to reflux disease in our study cohort. However the DeMRQ score (mean 1.16) suggest better health in majority of the participants.

The main reason for the disparity is not entirely clear but may be related to the inherent nature of the questionnaires, the disease condition studied and the complex interaction between cough and breathlessness in patients with IPF. RSI is designed to assess the possibility of laryngo-pharyngeal reflux in patients presenting with cough (with or without throat symptoms) thereby prompting further definitive investigations. GIQLI is an extensive questionnaire developed to assess quality of life in patients with gastro-intestinal diseases. They are sensitive hence might have identified patients with mild reflux disease (associated with no or minimal symptoms) among the study participants. DeMRQ was not developed to be used in IPF patients and it is possible that it does not truly reflect impact of reflux disease in these patients.

Breathlessness and cough are predominant symptoms in IPF. Reflux disease in turn can cause cough (specially after meals or lying down), throat and/or choking sensation, heartburn, chest tightness leading to sensation of breathlessness. Therefore symptoms (cough and breathlessness) could be attributed to both IPF and/or reflux disease. Patients with self-reported cough were enrolled for the study. Hence it is possible that patients have marked high scores on the following items of the RSI questionnaire (attached Appendix 1): presence of “troublesome cough”, “breathing difficulty or choking sensation”, “ cough after meals or on lying down”, “throat clearing” (difficult/unable to distinguish between cough and breathlessness related to IPF or reflux disease). Further, DeMRQ is a short questionnaire (attached Appendix 1) comprising of 3 items associated with a typical reflux symptom i.e. heartburn, regurgitation, dysphagia (no cough or breathlessness related item or question). As outlined previously, it is well established from published literature that there is increased prevalence of reflux disease in IPF patients compared to healthy population and patients with other chronic respiratory diseases but only a minority of these patients suffer/report classical reflux related symptoms.

4.4 Objective assessment of cough in patients with IPF

Objective assessment of cough severity in participants was performed with help of a 24-hour cough recording device as outlined in the section 3.6.5. Dr J Smith and her team provided us with the "cough count" data. I have attached an example (Table 9 below) of 24-hour cough count result (of participant ID 003 at baseline) as provided by colleagues at Manchester for the purpose of our study.

Table 9: Example of 24-hour cough count data at baseline (participant ID 003)

T:\Newcastle\IPF & Omeprazole Study\PPIPF_027\PPIPF_027_V1

Study	Newcastle IPF & Omeprazole Study
Subject ID	PPIPF_027
Visit number	1
Randomisation number	
Report date	31-Jul-14

Hour 0 cough count	6
Hour 1 cough count	2
Hour 2 cough count	7
Hour 3 cough count	24
Hour 4 cough count	6
Hour 5 cough count	21
Hour 6 cough count	14
Hour 7 cough count	8
Hour 8 cough count	3
Hour 9 cough count	26
Hour 10 cough count	20
Hour 11 cough count	6
Hour 12 cough count	6
Hour 13 cough count	0
Hour 14 cough count	0
Hour 15 cough count	0
Hour 16 cough count	0
Hour 17 cough count	0
Hour 18 cough count	0
Hour 19 cough count	0
Hour 20 cough count	4
Hour 21 cough count	17
Hour 22 cough count	5
Hour 23 cough count	0
Total coughs (Awake)	171
Total coughs (Asleep)	4
Total coughs 24 hr	175
Coughs per hour (Awake)	10.34
Coughs per hour (Asleep)	0.54
Coughs per hour (24 hr)	7.29

Sleep start (sample points)	348093422
Sleep end (sample points)	563005038
Sleep start (hh:mm:ss)	12:05:12
Sleep end (hh:mm:ss)	19:32:56

The data show total number of coughs recorded over a period of 24 hours with the help of which the final cough count per hour is calculated (total number of coughs/total recording time in hours). In the above example cough frequency is 7.29 per hour. It also shows daytime cough rate [cough per hour (awake)] and night-time cough rate [cough per hour (asleep)] which are 10.34/hr. and 0.54/hr. respectively.

Table 10 below summarises 24-hour cough frequency (cough count/hour) of the participants at baseline. Table also includes daytime and nocturnal cough counts.

Table 10: Baseline cough frequency (cough/hour) of participants (n=45)

Objective cough assessment at baseline (cough/hour)	Cohort N=45 Mean (SD) [Range]
24-hour cough frequency	11.99 (10.18) [1.63-52.29]
Daytime cough frequency	15.68 (13.72) [2.52-75.10]
Nocturnal cough frequency	4.62 (5.74) [0.00-21.00]
Total duration of cough recording (hours)	23.55 (0.92) [19.03-24.00]

24-hour cough recording data show mean cough frequency of 11.99/hour. Diurnal cough count is significantly higher than nocturnal cough counts (15.6/hr. compared to 4.6/hr.). These suggest IPF patients, who participated in our study, could cough up to 12 times in an hour. Their cough was particularly worse through out the day when they are awake. Good quality recording over 24-hour period was obtained from majority of the participants.

Hsu et al., (1994) studied cough frequency and diurnal variation in cough in 12 normal subjects, 21 stable asthmatics and 14 patients with chronic cough. They demonstrated only 0-16 coughs over 24-hour period in the normal subjects compared to median cough of 282 (range 45-1577) in asthma patients and median cough of 794

(range 64-3639) in patients with chronic cough. They also demonstrated cough most frequently occurred between 11:00 am – 02:00 pm and least between 02:00 am – 05:00 am (less than 3% in total). Cough during sleep was equally reduced in asthmatics and chronic coughers. Similar low cough counts (less than 2 per hour) in healthy individuals and diurnal variation in cough have been shown in further studies with patients suffering from chronic cough (Birring *et al*., 2006) and asthma (Marsden *et al*., 2008)

Key et al., (2010) measured significant high cough count (median 9.4/hr., range 1.5–39.4) in 19 IPF patients when compared to healthy subjects and asthmatics but similar to patients with chronic cough. Day-time cough rate (median 14.6/hr.) in these patients was much higher than night-time cough rate (median 1.9/hr.).

Diurnal variation in cough frequency among young adults during common cold was noted by Kuhn et al., (1982). Suppression of cough during sleep in patients with chronic bronchitis was demonstrated by Powers et al., (1984). The exact mechanism of cough suppression during sleep in not well known. Cough is a defence reflex therefore reduced exposure to stimuli in association with decrease cough reflex sensitivity at night might account for reduced cough during sleep (Lee *et al*., 2010).

The cough frequency noted in our study participants are significantly higher and consistent with previously published data.

4.4.1 Relation between subjective and objective assessment of cough

Previous studies have shown strong correlations between objective cough counts and cough related quality of life questionnaires (i.e. LCQ) in patients with chronic cough (Key *et al*, 2010; Kelsall *et al*., 2011). A high cough frequency negatively correlates with LCQ (high score implies better health quality).

Figure 12 below shows scatterplot (with regression) of cough frequency (cough count/hour) and LCQ-total score of our study patients (n=45). Analysis was performed with Minitab 17 statistical software.

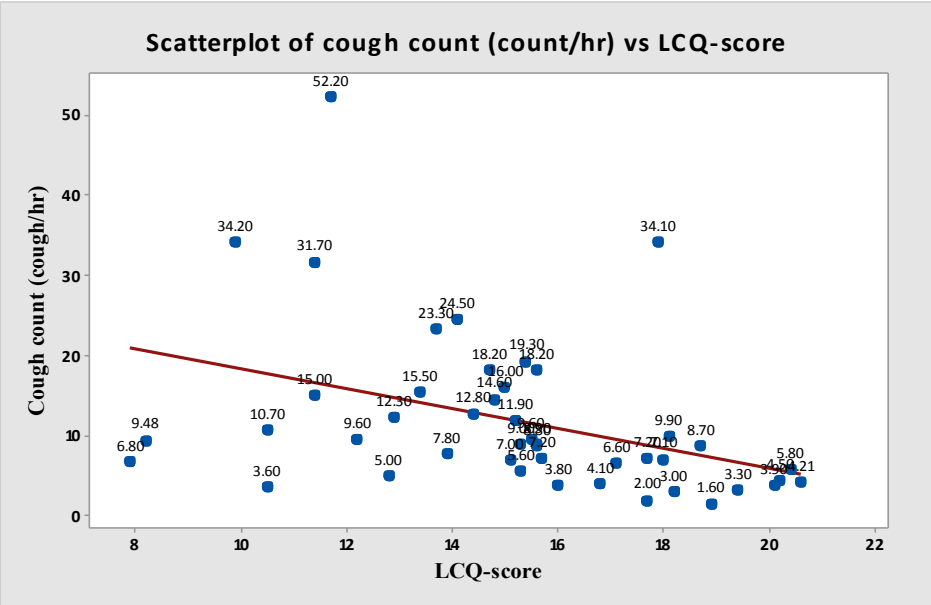


Figure 12: scatterplot showing negative correlation between cough count per hour and LCQ-total score (R – Sq.= 14.9%). Data points in blue represent cough counts (per hour) of our study participants.

In keeping with published literatures, data from our study show negative correlation between objective cough counts and LCQ-total score in patients with IPF.

4.5 Objective assessment of gastro-oesophageal reflux disease in patients with IPF

13 (28.9%) of the total recruited 45 participants consented for the GI physiology studies but baseline assessments were performed only on **9** (**69.2%** of those who consented) participants.

1 participant declined GI study during initial visit. Procedure was abandoned in 3 participants, as 2 were unable to tolerate manometry and/or pH-impedance catheter (for the tests to be completed), while I was not able to insert GI catheter in 1 participant.

All the participants who underwent GI physiology assessment successfully completed both the HRM and the 24-hour pH-impedance studies. Table 11 below summarises the findings on HRM oesophagus study at baseline.

Table 11: HRM oesophageal study at baseline (n=9)

Patient ID	HRM – oesophagus results
001	Normal study
002	Normal study
003	Normal study
005	Oesophago-gastric junction outflow obstruction
013	Oesophago-gastric junction outflow obstruction
020	Normal study
022	Ineffective oesophageal motility
030	Normal study
032	Oesophago-gastric junction outflow obstruction

In summary, **4 (44%)** out of the 9 patients with IPF demonstrated oesophageal motility disorder in the HRM study. HRM study is usually performed prior to pH-impedance study to assess integrity of oesophageal peristalsis and to exclude additional oesophageal motility disorder. The study results influence further decisions regarding anti-reflux surgery in patients with significant reflux disease. Presence of oesophageal dysmotility in IPF patients can predispose to reflux disease. Alternatively fibrosis of the lung itself can cause oesophageal motility disorder (disruption of normal anatomy by mechanical stretching) which in turn slows bolus clearance, increases exposure time to refluxate thereby accounting for increased prevalence of reflux disease in IPF patients.

I have listed the individual ambulatory pH-impedance test results of the participants in the table below (Table 12). The data presented is corrected for 24-hour study [i.e. raw data x (time on study in hours/24 hours)].

Table 12: Baseline data of 24-hour pH-impedance study (n=9)

Study parameters (normal values/ range)	**Patient ID**								
	001	002	003	005	013	020	022	030	032
Time pH <4 (<4.2%)	**6.4**	**12.8**	**7.6**	**8.4**	1.0	**4.2**	0.7	**22.4**	1.6
Number of times pH <4 (<50)	36.0	**71.0**	**54.1**	**51.0**	13.0	23.0	11.0	**65.0**	14.0
Number of long reflux episodes (<4.0)	**4.0**	**6.0**	**4.0**	**7.0**	0.0	2.0	0.0	**8.0**	0.0
Longest reflux episodes (2.2 min)	**25.2**	**33.0**	**14.6**	**13.1**	**3.2**	**26.3**	1.7	**16.9**	**4.0**
Number of acid refluxes (10-35)	35.0	**48.0**	23.0	**36.0**	15.0	29.0	1.0	18.6	12.0
Number of weak acid refluxes (5-18)	**24.0**	17.0	15.0	9.0	**38.0**	5.0	**58.0**	2.7	**19.0**
Number of proximal reflux events (4-17)	10.0	13.0	2.0	**20.0**	13.0	**21.0**	13.0	**17.0**	3.0
DeMeester score (<14.72)	**18.4**	**46.4**	**22.9**	**25.4**	3.8	**17.6**	3.0	**57.8**	4.2
Total number of reflux episodes (25-58)	**73.1**	**65.9**	38.0	46.0	55.0	36.0	**61.0**	30.8	31.0
Bolus clearance time (8-13 s)	9.0	10.0	13.0	12.0	8.0	13.0	**14.0**	**16.0**	11.0
Symptom associated probability (<95%)	0.0	**99.9**	**99.9**	0.0	0.0	0.0	0.0	0.0	0.0

The numbers highlighted (or in bold) represent values that are above the known normal range for the particular study parameter.

24-hour pH-impedance study confirmed presence of gastro-oesophageal reflux disease in most of the participants (**6** out of the 9 patients) as evidenced by high DeMeester scores (mean score: 22.1, range: 3.0, 57.8). I have highlighted the elevated DeMeester scores in the table above. Data also show higher number of weak acid reflux events among the participants (mean number of weak acid reflux events: 21.0, range 5.0, 58.0). Participants 013 and 022 suffered from significantly higher episodes of weak acid reflux events (number of events highlighted in the table above) although their DeMeester scores were within normal range. 3 out of the 6 patients (whose DeMeester scores were also elevated) demonstrated significant numbers of proximal reflux events (highlighted in the table above).

Interestingly only **2** patients (participant ID 002 and 003) with objective evidence of reflux disease reported clinical symptoms, which indicate symptoms, were related to reflux disease (SAP 99.9%). None of the other patients (with evidence of reflux in pH and/or impedance study) including those with significant amount of proximal reflux reported any symptoms during the study period suggesting silent or asymptomatic reflux disease in most of the patients.

Therefore, the 24-hour pH-impedance study data confirmed presence of significant reflux disease in majority of the participants within this study cohort.

However acceptability of GI physiology studies was low amongst the study participants. Although our study primarily focussed on cough, less than 30% of the participants consented for the GI studies. The invasive nature of the tests compounded by mandatory, multiple hospital visits and pre-existing medical co-morbidities made the tests undesirable among study participants. In addition, GI physiology assessments can be technically demanding both for patients and clinicians. 1 patient failed GI catheter insertion and 2 patients were unable to tolerate catheter in situ for completion of study. Therefore, even a smaller number of patients completed GI studies (9 out of 13 patients who initially consented, 1 participant declined during initial visit). I have discussed further regarding low rate of consent and completion of GI studies in subsequent section (section 5.3).

4.6 Relation between cough and reflux disease in patients with IPF

Only 9 out of 45 participants underwent formal GI physiology study hence it is difficult to establish any significant correlation between subjective and objective assessment of reflux disease. For similar reasons it is difficult to explore any association between increased cough count and presence of reflux disease in this cohort of IPF patients.

The following table (Table 13) compares baseline GI physiology studies of those 9 participants with their respective cough counts (per hour) and reported quality of life scores.

Table 13: Baseline GI physiology studies with respective cough counts (per hour) and reported quality of life scores (n=9)

Patient ID	GI test:HRM 24-hour pH-impedance (DeMeester score)	Cough count (per hour)	LCQ – score (total)	RSI - score	GIQLI - score	DeMRQ - score
001	HRM: normal DeMeester: **18.4**	8.67	18.70	04	129	0
002	HRM: normal DeMeester: **46.4**	10.67	10.50	28	62	2
003	HRM: normal DeMeester: **22.9**	8.77	15.60	14	119	1
005	HRM: O-GJO* DeMeester: **25.4**	19.38	15.40	06	120	0
013	HRM: O-GJO* DeMeester: 3.8	15.55	13.38	24	102	0
020	HRM: normal DeMeester: **17.6**	23.33	13.68	17	120	0
022	HRM: IOM** DeMeester: 3.0	3.33	19.36	4	111	2
030	HRM: normal DeMeester: **57.8**	14.56	14.85	4	100	1

032	HRM: O-GJO* DeMeester: 4.2	2.00	17.73	11	96	1

*O-GJO: Oesophago-gastric junction outflow obstruction
**IOM: Ineffective oesophageal motility

Patients who recorded high DeMeester score (above the accepted normal score of 14.7) on 24-hour pH-impedance study also demonstrated increased cough frequency on 24-hour ambulatory cough recording. Participants ID 005, 020, 030 demonstrated significantly greater cough frequency (above the calculated mean cough rate of 11.9/hr. in this study) also recorded comparable higher DeMeester score. This trend provides further support to well-accepted theory that acid reflux disease can cause persistent/chronic cough. The RSI score was appropriately raised in 3 participants with objective evidence of reflux disease (participants ID 002, 003 and 020) but no positive correlation demonstrated in other participants. Based on the data above, no significant trends were identified with regards to the GIQLI and DeMRQ quality of life scores when compared to GI physiology studies. This might reflect higher incidence of "silent reflux" disease in our study population but it is difficult to draw any firm conclusions given the limited number of studies performed.

Given the small number of studies available, no meaningful and/or valid relations might be demonstrated, however, exploring various plausible correlations is one of the main reasons for undertaking a research study. Therefore to explore further, I have plotted (scatter plot with regression) cough count (per hour) against DeMesster scores and reflux related health questionnaire scores. Analysis was performed with Minitab 17 statistical software.

Figure 13 below shows scatter plot with regression of cough count (per hour) and DeMeester scores of our study participants (n=9).

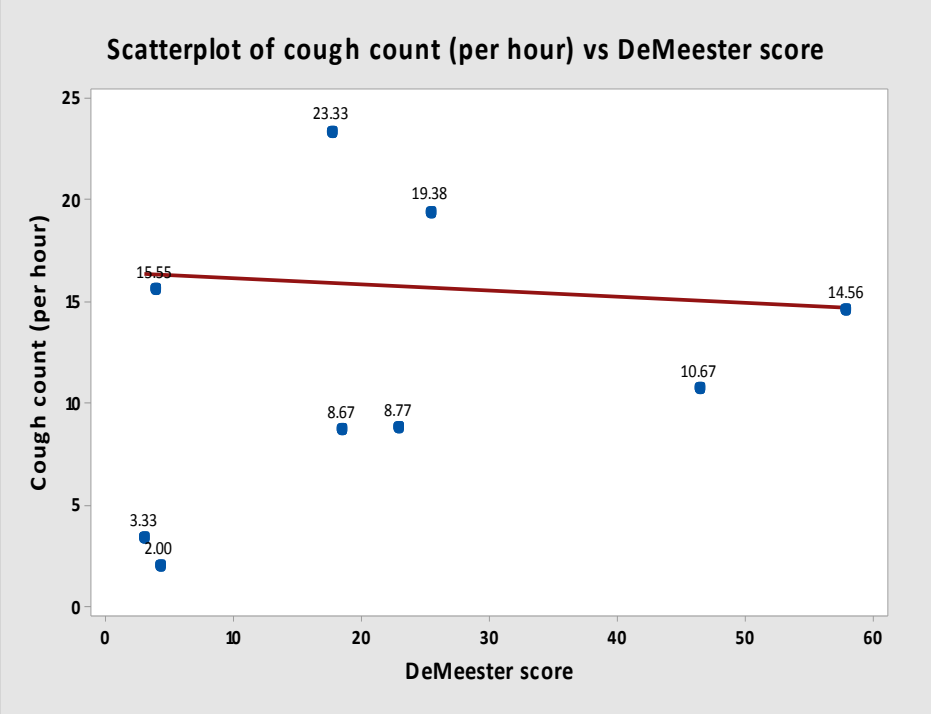


Figure 13 : scatterplot showing no significant correlation between cough count (per hour) and DeMeester scores (R-Sq=100%). Data points in blue represent cough counts (per hour) of our study participants.

Scatterplot does not show any significant correlation between cough frequency and DeMeester scores, as opposed to the well known fact that reflux causes cough. However, data were available only from 9 participants hence it is difficult to draw any firm conclusion. Conversely, it is possible that cough can also be induced by non-acid reflux. DeMeester score (which is a composite score taking into account pH measurements) will not be high in patients with predominantly non-acid reflux disease.

Figure 14 below shows scatter plot with regression of cough count (per hour) and RSI scores of our study participants (n=9).

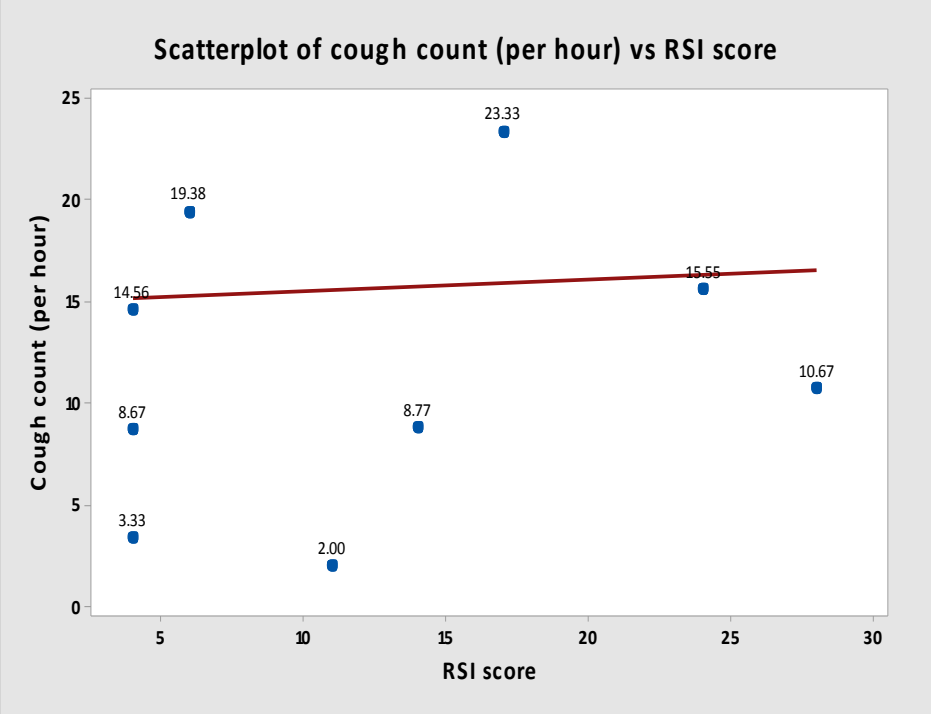


Figure 14: scatterplot showing no significant correlation between cough count (per hour) and RSI scores (R-Sq=84%). Data points in blue represent cough counts (per hour) of our study participants

Scatterplot does not show any significant positive correlation between cough frequency and RSI scores, as was expected. This is difficult to explain, but might be related to the limited amount of data available. In addition, RSI was not developed to be used in patients with IPF and it is possible that it does not actually reflect the impact of cough in these patients.

Figure 15 below shows scatter plot with regression of cough count (per hour) and GIQLI scores of our study participants (n=9).

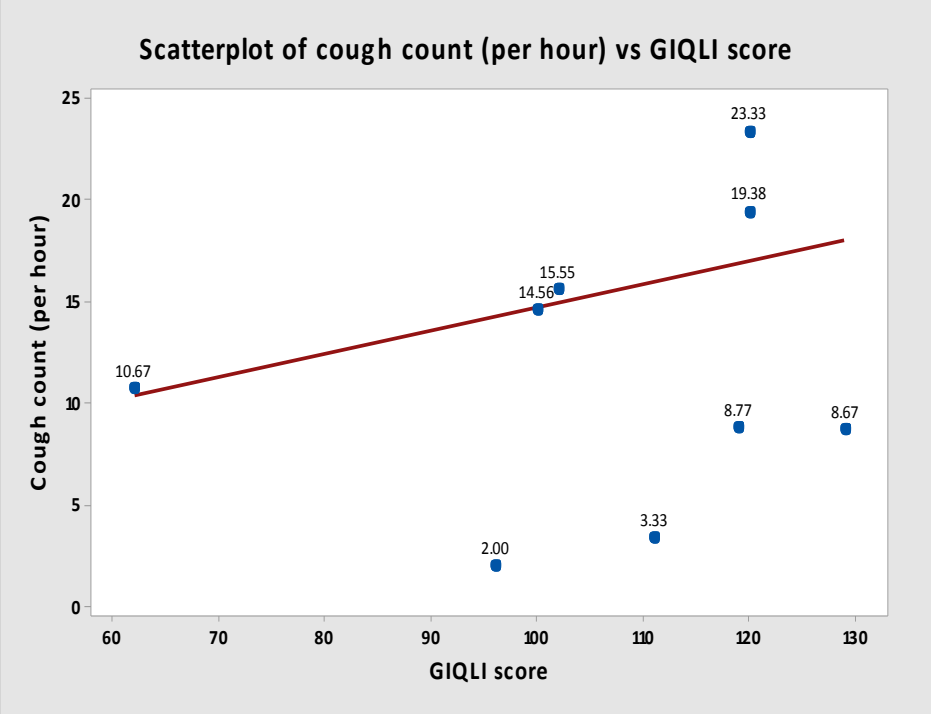


Figure 15: scatterplot showing no significant correlation between cough count (per hour) and GIQLI scores (R-Sq=98%). Data points in blue represent cough counts (per hour) of our study participants

Figure 16 below shows scatter plot with regression of cough count (per hour) and DeMRQ scores of our study participants (n=9).

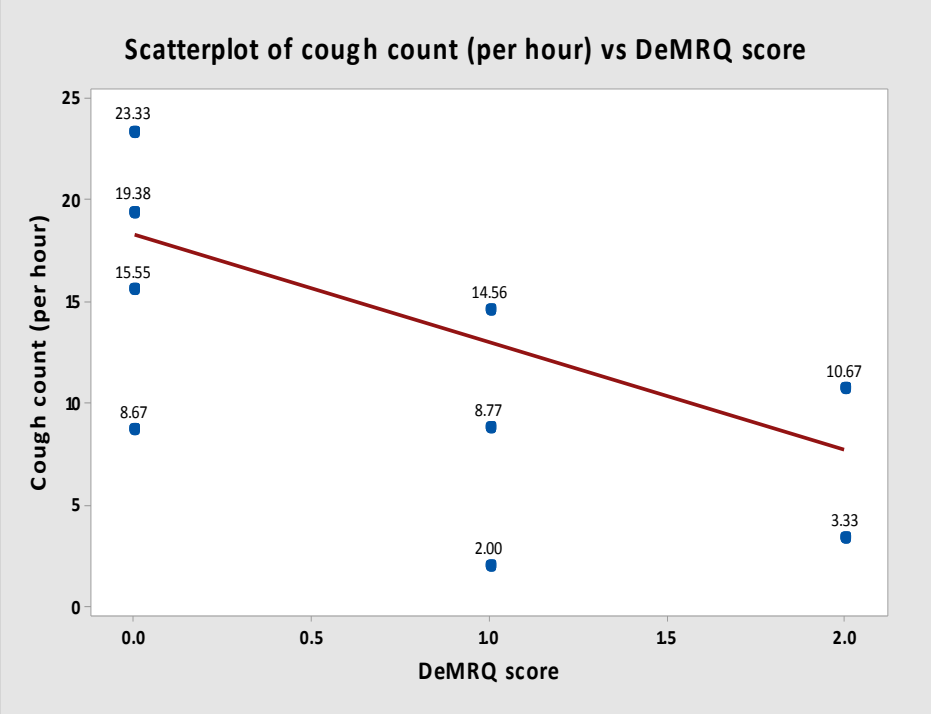


Figure 16: scatterplot showing no significant correlation between cough count (per hour) and DeMRQ scores (R-Sq=44%). Data points in blue represent cough counts (per hour) of our study participants

Scatterplots for both the GIQLI and DeMRQ scores show unexpected trends. It shows a trend towards a positive correlation between cough frequency and GIQLI scores and a trend towards a negative correlation between cough frequency and DeMRQ scores. Higher GIQLI score implies a better health status, conversely lower DeMRQ score imply better health status. Therefore, high cough frequency should negatively correlate with GIQLI scores and positively correlate with DeMRQ scores. This discrepancy is difficult to explain by the limited amount of data available. This could be related to the high incidence of asymptomatic or silent reflux disease in our study cohort. Also, these questionnaires were not developed to be used in IPF patients and it is possible that this does not really capture the impact of cough in IPF patients.

4.7 Bronchoscope and BAL samples

13 (28.9%) out of the recruited 45 participants consented for bronchoscopy at randomisation. Of them **8 (61.5%)** had procedure performed.

1 participant was withdrawn from the study (prior to completion of assessments) while another participant was not keen to discontinue his regular anticoagulant medication prior to bronchoscopy test. 2 participants were unable to attend on the planned date (1 due to accidental fall leading to severe back pain, 1 was admitted to hospital due to an unrelated illness). Procedure was not performed on 1 participant due to low resting oxygen saturations levels (procedure deemed unsafe on clinical grounds).

Table 14 below shows results of bronchoscopy and BAL sample analysis. It outlines the macroscopic features, the total and differential cell counts and the microbiology culture (for bacteria, acid fast bacillus, fungus) results.

Table 14: Results of bronchoscopy and BAL samples analysis (n=8)

Patient ID	Bronchoscopy	Total cell count (10^4/ml BAL)	Differential count [count (%)]	Microbial cultures
003	Normal appearance	12.4	Macrophages: 490 (98%) Neutrophil: 006 (1.2%) Lymphocytes: 004 (0.8%) Eosinophils: 000 (0%) Ciliated epithelial: 001 (0.2%) Metaplastic epithelial: 011 (2.1%)	No pathogens
005	Normal appearance	301.8	Macrophages: 086 (17.2%) Neutrophil: 398 (79.6%) Lymphocytes: 016 (3.2%) Eosinophils: 000 (0%) Ciliated epithelial: 004 (0.8%) Metaplastic epithelial: 000 (0%)	*Pseudomonas aeruginosa*
013	Normal appearance	24.5	Macrophages: 197 (39%) Neutrophil: 241 (48%) Lymphocytes: 062 (13%) Eosinophils: 000 (0%) Ciliated epithelial: 009 (2%) Metaplastic epithelial: 015 (3%)	*Staphylococcus aureus*
016	Normal appearance	83.0	Macrophages: 359 (72%) Neutrophils: 091 (18%) Lymphocytes: 050 (10%)	No pathogens

			Eosinophils: 000 (0%) Ciliated epithelial: 022 (4%) Metaplastic eplithelial: 005 (0.9%)	
020	Normal appearance	17.3	Macrophages: 344 (69%) Neutrophils: 126 (25%) Lymphocytes: 030 (6%) Eosinophils: 000 (0%) Ciliated epithelial: 006 (1.2%) Metaplastic epithelial: 004 (0.8%)	No pathogens
030	Bronchitic mucosa	36.3	Macrophages: 490 (98%) Neutrophils: 001 (0.2%) Lymphocytes: 009 (1.8%) Eosinophils: 000 (0%) Ciliated epithelial: 005 (1%) Metaplastic epithelial: 001 (0.2%)	No pathogens
032	Normal appearance	14.8	Macrophages: 462 (92.4%) Neutrophils: 033 (6.6%) Lymphocytes: 004 (0.8%) Eosinophils: 001 (0.2%) Ciliated epithelial: 015 (2.9%) Metaplastic epithelial: 000 (0%)	No pathogens
044	Normal appearance	27.3	Macrophages: 492 (98%) Neutrophils: 002 (0.4%) Lymphocytes: 006	No pathogens

			(1.2%) Eosinophils: 000 (0%) Ciliated epithelial: 002 (0.4%) Metaplastic epithelial: 000 (0%)	

In summary, bronchoscopy was macroscopically normal in all patients except participant ID 030. As expected, BAL samples showed leucocytosis with macrophages being the predominant cells. Only 2 patients (participant ID 005, 013) showed increased neutrophil counts in their BAL, both have shown bacterial pathogens on cultures probably indicating on-going chest infection. Only a limited number of patients in the study underwent bronchoscopy test hence it is difficult to draw any firm conclusions from the above results with regards to the differential cell counts and the rate of infection in patients with IPF.

Similar to the GI physiology study, bronchoscopy was not favoured by the study participants. Less than a third of the patients consented to undergo the procedure and only 8 completed the assessment. The procedure was undesirable amongst the participants due to the invasive nature of the test, associated mandatory hospital attendance and associated risks especially with lung fibrosis.

Chapter 5. Discussion

This study was designed to comprehensively assess impact of cough and reflux in patients with IPF. Advanced techniques were used to objectively assess cough and reflux disease. The trial focuses on a current major health problem and if the results are positive, is likely to provide further evidence in favour of a commonly used drug (omeprazole) treatment for the unmet health problem.

As outlined in the Consort diagram (Figure 11), I have screened a considerable number (n=280) of identified IPF patients to successfully recruit 45 participants for the study. Long-term PPI therapy was one of the predominant barriers to patient recruitment. I have discussed difficulties/challenges relating to screening and recruitment in details in the following sections. Despite multiple measures (as described in the subsequent sections) to improve recruitment remained below expected through out the study period. Approximately 2 participants were randomised every month.

In addition, acceptance of study related procedures was lower than expected. Of the recruited 45 participants, 13 (approximately 29%) consented for the GI physiology and/or bronchoscopy studies at baseline but GI study and bronchoscopy was actually performed only in 9 (69%) and 8 (61%) participants respectively. I have already outlined clinical and/or practical constraints regarding performing GI and bronchoscopy procedures in Chapter 4 (sections 4.5, 4.7). I have discussed "acceptance of study procedures" among the participants in further details in subsequent sections.

Participants recruited for the study had a secure diagnosis of IPF (history, radiology, and lung function test reviewed in the regional ILD-MDT and clinic at RVI). The majority of the patients suffered from moderate severity of disease (based on baseline patient characteristics) and were male, and consistent with the gender prevalence in IPF.

Consent and completion rate of non-invasive assessments among the study participants was high (one patient was unable to perform gas transfer factor tests due to poor technique and another patient, who had above knee amputation, were unable to perform 6MWT). All the participants completed baseline quality of life assessments (questionnaires) and 24-hour ambulatory cough recording. Participants tolerated the ambulatory cough recording well through out the study period with no major problems reported or identified.

Baseline data demonstrated significantly high cough frequency in IPF patients (mean 11.99/hour, range 1.63 – 52.29). Daytime cough frequency was markedly elevated than nocturnal cough frequency (Table 10). The cough rate in IPF patients demonstrated in our study is slightly higher than that published in a previous similar study (9.4/hour). (Key *et. al*. 2010).

Cough was associated with significant impairment of daily lives of patients with IPF as indicated by the LCQ-total score (mean15.22, range 7.92-20.63). Low scores were seen across all domains of LCQ providing further evidence that cough is a disabling symptom in patients with IPF with huge physical and social impact. Data also suggested a negative correlation (as expected) between subjective (LCQ-total score) and objective assessment of cough (cough count per hour) in patients with IPF.

Reflux related health quality questionnaires (RSI, GIQLI and DeMRQ) demonstrated interesting results. The RSI (mean score 15.69) suggested an increased incidence of reflux disease in our study cohort. The GIQLI (mean score 105.56) further confirmed impaired quality of life, possibly due to reflux disease in these patients. In contrast, DeMRQ (mean score 1.16) suggested a better health in the majority of the patients. The reason for this discrepancy is not entirely clear, but I have already discussed possible explanations in the previous chapter (Chapter 4).

Only a small proportion of patients consented for GI physiology and bronchoscopy studies for the inherent “invasive” nature of the procedures. I have briefly discussed

"acceptability" of these procedures among the study participants in the previous chapter. I have further discussed regarding the low rate of consent and completion of GI studies and bronchoscopy in the following section. 4 out of the 9 patients with IPF demonstrated peristaltic disorder in HRM study of the oesophagus (Table 11). 6 out of the 9 participants showed objective evidence of significant reflux study in 24-hour pH impedance study. In addition to acid reflux, considerable numbers of weak acid reflux events were noted in the impedance studies (Table 12). Participants ID 013, 022, 032 (with normal DeMeester score) showed high number of weak acid reflux events. 3 patients with objective evidence of reflux disease (in the pH-impedance study) demonstrated a significant number of proximal reflux events. Patients who showed evidence of reflux disease also demonstrated raised cough count on ambulatory cough recording (Table 13).

However only 2 participants with reflux disease reported associated clinical symptoms. No definite correlation was noted between the reflux related quality of life questionnaires in patients with evidence of reflux disease on impedance study.

Overall results suggest increased incidence of reflux disease among the IPF patients in our study (as evidenced by impedance study). Presence of reflux disease was associated with higher cough frequency. Majority of patients (with objective evidence of reflux in impedance study) did not report any symptoms suggesting increased incidence of "silent" or asymptomatic reflux disease in our patients. However these observations (regarding GI studies, cough, reflux related questionnaires) are based on only small numbers of studies and/or participants hence it is not possible to draw any firm conclusions.

Similarly limited bronchoscopy studies were performed. As expected, BAL samples showed leucocytosis with macrophages predominant. Only 2 participants showed signs of possible infection hence it is difficult to comment on BAL cell count or rate of infections (in relation to IPF and/or trial drug therapy).

In future, further BAL sample analysis can be performed to look for markers of inflammation and aspiration (as outlined in Chapter 3, section 3.6.7) and comparison made with GI physiology study and cough counts. This could provide further evidence in favour of reflux-associated cough in patients with IPF.

Given the interesting baseline findings, I am looking forward to the final analysis of the trial data especially relating to cough. In total 45 patients were recruited. We have achieved our initial aim of randomising at least 40 participants for the study. I believe trial results will provide useful information with regards to the role of antacid therapy in patients with IPF and cough. If results are positive, it will help plan further multi-center definitive trials, If not it will guide future researches in treatment and pathogenesis of IPF.

5.1 Difficulties/challenges encountered in the PPIPF study

The initial study plan was to recruit 60 patients over a period of 21 months (starting from August 2013), which meant a target recruitment rate of 3 participants per month.

At the time of my appointment in August 2013, Ethics Committee and MHRA approvals for the study were already in place. The Research Ethics Committee (REC) and MHRA granted approval for the study by September 2013.

Newcastle Clinical Trials Unit subsequently scrutinised the submission again, and noted that some of the original submission was based on a template used by a different Clinical Trials Unit (CTU). For internal consistency and clarity, they suggested that improvements could be incorporated around sections on patient recruitment, randomisation, statistical analysis, pharmacovigilance, and reporting of adverse events during the study period. Therefore the requested changes were made. The overall study design and the end-points to be assessed did not change. This meant substantial amendments, and all other related documents e.g. Patient Information Sheet and Consent Forms had to be amended to coincide with changes in the protocol

and re-submitted to Ethics and MHRA. As a result recruitment for the study was delayed until March 2014 (recruitment commenced 6 months later than initially planned).

Up until July, recruitment was slower than expected (only 5 patients randomised for the study procedure by the end of July 2014). Feedback from eligible participants highlighted that multiple hospital visits and invasive tests (namely GI studies/bronchoscopy) were the main things dissuading them from taking part.

In addition, with the new aero-digestive approach to patient management of IPF and the recently concluded Characterisation Study (a separate study of the biology for reflux in IPF patients), a number of eligible patients had already undergone research-based GI studies and/or bronchoscopy examination.

Due to unforeseen circumstances, TEVA changed the colour of their omeprazole capsules. In order to maintain blinding, Victoria Pharmaceuticals had to source an alternative UK-licensed generic omeprazole capsule to match the yellow placebo capsules for the study. Hence a second notice of substantial amendments had to be filed for approval to MHRA.

5.2 Action/Measures put in place

Listening to feedback from eligible participants and following recommendations from the DMSC chair, a range of measures was put in place to address the low rate of recruitment to the study. The main things were:

1. **Alterations to the Patient Information Sheet (PIS):** The patient information sheet was amended to make it clear to the participants that the only parts of the study that were essential were taking the study drug and having the (non-invasive) cough monitoring performed twice. The more invasive tests were optional. A short summary of the study was added on page 1 of the PIS and the "cough monitoring and drug only" part of study on page 3 was highlighted (attached Appendix 4).

Again, the overall study design and the end-points to be assessed did not change. An application was made to the REC for approval of these amendments.

2. **Addition of Participant Identification Centre (PIC) sites**: We identified and added 3 PIC sites namely Sunderland Royal Hospital, James Cook University Hospital in Middlesbrough and North Tees Hospital in Stockton-on-Tees. Over the preceding 12 months, an increasing number of IPF patients were referred to our regional ILD clinic at RVI from these hospitals, especially for pirfenidone therapy. However the patients referred represent a selected group (considered eligible for pirfenidone therapy) and represent only a small proportion of IPF patients, hence inclusion of these centres as PIC sites was expected to "unlock" further IPF patients potentially eligible for the study around the North East region. Eligible patients were identified and invited to take part in the study by clinicians involved in their usual care. I followed up those who expressed interest in the study. Amendments were made in the study protocol (attached Appendix 2) to allow inclusion of the PIC sites.

5.3 Challenges in recruitment to the study

Recruitment to the study remained slower than expected or initially estimated in spite of the above-mentioned measures. In addition, the BLF granted us a 12-month no-cost extension to achieve target recruitment. While these measures gave access to more IPF patients across the region, recruitment to the study remained challenging throughout, for the following reasons (in my view):

5.3.1 IPF patients prescribed PPI therapy

Of the 280 patients screened (Consort diagram), 150 (54%) were already on long-term PPI therapy. Indications for the therapy were peptic ulcer disease (with or without GI bleed), upper GI endoscopy (performed for dysphagia, dyspepsia) showing signs of gastritis/oesophagitis assumed secondary to reflux disease, and/or Barrett's oesophagus. However a major proportion of patients were started on PPI therapy either empirically for possible gastro-oesophageal reflux disease or as secondary

prophylaxis for drug-induced gastritis (because they had trial therapy of high dose prednisolone for their ILD or in a few cases non-steroidal anti-inflammatory drugs as analgesics). In most cases, PPI therapy continued even after withdrawal of corticosteroids and/or analgesics. Once established on therapy it is difficult to take patients off PPI therapy for research, especially when they are suffering from a chronic progressive disease with no cure.

Of the patients on long term PPI therapy, 25 agreed to come off PPI for a "period of 2 weeks of supervised discontinuation" for the purpose of the research. Of these, 16 participants consented and were randomised for the study while 9 had recurrence of symptoms and were excluded from the study.

Despite few indications for long term PPI therapy, a significantly high proportion of IPF patients were prescribed prolonged antacid therapy. Often it has been difficult to discontinue antacid therapy in these patients, which remained one of the predominant reasons for persistent slow recruitment.

5.3.2 IPF severity and comorbidities

IPF is a chronic progressive disease. Often there are associated significant cardiovascular and other systemic co-morbidities (for example diabetes mellitus, osteoarthritis, stroke). Although a median survival of 3 to 5 years from diagnosis is often quoted, a subgroup of patients has a rapidly progressive course with shortened survival. Also 5-20% of patients suffer from episodes of "acute exacerbation" where they suffer from rapid progression of the disease without any identifiable cause i.e. infection, infarction, heart failure, embolism etc. Poor lung function at diagnosis, resting oxygen saturation and walk- distance are some of the markers for poor prognosis, but these are not reliable predictors for these events in any given individual.

Of the patients I screened for research (this is not a definitive list but a few representative examples):

"Participant Screen 124" was reviewed and started on treatment with pirfenidone in October 2014, consented, and study visits were booked on 24.2.15. However the patient was admitted to hospital on 17.2.15 and died most probably secondary to an episode of acute exacerbation.

"Participant Screen 107" had advanced disease by the time of review in the clinic, was referred for lung transplant assessment, and had undergone lung transplant within 2 months of referral. The participant had expressed interest in our research.

"Participant Screens 115 and 153" both expressed interest in the research, however both suffered from rapid progression of disease and were accepted on the active lung transplant list (both were prescribed PPI therapy due to the possible link between reflux and IPF).

Hence, in spite of initial expression of interest and/or consent, I was not able to actually enrol the above patients for the research study, as a result of the inherently progressive nature of the condition being studied.

As tabulated in the Consort diagram, "Patients considered unsuitable for study specific procedures due to clinical reasons" includes some of the potential participants with progressive/advanced IPF who were deemed appropriate for symptomatic or palliative treatment.

Therefore, often it has been a challenge to recruit IPF patients with severe and/or advanced disease. However, our study population is comparable to most of the IPF patients enrolled in other clinic trials in terms of mean age and baseline characteristics, although we recruited a higher proportion of male participants than usual.

5.3.3 Study procedure-related

The study was planned to provide an assessment of the prevalence of acid and non-acid reflux in patients with IPF and of the role of acid suppression therapy in management of these patients. The study entailed 6 visits to the RVI chest clinic (for 2

x 24 hour ambulatory cough monitor studies, 2 x GI physiology studies, 1 x trial medication-related and 1 bronchoscopy study).

Feedback from the eligible/potentially eligible participants was that the invasive nature of the GI studies and the bronchoscopy, as well as the number of visits, had put them off the study. This was compounded by the fact that most of the patients had other significant medical co-morbidities and they often had to travel a considerable distance (e.g. from Morpeth, Hexham, Cleveland, Middlesbrough or Darlington).

In addition, as stated above, patients were often on PPI therapy.

Also, a few of the IPF patients (10 in total) in our clinic had already undergone GI studies with bronchoscopy as part of a recently concluded Characterisation Study (looking into the incidence and nature of reflux in IPF patients) as part of a new Aero-Digestive MDT approach towards managing ILD patients. Subsequent to their study they were advised to take PPI therapy if reflux was present. Those without evidence for reflux were understandably not keen for a repeat study.

Hence, consent and completion rate of GI studies and bronchoscopy remained low through out the study period. Also, our study primarily focused on cough (and the impact of antacid and/or placebo on cough) in IPF patients. Nonetheless, data suggested invasive tests are not desirable among IPF patients.

5.3.4 Mandatory compliance with regulatory framework around clinical trials with an IMP

As outlined earlier, working through the necessary regulatory paperwork and sorting out the initial teething problems of setting up a clinical trial meant a delayed start to the study (by 6 months). After starting, the study underwent 3 Major Amendments and 1 Minor Amendment. I have tabulated the reasons, and the timeline of the filing and approval of the amendments (Table 15).

Table 15: Dates of submission of amendments with reasons and approval dates:

Protocol version	**Amendment**	**Details**	**Date of approval**
1.0, dated 22/07/2013	Original submission		REC – 20/09/2013 (conditional) MHRA – 13/09/2013
2.0, dated 15/09/2013	Protocol changes	Changes made for unconditional REC favourable opinion – updated correct Funding Ref.	REC only 20/09/2013
3.0, dated 20/11/2013	Protocol changes	Amendments to update protocol for internal consistency as suggested by NCTU. Amendments made in sections of Patient Recruitment, Randomisation, Statistical Analysis, Pharmacovigilence, Adverse Event Report Reporting. Also updated contact numbers of study members. New document added: Patient Diary Card – to help assess adverse events in participants, Patient Information Sheet, GP information letters, Consent Forms: updated with appropriate changes in version number and date to coincide with amended protocol.	REC – 10/01/2014 MHRA – 15/01/2014

4.0, dated 03/07/2014	Protocol changes	Amendments in protocol to allow inclusion of PIC sites to help improve recruitment for the study. Also a summary page was added to the Participant Information Sheet to make it plain to participants that the only essential part of the study was cough monitoring and taking the drug. All other tests were non-essential. Amendments in the CTA form due to changes in colour of the TEVA omeprazole capsules. To maintain blinding Victoria Pharmaceuticals had to source an alternative generic UK-licensed omeprazole for the study. Consent Forms and PIS were updated to correspond changes in Protocol.	REC – 26/08/2014 MHRA – 02/09/2014 R&D – 11/09/2014
4.0, dated 03/07/2014 (Nil changes in protocol)	Minor Amendment: PIS: version 3.0 dated 1/10/14 GP letter: version 2.0 dated 1/10/14 Consent Forms: version 3.0 dated 1/10/14	A participant was prescribed regular antacid (PPI) by his general practitioner during the study period when the participant was taking trial medication. Neither the patient nor his doctor informed the study team. This was picked up by the study team on review of notes prior to booking his subsequent study visits. Participants cannot be on a regular PPI as this is a double-blinded trial comparing omeprazole with placebo. As a result of this, after discussion with the Sponsor and NCTU, modification was made in the PIS and GP letter to make it clear to patients and their GPs that they should not be prescribed regular PPI or	REC – acknowledgement 12/11/2014

		antacid while they are in the trial. If prescription of PPI or antacid was necessary for clinical reasons they should contact the trial team prior to the start of medication. Prof Simpson (CI) confirmed with the Chair of the REC that these modifications should be minor amendments. Consent Forms were updated to correspond to changes in the PIS.	
5.0, dated 07/03/2016	Substantial Amendment	Dr. Forrest was listed as co-investigator Change of Senior Trial Manager – Dr. Lesley Hall replaced Dr. Jennifer Wilkinson Change of Trial Manager – Mr. Mark Palmer replaced Jessica Qian. New Clinical Research Fellow – Dr. Wendy Funston replaced me. Changes to randomisation section were made to bring in line with current procedures. Addition of reference to Summary of Product Characteristics (SmPC) in section 3.5 of Protocol. Update to increase clarity in section 5.3 (Withdrawal of Consent of Protocol) and to include 2 options for withdrawal. Addition of Expectedness as per section 7.3 in Protocol. Addition of nintedanib to list of active trial of treatment in exclusion criteria.	REC – 12/07/2016

After starting recruitment I attended the following mandatory meetings relating to the study (until July 2015):

1. TMG meeting – every month

2. DMSC meetings – 4

3. TSG meetings – 1

The NCTU monitored the study twice during the corresponding period: once at the start (informally) and then in January 2015 (formally).

Subsequently, the sponsor (NuTH) also audited the trial in March 2015 (mandatory for clinical trials with an IMP).

These regulations are in place to ensure patient safety, to ensure clinical trials/research are conducted in accordance to mandatory guidelines, and to ensure research yields high quality data/results. They are also to ensure that any problems relating to the study are identified early and measures put into place as appropriate. The suggested amendments to my trial were sensible, positive and in one instance unavoidable (the change in colour of TEVA omeprazole capsules). Unfortunately filing amendments, preparation, organisation of meetings (with which I had invaluable help from NCTU) and managing the trail of paperwork subsequently generated (in addition to the mandatory paperwork directly related to study and recruitment i.e. amendments/consent forms/screening logs/randomisation logs/trial files/SAE logs etc.) did take up considerable amounts of effort and time. In a trial like mine (limited by time) on occasions it proved difficult to prioritise screening and recruitment of potential patients while keeping "the momentum going" (the dilemma of whether to keep trying to recruit more participants or whether to keep mandatory paperwork up-to-the-minute/file more paperwork to help recruit).

This is not a criticism of any department or individual, but rather a realisation that the current statutes regulating conduct of clinical trials can result in time pressures felt by clinical researchers.

Interestingly, the Association of British Insurers has guidelines for healthy volunteers as well as patients considering taking part in clinical trials. In summary the guidelines advise potential participants to inform their insurers about clinical trials they are interested in taking part in, as there are implications for their travel, income, critical illness and private medical insurance covers. I have copied a paragraph outlining an insurer's advice with regard to clinical trials and travel insurance from the association's published guidelines (taken from the internet – Google search).

"*If you are not a healthy volunteer the cover you could get will depend on the type of insurance and what is wrong with you*

Travel insurance

*You **might get cover** if you take part in a clinical research trial of **established**[2] **drugs**, research, imaging studies or comparisons of established surgical interventions (to identify which treatment is better in certain circumstances) that are prescribed by your doctor.*

*It is **unlikely that you would get cover** if you take part in a clinical research trial of: Drugs that are in the experimental/unproven phase, even if you are healthy with no pre-existing conditions*[3]

New or innovative surgical procedures."

In the above context, "getting cover" often means an increase in the premium. Also, Participant ID 012 informed me that his usual insurer refused him travel insurance while in the study. Understandably he was not impressed.

I was not aware of this issue prior to initiation of the study. Subsequently, I had to inform potential participants about their insurance implications, especially if they had travel plans while on the trial IMP.

There is no doubt that the issues illustrated above make recruitment to any clinical trial challenging. Some of them are unavoidable but some can be modified. Hopefully with sanction of the proposed new EU Clinical Trials Regulations (May 2014) a single clinical trials approval will replace the current separate approvals from REC, MHRA etc. This should ease and expedite negotiation of mandatory regulations.

5.4 Strengths and weaknesses of the study

5.4.1 Strengths

This was a carefully designed pilot randomised controlled trial to assess cough and reflux in patients with IPF. State of the art techniques were utilised to accurately assess cough and quantify reflux in patients. The diagnosis of IPF in the study population was well defined as they undergo robust assessments (including lung function tests, high resolution CT scans, and very occasionally surgical biopsy if indicated) by the regional ILD team at RVI, as part of their clinical care. Patients recruited for the study are comparable to the IPF patients enrolled in other clinical trials. All the participants completed ambulatory 24-hour cough recording without any major problems (the primary focus of the trial was cough and the change of cough with omeprazole and/or placebo therapy).

The study provided a detailed characterisation of both acid and non-acid reflux in patients with IPF. In addition it assessed the impact of omeprazole therapy in improving cough and reflux in patients. If the final analysis of the study shows that omeprazole does significantly improve cough in a safe manner, then it will provide further evidence for an effective and inexpensive solution to a long-standing unmet health problem. It will help plan further multi-centre definitive trials. If omeprazole does not improve symptoms, the study will provide new insights into the role of non-acid reflux in pathogenesis of IPF and/or symptoms in IPF. This will help determine future researches into treatment and pathogenesis of IPF. Therefore the study should provide practical benefits either way.

5.4.2 Weaknesses

As discussed previously recruitment to the study was challenging throughout. The majority of the patients were already established on empirical anti-acid therapy. In addition, the study population mostly included elderly patients with multiple co-morbidities, which limited their suitability for multiple invasive procedures.

Patients with subjective and/or clinical symptoms of reflux (as opposed to those with no symptoms or "silent reflux") are more likely to consent for the study procedures. Multiple study visits and study procedures had the potential to introduce selection bias towards a "less severe" disease group. Hence the study population may mostly comprise patients with mild to moderate IPF and/or patients with gastro-oesophageal reflux disease. However, given the nature of the diagnostic tests for reflux disease, and those involved in the study project, the potential for recruitment bias were unavoidable. Moreover, patients who could not tolerate discontinuation of antacid therapy were excluded from the study.

Omeprazole is a widely used acid suppressant therapy. It achieves excellent 24 hour acid suppression, especially with the twice daily regime. Therefore, 3 months duration of twice daily omeprazole therapy (for the treatment arm) was chosen for the trial mostly for practical reasons. However, omeprazole could reduce circulating pirfenidone levels in IPF patients as it induces hepatic enzymes. Study participants were informed of the potential drug interaction prior to recruitment. For future trials (depending on the final analysis of this study data) alternative PPIs and/or antacid therapy should be considered to avoid such drug interaction.

Research conducted in association with patients and/or their representatives to address specific clinical needs are more likely to yield results that can improve health and well being. Researchers can design relevant, patient-friendly trials by taking account of the patient's and/or their relative's unique insight into their condition. The potential benefits of involving patient and public in clinical research/trials have long been identified and currently increasingly a funder requirement. Patients and/or relatives can be involved early as a "driving force" or co-researcher (pre-approval and design stages) to help ensure efficient trial design, help raise funds and address issues regarding recruitment and compliance. They can also be engaged later in the study as a "reviewer", "advisor", "information provider" and "research subject" to help smooth delivery of clinical trials (adapted from Patient Partner, Patient involvement in clinical research, a guide for sponsors and investigators, produced by the Patient Partner

project funded by the 7th Framework Programme of the European Commission, taken from the internet – Google search).

No patient and/or public was involved in our trial study design (to the best of my knowledge). I am grateful to Mr. Ian Perry (lay patient member) for his help and advice with the trial as an independent TSG member (involved as a reviewer, advisor and information provider). As outlined previously, recruitment to our study has been slower than expected in spite of multiple measures put in place. In retrospect, early involvement of patients and their relatives could have been helpful to address some of the identified issues.

Chapter 6. Efficacy of pulsed cyclophosphamide and methylprednisolone therapy in patients with progressive interstitial lung disease (ILD) – a retrospective study

6.1 Background

ILDs diffusely affect lungs and are often relentlessly progressive with healthy lung tissue replaced by fibrous tissue, which reduces lung compliance leading to breathlessness and hypoxia on exertion and/or rest, ultimately leading to respiratory failure. The exact mechanism of progression of ILD is not known but a current accepted theory is that a hyperactive immune stimulation (often due to an unknown trigger) leads to inflammation and fibrosis. Lung fibrosis is generally considered irreversible, hence therapeutic strategies aim to reduce inflammation.

However, therapeutic options are limited, as no pharmacological agent has shown to significantly reduce mortality in ILD. Despite this it is often difficult to withhold potential treatments in a seriously ill, deteriorating patient due to lack of robust evidence to suggest any individual therapy.

The drug cyclophosphamide is an alkylating agent that prevents cell division by inhibiting intracellular DNA synthesis (Fleer *et al*, 1982). It is also a potent immunosuppressant. Its beneficial immunomodulatory effects are mediated via cytotoxic effects on both resting and dividing lymphocytes. Cyclophosphamide therapy induces reduction in the numbers and functions of T and B-lymphocytes thereby impairing both cellular and humoral immunity, which potentially helps to reduce lung injury, and therefore theoretically may inhibit further fibrosis and decline in lung function (Cupps *et al*, 1982).

Intravenous pulsed cyclophosphamide therapy in association with methylprednisolone is employed as a therapeutic strategy in selected centres, especially in a setting of rapidly progressive ILD, as a few studies have shown small improvements in lung function with this therapy, especially in ILD associated with systemic sclerosis

(Griffiths *et al*, 2002; Hoyles *et al*., 2006; Poormoghim *et al*., 2012) and in some cases of non specific interstitial pneumonia (NSIP) (Naki *et al*, 2002; Kondoh *et al*., 2005; Brummaier *et al*, 2013)].

However cyclophosphamide therapy is potentially associated with significant side effects namely bone marrow suppression, worsening of liver and kidney function, haemorrhagic cystitis (Monarch *et al*., 2010) and increased risk of infections.

In our centre, cyclophosphamide therapy is administered via a dedicated Rheumatology Day Unit at Freeman Hospital (with Dr. Bridget Griffiths, Consultant Rheumatologist, as clinical lead). Therapy has been given to patients with a wide variety of rapidly progressive ILD. It was considered important to review whether the treatment had overall beneficial or detrimental effect.

Therefore, I undertook a retrospective case series study to assess the outcome of cyclophosphamide therapy in patients with ILD, regardless of aetiology.

6.2 Treatment protocol

Usually, patients receive 6 pulses of intravenous (IV) methylprednisolone 10 mg/kg and IV cyclophosphamide 15 mg/kg. The first 3 pulses are given at 3 weekly intervals and the last 3 pulses at 4 weekly intervals. The dose of IV methylprednisolone is usually reduced in patients with diabetes mellitus or known osteoporosis. The dose of IV cyclophosphamide is reduced if the patient is over 70 years of age to 10 mg/kg and if they have renal impairment. The patients receive Mesna 400mg orally 3 times on the day of IV cyclophosphamide to reduce the risk of haemorrhagic cystitis, co-trimoxazole 960mg Monday, Wednesday and Friday, a proton pump inhibitor and bone protection therapy. If a patient is on oral prednisolone, then this is gradually reduced according to response with each IV pulse. Depending upon a patient's response, they may receive an additional 3 pulses of treatment (i.e. 9 pulses in total). After completing the course of IV methylprednisolone and cyclophosphamide, patients are usually started on maintenance immunosuppressant therapy in the form of mycophenolate mofetil.

6.3 Study aims

The primary aim of the study was to assess the change in lung function between the 3-6 months pre-therapy and the 3-6 months post-therapy (up to 12 months pre- and post-therapy if results were available).

The secondary aims of the study were to:

1. Assess the rate of change in lung function 3-6 months pre-therapy to that in the 3-6 months post-therapy (especially in patients who had not responded, or who had shown deterioration in lung function)

2. Assess any significant change in oral prednisolone dose before and after pulsed therapy.

3. Assess any significant change in weight post-pulsed therapy.

4. Assess any significant change in oxygen requirement post-pulsed therapy.

5. Any cyclophosphamide-related side effects, in particular bone marrow suppression, haemorrhagic cystitis, and infection.

6. Review the most recent pre-therapy chest CT scans to assess for the predominant radiological features regardless of the working/clinical diagnosis.

Chapter 7. Methods and data collection

7.1 Patient identification

A database of patients receiving pulsed intravenous cyclophosphamide therapy is maintained by the Rheumatology Department at Freeman Hospital, as part of routine clinical care. Patients were identified from the database and data were collected from the identified patients' case notes. My aim was to study all patients with ILD who had received cyclophosphamide therapy from January 2010 to August 2014.

7.2 Data collection

Data were collected in an anonymised audit proforma sheet. With help from my supervisors and Dr Bridget Griffiths I designed the audit proforma and also prepared a study protocol mainly to set study parameters and outcome definitions to help guide data collection and subsequent analysis.

7.3 Pre-determined study parameters and outcome definitions for data analysis

The study team agreed on the following definitions (especially with regards to changes in lung function) prior to data collection to help guide subsequent interpretation/analysis:

1. Primary aim/outcome definitions:

    a) Response or improvement to therapy: if post-treatment lung function increased by more than or equal to 10% when compared to pre-treatment lung function
    b) No Response to therapy or Static: if post-treatment lung function was unchanged, or if it improved but by less than 10%, or if declined but by less than 10% when compared to pre-treatment lung function
    c) Deterioration despite therapy: if post-treatment lung function declined by more than or equal to 10%. when compared to pre-treatment lung function

2. Secondary aim/outcome definitions:

a) If the rate of decline in post treatment lung function was reduced by more than or equal to 10% – "significant decline prevented by the therapy"

b) If the rate of decline in post treatment lung function reduced but by less than 10%, or if the rate of decline increased – "no significant decline induced by the therapy"

3. Echocardiogram study: results of echocardiography performed up to 6 months pre or post therapy were recorded.

4. Blood tests for autoantibodies: results from the most recent panel (pre-therapy) were accepted. Results from diagnosis headings in clinic letters were included as well, if no autoantibody results could be found in the notes or on electronic records.

5. Definition of impaired liver function tests: bilirubin, alanine transaminase (ALT), alkaline phosphatase (ALP) or gamma-glutamyl transferase (GGT) enzyme levels >/= 3 times the normal limit.

6. Definition of impaired renal function: creatinine above the normal range for age/wt. or increase of more than 10% from baseline (last test prior to starting therapy).

7. Definition of bone marrow suppression: any from haemoglobin less than 9.0 g/dl (new onset), neutrophil count< 2.0×10^9 /L (new onset), lymphocyte count <0.6×10^9 /L (new onset), platelet count < 140×10^9 /L (new onset).

Chapter 8. Results

Leo Mansell, an undergraduate Pharmacy student in Newcastle University showed interest in taking up the cyclophosphamide audit for his mandatory research topic. Prof Simpson was happy for me to lead Leo's supervision.

Unfortunately, complying with Research/Information Governance guidelines meant Leo was not allowed direct access to NHS clinic notes, records or tests. Also, given the limited time allotted for him to complete the project it was deemed not practical to get a "research passport" sorted. Hence I collected all the data in the anonymised audit proforma and then he analysed the data (using Minitab 17 statistical software). He has submitted his dissertation. Some of the results presented here have been taken from his work.

The collected data were scrutinised further with the help of Dr. Peter Avery (Senior Lecturer, Institutes of Statistics and Mathematics, Newcastle University) to extract further evidence with regard to the administered therapy (as a host of other information was collected i.e. change in prednisolone therapy, change in weight, cyclophosphamide dosing schedule). He has kindly helped me with the statistical analysis especially relating to the assessment of change of lung function pre- and post-pulsed cyclophosphamide and methylprednisolone therapy.

In addition, Dr. Anna Beattie, Dr. Sylvia Worthy (Consultant Thoracic Radiologists at Royal Victoria Infirmary) and Prof John Simpson reviewed chest CT scans of the patients.

Results of the study were presented as a poster at the British Thoracic Society Winter Meeting on 02/12/2015 (Efficacy of pulsed cyclophosphamide and methylprednisolone therapy in patients with progressive interstitial lung disease).

8.1 Patient demographics

60 patients were identified from the database. Of these 53 patients' notes were available and transcribed.

The median age of patients was 60 years (range 39 – 81 years); 29 (55%) were male. 53% of the patients were either former or current smokers (ex-smokers 26, current smokers 2, non smokers 22, unknown 3).

In addition to the primary diagnosis of ILD, the major co-existing medical conditions were diabetes mellitus in 10 (19.2%) patients, hypertension in 13 (25%), ischaemic heart disease in 7 (13.2%), heart failure in 2 (3.8%) and osteoporosis in 3 (5.7%). 1 patient had previously had a stroke.

Echocardiography data were available from 27 patients. Of these, 10 showed right ventricular enlargement with mild impairment of right ventricular function in 4 patients, moderate impairment of function in 9 patients and severe impairment in 3 patients.

8.2 Diagnosis of ILD at initiation of therapy

Diagnosis at initiation of therapy included connective tissue disease related – ILD (CTD-ILD 21, 40%), idiopathic NSIP (12, 23%), chronic hypersensitivity pneumonitis (8, 15%), IPF (6, 11%), unclassifiable ILD (6, 11%).
Of the 21 patients with CTD – ILD, the nature of the underlying connective tissue disease was: Undifferentiated CTD (8), systemic sclerosis (7), rheumatoid arthritis (2), inflammatory arthritis (2), polymyositis (1), primary Sjogren's syndrome (1).

8.3 Dosage of cyclophosphamide and methylprednisolone delivered and follow on immunosuppression

The median number of cyclophosphamide and methylprednisolone pulses received by patients was 6 (range 1 – 23). This meant a median cumulative dose of 7085 mg of cyclophosphamide (min 840mg; max 25000 mg, N=50) received by patients. 42 (79%) out of the 53 ILD patients received at least 6 pulses of therapy. 12 (23%) received upto 9 pulses.

The dose of methylprednisolone therapy was reduced (less than 10mg/kg) in 19 patients, mostly due to diabetes mellitus. Eight patients had a reduced dose of cyclophosphamide therapy (10 mg/kg) due to age (most common reason) and/or renal impairment or thrombocytopenia at presentation.

As per the protocol, all patients received Mesna (to prevent haemorrhagic cystitis) during pulsed cyclophosphamide and methylprednisolone therapy.
46 (87%) patients were prescribed co-trimoxazole prophylaxis during therapy. 2 patients were already on azithromycin therapy, 2 patients had previously documented allergy/adverse reaction to co-trimoxazole and 1 patient suffered from deterioration of liver function during therapy – hence they were not prescribed additional co-trimoxazole (data were missing on 2 patients).

After completion of cyclophosphamide and methylprednisolone therapy, 41 (77%) patients received follow on immunosuppressive therapy in the form of mycophenolate mofetil (37), azathioprine (2) or rituximab (2).

8.4 Assessment of change in lung function post-therapy

I am extremely grateful to Dr Peter Avery for his help with the statistical analysis with regards to the rate of change of lung function pre- and post-therapy.

Lung function data (including spirometry and gas transfer data) were collected up to 12 months (or even up to 24 months if available) prior and subsequent to completion of cyclophosphamide therapy. Forced vital capacity (FVC) is the volume of air that can be forcibly expired after a full inspiration. It is measured in litres. Transfer factor of lung for carbon monoxide (TLco) test determines the extent to which diffusible gas passes from lung alveoli to blood. It is measured with the help of carbon monoxide gas and expressed in mmol/min/kPa

Previous studies have shown that FVC and TLco are the two best predictors to determine any subtle progression in ILD, hence those two parameters were chosen for analysis.

Statistical analysis was done with Minitab 17 software. Rate of decline of lung function pre- and post-cyclophosphamide therapy was compared using a random intercept model.

Figure 17 shows data plots and average fitted quadratic curve for FVC of patients who received pulsed cyclophosphamide and methylprednisolone therapy

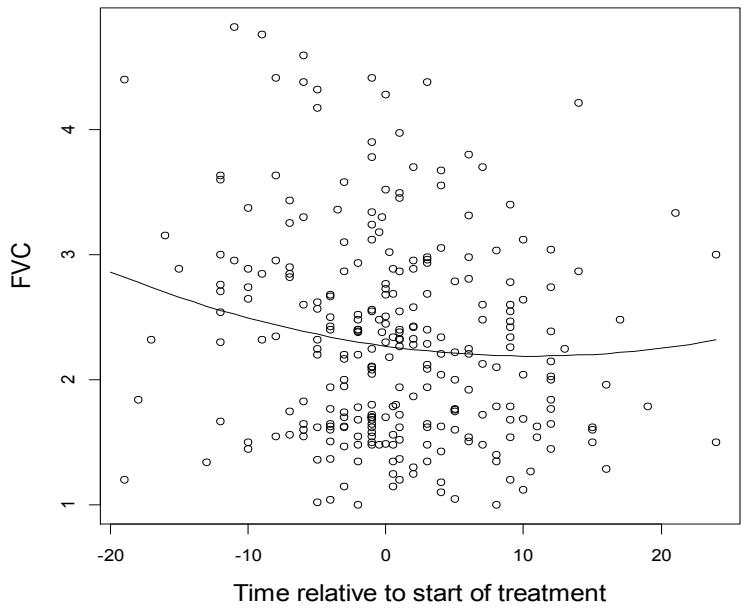


Figure 17: Average fitted quadratic curve for FVC; FVC (litres);
Time relative to start of treatment (months); p=0.0004

The graph shows a mean decline in FVC from 2.9 litres to approximately 2.4 litres over a period of 20 months prior to start of therapy. This means a decline in lung volume of more than 400 ml in absolute terms (i.e. more than 10% decline from baseline). This is considered clinically significant, as this would suggest a reduced life expectancy. Post-therapy there is a significant reduction in the rate of decline of FVC (highly significant quadratic term in time p=0.0004).

Figure 18 below shows data plots and average fitted quadratic curve for TLco of patients who received pulsed cyclophosphamide and methylprednisolone therapy

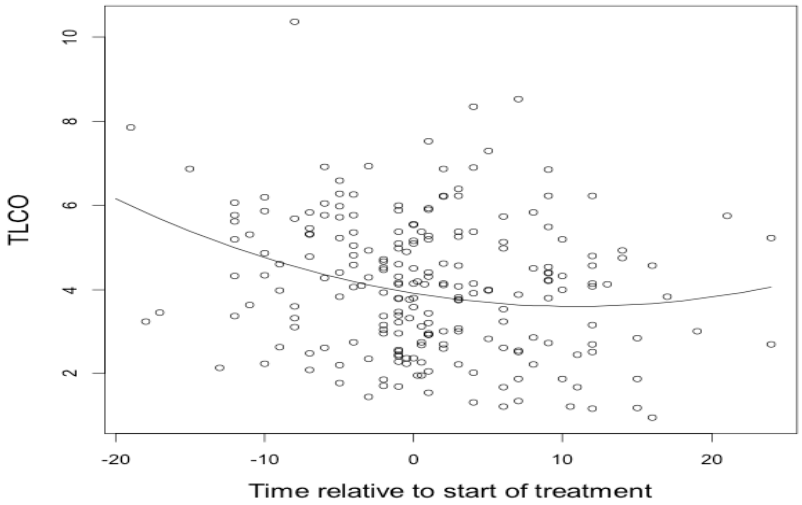


Figure 18: Average fitted quadratic curve for TLCO; TLco (mmol/min/kPa); Time relative to start of treatment (months); p = 0.00015

Similar to FVC, the above graph shows a decrease in the rate of decline of TLco post-cyclophosphamide and methyl-prednisolone therapy, the quadratic term in time is highly significant p=0.00015.

Not all patients who received therapy (at least 1 pulse of cyclophosphamide and methyl-prednisolone) had lung function tests performed at regular intervals post-therapy (due to death, advanced disease or other reasons as outlined below).

1 patient underwent lung transplantation after receiving 2 pulses of therapy. Only 1 lung function test (at 3 months post-therapy) was available for him. Another patient who was switched to mycophenolate therapy after a couple of pulses (poorly tolerated pulsed therapy) struggled to perform lung function tests post-therapy. 1 patient, who had previously received 6 pulses of therapy but died 11 months post-therapy due to malignancy of gynaecological origin, had only 1 lung function test (4 months after therapy) available for analysis. Another patient who died 16 months after receiving 6 pulses had only one lung function test performed 8 months post-therapy.

In total, 13 patients (at the time of review) had died during or after completion of pulsed therapy. Of them 7 had no post-therapy lung function tests performed. Therefore the data available from the study was randomly clustered or grouped at different time intervals. Hence the random intercept model with average fitted quadratic curve (process of constructing a curve that approximately/best fits the data) was chosen for analysis of the collected data (as advised by Dr Peter Avery).

In a selected group of patients (who had 0 – 6 months pre- and post-therapy lung function tests available) rate of decline in lung function was assessed before and after therapy.
Rate of decline was calculated for each patient by dividing their total decline pre- or post-therapy by the number of months over which the decline took place.
(This work was carried out by Leo Mansell and reproduced with his kind permission)

Figure 19 shows change in rate of decline in FVC 6-0 months pre-therapy to 0-6 months post-therapy (data available from 17 patients).

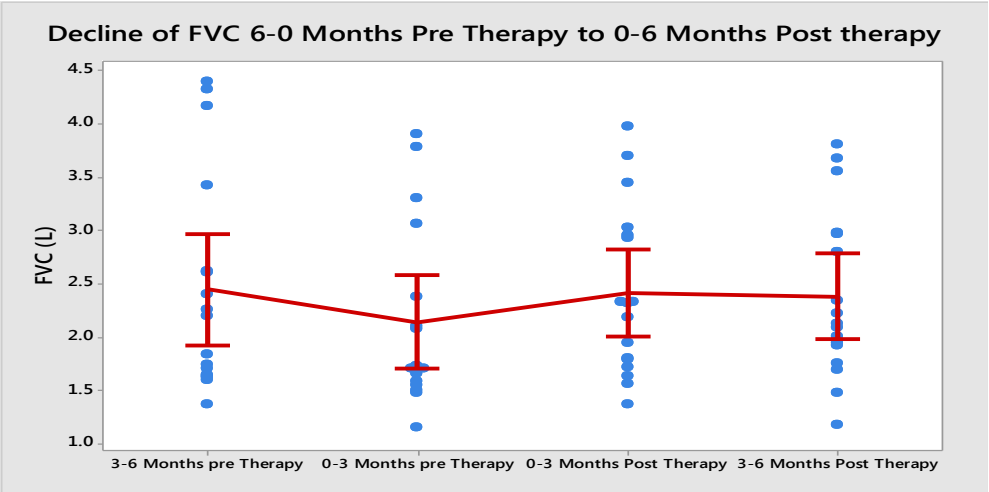


Figure 19: Change in FVC 6 months pre- and post-therapy: FVC (litres), Time relative to start of treatment (months). The red line depicted on the graph connects the mean FVC of each time period (paired t test, n=17, p=0.002).

The graph shows steady decline in FVC of 17 patients pre therapy (mean decline of 0.303 litres in 3 – 6 months pre-therapy). There was significant reduction in rate of

decline of FVC in 3 – 6 months post-therapy (mean decline of 0.003 litres, p=0.002, paired t test).

Significant decline in FVC was prevented in 13 out of 17 patients (defined as greater than or equal to 10% reduction in the rate of decline in secondary outcome/aim definition) after therapy.

Figure 20 shows change in rate of decline in TLco 6-0 months pre-therapy to 0-6 months post-therapy (data available from 10 patients).

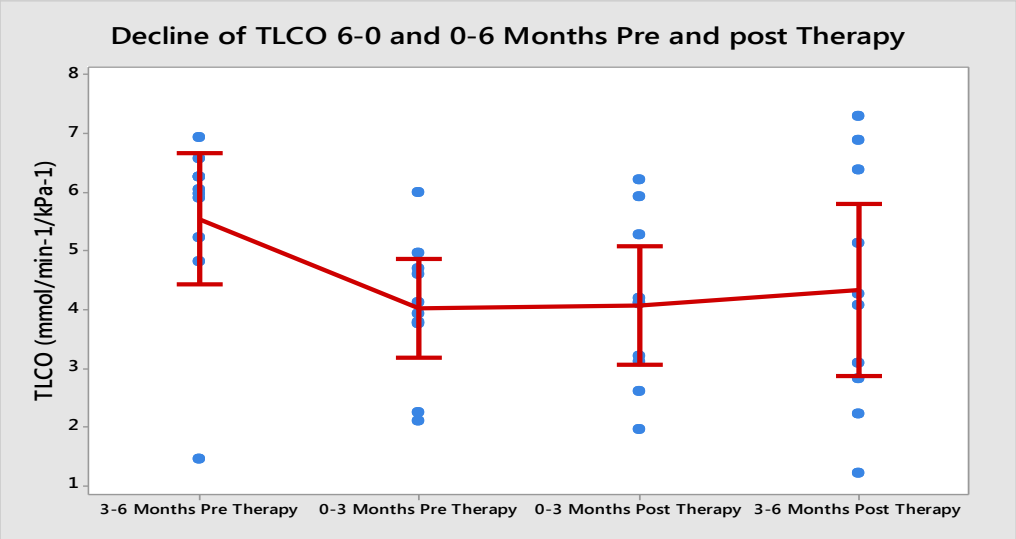


Figure 20: Change in TLco 6 months pre- and post-therapy: TLco (mmol/min/kpa), Time relative to start of treatment (months). The red line depicted on the graph connects the mean TLco of each test time period (paired t test, n=10, p=0.001).

Similar to FVC, this graph shows steep decline in TLco of 10 patients in 3 – 6 months pre-therapy (mean decline of 1.53 mmol/min/kpa). Following therapy, TLco shows a small amount of incline, which continued upto 3 – 6 months, post-treatment (mean increase in TLco by 0.27 mmol/min/kpa).

Significant decline in TLco was prevented in 8 out of 10 patients after therapy (p=0.001, paired t test).

8.5 Assessment of any significant change in pre-therapy maintenance prednisolone dose

Post-therapy, the dose of oral prednisolone was decreased in 15 patients but increased in 6 patients. 28 patients had no change.
Mean dose of prednisolone pre-therapy was 11.5 mg compared to 7.5 mg post-therapy. Wilcoxon signed rank test was performed as data were non-parametric but symmetrical.

•	**Wilcoxon Signed Rank Test: C3**				
•	Test of median = 0.000000 versus median ≠ 0.000000				
•					
•		N for	Wilcoxon		Estimated
•		N	Test	Statistic	**P**	Median
•	C3	49	25	242.5	**0.032**	1.000

4 patients were not included in the test as pre- and post-data (for prednisolone dose) were not available. The test showed a p value of 0.032 when comparing prednisolone dose pre- and post-therapy, implying a significant reduction in maintenance prednisolone dose post-cyclophosphamide and methylprednisolone therapy.

8.6 Assessment of any change in weight post-therapy

46 patients were included in the analysis as a full set of data was not available in 7. Paired t test was performed as the data were normally distributed. Mean weight of the patients pre-therapy was 83.4 kg compared to 83.5 kg post therapy.

•	**Paired T-Test and CI: C5 (pre therapy), C6 (post therapy)**				
•	Paired T for C5 - C6				
•		N	Mean	StDev	SE Mean
•	C5	46	83.44	20.74	3.06
•	C6	46	83.50	19.32	2.85
•	Difference	46	-0.063	6.374	0.940
•	95% CI for mean difference: (-1.956, 1.830)				
•	T-Test of mean difference = 0 (vs ≠ 0): T-Value = -0.07 **P-Value = 0.947**				

The test shows a p value of 0.947, implying no significant change in weight post cyclophosphamide and methylprednisolone therapy.

8.7 Assessment of any significant change in oxygen prescription post-therapy

6 patients had a documented increase in oxygen therapy while 1 patient had a decrease in oxygen therapy (from 3 litres/min to 2 litres/min). Data were missing in 11 patients.

Many of the patients who have received therapy were referred to the regional Newcastle ILD clinic from surrounding district general hospitals. Usually, the local oxygen team manages the oxygen therapy and there is often a delay in documentation and/or notification of update in prescription. Patients (who are often significantly ill and undergoing toxic treatment) might not always recollect their oxygen use accurately (i.e. use as short burst therapy and/or ambulatory only or long-term oxygen therapy).

Given these limitations and the low numbers, no statistical test has been carried out. There appeared to have been no change in oxygen prescription in 35 patients (this included patients who never had oxygen prescribed prior or at end of therapy).

8.8 Adverse events during therapy

Table 16 below outlines adverse events suffered by patients whist on pulsed cyclophosphamide and methyl-prednisolone therapy.

Table 16: Adverse events during pulsed cyclophosphamide and methyl-prednisolone therapy

Adverse events	Number of patients	Percentage (%)
Bone marrow suppression	8	15
Impaired liver functions	4	7
Impaired renal functions	3	6
Infections (any)	27	51
Microscopic haematuria	1	2

32 episodes of infection (assessed from medical notes) were reported in 27 ILD patients' receiving pulsed therapy. Majority (21, 66%) were of respiratory origin. Table 17 below outlines sites of infections reported (in addition to chest infections) whilst on therapy.

Table 17: Types of infection reported during pulsed therapy

Site/type of infection	Number of episodes	Percentage (%)
Chest/Respiratory	21	66
Urinary tract infection	4	13
Fungal nail infection	2	6
Tooth abscess	1	3
Oral candidiasis	1	3
Viral infection (flu)	1	3
Tonsillitis	1	3
Ear infection	1	3

In total, 13 episodes of hospital admission were documented amongst the patients during therapy. Of these 8 were considered likely to be related to cyclophosphamide therapy (with pneumonia being the most common diagnosis). Other causes of hospital

admission were chest pain (leading to a diagnosis of pulmonary embolism), bleeding from gastric ulcer, deep venous thrombosis, hyperkalemia and exacerbation of ILD.

25 (71%) of the 35 patients who suffered from an adverse event were able to complete the therapy.

8.9 Review of pre-therapy chest CT scan features

Prof John Simpson, Dr Sylvia Worthy and Dr A Beattie independently reviewed the most recent pre-therapy chest CT scans of the patients who received the cyclophosphamide therapy to assess the predominant radiological feature blinded to the clinical diagnosis. I assessed the concordance/agreement between the chest physician and the radiologists' interpretation (based on pre determined categories/descriptors on CT scan features: a) predominant fibrotic changes with secondary bronchial dilatation; b) predominant ground glass changes; c) predominant fibrotic changes with honeycombing).

Chest CT scans showed predominant fibrotic changes with secondary bronchial dilatation in 30 (58%) patients; predominant ground glass changes in 16 (31%) patients and predominant fibrotic changes with honeycombing in 6 (11%) patients. (scans were not available for one patient).

There was concordance between the members on most occasions (44 out of the 52 scans reviewed).

Chapter 9. Discussion

This was a single centre, retrospective study. It showed pulsed intravenous cyclophosphamide and methylprednisolone therapy was associated with stabilisation of lung function in a mixed cohort of patients with progressive ILD. There was a significant reduction of maintenance steroid dose post therapy but no significant change in weight. Adverse events were common, most frequently in the form of respiratory tract infections. The adverse events were however transient and managed with dose reduction and/or delay in the schedule of cyclophosphamide and methyl-prednisolone therapy. After completion of therapy the majority of patients were able to continue with follow on immunosuppression therapy in the form of mycophenolate mofetil.

An inherent issue in retrospective studies are missing data. This study was not an exception especially in regard to oxygen therapy, echocardiography studies and on occasions lung function tests (as previously outlined in section 8.4).

A minority of patients declined to continue with cyclophosphamide therapy and in a few patients therapy was discontinued due to clinical reasons (due to adverse events or due to rapid decline in lung disease necessitating palliative measures). In addition, patients with severe ILD often find it difficult to perform a full set of lung function tests. 7 patients who received therapy (at least one pulse) had no post-therapy lung function test performed. A further 7 patients had only 1 post therapy lung function test performed after completion of therapy. Therefore, it has been challenging to account for these “dropped out” lung function tests in the analysis and assess what effect they would have had on the overall study results. In theory, it is possible that the study results have been positively influenced by the patients who were able to tolerate the therapy better (i.e. complete treatment as per protocol thereby inducing “survivor bias”).

However subsequent scrutiny/analysis of data (where both pre- and post-therapy lung function tests were available at stipulated intervals) showed pulsed cyclophosphamide and methyl-prednisolone therapy was associated with stabilisation of lung function in a mixed cohort of patients with rapidly progressive ILD (although the number of patients/test results available were low, as detailed in section 8.4).
Some of the patients showed significant improvement (greater than 10%) in their lung function post-therapy. Given the nature of the study, it is difficult to predict the "phenotype" of the patients who would benefit the most from therapy.

The study is in concordance with previously published papers showing small improvements in lung function with the therapy in patients with ILD (as outlined in section 6.1). I hope the study has provided further important evidence for the team at the Newcastle regional ILD clinic, contributing to improvements in the clinical care of patients with progressive ILD.

References

Arai, T., Inoue, Y., Sasaki, Y., Tachibana, K., Nakao, K., Sugimoto, C., Okuma, T., Akira, M., Kitachi, M., Hayashi, S., (2014), 'Predictors of the clinical effects of Pirfenidone on idiopathic pulmonary fibrosis', *Respiratory Investigation*, 52(2), pp. 136-143

Arnold, R., (1994), 'Safety of proton pump inhibitors – an overview', *Alimentary Pharmacology and Therapeutics*, 8 (Suppl 1), pp. 65-70

ATS Board of Directors, (2002), 'ATS Statement: Guidelines for the Six-Minute Walk Test', *American Journal of Respiratory and Critical Care Medicine*, 166, pp. 111-117

Belafsky, P. C., Postma, G. N., Kaufman, J. A. (2002), 'Validity and reliability of reflux symptom index (RSI)', *Journal of Voice*, 16(2), pp. 274-277

Belcher, J. R., (1949), 'The pulmonary complications of dysphagia', *Thorax*, 4, pp. 44-56

Benson, V. S., Mullerova, H., Vestbo, J., Wedzicha, J. A., Patel, A., Hurst, J. R., (2015), 'Association between gastro-oesopheageal reflux, its management and exacerbation of chronic obstructive pulmonary disease', *Respiratory Medicine*, 109, pp. 1147-1154

Berkhof, F., F., Boom, L., N., ten Hertog, N., E., Uil, S., M., Kerstjens, H., A., M., van den Berg, J., W., K., (2012), 'The validity and precision of the leicester cough questionnaire in COPD patients with chronic cough', *Health and quality of life outcomes*, 10(4), doi: 10.1186/1477-7525-10-4

Birring, S. S., (2011), 'New concepts in the management of chronic cough', *Pulmonary Pharmacology & Therapeutics*, 24(3), pp.334-338

Birring, S. S., Matos, S., Patel, R. B., Prudon, B., Evans, D. H., Pavord, I. D., (2006),

'Cough frequency, cough sensitivity and health status in patients with chronic cough', *Respiratory Medicine*, 100, pp. 1105-1109

Birring, S. S, Prudon, B., Carr, A. J., Singh, S. J., Morgan, M. D. L., Pavord, I. D., (2003), 'Development of a symptom specific health status measure for patients with chronic cough: Leicester Cough Questionnaire (LCQ)', *Thorax,* 58, pp. 339-343

Bradley, B., Branley, H. M., Egan, J. J., Greaves, M. S., Hansell, D. M., Harrison, N. K., Hirani, N., Hubbard. R, Lake, F., Millar, A. B., Wallace, W. A., Wells, A. U., Whyte, M. K., Wilsher, M. L., (2008), 'Interstitial lung disease guideline: the British Thoracic Society in collaboration with the Thoracic Society of Australia and New Zealand and the Irish Thoracic Society', *Thorax*, 63(Suppl 5: v1-58)

Bredenoord., A. J., Fox, M., Kahrilas, P. J., Pandolfino, J. E., Schwizer, W., Smout, A. J. P. M., Conklin, J. L., Cook, I, J., Gyawali, P., Hebbard, G., Holloway, R. H., Ke, M., Keller, J., Mittal, R. K., Peters, J., Richter, J, Roman, S., Rommel, N., Sifrim, D., Tutuian, R., Valdovinos, M., Vela, M. F., Zerbib, F. (2012), 'Chicago classification criteria of Esophageal motility disorder defined in high resolution esophageal pressure topography (EPT)', *Neurogastroenterology and motility*, 24(Suppl 1), pp. 57-65

Bredenoord, A. J., Pandolfino, J. E., Smout, A. J. P. M., (2013), 'Gastro-oesophageal reflux disease', *The Lancet*, 381, pp. 1933-1942

Brummaier, T., Pohanka, E., Studnika-benke, A., Pieringer, H., (2013), 'Using cyclophosphamide in inflammatory rheumatic diseases', *European Journal of Internal Medicine*, 24(7), pp. 590-596

Casanova, C., Baudet, J. S., del Valle Velasco, M., Martin, J. M., Aguirre-Jaime, A., de Torres, J. P., Celli, B. R., (2004), 'Increased gastro-oesophageal reflux disease in patients with severe COPD', *European Respiratory Journal*, 23, pp. 841-845

Catarina, M. and Julius, D., (1999), 'Sense and specificity: a molecular identity for nociceptors', *Current opinion in Neurobiology*, 9, pp. 525-530

Catarina, M., Schumacher, M., Tominaga, M., Rossen, T., Levine, J. and Julius, D., (1997), 'The capsaicin receptor: a heat activated ion channel in the pain pathway', *Nature*, 389, pp. 816-823

Chambers, R. C. and Scotton, C. J., (2012), 'Coagulation cascade proteinases in lung injury and fibrosis', *Proceedings of the American Thoracic Society*, 9(3), pp. 96-101

Chung, J. H., Chawla, A., Peljto, A., L., Cool, C., Gorshong, S. D., Talbert, J. L., McKean, D., Brown, K. K., Fingerlin, T. E., Schwarz, M. I., Schwarz, D. A., Lynch, D. A., (2015), ' CT finding of probable UIP have a high predictive value for histologic UIP', *Chest*, 147(2), pp. 450-459

Chung, K. F., McGarvey, L., Mazzone, S. B, (2013), 'Chronic cough as a neuropathic discorder', *Lancet Respiratory Medicine*, 1(5), pp. 414-422

Chung, K. F., Pavord, I. D., (2008), 'Prevalence, pathogenesis, and causes of chronic cough', *The Lancet*, 371, pp. 1364-1374

Cohen, J. T., Gil, Z., Fliss, D. M., (2005), 'The reflux symptom index-a clinical tool for the diagnosis of laryngopharyngeal reflux', *Harefuah*, 144(12), pp. 826-829

Cronkhite, J. T., Xing, C., Raghu, G., Chin, K. M., Torres, F., Rosenblatt, R. L., Garcia, C. K., (2008), 'Telomere shortening in familial and sporadic pulmonary fibrosis', *American Journal of Respiratory and Critical Care Medicine*, 178, pp. 729-737

Crooks, M. G. and Hart, S. P., (2015), 'Coagulation and anticoagulation in idiopathic pulmonary fibrosis', *European Respiratory Review*, 24, pp. 392-399

Crystal, R. G., Fulmer, J. D., Roberts, W. C., Moss, M. L., Line, B. R., Reynolds, H. Y., (1976), 'Idiopathic Pulmonary Fibrosis: clinical, histological, radiographic, physiological, scintigraphic, cytological and biochemical aspects', *Annals of Internal Medicine*, 85(6), pp. 769-788

Cupps, T. R., Edgar, L. C., and Fauci, A. S. (1982), 'Suppression of human B lymphocyte function by cyclophosphamide', *Journal of Immunology*, 128(6), pp. 2453-2457

DAMOCLES Study Group, (2005), 'A proposed charter for clinical trial data monitoring committees: helping them to do their job well', *The Lancet*, 365, pp. 71-722

Decalmer, S. C., Webster, D., Kelsall, A. A., McGuiness, K., Woodcock, A. A., Smith, J. A., (2007), 'Chronic cough: how do cough reflex sensitivity and subjective assessments correlate with objective cough count during ambulatory monitoring', *Thorax*, 62, pp. 329-334

DeMeester, T., Johnson, L. F, Joseph, G. J., Toscano, M. S., Hall. A. W., Skinner, D. B., (1976)' 'Patterns of gastroesophageal reflux in health and disease', Annals of Surgery, 184(4), pp. 459-470

Dicpinigaitis, P. V, (2008), 'Currently available antitussives', Pulmonary *Pharmacology & Therapeutics*, 22(2), pp. 148-151

Doherty, M. J., Mister, R., Pearson, M. G. and Calverley, P. M. A., (2000), 'Capsaicin induced cough in cryptogenic fibrosing alveolitis', *Thorax*, 55, pp. 1028-1032

Du Bois., R., M., Weycker, D., Albera, C., Bradford, W., Z., Costabel,. U., Kartasov, A., Lancaster, L., Nobel, P. W., Sahn, S., A., Szwarcberg, J., Thomeer, M., Valeyre, D., King Jr., T., E., (2010), ' Six-Minute-Walk Test in Idiopathic Pulmonary Fibrosis Test validation and Minimal Clinically Important Difference', *American Journal of Respiratory and Critical Care Medicine*, 183, pp. 1231-1237

Enright, P., L. and Sherrill, D., L., (1998), 'Reference equations for the six-minute walk in healthy adults', *American Journal of Respiratory and Critical Care Medicine*, 158, pp. 1384-1387

Eypasch, E., Williams, J. I., Wood-Dauphinee, S., Ure, B. M., Schmulling, C.,

Neugebaur, E., Troild, H., (1995), 'Gastrointestinal Quality of Life Index: development, validation and application of a new instrument', *British Journal of Surgery*, 86, pp. 216-222

Fahim, A., Crooks, M., Hart, S. P, (2011), 'Gastroesophageal reflux and Idiopathic Pulmonary Fibrosis: a review', *Pulmonary Medicine*, 2011, Article ID 634613, pp. 7 pages, doi. 10.1155/2011/634613

Fleer, R., and Brendel, M., (1982), 'Toxicity, interstrand cross-links and DNA fragmentation induced by 'activated' cyclophosphamide in yeast: comparative studies on 4- hydroperoxy-cyclophosphamide, its monofunctional analogon, acrolein, phosphoramide mustard, and nor- nitrogen mustard', *Chemico Biological Interactions,* 39(1), pp.1-15

Garcia, C. K., (2011), 'Idiopathic pulmonary fibrosis update on genetic discoveries', *Proceedings of the American Thoracic Society*, 8, pp. 158-162

Griffiths, B., Miles, S., Moss, H., Robertson, R., Veale., D., Emery, P., (2002), 'Systemic sclerosis and interstitial lung disease: a pilot study using pulse intravenous methylprednisolone and cyclophosphamide to assess the effect on high resolution computed tomography scan and lung function', *The Journal of Rheumatology,* 29(11), pp. 2371-2378

Groneberg, D. A., Niimi, A., Dinh, Q.T., Casio, B., Hew, M., Fischer, A. and Chung, K. F., (2004), 'Increased expression of transient receptor potential vanilloid – 1 in airway nerves of chronic cough', *American Journal of Respiratory and Critical Care Medicine*, 170 (12), pp. 1276-1280

Harrison, N. K., (2013), 'Cough, sarcoidosis and idiopathic pulmonary fibrosis: raw nerves and bad vibrations', *Cough*, 9:9, doi: 10.1186.1745-9974-9-9

Hertzog, M. A., (2008), 'Considerations in determining sample size for pilot studies', *Research in Nursing and Health*, 31, pp. 180-191

Hope-Gill, B., Hilldrup, S., Davies, C., Newton, R. and Harrison, N. K., (2003), 'A study of the cough reflex in idiopathic pulmonary fibrosis', *American Journal of Respiratory and Critical Care Medicine*, 168 (8), pp. 995-1002

Horton, M. R., Santropietro, V., Mathew, L., Horton, K. M., Polito, A. J., Liu, M. C., Danoff, S. K., Lechtzin, N., (2012), 'Thalidomide for the treatment of cough in idiopathic pulmonary fibrosis: a randomized trial', *Annals of Internal Medicine*, 157(6), pp. 398-406

Hoyles, R. K., Ellis, R. W., Wellsbury, J., Lees, B., Newlands, P., Goh, N. S., Roberts, C., Desai, S., Herrick, A. L., McHugh, N. J., Foley, N. M., Pearson, S. B., Emery, P., Veale, D. J., Denton, C. P., Wells, A. U., Black, C. M., du Bois, R. M., (2006), 'A multicentre, prospective, randomised double-blind, placebo-controlled trial of corticosteroids and intravenous cyclophosphamide followed by oral Azathioprine for the treatment of pulmonary fibrosis in scleroderma', *Arthritis and Rheumatism*, 54(12), pp. 3962-3970

Hsu, J., Y., Stone, R., A., Logan-Sinclair, R., B., Worsdell, M., Busst, C., M., Chung, K., F., (1994), 'Cough frequency in patients with persistent cough: assessment using a 24 hour ambulatory recorder', *European Respiratory Journal*, 7, pp. 1246-1253

Johnson, W.C., Raghu, G. (2005), 'Clinical trials in idiopathic pulmonary fibrosis: a word of caution concerning choice of outcome measures', *European Respiratory Journal*, 26, pp. 755-758

Jones, R. M., (2012), 'Studies on the pathogenesis of the cough reflex in idiopathic pulmonary fibrosis', *MD thesis*, University of Swansea

Kaur, A., Mathai, S. K., Schwartz, D. A., (2017), 'Genetics in idiopathic pulmonary fibrosis pathogenesis, prognosis and treatment', *Frontiers in Medicine*, 4, 154, doi:10.3389/fmed.2017.00154

Kellsall, A., Houghton, L. A., Jones, H., Decalmer, S., McGuiness, K., Smith, J. A.,

(2011), ‘A novel approach to studying the relationship between subjective and objective measures of cough’, *Chest*, 139(3), pp. 569-575

Kessing, B. F., Smout, A. J. P., Brendenoord, A. J., (2012), ‘Clinical applications of Esophageal Impedance Monitoring and High-Resolution Manometry’, *Current Gastroenterology Reports*, 14, pp. 197-205

Key, A. L., Holt, K., Hamilton, A., Smith, J. A., and Earis, J. E., (2010); ‘Objective cough frequency in Idiopathic Pulmonary Fibrosis’, *Cough*, 6:4. doi: 10.1186/1745-9974-6-4.

Kilduff, C. E., Counter, M. J., Thomas, G. A., Harrison, N. K., Hope-Gill, B. D., (2014), ‘Effect of acid suppression therapy on gastroesophageal reflux and cough in idiopathic pulmonary fibrosis: an intervention study’, *Cough*, 10:4. doi. 10.1186/1745-9974-10-4.

King, T. E. Jr, Bradford, W. Z., Castro-Bernaedini, S., Fagan, E. A., Glaspole, I., Glassberg, M. K., Gorina, E., Hopkins, P. M., Kardatzke, D., Lancaster, L., Lederer, D. J., Nathan, S. D., Pereira, C. A., Sahn, S. A., Sussman, R., Swigris, J. J., Noble.P.W., (2014), ‘ A phase 3 trial of Pirfenidone in patients with Idiopathic Pulmonary Fibrosis’, *The New England Journal of Medicine*, 370, pp. 2083-2092

King, T. E. Jr, Pardo, A., Selman, M., (2011) ‘ Idiopathic pulmonary fibrosis’, *The Lancet*, 378, pp. 1949-1961

Kobayashi, A., Okamoto, H., (2012), ‘Treatment of interstitial lung diseases associated with connective tissue diseases’, *Expert Review Clinical Pharmacology.* 5(2), pp. 219-227

Kondoh, Y., Taniguchi, H., Yokoi, T., Nashiyama, O., Ohishi, T., Kato, T., Suzuki, K., Suzuki, R., (2005), ‘Cyclophosphamide and low-dose prednisolone in idiopathic pulmonary fibrosis and fibrosing nonspecific interstitial pneumonia’, *European Respiratory Journal*, 25(3), pp. 528-533

Kuhn, J. J., Hendley, J. O., Adams, K. F., Clark, J. W., Gwaltney, J. M. Jr., (1982), 'Antitussive effect of guaifenesin in young adults with natural colds: objective and subjective assessment', *Chest*, 82(6), pp. 713-718

Lancaster, G. A., Dodd., S., Williamson, P. R., (2002), 'Design and analysis of pilot studies: recommendations for good practice', *Journal of Evaluation in Clinical Practice*, 10(2), pp. 307-312

Lee, A. L., Button, B. M., Denehy, L., Wilson, J. W., (2011), 'Gastro-oesophageal reflux in noncystic fibrosis bronchiectasis', *Pulmonary Medicine*, 2011 doi:10.1155/2011/395020

Lee, J. S., Collard, H. R., Anstrom, K. J., Martinez, F. J., Noth, I., Roberts, R. S., Yow, E., Raghu, G., (2013), 'Anti-acid treatment and disease progression in idiopathic pulmonary fibrosis: an analysis of data from three randomized controlled trials', *The Lancet*, 1, pp. 369-376

Lee, J. S., Ryu, J. H., Elicker, B. M., Lydell, C. P., Jones, K. D., Wolters, P. J., King, T. E. Jr, Collard, H. R., (2011), 'Gastroesophageal reflux therapy is associated with longer survival in patients with idiopathic pulmonary fibrosis', *American Journal of Respiratory and Critical Care Medicine*, 184(12), pp. 1390-1394

Lee, J. S., Song, J. W., Wolters, P. J., Ellcker, B. M., King, T. E. Jr, Kim, D. S., Collard, H. R., (2012), 'Bronchoalveolar lavage pepsin in acute exacerbation of idiopathic pulmonary fibrosis', *European Respiratory Journal*, 39, pp. 352-358

Lee, K. K, Birring, S. S., (2010), 'Cough and Sleep', *Lung*, 188(1), pp. 91-94 doi: 10.1007/s00408-009-9176-0

Leo Mansell, (2015), 'Cyclophosphamide audit: an audit to assess the efficacy and safety of cyclophosphamide therapy with progressive lung fibrosis', Dissertation submitted to Newcastle University for degree course in Pharmacy

Linden, P. A., Gilbert, R. J., Yeap, B. Y., Boyle, K. Deykin, A., Jaklitsch, M. T.,

Sugarbaker, D. J., Bueno, R., (2006), 'Laparoscopic fundoplication in patients with end-stage lung disease awaiting transplantation', *Journal of Thoracic and Cardiovascular Surgery*, 131(2), pp. 438-446

Lutherer, L. O., Nugent, K. M., Schoettle, B. W., Cummins, M. J., Raj, R., Birring, S. S., Jumper, C. A., (2011), 'Low dose oral interferon-alpha probably retards the progression of idiopathic pulmonary fibrosis and alleviates associated cough in some patients', *Thorax*, 66, pp. 446-447

Macintyre, N., Crapo, R. O., Viegi, G., Johnson, D. C., van der Griten, C. P., Brusasco, V., Burgos, F., Casaburi, R., Coates, A., Enright, P., Gustafsson, P., Hankinson, J., Jensen, R., McKay, R., Miller, M. R., Navajas, D., Pedersen, O. F., Pellegrino, R. and Wanger, J., (2005), 'Standardisation of the single-breath determination of carbon monoxide uptake in the lung', *European Respiratory Journal,* 26(4), pp. 720-735

Marsden, P. A., Smith, J. A., Kelsall, A. A., Owen, E., Naylor, J. R., Webster, D., Sumner, H., Alam, U., McGuinness, K., Woodcock, A. A., (2008), 'A comparison of objective and subjective measure of cough in asthma', *Journal of Allergy and Clinical Immunology*, 122(5), pp. 903-907

Mays, E. E., Dubois, J. J., Hamilton, G. B., (1976), 'Pulmonary fibrosis associated with tracheobronchial aspiration. A study of the frequency of hiatal hernia and gastroesophageal reflux in interstitial fibrosis of obscure etiology', *Chest*, 69(4), pp. 512-515

Mcgarvey, L. P. A., Polley, L., MacMohan, J., (2007), 'common causes and current guidelines', *Chronic Respiratory Disease*, 4, pp. 215-223

McGuinnes, K., Holt, K., Dockry, R., Smith, J., (2012), 'Validation of the Vitalojak™ 24 hour ambulatory cough monitor', *Thorax*, 67. doi: 10.1136/thoraxjnl-2012-202678.220.

Miller, M.R., Hankinson, J., Brusasco, V., Casaburi, R., Coates, A., Crapo, R., Enright, P., van der Griten, C. P., Gustafsson, P., Jensen, R., Johnson, D. C., Macintyre, N., McKay, R., Navajas, D., Pedersen, O. F., Pellegrino, R., Viegi, G. and Wanger, J., (2005), 'Standardisation of spirometry', *European Respiratory Journal*, 26(2), pp. 319-338

Miner, P. Jr., Katz, P. O., Chen, Y. and Sostek, M., (2003), 'Gastric acid control with esomeprazole, lansoprazole, omeprazole, pantoprazole, and rabeprazole: a five-way crossover study', *The American Journal of Gastroenterology*, 98(12), pp. 2616-2620

Monarch, P. A., Arnold, N. M., Merkel, P. A., (2010), 'Incidence and prevention of bladder toxicity from cyclophosphamide in the treatment of rheumatic diseases: a data driven review,' *Arthritis and Rheumatism*, 62(1), pp. 9-21

Murray, M., P., Turnbull, K., MacQuarrie, S., Pentland, J., L., Hill, A., T., (2009), 'Validation of the Leicester Cough Questionnaire in non-cystic fibrosis bronchiectasis', *European Respiratory Journal*, 34, pp. 125-131

Nanki, N., Fujita, J., Yamaji, Y., Maeda, H., Kurose, T., Kaji, M., Satoh, K., Miyatani, K., Yamadori, I., Ohtsuki, Y., Ishida, T., (2002), 'Nonspecific interstitial pneumonia/fibrosis completely recovered by adding cyclophosphamide to corticosteroids', *Internal Medicine (Tokyo, Japan*), 41(10), pp. 867-870

Navaratnam, V., Fogarty, A. W., Mckeever, T., Thompson, N., Jenkins, G., Johnson, S. R., Doland, G., Kumaran, M., Pointon, K., Hubbard, R. B., (2014), 'Presence of a prothrombotic state in people with idiopathic pulmonary fibrosis: a population based case-control study', *Thorax*, 69, pp. 207-215

Noth, I., Anstrom, K. J., Calvert, S. B., de Andrade, J., Flaherty, K. R., Glazer, C., Kaner, R. J. and Olman, M. A., (2012), 'A placebo-controlled randomized trial of warfarin in idiopathic pulmonary fibrosis', *American Journal of Respiratory and Critical Care Medicine*, 186(1), pp. 88-95

Pavord, I. A., Chung, K. F., (2008), 'Management of chronic cough', *The Lancet*, 371, pp. 1375-1384

Pearson, J. E. G. and Wilson, R., S. E., (1971), 'Diffuse pulmonary fibrosis and hiatus hernia', *Thorax,* 26, pp. 300-305

Poormoghim, H., Lakeh, M. M., Mohammadipour, M., Sodagari, F., Toofaninjed, N., (2012), 'Cyclophosphamide for scleroderma lung disease: a systematic review and meta-analysis', *Rheumatology International,* 32, pp.2431-2444

Power J., T., Stewart, I. C., Connaughton, J. J., Brash, H. M., Shapiro, C. M., Flenley, D. C., Douglas, N. J., (1984), 'Nocturnal cough in patients with chronic bronchitis and emphysema,' *American review of respiratory disease*, 130(6), pp. 999-1001

Raghu, G., Collard, H. R., Egan, J. J., Martinez, F. J., Behr, J., Brown, K. K., Colby, T. V., Cordier, J. F., Flaherty, K. R., Lasky, J. A., Lynch, D. A, Ryu, J. H., Swigris, J. J., Wells, A.U, Ancochea, J., Bouros, D., Cavalho C., Costabel U, Ebina, M, Hansell, D. M., Johkoh, T., Kim, D. S., King, T. E. Jr, Kondoh, Y., Myers, J., Muller, N. L., Nicholson, A. G., Richeldi, L., Selman, M., Dudden, R. F, Griss, B. S., Protzko, S. L., Schunemann, H. J., (2011), 'An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence based guidelines for diagnosis and management', *American Journal of Respiratory and Critical Care Medicine*, 183(6), pp. 788-824

Raghu, G., Freudenberger, T. D., Yang, S., Curtis, J. R., Spada, C., Hayes, J. Sillery, J. K, Pope, C. E. 2nd, Pellerini, C. A., (2006), 'High prevalence of abnormal acid gastro-oesophageal reflux in idiopathic pulmonary fibrosis', *European Respiratory Journal*, 27(1), pp. 136-142

Raghu, G., Meyer, K. C., (2012), 'Silent gastro-oesophageal reflux and microaspiration in IPF: mounting evidence for anti-reflux therapy,' *European Respiratory Journal*, 39(2), 242-245

Raghu, G., Yang, S. T., Spada, C., Hayes, J., Pellegrini, C. A., (2006), 'Sole treatment

of acid gastro-oesophageal reflux in idiopathic pulmonary fibrosis', *Chest*, 129(3), pp. 794-800

Raj, A. A., Pavord, D. I., Birring, S. S., (2009), 'Clinical cough IV: what is the minimal important difference for the Leicester Cough Questionnaire', *Handbook of experimental pharmacology*, 187, pp. 311-320, doi: 10.1007/978-3-540-79842-2_16

Redelmeier, D., A., Bayoumi, A., M., Goldstein, R., S., Guyatt, G., H., (1997), 'Interpreting small differences in functional status: The six minute walk test in chronic lung disease patients', *American Journal of Respiratory and Critical Care Medicine,* 155, pp.1278–1282

Reilly, J. P., (1999), 'Safety profile of the proton-pump inhibitors', *American Journal Health-System Pharmacy*, 56(23 Suppl 4), S11-S17

Ricci, A., Graziano, O., Bronzetti, E., Saltini, C., Sciacchitano, S. and Cherubini, E., (2007), 'Increased pulmonary neurotrophin protein expression in idiopathic interstitial pneumonias', *Sarcoidosis Vasculitis and Diffuse Lung Disease*, 24 (1), pp. 13-23

Richeldi, L., Collard, H. R., Jones, M.G. (2017) 'Idiopathic pulmonary fibrosis', *The Lancet*, 389, pp. 1941-1952

Richeldi, L., Du Bois, R. M., Raghu, G., Azuma, A., Brown, K. K., Costabel, U., Cottin, V., Flaherty, K. R., Hansell, D. M., Inoue, Y., Kim, D. S., Kolb, M., Nicholson, A. G., Noble, P. W., Selman, M., Tanighuchi, H., Brun, M., Le Maulf, F., Girarad, M., Stowasser, S., Schlenker-Herceg, R., Disse, B., Collard, H. R., (2014) 'Efficacy and Safety of Nintedanib in Idiopathic Pulmonary Fibrosis', *The New England Journal of Medicine*, 370 (22), pp. 2071-2082

Robinson, M., (2004), 'Review article: the pharmacodynamics and pharmacokinetics of proton pump inhibitors – overview and clinical implications', *Alimentary Pharmacology and Therapeutics*, 20 (Suppl 6), pp. 1-10

Robinson, N. B. and DiMango E., (2014), 'Prevalence of gastro-oesophageal reflux in

cystic fibrosis and implications for lung disease', *Annals of the American Thoracic Society*, 11(6), pp. 964-968

Ryan, N. M., Birring, S. S, Gibson, P. G., (2012), 'Gabapentin for refractory chronic cough: a randomized, double-blind, placebo-controlled trial', *The Lancet,* 380(9853), pp. 1583-1589

Savarino, E. Carbone. R, Marabotto, E., Furnari, M., Sconfienza, L., Ghio, M., Zentilin, P., Savarino, V., (2013), 'Gastro-oesophageal reflux and gastric aspiration in idiopathic pulmonary fibrosis patients', European Respiratory Journal, 42, pp. 1322-1331

Schelling, F.A., (2003), 'Clinical trials: deliberations on their essence and value', *Journal of Evaluation in Clinical Practice*, 10(2), pp. 291-296

Stovold, R., Forrest, I. A., Corris, P. A., Murphy, D. M., Smith, J. A., Decalmer, S., Johnson, G. E., Dark, J. H., Pearson, J. P., Ward, C., (2007), 'Pepsin a biomarker of gastric aspiration in lung allografts: a putative association with rejection', *American Journal of Respiratory and Critical Care Medicine,* 175(12), pp. 1298-1303

Summary of product characteristics (SmPC): omeprazole, version 4, June 2013, Bristol Laboratories Ltd.

Swigris, J. J., Wamboldt, F., S., Behr, J., du Bois, R., M., King, T. E., Raghu, G., Brown, K. K., (2010), ' The 6 minute walk in idiopathic pulmonary fibrosis: longitudinal changes and minimum important difference', *Thorax*, 65 pp. 173-177

The British Thoracic Society Interstitial Lung Disease Registry Programme: Annual Report 2013/2014. Available at: https://www.brit-thoracic.org.uk/…/bts-ild-registry-programme-annual-report-201314/

Terada, K., Muro, S., Sato, S., Ohara, T., Haruna, A., Marumo, S., Kinose, D., Ogawa, E., Hoshino, Y., Niimi, A., Terada, T., Mishimo, M., (2008), 'Impact of gastro-oesophageal reflux disease symptoms on COPD exacerbation', *Thorax*, 63, pp.

951-955

Tobin, R. W., Pope, C. E., Pellegrini, C. A., Edmond, M. J., Sillery, J., Raghu, G., (1998), 'Increased prevalence of gastroesophageal reflux in patients with idiopathic pulmonary fibrosis', *American Journal of Respiratory and Critical Care Medicine*, 158, pp. 1804-1808

Turner-Warwick, M., Burrows, B., Johnson, A., (1980), 'Cryptogenic Fibrosing alveolitis: clinical features and their influence on survival', *Thorax*, 35, pp. 171-180

Vanderhoff, B. T. and Tahboub, R. M., (2002), 'Proton pump inhibitors: an update', *American Family Physician*, 66(2), pp. 273-280

Vanella, K. M., Moore, B. B., (2008), 'Viruses as co-factors for the initiation or exacerbations of lung fibrosis', *Fibrogenesis and Tissue repair*, 1:2, doi.org/10.1186/1755-1536-1-2

Welage, L. S. and Berardi, R. R., (2000), 'Evaluation of omeprazole, lansoprazole, pantoprazole, and rabeprazole in the treatment of acid-related disease', *Journal of the American Pharmaceutical Association (Washington, D.C. : 1996),* 40(1), pp. 52-62

Woodcock, A., Young, E. C., Smith, J. A., Hilton, E., (2010), 'New insights in cough', *British Medical Bulletin*, 96(1), pp. 61-73

Yano, F., Sherif, A. E., Turaga, K., Stadlhuber, R. J., Tsuboi, K., Ramaswamy, S., Mittal, S. K., (2009), 'Gastrointestinal quality of life questionnaire in patients after ant-reflux surgery', *Diseases of the Esophagus*, 22, pp. 177-184

Yousaf, N., Lee, K. K., Jayaraman, B., Pavord. I. D., Birring, S. S., (2011), 'The assessment of quality of life in acute cough with the Leicester Cough Questionnaire (LCQ-acute)', *Cough*, 7(4), doi: 10.1186/1745-9974-7-4

Zerbib, F., des Varannes, S. B., Roman, S., Pouderoux, P., Artigue, F., Chaput, U., Mion, F., Caillol, F., Verin, E., Bommelaer, G., Ducrotte, P., Galmiche, J. P., Sifrim,

D., (2005), ‘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 and Therapeutics*, 22(10), pp.1011-1021

Appendix 1: PPIPF study: Questionnaires

PPIPF STUDY: RCT of Omeprazole/Placebo in IPF
(PI: Prof John Simpson, Rec: 13/YH/0284)

Subject Initial:_____ Study ID/Trial ID_____ Date:___/___/_____

LEICESTER COUGH QUESTIONNAIRE (LCQ)

This questionnaire is designed to assess the impact of cough on various aspects of your life.

Read each question carefully and answer by CIRCLING the response that best applies to you.
Please answer ALL questions, as honestly as you can.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Thank you for completing this questionnaire.

PPIPF STUDY: RCT of Omeprazole/Placebo in IPF
(PI: Prof John Simpson, Rec: 13/YH/0284)

Subject Initial:_____ Study ID/Trial ID_____ Date:___/___/_____

The Reflux Symptom Index (RSI)

Reflux Symptom Index Scale Test: Rate the following items on a scale of 0-5.

The Reflux Symptom Index

Within the past month, how did the following affect you?
0 = No problem 5 = Severe problem
(Please put a tick in the score box as applicable to your symptom)

Hoarseness or a problem with your voice?	**0**	**1**	**2**	**3**	**4**	**5**
Clearing your throat?						
Excess throat mucus or postnasal drip?						
Difficulty swallowing food, liquids or pills?						
Coughing after you ate or lie down?						
Breathing difficulties or choking episodes?						
Troublesome or annoying cough?						
Sensations of something sticking in your throat or a lump in your throat?						
Heartburn, chest pain, indigestion, or stomach acid coming up?						

Thank you for completing the questionnaire.

PPIPF STUDY: RCT of Omeprazole/Placebo in IPF
(PI: Prof John Simpson, Rec: 13/YH/0284)

Subject Initial:_____ Study ID/Trial ID_____ Date:___/___/_____

Gastrointestinal Quality of Life Index Questionnaire (GIQLI)

This questionnaire is designed to assess the impact of GI symptoms on various aspects of your life.

Read each question carefully and answer by circling the response that best applies to you.

1. How often during the past 2 weeks have you had pain in the abdomen?

All of the time	Most of the time	Some of the time	A little of the time	Never

2. How often during the past 2 weeks have you had a feeling of fullness in the upper abdomen?

All of the time	Most of the time	Some of the time	A little of the time	Never

3. How often during the past 2 weeks have you had bloating (sensation of too much gas in the abdomen)?

All of the time	Most of the time	Some of the time	A little of the time	Never

4. How often during the past 2 weeks have you been troubled by excessive passage of gas through the anus?

All of the time	Most of the time	Some of the time	A little of the time	Never

5. How often during the past 2 weeks have you been troubled by strong burping or belching?

All of the time	Most of the time	Some of the time	A little of the time	Never

6. How often during the past 2 weeks have you been troubled by gurgling noises from the abdomen?

All of the time	Most of the time	Some of the time	A little of the time	Never

7. How often during the past 2 weeks have you been troubled by frequent bowel movements?

All of the time	Most of the time	Some of the time	A little of the time	Never

8. How often during the past 2 weeks have you found eating to be a pleasure?

All of the time	Most of the time	Some of the time	A little of the time	Never

9. Because of your illness, to what extent have you restricted the kinds of food you eat?

Very Much	Much	somewhat	A little	Not at all

10. During the past 2 weeks, how well have you been able to cope with everyday stresses?

Extremely poorly	Poorly	Moderately	Well	Extremely Well

11. How often during the past 2 weeks have you been sad about being ill?

All of the time	Most of the time	Some of the time	A little of the time	Never

12. How often during the past 2 weeks have you been nervous or anxious about your illness?

All of the time	Most of the time	Some of the time	A little of the time	Never

13. How often during the past 2 weeks have you been happy with life in general?

Never	A little of the time	Some of the time	Most of the time	All of the time

14. How often during the past 2 weeks have you been frustrated about your illness?

All of the time	Most of the time	Some of the time	A little of the time	Never

15. How often during the past 2 weeks have you been tired or fatigued?

All of the time	Most of the time	Some of the time	A little of the time	Never

16. How often during the past 2 weeks have you felt unwell?

All of the time	Most of the time	Some of the time	A little of the time	Never

17. Over the past week, have you woken up in the night?

Every night	5-6 nights	3-4 nights	1-2 nights	Never

18. Since becoming ill, have you been troubled by changes in your appearance?

A great deal	A moderate amount	Somewhat	A little bit	Not at all

19. Because of your illness, how much physical strength have you lost?

A great deal	A moderate amount	Somewhat	A little bit	None

20. Because of your illness, to what extent have you lost your endurance?

A great deal	A moderate amount	Somewhat	A little bit	None

21. Because of your illness, to what extent do you feel unfit?

Extremely Unfit	Moderately Unfit	Somewhat Unfit	A little Unfit	Fit

22. During the past 2 weeks, how often have you been able to complete your normal daily activities (school, work, household)?

All of the time	Most of the time	Some of the time	A little of the time	Never

23. During the past 2 weeks, how often have you been able to take part in your usual patterns of leisure or recreational activities?

All of the time	Most of the time	Some of the time	A little of the time	Never

24. During the past 2 weeks, how much have you been troubled by the medical treatment of your illness?

Very Much	Much	Somewhat	A little	Not at all

25. To what extent have your personal relations with people close to you (family or friends) worsened because of your illness?

Very Much	Much	Somewhat	A little	Not at all

26. To what extent has your sexual life been impaired (harmed) because of your illness?

Very Much	Much	Somewhat	A little	Not at all

27. How often during the past 2 week, have you been troubled by fluid or food coming up into your mouth (regurgitation)?

All of the time	Most of the time	Some of the time	A little of the time	Never

28. How often during the past 2 weeks have you felt uncomfortable because of your slow speed of eating?

All of the time	Most of the time	Some of the time	A little of the time	Never

29. How often during the past 2 weeks have you had trouble swallowing your food?

All of the time	Most of the time	Some of the time	A little of the time	Never

30. How often during the past 2 weeks have you been troubled by urgent bowel movements?

All of the time	Most of the time	Some of the time	A little of the time	Never

31. How often during the past 2 weeks have you been troubled by diarrhoea?

All of the time	Most of the time	Some of the time	A little of the time	Never

32. How often during the past 2 weeks have you been troubled by constipation?

All of the time	Most of the time	Some of the time	A little of the time	Never

33. How often during the past 2 weeks have you been troubled by nausea?

All of the time	Most of the time	Some of the time	A little of the time	Never

34. How often during the past 2 weeks have you been troubled by blood in the stool?

All of the time	Most of the time	Some of the time	A little of the time	Never

35. How often during the past 2 weeks have you been troubled by heartburn?

All of the time	Most of the time	Some of the time	A little of the time	Never

36. How often during the past 2 weeks have you been troubled by uncontrolled stools?

All of the time	Most of the time	Some of the time	A little of the time	Never

Thank you for completing this questionnaire.

PPIPF STUDY: RCT of Omeprazole/Placebo in IPF
(PI: Prof John Simpson, Rec: 13/YH/0284)
Subject Initial:_____ Study ID/Trial ID_____ Date:___/___/_____

DeMeester Reflux Associated Questionnaire (DeMRQ)

This questionnaire is designed to assess the impact of reflux symptom in your activities of daily life.
Please circle the response applicable to your symptoms.
In the **last 2 weeks** have you suffered from:

1) Heartburn (burning sensation in chest): [Flow of Gastric Contents into Oesophagus]

None - 0 - No heartburn

Minimal - 1 - Occasional episodes

Moderate - 2 - Reason for medical visit

Severe - 3 - Interference with daily activities

2) Regurgitation - (Flow of Gastric Contents into Mouth)

None - 0 - No regurgitation

Minimal - 1 - Occasional episodes

Moderate - 2 - Predictable on position or straining

Severe - 3 - Episodes of Pulmonary Aspiration (nocturnal cough, recurrent pneumonia)

3) Dysphagia – (Difficulty swallowing or food getting stuck)

None - 0 - No dysphagia

Minimal - 1 - Occasional episodes

Moderate - 2 - Required liquids to clear

Severe - 3 - Episode of meat impaction requiring medical treatment

Total symptom score (heartburn + regurgitation + dysphagia) = _____

Thank you for completing the questionnaire.

Appendix 2: PPIPF study: Protocol

STUDY PROTOCOL

A randomised placebo-controlled pilot trial of omeprazole in idiopathic pulmonary fibrosis (IPF) (Acronym: PPIPF Study)

Sponsor	The Newcastle upon Tyne Hospitals NHS Foundation Trust
Sponsor Protocol number:	IAFIPF001
EudraCT Number:	2013-003301-26
Funder	British Lung Foundation
Funding Reference Number	IPFPSG12-7
Chief Investigator	Prof John Simpson Professor of Respiratory Medicine Institute of Cellular Medicine 4th Floor, William Leech Building Medical School, Newcastle University Framlington Place Newcastle upon Tyne NE2 4HH
REC Number	13/YH/0284
Version Number and Date	Version 5.0, 07 March 2016

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AE	Adverse event
AR	Adverse reaction
BAL	Bronchoalveolar lavage
CI	Chief investigator
CONSORT	Consolidated standards of reporting trials
CRF	Case report form
CTU	Clinical trials unit
CYP	Cytochrome P450
DMSC	Data monitoring and safety committee
GCP	Good clinical practice
GIQOLI	Gastrointestinal quality of life index
GP	General practitioner
H_2-antagonists	Histamine receptor antagonists
H/K-ATPase	Hydrogen-potassium adenosinetriphosphatase
HRCT	High resolution computed tomography
IIP	Idiopathic interstitial pneumonia
ILD	Interstitial lung disease
ILD-MDT	Interstitial lung disease multidisciplinary team
IPF	Idiopathic pulmonary fibrosis
LCQ	Leicester cough questionnaire
MHRA	Medicines and Healthcare products Regulatory Agency
NuTH	Newcastle upon Tyne Hospitals
PI	Principal investigator
PPI	Proton pump inhibitor
REC	Research ethics committee
RSI	Reflux symptoms index
SAE	Serious adverse event
SAR	Serious adverse reaction
SmPC	Summary of product characteristics
SOP	Standard operating procedure
SUSAR	Suspected unexpected serious adverse reaction
Tco	Transfer factor for carbon monoxide
UAR	Unexpected adverse reaction
VC	Vital capacity

Protocol Authorisation

Full protocol title: A randomised placebo-controlled pilot trial of omeprazole in idiopathic pulmonary fibrosis (IPF)

Protocol Number 1
Version/Date Version 5.0, 27th October 2015

A review of the protocol has been completed and is understood and approved by the following:

Designation	Name	Signature	Date
Chief Investigator/Principle Investigator	Prof John Simpson		
Sponsor's Representative	Ms Jillian Peacock		
Co-Investigator	Dr Ian Forrest		
Co-Investigator	Dr Chris Ward		
Co - Investigator	Dr Jacky Smith		
Trial Statistician	Ms Vicky Ryan		
Senior Trial Manager	Dr Lesley Hall		
Trial Manager	Mr Mark Palmer		

Study Team

Chief Investigator/Principle Investigator	Prof John Simpson
Co-Investigator	Dr Ian Forrest*
Co-Investigators	Dr Chris Ward
	Professor Michael Griffin
	Dr Jacky Smith**
	Prof Jeff Pearson
Clinical Research Fellows	Dr Wendy Funston
	Mr Rhys Jones
Research Nurse	to be assigned by CLRN team*
Newcastle Clinical Trials Unit representative	Prof Elaine McColl
Sponsor's representative	Mr Andrew Johnston
Trial statistician	Ms Vicky Ryan
Senior Trial Manager	Dr Lesley Hall
Trial Manager	Mr Mark Palmer

All Newcastle University except: *The Newcastle upon Tyne Hospitals NHS Foundation Trust, ** University of Manchester

1. Lay summary

Idiopathic pulmonary fibrosis (IPF) is a disease of unknown cause in which areas of normal lung tissue are replaced by scars. As a result it becomes harder for the lungs to extract oxygen from the air. IPF is commonly progressive, and around 50% of patients diagnosed with the disease die after approximately 3 years. The most common, troublesome symptoms of IPF are breathlessness on exertion, and cough. No drug treatments have been unequivocally shown to improve the death rate, or to significantly impact upon symptoms, in IPF.

In recent years it has been recognised that cough can be caused by small amounts of liquid coming up from the stomach and "going down the wrong way" into the lungs, a process commonly known as "reflux". As liquid in the stomach is usually acidic, patients' lungs may repeatedly be exposed to small amounts of acid. Reflux is unusually common in IPF and could potentially contribute to the debilitating cough found with the disease. However there are many potential causes for cough in IPF.

Stomach acid can be efficiently "switched off" by drugs called "proton pump inhibitors", one of which is called omeprazole. If reflux of stomach acid does contribute to cough in IPF, omeprazole might be expected to reduce cough. ***The purpose of this study is therefore to test whether omeprazole does reduce cough in patients with IPF***. Sixty patients with IPF will be randomly allocated to have 3 months of omeprazole or a placebo. Neither the patient nor the doctor will be aware which treatment has been given, ie this is a randomised "double-blind", placebo-controlled trial. Patients' cough frequency will be measured before and after treatment and the change in cough frequency compared in those receiving omeprazole and those receiving placebo. Change in cough frequency is the main thing we aim to compare, but a range of other measurements will be assessed such as the numbers of patients eligible to take part, agreeing to randomization and providing outcome data, patients' lung function, symptom scores, the amount of reflux, and the amount of inflammation in the lungs.

2. Background

Idiopathic pulmonary fibrosis (IPF) is the commonest of the idiopathic interstitial pneumonias (IIPs).[1,2] Clinically the disease is characterised by exertional breathlessness and cough, both of which may be debilitating for patients.[1,2] Pathological features include the deposition of excessive fibrotic matrix in the alveolar regions of the lung, usually in a predominantly basal and subpleural distribution.[3] IPF tends to become progressively worse, and median survival from time of diagnosis is typically estimated at 3 years.[1,2,4] No pharmacological treatments have significantly impacted upon mortality in IPF, and increasingly attention is focusing on more patient-centred end-points in clinical trials. However the treatment of cough associated with IPF remains notoriously difficult, and new approaches are required.

2.1. Pathogenesis of IPF

The pathogenesis of IPF remains poorly defined. The main prevailing theory is that the alveolar epithelium sustains damage from an environmental cause(s), probably on a repeated or perpetual basis. In genetically predisposed individuals, the injury leads to an aberrant repair process where myofibroblasts release inappropriately high amounts of interstitial collagen. The rate of deposition of collagen exceeds the rate of resorption, and fibrosis ensues. The histological hallmark of IPF is thought to be the "fibroblastic focus", a characteristic lesion in which myofibroblasts generate – and sit within – a loose stroma of immature collagen, typically right beneath abnormal alveolar epithelium.[5,6] This histological appearance broadly supports the prevailing view of pathogenesis.

The nature of environmental insults initiating or perpetuating the cycle of damage and aberrant repair remains elusive. Several candidates, most notably viruses, have been proposed.[7] However, in recent years, a contribution from aspirated gastrointestinal contents has received a great deal of attention. Under physiological conditions, stomach contents are acidic, and it is recognised that "micro-aspiration" of stomach content into the lung is a common occurrence. Bile acids may also be aspirated into the lungs. Stomach acid and bile acid can induce injury in alveolar epithelial cells, and the suggestion has emerged that micro-aspiration of gastrointestinal contents may contribute to the pathogenesis of IPF through repeated epithelial injury.

2.2. Reflux in IPF

Gastro-oesophageal reflux is significantly more frequent in patients with IPF than in age- and sex-matched controls.[8-13] Certainly the accumulated evidence suggests that over 50% of patients with IPF have "distal" gastro-oesophageal reflux. Studies have

suggested that surgical correction of reflux in IPF reduces disease progression.[14] However, many patients with IPF are unsuitable for significant surgical intervention. Effective medical interventions are likely to prove more useful in this context.

3. Omeprazole

3.1. Drug class and licensed indications

Omeprazole is a substituted benzimidazole, and belongs to the "proton pump inhibitor" (PPI) class of drugs. Omeprazole specifically inhibits the hydrogen-potassium-ATPase (H/K-ATPase) enzyme at the apical surface of gastric parietal cells. H/K-ATPase is responsible for delivering hydrogen ions to the lumen of the stomach, thus acidifying the contents. Omeprazole's dose-dependent and specific inhibition of the enzyme therefore neutralizes gastric acid contents. Omeprazole inhibits basal and induced gastric acid release.

Omeprazole has been used clinically for many years. It is licensed for use in gastro-oesophageal reflux, erosive oesophagitis, duodenal ulcer, gastric ulcer, eradication of *Helicobacter pylori* (in combination with antibiotics), and Zollinger-Ellison syndrome. It is also used for the prevention of gastric adverse events associated with use of non-steroidal anti-inflammatory drugs.

3.2. Studies of PPI in IPF

Randomised controlled trials directly comparing omeprazole and placebo in IPF are lacking. A search using "omeprazole AND idiopathic pulmonary fibrosis" on clinicaltrials.gov reveals no matches. Small studies have reported a reduced rate of disease progression in patients treated with PPI with or without fundoplication.[15] A retrospective study suggested that patients with IPF on PPI had improved survival and less marked fibrosis than patients not taking PPI.[16]

3.3. Dose and duration

Omeprazole is generally prescribed in doses of 20mg or 40mg daily. Short-term use (eg for 2 months) and long-term use are both commonly employed in clinical practice. Omeprazole is effective at suppressing stomach acid within 2 hours in most individuals.[17, 18] A daily dose of 20mg results in gastric pH levels >4 for approximately 12 hours.[19] This influenced our choice of omeprazole 20mg bd for the current study.

3.4. Clinical pharmacology

Omeprazole is rapidly absorbed from the gastrointestinal tract and is highly bound to plasma proteins. An intravenous formulation is available, but the good bioavailability

of omeprazole has led us to favour oral administration. Omeprazole is principally metabolized in the hepatic cytochrome P450 (CYP) enzyme system, and eliminated principally in the kidneys (the remainder being eliminated by the faecal route).

As a result, omeprazole has a longer half-life in patients with advanced liver disease. It may interact with drugs that are metabolized by the P450 system. Arguably the most important interactions are with

- warfarin
- diazepam
- phenytoin
- ketoconazole or other azoles

For the purposes of this study, warfarin will be contraindicated as it has been associated with adverse effects in IPF.[20] Patients on phenytoin and long term diazepam will be excluded. Patients on azole drugs will be excluded (but allowed to enter the study once azoles are discontinued). Patients with documented cirrhosis will be excluded. These points are reiterated in the Exclusion Criteria section (section 4.6, page 12).

3.5. Adverse effects

Omeprazole is widely used and has a good safety record. In general, adverse events have been recorded in under 5% of patients, with rates approximating closely to those in patients taking H_2-antagonists or placebo.[21-24]

The most common adverse events are diarrhoea, headache, abdominal discomfort and nausea.

Important, rare adverse events include: skin reactions, anaemia, agranulocytosis, haematuria, proteinuria, and urinary tract infection.

For details please refer to section 4.8 in SmPC.

3.6. Administration schedule chosen for this trial

Omeprazole 20mg bd, to be taken before food.

3.7. Manufacture and supply

Omeprazole and matched placebo will be supplied by Victoria Pharmaceuticals, Royal Hospitals, Belfast BT12 6BA.

4. Study Design

4.1. Hypothesis

Omeprazole reduces cough in IPF.

4.2. Trial Description

Prospective, randomised, double-blind, allocation-concealed, single-centre pilot trial of omeprazole in patients with IPF. The trial is designated a 'pilot' trial in that the trial seeks to provide proof of concept with respect to reduction in cough, with a view to designing definitive multi-centre trials in IPF. To this end, a key focus of the pilot trial will be on rates of participant eligibility, recruitment and retention, and on the yield and quality of data in respect of the proposed secondary outcomes for those future definitive trials.

4.3. Patient Population and Recruitment

Patients fulfilling pre-defined criteria for IPF aged between 40 and 85.
The study seeks to recruit prevalent (rather than only incident) IPF cases. At the time of writing the Newcastle regional ILD clinic sees approximately 18 patients per week and records suggest that approximately six different IPF patients are seen per week. We estimate that 50% of these patients may be eligible for inclusion in the trial (exclusion expected to be largely due to existing PPI use) and that 50% of eligible patients will consent to take part, resulting with randomising on average 1.5 patients per week. Therefore, over a 21 month recruitment phase, we estimate that 136 patients would be available of whom we aim to recruit and randomise 60. However we recognize that these are approximations and that elements of these estimate may change; estimation of actual rates of eligibility, randomization and retention will be made, in line with the pilot nature of this trial.
Patients can also be identified and referred for participation in trial from Participant Identification Centres (PICs) by their treating clinicians. Potential eligible patients would be sent a Patient Invitation Letter and Patient Information Sheet by clinicians who are responsible for their usual clinical care. The study team will follow up patients who have expressed their interest in the study.

4.4. Sample size

Sixty patients will be randomised 1:1 to omeprazole or placebo. In keeping with the principles of a pilot study no formal sample size calculations have been performed as analyses of outcome data will be, by definition, exploratory in nature. However, recommendations for good practice are that 20-30 patients per treatment arm should provide sufficient data to assess the feasibility of the trial, investigate the

distribution of outcome measures and estimate with adequate precision standard deviations of key study parameters.[25,26] The attrition rate for randomised patients in this study is not known (and is part of the feasibility assessment). Randomising 60 patients, however, will allow for up to 33% attrition while achieving the minimum recommended sample size of 20 patients per treatment arm with complete study follow-up.

4.5. Inclusion criteria

A pragmatic clinical definition of IPF will be used, in which recruited patients must fulfill all of the following criteria

- IPF is considered the most likely diagnosis by the regional interstitial lung disease multidisciplinary team meeting (ILD-MDT)
- history of cough, with or without exertional dyspnoea
- high resolution computed tomography (HRCT) scan features of honeycombing in a predominantly basal and subpleural distribution
- bibasal crackles on auscultation
- features of a restrictive ventilatory defect (vital capacity (VC) <90% predicted and/or diffusion factor for carbon monoxide (Tco) <90% predicted)
- aged 40-85 years

Patients with radiological emphysema will be eligible so long as the diagnosis of IPF is secure, ie all the features above are satisfied.

If the regional ILD-MDT cannot reach a clear consensus as to the diagnosis, the case will be referred to 2 experts in ILD from outside the region, and the patient will be eligible if both consider IPF to be the most likely diagnosis.

Patients taking a PPI during screening will potentially be eligible. In these cases the indication for on-going treatment will be reviewed.

- Patients taking short courses (eg 2 months) of PPI will be eligible once the treatment has been discontinued for a minimum of 1 month.
- There are few licensed indications for long-term omeprazole other than Zollinger-Ellison syndrome. Therefore, unless there is a known diagnosis of Zollinger-Ellison or a history of significant dyspepsia or gastrointestinal bleeding during a previous discontinuation of PPI, patients on long-term PPI will be asked to consider a trial of supervised discontinuation.

If patients taking omeprazole (or a related stomach treatment) wish to take part, we shall contact the GP to check that the GP is aware and in agreement. The patient will then be asked to sign a consent form to agree to try a period off treatment for 2 weeks. If symptoms return during that 2- week period the patient should go back on treatment and not take part in the study. If patients manage well without the

treatment for 2 weeks, they will be asked to sign a second consent form before starting the study.

Patients taking antacids, prokinetics or raft alginates at the time of screening will be eligible if they have been off these treatments for a period of at least 2 weeks.

4.6. Exclusion criteria

- known allergy to omeprazole or other PPI
- concomitant use of warfarin, diazepam, phenytoin or ketoconazole
- concomitant use of a regular PPI, antacid, prokinetic or raft alginate during the trial period.
- history of upper respiratory tract infection, lower respiratory tract infection or exacerbation of IPF in the 4 weeks before starting study drugs
- active trial of treatment for IPF (eg prednisolone, pirfenidone, nintedanib, N-acetylcysteine) started in the 4 weeks before starting study drugs
- documented history of hepatic cirrhosis
- pregnancy or lactation
- ILD-MDT considers the most likely cause of the patient's ILD to be a condition other than IPF, for example rheumatoid lung, systemic sclerosis ILD, asbestosis, chronic hypersensitivity pneumonitis, sarcoidosis, etc.
- concurrent enrolment in a trial of a CTIMP for IPF

4.7. Intervention

Patients will be randomised 1:1 to omeprazole 20mg twice daily or matching placebo, to be taken orally before food for 90 days.

4.8. Primary outcomes

4.8.1 Primary efficacy outcome

The change in frequency of objectively measured cough from beginning of the study (i.e.baseline) to the end of treatment (ie within the last 2 weeks of last treatment). This will be compared in the two groups.

4.8.2 Feasibility outcomes

- Rates of eligibility, recruitment, randomization and study completion
- Feasibility and acceptability of trial procedures

4.9. Secondary outcomes

The following outcomes, proposed as secondary efficacy outcomes for any future trial, will be measured, with the focus of analysis being on data yield and quality

- Change in symptoms of cough at the end of treatment (as measured by validated questionnaire)
- Change in symptoms of reflux at the end of treatment (as measured by validated questionnaire)
- Change in acid and non-acid reflux after treatment
- Change in VC and Tco at the end of treatment
- Change in 6 minute walk distance at the end of treatment
- Markers of lung inflammation in bronchoalveolar lavage (BAL) fluid at the end of treatment (eg concentration of transforming growth factor beta, interleukin-8 etc)
- Change in lung infection in BAL fluid at the end of treatment
- Patient-reported adverse events

4.10 Statistical analysis

The primary outcome measure is change in cough frequency when comparing the objective assessments before treatment (baseline) and in the last 2 weeks of treatment. Exploratory statistical analyses will be conducted around this end-point. In particular, using analysis of covariance, we will estimate the mean difference in the change in objective cough frequency (adjusting for baseline objective cough frequency) and report this estimate with a 95% confidence interval. Further, both baseline cough frequency and PPI naivity have been identified as possibly prognostic for the primary outcome, we will therefore also consider, if feasible, an analysis which adjusts for both baseline cough frequency and PPI naivity.[34]

The analyses of all other outcomes will be mainly descriptive, with 95% confidence intervals reported where appropriate. Confidence limits for the estimated standard deviations of key study parameters will also be calculated and used in sensitivity analyses for sample size calculations for future definitive trial applications.

5. Trial procedures

5.1. Screening

Medical records of patients attending the regional interstitial lung disease in Newcastle will be screened by members of the clinical team (Dr Forrest, Prof Simpson, Dr Funston, Mr Jones) or the research nurse assigned to the study. Eligible patients will be provided with information relating to the study either in person (when they attend the clinic), or by letter. Patients will be informed that they may take as long as they like to consider the information provided, and to answer any questions. Patients on a PPI will be considered as in section 4.5, page 12. A screening log will be maintained.

5.2. Informed consent

The Chief Investigator is responsible for ensuring that informed consent for trial participation is given by each subject. An appropriately trained doctor may take consent. If no consent is given a subject cannot be randomised and entered into the trial. The subject will be asked to sign the consent form which will then be countersigned by the person taking consent and will be retained in the trial site file.

5.3. Withdrawal of consent

Participants have the right to withdraw from the study at any time for any reason, and without giving a reason. The investigator also has the right to withdraw patients from the study intervention if it is judged to be in the patient's best interests. It is understood by all concerned that an excessive rate of withdrawals can render the study uninterpretable and therefore, unnecessary withdrawal of patients should be avoided. Should a patient decide to withdraw from the study, all efforts will be made to report the reason for withdrawal as thoroughly as possible.
There are three withdrawal options:

1. Withdrawing completely (i.e. withdrawal from allocated treatment and provision of follow-up data)
2. Withdrawing active participation in trial but allowing continued review by research team of healthcare records
3. Withdrawing partially but continuing to provide follow-up data by attending clinic.

We will encourage participants that decide to withdraw to choose option 2 or 3 but if they wish to withdraw completely we will retain data collected up to the point of withdrawal. Participants will be asked if they would be happy for the reason for the decision to withdraw to be recorded. Participants who withdraw completely will not be replaced but the rate of withdrawal will be monitored and reported to the DMC.

5.4. Randomization

Participants will be randomized to omeprazole or placebo in a 1:1 ratio, using random permuted blocks. The randomization allocation schedule will be generated by a statistician with no other involvement in the study to achieve concealment of allocation. Randomization will be performed by a member of site staff, appropriately trained and identified on the delegation log, using a secure password-protected web-based system administered by Newcastle Clinical Trials Unit.

Contact details for Randomization: https://apps.ncl.ac.uk/random/

(Available 24 hours a day)

Assignment to either omeprazole or placebo will be blinded to both the participant and the research team (double blinded). Randomization will generate two numbers: a unique 3-digit "Study ID" number for each participant and a unique 2-digit "Bottle" number (which will match a "Trial patient no.(Pack number)" on a medication pack held in Pharmacy at the Royal Victoria Infirmary). Both numbers will be entered on to the prescription which is delivered to Pharmacy.

5.5. Study Drug Termination Criteria

The study drug (omeprazole or placebo) will be continued until one of the following conditions are met (whichever comes first):

- 90 days of treatment
- study drug-related serious adverse reaction
- decision by the patient's GP or a consultant he/she is attending that a PPI should be prescribed on clinical grounds
- the patient withdraws from the study
- death or discontinuation of active medical treatment

5.6. Clinical Management of Patients in the Study

Administration of either omeprazole or placebo will be outside of usual clinical care for all patients entered into the study.

Lung function (VC and Tco) is a routine part of patient care, and patients' entry in to the study will be timed to correspond to routine measurement of lung function wherever possible.

Six minute walk test and bronchoscopy with BAL are used in clinical practice for ILD when indicated, but are not considered routine. Cough and reflux questionnaires, objective cough monitoring, pH impedance manometry, and issue of adverse event diaries are outside of usual care for patients entered into the study.

All other aspects of usual patient care will be delivered to patients prior to, during and after completion of the study.

5.7. Study Procedures for Unblinding

The investigator or treating physician may unblind a participant's treatment assignment in the case of an emergency, when knowledge of the study treatment is essential for the appropriate clinical management or welfare of the subject. Should a treating clinician require emergency unblinding, the investigator will be contacted via an emergency contact telephone number (0191 208 7770, mobile: 07765920130). The investigator will contact the clinical trials pharmacist at the Royal Victoria Infirmary during working hours or if out of working hours the on call pharmacist for the Newcastle upon Tyne Hospitals NHS Foundation Trust for emergency unblinding. The date and reason for the unblinding must be recorded in the case report form (CRF).

6. Study Assessments

A summary of study assessments and patient visits is shown in the Table below.

	Days -7 to 0 (ie any 3 days in the lead up to starting study medication			Days 1-87	Days 88-90 **§§**		
Confirm eligibility	*						
Consent	*						
Demographics	*						
VC and TLco	*				*		
6 minute walk test	*				*		
Cough questionnaire	*				*		
Reflux questionnaire	*				*		
Commence 24h cough monitoring	*				*		
Commence 24h oesophageal physiology tests		*				*	
Randomization			*				

Issue omeprazole or placebo			*				
Issue adverse event diary	*						
Take study medication				*	*	*	*
Bronchoscopy and BAL							*

§§ - the tests scheduled for "days 88-90" should ideally be performed on these days, but can be performed at any point in the 2 weeks before completing omeprazole/placebo.

6.1. Demographics

Patients' age, gender, smoking history and current medications will be recorded.

6.2. Vital capacity and transfer factor

These will be performed in the Chest Clinic, Royal Victoria Infirmary, to standards laid out in international guidelines.[27,28] It is expected that all patients will have performed lung function tests before, and be accustomed to the procedures.

6.3. Six-minute walk distance

This will be performed according to international guidelines,[29] under medical supervision at the Royal Victoria Infirmary. By definition, patients can stop the test at any point if they feel too breathless or unwell to continue.

6.4. Cough and reflux questionnaires

Validated questionnaires will be administered at the Royal Victoria Infirmary, and may include The De Meester reflux-related symptoms questionnaire, The Reflux Symptoms Index (RSI), The Gastrointestinal Quality of Life Index (GIQOLI) and the Leicester Cough Questionnaire (LCQ).

6.5. Objective cough monitoring

24-hour ambulatory cough sound recording involves patients being fitted with a CE marked lightweight sound recording apparatus (Vitalojak, Vitalograph Ltd, Buckingham, UK). These are generally well tolerated by patients without discomfort.[30] The cough frequency is calculated from a standard sound recording with the assistance of validated custom-written software.[31] Anonymised sound

recordings will be transferred to a secure server at the University of Manchester site. Files will be compressed using software to remove silences, background noise and the majority of speech. The resulting files will then be listened to and the location of cough sounds electronically tagged. A report documenting the number of coughs per hour will then be generated and returned to the chief investigator.
Cough counting will be done by Dr Jacky Smith (Co-Investigator, University of Manchester) and her team, which consists of 3 trained cough counters. To reduce the impact of variability between counters, for any individual subject, all cough recordings are counted by the same cough counter. Blind re-count of 10% of the recording data is done by a second counter, and the differences between cough counters are calculated. It is then compared to 95% limits of agreement based on a large dataset counted by the most experienced cough counters, to make sure the agreement is good and consistent with previous work.

6.6. Oesophageal physiology

Oesophageal physiology testing has two parts - oesophageal manometry and ambulatory pH/impedance testing. Oesophageal manometry uses pressure sensors to measure the oesophageal muscular contraction and relaxation of the gasto-oesophageal sphincter. pH/impedance testing uses sensors of acidity and electrical resistance to measure "reflux" of material from the stomach. The test runs over a 24-hour period but hospital admission is not required. The tests are performed in the Endoscopy Department at the Royal Victoria Infirmary. A fine nasogastric tube is passed for the manometry test, which takes around twenty minutes and involves the patient performing a number of swallows. The tube is then removed and a second, finer nasogastric tube is passed and left in place for 24 hours, connected to a data-recording box. The test does not significantly restrict normal activities. Insertion of the tube can be slightly uncomfortable but pain is unusual. The tube is removed, in the Royal Victoria Infirmary, the following day.

6.7. Bronchoscopy and BAL

Bronchoscopy and BAL will be performed at the Royal Victoria Infirmary or the Freeman Hospital, according to standard practice and international guidelines.[32,33] All patients will have an intravenous cannula placed, and ECG monitoring and oxygen saturation monitoring will be performed throughout. Patients will receive topical local anaesthetic to the nose, mouth, vocal cords and respiratory tract as required. Patients will receive supplemental oxygen. Patients will also be offered intravenous sedation, usually in the form of midazolam. The bronchoscopist will review previous HRCT scans and where possible will perform BAL in a segment with changes of IPF but without advanced honeycombing, in order to minimise the small theoretical risk of pneumothorax. Three aliquots of saline (60ml) each will be instilled and aspirated. After lavage has been performed, endobronchial biopsies will be taken for later

analysis of cough nerve fibre content. The patient will be monitored for at least 2 hours after the procedure. Patients who have had sedation will be advised that they may not drive, work, drink alchohol, operate moving machinery (including drills etc at home), or sign legal documents for the remainder of the day, and must return home accompanied by a responsible adult.

6.8. Assessment of BAL fluid

The quantity of BAL fluid retrieved will be recorded. An aliquot of BAL fluid will be sent to the NHS Microbiology Laboratory to test for culture, with the aim of detecting potentially pathogenic bacteria. The remainder of the sample will be taken to research labs at Newcastle University. Samples will be centrifuged. The cell pellet will be reconstituted, and used to make an estimation of total cell count. Cytospin preparations will be made on glass slides for future examination. Cell-free supernatant will be stored frozen until further use. Supernatant will be assessed for the levels of mediators including (but not limited to) transforming growth factor beta, pepsin, bile acids, matrix metalloproteinases, and interleukins.

7. Pharmacovigilance

7.1. Definitions

A**dverse event (AE):** any untoward medical occurrence which does not necessarily have a causal relationship with the treatment. "Treatment" includes all investigational agents (including comparative agents) administered during the course of the study. Medical conditions/diseases present before starting study treatment are only considered adverse events if they worsen after starting study treatment.

A**dverse reaction (AR):** any untoward and unintended response to an Investigational Medicinal Product related to any dose administered. All AEs judged by either the reporting investigator or the sponsor as having reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression "reasonable causal relationship" means to convey in general that there is evidence or argument to suggest a causal relationship.

Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR): any untoward medical occurrence or effect that at any dose:

- results in death
- is life threatening (i.e. the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- requires prolongation of existing hospitalisation

- results in persistent or significant disability or incapacity.
- is a congenital anomaly or birth defect.

Medical judgement should be exercised in deciding whether an AE/AR is serious in other situations. Important AE/ARs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

7.2. Assessment of Causality

Each AE should be clinically assessed for causality based on the information available, i.e. the relationship of the AE to the study drug. The assignment of the causality should be made by the Chief Investigator or the Principal Investigator responsible for the care of the participant using the definitions in the table below. All adverse events judged as having a reasonable suspected causal relationship to the study drug (i.e definitely, probably or possibly related) are considered to be adverse reactions. If any doubt about the causality exists, the Principal Investigator should consult the Chief Investigator. In the case of discrepant views on causality between the Principal Investigator and others, all parties will discuss the case and will refer as necessary to the Data Monitoring and Safety Committee (DMSC). In the event that no agreement is reached the MHRA, Research Ethics Committee (REC) and other bodies will be informed of both points of view.

Relationship	**Description**
Unrelated	There is no evidence of any causal relationship. The clinical event has an incompatible time relationship to the study administration drug, and could be explained by underlying disease, or other drugs or chemicals.
Unlikely	There is little evidence to suggest there is a causal relationship (eg. the event did not occur within a reasonable time after study drug administration). There is another reasonable explanation for the event (eg. the participant's clinical condition).
Possible	There is some evidence to suggest a causal relationship (eg. because the event occurs within a reasonable time after the study procedure). However the influence of other factors may have contributed to the event (eg. the participant's clinical condition).
Probable	There is evidence to suggest a causal relationship, including a reasonable time relationship with the study drug administration, and the influence of other factors is unlikely.
Definitely	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.

Not assessable	There is insufficient or incomplete evidence to make a clinical judgement of the casual relationship.

Suspected, Unexpected Serious Adverse Reaction (SUSAR): an adverse reaction that is both unexpected and serious. An adverse reaction is 'unexpected' if its nature or severity is not consistent with the applicable product information.

7.3. Assessment of expectedness

The assessment of expectedness will be performed by the CI against the Reference Safety Information (RSI) for the trial. The RSI is [***enter the chapter of IB or section of SmPC here***]. As this is a double-blinded study, the blind should be maintained as far as possible. Participants experiencing events that are serious, related and unexpected (i.e. meet the criteria of a SUSAR) must be unblinded (as per section 5.7) as only those events related to omeprazole would be considered SUSARs and require expedited reporting.

7.4. Adverse Event Reporting Period

The AE reporting period for this trial begins upon enrolment into the trial and ends 7 days following administration of the last dose of study drug. All AEs assessed by the PI as possibly related to the study drug and all SAEs that occur during this time will be followed until they are resolved or are clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es).

7.5. Adverse Event Reporting Requirements

AEs should be reported and documented on the relevant pages of the CRF, in accordance with the procedures outlined below. The PI at each site will also evaluate all AEs for expectedness in addition to causality.

7.6. Reporting AEs (including ARs)

Because this trial is recruiting a population that has cough and breathlessness, it is expected that many of the participants will experience AEs. Events that are expected in this population (ie events in keeping with the patient's underlying medical condition) should not be reported as AEs. An adverse reaction (AR) is an AE which is related to the administration of the study drug. If any AEs are related to the study drug (i.e. are ARs) they must be reported on the AE form within the CRF. It is the responsibility of the PI to record all relevant information in the CRF. Any questions

concerning adverse event reporting should be directed to the Chief Investigator (or Trial Manager in the absence of the Chief Investigator) in the first instance.

As trial participants are suffering from progressive disease (IPF) it is expected that many participants will experience worsening cough and breathlessness. Hence subjective worsening of cough and breathlessness should not be reported as AE unless they:

- required hospital admission for assessment and treatment
- required antibiotic treatment via GP surgery or chest clinic

Severity of AEs will be graded on a three-point scale (mild, moderate, severe). Relation of the AE to the treatment (causality) should be assessed by the investigator at site.

7.7. Reporting Serious Adverse Events (SAEs), Serious Adverse Reactions (SARs) and Suspected, Unexpected Serious Adverse Reactions (SUSARs)

Please see Flowchart 1 below to aid with reporting procedures

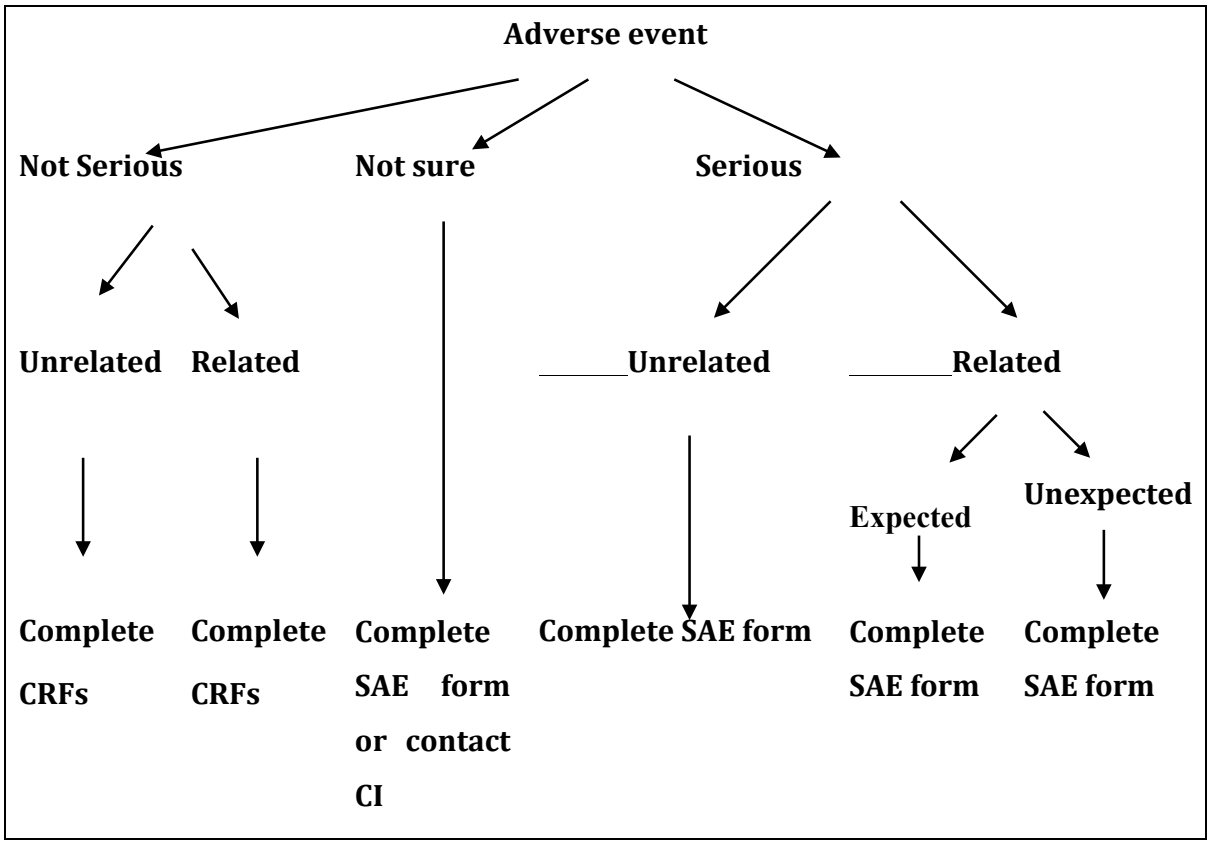
Serious Adverse Events (SAEs) and Serious Adverse Reactions (SARs, including SUSARS and expected SARs) will be reported to the Chief Investigator and Trial Management Team within 24 hours of the site Principal Investigator becoming aware of the SAE/SAR/SUSAR. Reporting of SAEs will be via SOHO66 system.

If an SAE occurs, reporting will follow the regulatory requirements as appropriate and all SUSARs will be the subject of expedited reporting. SAEs will be evaluated by the PI for causality (i.e. their relationship to the study drug) and expectedness. Once the PI becomes aware that an SAE has occurred in a study patient, they must complete the SAE form in the CRF and report the information to the CTU within 24 hours. The SAE form must be completed as thoroughly as possible with all available details of the event, signed by the PI or designee. If the PI does not have all information regarding an SAE, they will not wait for this additional information before notifying CTU. The form can be updated when the additional information is received. Follow up information should include whether the event has resolved, if and how it was treated, whether the patient continues in the study or has been withdrawn from treatment and information regarding unblinding of any patients. The SAE form should be transmitted by fax to the Newcastle CTU on 0191 5800400

The CTU is responsible for reporting SAEs that are considered to be related and unexpected to the Sponsor. The Sponsor will report any SUSARs to REC, and the MHRA within 15 days of becoming aware of the event (as per Joint Research Office

Standard Operating Procedure 03). In the event of a fatal or life threatening SUSAR reporting to the relevant regulatory authorities should take place within 7 days.

The Co-ordinator of the main REC should acknowledge receipt of related, unexpected safety report within 30 days.



7.8. Reporting SAEs to the DMSC

SAEs will be reported to the DMSC within the same timelines as for regulatory reporting. A copy of each report will be sent to the DMSC.

7.9. Pregnancies

7.9.1. Time period for collecting pregnancy information

All pregnancies in female participants and female partners of male participants will be collected after the start of dosing and until last follow-up visit.

7.9.2. Action to be taken if pregnancy occurs

The investigator will collect pregnancy information on any female participant who becomes pregnant while participating in this study. The investigator will record pregnancy information on the appropriate form, and submit it to the Chief Investigator within 2 weeks of learning of a participant's pregnancy. The participant will also be followed-up to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the Chief Investigator. Generally, follow-up will be no longer than 8-12 weeks following the estimated delivery date.

Any premature termination of the pregnancy will be reported. While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded as an AE or SAE (see AE/SAE section of the protocol for definitions and a description of follow-up). A spontaneous abortion is always considered to be an SAE and will be reported as such.

Furthermore, any SAE occurring as a result of a post-study pregnancy and considered reasonably related to the investigational product by the investigator, will be reported to the Chief Investigator. While the investigator is not obliged actively to seek this information in former study participants, he or she may learn of an SAE through spontaneous reporting.

Any female participant who becomes pregnant while participating will be withdrawn from the study.

Any female participant who becomes pregnant during dosing, will stop dosing immediately.

8. **Regulations, ethics and governance**

8.1. End of Trial

The trial will end when the completed number of patients have been recruited and completed follow-up.
The trial will be stopped prematurely if:

- Mandated by the Ethics Committee
- Mandated by the MHRA
- Mandated by the sponsor eg following recommendations from the DMSC

The REC that originally gave a favourable opinion of the trial and the MHRA that issued the Clinical Trial Authorisation will be notified in writing if the trial has been concluded or terminated early.

8.2. Research governance and regulatory approvals

The trial will comply with the principles, requirements and standards set out in the Research Governance Framework and The Medicines for Human Use (Clinical Trials) Regulations 2004 and subsequent amendments. A favourable ethical opinion from a NHS Research Ethics Committee and a Clinical Trial Authorisation from the MHRA will be obtained before the start of the trial.
The trial will be registered with the International Standard Randomised Controlled Trial Number register.

8.3. Sponsorship

The Newcastle upon Tyne Hospitals (NuTH) NHS Foundation Trust will act as sponsor.

8.4. Ethics

The study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. Following detailed discussion of the study, written, informed consent will be obtained from the participant.

8.5. Patient Confidentiality

Patient confidentiality will be maintained at every stage and compliance with the Data Protection Act (1998).

8.6. Good Clinical Practice (GCP)

The trial will be carried out in accordance with the principles of the International Conference on Harmonisation Good Clinical Practice (ICH-GCP) guidelines (www.ich.org).

8.7. Trial Monitoring

Site monitoring will be directed by the sponsor according to the study risk assessment. Site visits will be performed on a regular basis to ensure that all regulatory requirements are met and to monitor the quality of the data collected. The CRF will be used for source data verification.

8.8. Indemnity

The Newcastle upon Tyne Hospitals (NuTH) NHS Foundation Trust will provide indemnity for the management and conduct of the trial. Newcastle University is providing indemnity for trial design.

8.9. Funding

The study is funded by the British Lung Foundation, with additional support from the Newcastle Biomedical Research Centre.

8.10. Safety and well being of study participants

Participant safety and well-being are protected by implementation of the sponsor's standard operating procedures (SOP) as set out in the Research Governance Framework and The Medicines for Human Use (Clinical Trials) Regulations 2004. The sponsor, NuTH requires all research to be managed through a registered Clinical Trials Unit (NCTU for this trial). Systems are in place to ensure that all investigators are able to demonstrate that they are qualified by education, training or experience to fulfil their roles and those systems and procedures are in place which can assure the quality of every aspect of the trial.

If new safety information becomes available, then study participants will be informed of this and asked if they wish to continue in the study. If the subjects wish to continue in the study they will be formally asked to sign a revised approved participant information sheet and consent form.

8.11. Safety of investigators

NuTH and Newcastle University have Health and Safety Policies applicable to all employees. All personnel should also ensure they adhere to any other Health and Safety regulations relating to their area of work. The Chief investigator will ensure that all personnel have been trained appropriately to undertake their specific tasks. As the study fits closely to standard practice, there are few risks identified which are hazardous to the investigators. The study team will complete GCP training prior to start up.

9. Data Management, data collection and recording

9.1. Data Collection

All data for individual subjects will be collected by the chief investigator or by a delegated investigator and recorded in the electronic CRF. Due care will be taken to ensure data safety and compliance with the Data Protection Act 1998.

9.2. Data Storage

All essential documentation and trial records will be stored by the Chief Investigator in conformance with the applicable regulatory requirements and access to stored information will be restricted to authorised personnel.

9.3. Archiving

Trial documentation and data will be archived after completion of the trial in keeping with the applicable regulatory requirements.

9.4. Trial management

The Chief Investigator will take responsibility for the need to change the protocol for any reason, reviewing relevant information from other sources and considering recommendations from the DMSC. Day to day management will be undertaken via a trial management group composed of the Chief Investigator and supporting staff. They will meet regularly (approximately monthly) to discuss study issues.

9.5. Data Monitoring and Safety Committee (DMSC)

A DMSC will be appointed. The committee will be independent of the study team and will comprise a statistician and two clinicians with experience in undertaking clinical trials. The DMSC will meet to agree conduct and remit. The DMSC will convene soon after set up of the trial and may do so by teleconference if they wish. After the first meeting, the DMSC will decide on the frequency of subsequent meetings. In the event of an occurrence of an unexpected severe adverse reaction an additional unplanned DMEC meeting may be convened.

An interim analysis of efficacy is not planned although this issue can be discussed by the DMSC as required. The DMSC will function primarily as a check for safety, reviewing adverse events. They will report any issues pertaining to safety to the Chief Investigator. It will be the responsibility of the Chief Investigator to inform the sponsor who will take appropriate action to halt the trial if concerns exist about patient safety.

9.6. Dissemination

The trial will be reported in accordance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines (www.consort-statement.org). Dissemination will be achieved in several ways: (1) the findings will be presented at national and international meetings with open access abstracts on-line e.g. the American Thoracic Society annual meeting; and (2) in accordance with the open access policies proposed by the leading research funding bodies we aim to publish the findings in high quality peer-reviewed open access (via Pubmed) journals. This will secure a searchable compendium of these publications and make the results readily accessible to the public, health care professionals and scientists.

Where appropriate, research details will also be posted on institutional websites available to the general public. In addition, the most significant results will be communicated to the public through press releases.

10. References

1. Raghu G et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med 2011; 183: 788-824.
2. Bradley B et al. Interstitial lung disease guideline: the British Thoracic Society in collaboration with the Thoracic Society of Australia and New Zealand and the Irish Thoracic Society. Thorax 2008; 63 Suppl 5: v1-58.
3. Katzenstein AL, Myers JL. Idiopathic pulmonary fibrosis: clinical relevance of pathologic classification. Am J Respir Crit Care Med 1998; 157(4 Pt 1): 1301-1315.
4. Flaherty KR et al. Radiological versus histological diagnosis in UIP and NSIP: survival implications. Thorax 2003; 58: 143-148.
5. King TE Jr et al. Idiopathic pulmonary fibrosis: relationship between histopathologic features and mortality. Am J Respir Crit Care Med 2001; 164: 1025-1032.
6. Nicholson AG et al. The relationship between individual histologic features and disease progression in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2002; 166: 173-177.
7. Vannella KM, Moore BB. Viruses as co-factors for the initiation or exacerbations of lung fibrosis. Fibrogenesis Tissue Repair 2008; 1: 2.
8. Tobin RW et al. Increased prevalence of gastroesophageal reflux in patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 1998; 158: 1804-1808.
9. Patti MG et al. Idiopathic pulmonary fibrosis: how often is it really idiopathic? J Gastrointest Surg 2005; 9: 1053-1056.
10. Raghu G et al. High prevalence of abnormal acid gastro-oesophageal reflux in idiopathic pulmonary fibrosis. Eur Respir J 2006; 27: 136-142.
11. Salvioli B et al. Gastro-oesophageal reflux and interstitial lung disease. Dig Liver Dis 2006; 38: 879-884.
12. Sweet MP et al. Gastroesophageal reflux in patients with idiopathic pulmonary fibrosis referred for lung transplantation. J Thorac Cardiovasc Surg 2007; 133: 1078-1084.
13. Fahim A et al. Gastroesophageal reflux and idiopathic pulmonary fibrosis: a review. Pulm Med 2011: 2011: 634613.
14. Linden PA et al. Laparoscopic fundoplication in patients with end-stage lung disease awaiting transplantation. J Thorac Cardiovasc Surg 2006; 131: 438-446.
15. Raghu G et al. Sole treatment of acid gastroesophageal reflux in idiopathic pulmonary fibrosis. Chest 2006; 129: 794-800.
16. Lee JS et al. Gastroesophageal reflux therapy is associated with longer survival in patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2011; 184: 1390-1394.
17. Robinson M. Review article: the pharmacodynamics and pharmacokinetics of proton pump inhibitors – overview and clinical implications. Aliment Pharmacol Ther 2004; 20 (Suppl 6): 1-10.

18. Pantoflickova D et al. Acid inhibition on the first day of dosing: comparison of four proton pump inhibitors. Aliment Pharmacol Ther 2003; 17: 1507-1514.
19. Miner P Jr et al. Gastric acid control with esomeprazole, lansoprazole, omeprazole, pantoprazole, and rabeprazole: a five-way crossover study. Am J Gastroenterol 2003; 98: 2616-2620.
20. Noth I et al. A placebo-controlled randomized trial of warfarin in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2012; 186: 88-95.
21. Vanderhoff BT, Tahboub RM. Proton pump inhibitors: an update. Am Fam Physician 2002; 66: 273-280.
22. Arnold R. Safety of proton pump inhibitors – an overview. Aliment Pharmacol Ther 1994: 8 Suppl 1: 65-70.
23. Reilly JP. Safety profile of the proton-pump inhibitors. Am J Health Syst Pharm 1999; 56(23 Suppl 4): S11-17.
24. Welage LS, Berardi RR. Evaluation of omeprazole, lansoprazole, pantoprazole, and rabeprazole in the treatment of acid-related disease. J Am Pharm Assoc (Wash) 2000; 40: 52-62.
25. Hertzog MA. Considerations in determining sample size for pilot studies. Res Nurs Health 2008; 31: 180-191.
26. Lancaster GA et al. Design and analysis of pilot studies: recommendations for good practice. J Eval Clin Pract 2004; 10: 307-312.
27. Miller MR et al. Standardisation of spirometry. Eur Respir J 2005; 26: 319-338.
28. Macintyre N et al. Standardisation of the single-breath determination of carbon monoxide uptake in the lung. Eur Respir J 2005; 26: 720-735.
29. Crapo RO et al. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med 2002; 166: 111-117.
30. Key AL et al. Objective cough frequency in idiopathic pulmonary fibrosis. Cough 2010; 6: 4.
31. McGuinness K et al. Validation of the VitaloJAK™ 24 hour ambulatory cough monitor. *Thorax* 2012; 67(Suppl 2): A131.
32. Honeybourne D et al. British Thoracic Society guidelines on diagnostic flexible bronchoscopy. Thorax 2001; 56 (Suppl I): i1-i21.
33. Meyer KC et al. An official American Thoracic Society clinical practice guideline: the clinical utility of bronchoalveolar lavage cellular analysis in interstitial lung disease. Am J Respir Crit Care Med 2012; 185: 1004-1014.
34. Roberts C and Torgerson D, Baseline imbalance in randomised controlled trials. BMJ 1999; 319: 185

11. Amendment History

Protocol version	Amendment	Details	Approved Date
1.0, dated 22/07/2013	Original submission		REC – 20/09/2013 (conditional) MHRA – 13/09/2013
2.0, dated 15/09/2013	Protocol changes	Changes made for unconditional REC favourable opinion – updated correct Funding Ref.	REC only 20/09/2013
3.0, dated 20/11/2013	Protocol changes	Amendments to update protocol for internal consistency as suggested by NCTU. Amendments made in sections of Patient Recruitment, Randomisation, Statistical Analysis, Pharmacovigilence, Adverse Event Report Reporting. Also updated contact numbers of study members. New document added: Patient Diary Card – to help assess adverse events in participants, Patient Information Sheet, GP information letters, Consent Forms: updated with appropriate changes in version number and date to coincide with amended protocol.	REC – 10/01/2014 MHRA – 15/01/2014
4.0, dated 03/07/2014	Protocol changes	Amendments in protocol to allow inclusion of PIC sites to help improve recruitment for the study. Also a summary page has been added to Participant Information Sheet to make it plain to participants that the only essential part of the study is cough monitor and taking the drug. All other tests are optional. Amendments in CTA form due	REC – 26/08/2014 MHRA – 02/09/2014 R&D – 11/09/2014

		to changes in colour of the yellow TEVA omeprazole capsules. To maintain blinding Victoria Pharmaceuticals had to source an alternative generic UK licensed omeprazole from for the study. Consent Forms and PIS updated to correspond changes in Protocol.	
5.0 dated 21/09/2015	Substantial Amendment	Dr Forrest changed to be listed as co-investigator Change of Senior Trial Manager – Dr Lesley Hall replaced Dr Jennifer Wilkinson Change of Trial Manager – Mr Mark Palmer replaced Jessica Qian New Clinical Research Fellow – Dr Wendy Funston replaced Dr Prosenjit Dutta Changes to randomisation section to bring in line with current procedure. Addition of reference to SmPC in section 3.5 of Protocol Update to increase clarity to section 5.3 Withdrawal of Consent of Protocol and to include 3 options of withdrawal. Addition of Expectedness as section 7.3 in Protocol. Addition of nintedanib to list of active trial of treatment in exclusion criteria.	

Appendix 3: PPIPF study: Consent forms

CONSENT FORM

A pilot, randomised controlled trial of omeprazole in idiopathic pulmonary fibrosis

Chief Investigator, Prof John Simpson; Co-Investigator, Dr Ian Forrest, Co- Investigators, Dr Chris Ward, Prof Michael Griffin; Clinical Research Fellows, Dr Wendy Funston, Mr Rhys Jones.

	Please initial
1. I confirm I have read and understood the Patient Information Sheet (Version 3.1, dated 05/08/2015) and have had sufficient opportunity to ask questions.	[ ]
2. I confirm that study doctors have discussed with me the requirement to come off medications which affect stomach function if I am to participate in the study, and that they have discussed what I should do if my symptoms change.	[ ]
3. I agree to a trial of discontinuation of PATIENT TO COMPLETE	[ ]

_____	_____	_____
Name of Patient	Date	Signature
_____	_____	_____
Researcher	Date	Signature

1 for patient; 1 for researcher; 1 for case notes

CONSENT FORM

A randomised controlled trial of omeprazole in idiopathic pulmonary fibrosis

Chief Investigator, Prof John Simpson; Co-Investigator, Dr Ian Forrest

Co-Investigators Dr Chris Ward, Prof Michael Griffin; Clinical Research Fellows, Dr Wendy Funston, Mr Rhys Jones.

		Please Initial
1.	I confirm that I have read and understand the information sheet, version 3.1, dated 7th March 2016, for the above study. I have had the opportunity to consider the information, ask questions and have had these questions answered satisfactorily.	[ ]
2.	I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal rights being affected.	[ ]
3.	I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from: the study team; the Study Sponsor (The Newcastle upon Tyne Hospitals NHS Foundation Trust) or their representatives; and regulatory authorities, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records, even if I withdraw, and I understand that my records will only be reviewed for information related to my participation in the study.	[ ]
4.	I agree that the research team may record data from my case records solely for the purpose of this study.	[ ]
5.	I agree to be randomised to either omeprazole or placebo therapy.	[ ]
6.	I agree to complete lung function tests, a 6-minute walk test and study questionnaires.	[ ]
7.	I agree to have 24-hour cough monitoring.	[ ]
8.	I agree to have 24-hour oesophageal (gullet) physiology tests.	[ ]
9.	I agree to have a bronchoscopy test and "lavage" and that the lung fluid not required for NHS purposes can be used for this study.	[ ]
10.	I consent to the storage of my cough recordings in RaDAR. I understand that anonymised recordings may be made available to internal and external researchers for further research. Any recordings released for further research will be subject to an application process and approved by the RaDAR Management Team.	[ ]
11.	I agree that my lung fluid sample can be used in future studies on condition that my identity cannot be determined from the sample.	[ ]
12.	I agree that the research team may send a letter informing my general practitioner of my participation in this study.	[ ]

_____ _____ _____

Name of Patient	Date	Signature
_____	_____	_____
Researcher	Date	Signature

1 for patient; 1 for researcher; 1 for case notes

Appendix 4: PPIPF study: PIS

PARTICIPANT INFORMATION SHEET

SHORT SUMMARY

A pilot study in idiopathic pulmonary fibrosis
Does switching off stomach acid (with omeprazole tablets) reduce cough, and can it improve other features of the disease?

Study Team: Dr Ian Forrest (Co-Investigator), Prof John Simpson (CI), Dr Wendy Funston (Clinical Fellow)

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. This page gives a very short overview. The following pages provide more detail (Part 1 and Part 2 of Information Sheet). Please ask us if there is anything that is not clear or if you would like more information. Take as much time as you like to decide whether or not you wish to take part. Thank you.

Idiopathic pulmonary fibrosis (IPF) *causes breathlessness and* ***cough*** *because of scarring of the lungs, for which* ***we need new and better treatments****. Fluid or acid from the stomach may contribute to IPF. There are ways to reduce acid from the stomach (e.g. a widely used tablet called omeprazole).*

The study involves patients having a small recording device clipped on for 24 hours on two occasions, one before and one at the end of taking omeprazole or 'placebo' tablets for 3 months. The main aim is to see whether the treatment you receive improves cough or not. *These are the only parts of the research that are essential to complete.*

If patients wish, however, they can have additional tests performed as part of the study, which measure acid/fluid in the stomach, and whether any of this fluid has reached the lung.

The following pages provide detailed information to allow you to decide ***whether to have only the medicine and cough test****, whether to have all the tests, or whether not to take part.*

PARTICIPANT INFORMATION SHEET

PART 1

A pilot study in idiopathic pulmonary fibrosis.
Does switching off stomach acid (with omeprazole tablets) reduce cough, and can it improve other features of the disease?

Investigators: Dr Ian Forrest (Co-Investigator), Prof John Simpson (CI), Dr Wendy Funston, Dr Chris Ward, Prof Jeff Pearson, Dr Vicky Ryan, Prof Michael Griffin, Mr Rhys Jones, Prof Elaine McColl (all Newcastle), Dr Jacky Smith (Manchester)

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish.

- Part 1 tells you the purpose of this study and what will happen to you if you take part.
- Part 2 gives you more detailed information about the conduct of the study.

Ask us if there is anything that is not clear or if you would like more information. Take as much time as you like to decide whether or not you wish to take part.
Thank you for reading this.

What is the purpose of the study?

There is a distinct lack of effective treatments for idiopathic pulmonary fibrosis (IPF). The most common, and most distressing, symptoms of IPF tend to be breathlessness and cough. These symptoms are particularly hard to treat. There is some evidence that small amounts of acid coming up from the stomach might contribute to IPF. There is also evidence that stomach acid causes cough in some other lung conditions. It therefore seems reasonable that tablets that reduce stomach acid might improve cough in IPF. On the other hand, stomach acid is part of the digestion process, and some people believe it may help kill some bugs that are swallowed. The most scientific way to work out whether cough (and other symptoms of IPF) are improved by switching off stomach acid is to perform what we call a “double-blind randomised controlled trial (RCT)” of a tablet that switches off stomach acid. A tablet called omeprazole switches off stomach acid effectively, and has been commonly used in the UK for over 20 years.

In an RCT half the patients get omeprazole, half get a “placebo” (which has no active ingredients and does not affect stomach acid). Whether patients get omeprazole or

placebo is decided at random using a computer programme. The key point however is that while the patient is receiving treatment, neither the patient nor the doctors

know whether the patient is receiving omeprazole or placebo (ie the study is "double-blind"). Our team will only find out which treatment was which after the study results have been analysed.

Therefore, the aim of this study is to determine whether omeprazole can reduce cough (and other symptoms) in patients with IPF. If the results are encouraging, this would pave the way for larger studies to comprehensively assess the potential role of omeprazole as a new effective treatment for IPF.

Why have you been chosen?

You have been chosen because you have a diagnosis of IPF.

If you are already taking omeprazole (or a related treatment for the stomach) you may still be eligible to take part if you are willing to try a period off your treatment and if the doctors consider that this is a safe option.

Do you have to take part?

No. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to you if you take part?

Summary

The study will usually involve 3 short visits to hospital, then 3-months of taking tablets at home, then 3 visits to hospital again (2 short ones and one longer one) shortly before you stop the treatment.

Do you have to have all the tests and visits?

No. We would obviously prefer that all patients in the study have all the tests, in order to maximise the information available. However we recognise that not all patients will be able to attend all the visits, and that some patients may not wish to have specific tests. The bare minimum information that will provide enough information for the study is if every patient has 2 "cough monitor" tests (see page

3 and the Appendix). These have no side effects and are very simple to perform. In other words, you would be able to take part in the study if the only tests you wanted to be done were the 2 cough monitor tests.

Before the study starts

If you are not taking omeprazole (or a related stomach treatment) and wish to take part, you will be asked to sign one consent form before starting the study.

If you are taking omeprazole (or a related stomach treatment) and wish to take part, we shall contact your GP to check he/she is aware and in agreement. You will then be asked to sign a consent form to say you agree to try a period off the treatment for 2 weeks. If your symptoms return during that 2 weeks you should go back on the treatment and not take part in the study. If you manage well without the treatment for 2 weeks, then you will be asked to sign a second consent form before starting the study.

The study period

After providing consent you will be given tablets to take twice a day, preferably before food, for 3 months.

We also ask you to have a few medical tests performed before you start the tablets, and shortly before you come off them. One further test is done just before you stop the tablets. The tests generally involve 3 visits to RVI before the tablets start, and 3 just before the tablets finish. An outline of these tests and visits is described in the following paragraphs. More detailed descriptions of three of the tests are found in the Appendix accompanying this Patient Information Sheet.

Before the tablets start

VISIT 1. You would come to the Chest Clinic at RVI and have your usual breathing tests (in almost all cases this will be timed so that the breathing tests double up as your routine clinical test and your study test). The breathing tests are called a “vital capacity” (the amount of air you can blow out of your chest after a deep breath) and a “transfer factor” (an indication of how efficiently gas can pass from the air into your blood).

In addition you would be asked to perform a “6 minute walk test”, in which you walk for up to 6 minutes under medical supervision (you can of course stop if you are too breathless). Many patients in the study will have had a walk test done before.

You will also be asked to complete a number of questionnaires which provide us with information about how breathless you are, and how much you are bothered by cough.

Finally, you will be asked to wear a small device that records how much you cough over a 24-hour period. This is simply a small recording device which is attached to the chest, and is not uncomfortable. You will be given full instructions about how to wear the device, and you simply go home wearing the device, bringing it back the next day (see below) or whenever suits you best. Further details are in the Appendix.
We estimate that Visit 1 will take around 2 hours.

VISIT 2. For the second visit, you would bring back the cough monitor to the Chest Clinic at RVI. Ideally, visit 2 would therefore be the day after Visit 1, but it does not have to be.

At visit 2 you would have "oesophageal physiology tests", which are tests of how your stomach and gullet work, and how much acid is produced in your stomach. You need to miss your breakfast that morning. The test usually does not cause discomfort. A thin, flexible tube is passed through your nose to the back of the throat, where you swallow the end of it. A member of the team then makes a few measurements while you sit comfortably. The tube is then removed and replaced by a thinner tube which is left in place for 24 hours, ie you go home with it in place and bring it back the next day. More details of the test (and a picture of a patient having it done) are in the Appendix at the end of this document.
We estimate that Visit 2 will take up to around 2 hours.

VISIT 3. For the third visit, you would come to the Chest Clinic, RVI, to have the nasal tube removed. You would then be issued with your pack of tablets (omeprazole or placebo), to start the next day. You will also be issued with a "symptoms diary" in which we ask you to record any symptoms you have when on the treatment. You will also be issued with contact numbers through which you can get in touch with the study team if you have any questions.
We estimate that Visit 3 will take less than 1 hour.

As the tablets come to an end

The visits below would ideally be performed on the 3rd last, 2nd last and last day of treatment, but could be done any time in the last 2 weeks of treatment.

VISIT 4 (ideally 3rd last day of treatment). This is a duplicate of Visit 1, ie you will have breathing tests, a 6-minute walk test, complete questionnaires, and have the cough monitor fitted again for overnight use.

VISIT 5 (ideally the 2nd last day of your treatment). This is a duplicate of Visit 2, ie you will bring the cough monitor back to the Chest Clinic at RVI, and have the gullet and stomach tests, going home with the nasal tube in place for overnight use. You need to miss breakfast that morning.

VISIT 6 (ideally the final day of your treatment). You will come to the Chest Clinic at RVI having missed your breakfast that morning and the nasal tube will be removed. You will then go for a telescope test of the lungs, where we wash a small segment of the lungs to sample cells and fluid there (a "bronchoscopy and lavage"). Many patients in the study will have had a bronchoscopy before. Details of bronchoscopy and lavage, along with a picture of a patient having bronchoscopy, are found in the Appendix. Briefly, you are taken to the dedicated Bronchosopy Suite where the preparations will be as for any patient having bronchoscopy ie you will have some anaesthetic spray to numb your mouth and nose, you will be given some extra oxygen to breathe during the test, and a plastic cannula (tube) will be placed in a vein in your hand or arm. Your heart rate and oxygen saturations will be measured throughout. You will be offered a sedative medicine (to make you relaxed and sleepy). When you are comfortable and ready, a thin, flexible plastic "scope" (or camera) with a light on the end is passed through your nose or mouth and down into the lung. Once the tube is in the lung we place it in one "segment" of the lung (the lungs have approximately 23 segments and we seek to wash only one of these), and then we slowly squirt in saline (salty water) and suck it back through the scope. This effectively sucks up cells and liquid from the furthest areas of the "segment". After the washing procedure, we take up to 4 biopsies from one of your bronchial tubes – these are taken with forceps passed through the telescope. The biopsies are generally the size of the point of a ballpoint pen, ie approximately 1-2 millimetres. Because the lung generally contains "cough nerves" but not "pain nerves", and because we apply local anaesthetic to the area, the biopsies are painless. After the wash and the biopsies, we remove the "scope" and you simply rest for a couple of hours. You are not allowed to eat or drink until the local anaesthetic spray wears off (about 2-3 hours), and if you have received sedation you must be accompanied home by a responsible adult. Details are in the Appendix and will of course be gone over again before the test.

We estimate that Visit 6 will last about 4-6 hours (the test itself takes about half an hour, the rest of the time is preparing you for the test or observing you afterwards).

You should **stop** taking tablets 3 months (90days) after starting. We need you to return the trial medication pack with any remaining tablets **within 7days** of completion date to Chest Clinic RVI. It is essential to assess compliance as soon as possible after completion of study treatment. This visit will be very brief approximately 30 minutes.

During the study period

You should not be taking any regular antacid medication (namely Proton Pump Inhibitors like Omeprazole, Lansoprazole, Pantoprazole, Rabeprazole) during the study period while you are on the trial drug. If your GP or any other doctor prescribes you any regular antacid medication please inform one of study team members (contact details is on page 9) before taking the medication.

What are the possible risks and side effects of taking part?

The risks from taking part are considered to be low. Cough monitoring is a research tool, but is not associated with any known risks. All of the other tests are routinely carried out in clinical practice, and all are considered to be low-risk procedures. Omeprazole has been used in clinical practice by millions of patients and is regarded as having an extremely low rate of serious side effects.

Side effects from omeprazole are uncommon and it is estimated that >95% of patients have none. If patients do get side effects, the most common ones are diarrhoea, headache, abdominal discomfort and nausea. Rare side effects, affecting less than 1% of patients, can include skin reactions, low blood counts (including anaemia), blood or protein in the urine and urinary tract infection. In most studies to date, the rate of side effects has been very similar whether patients receive omeprazole or placebo.

Side effects with omeprazole are considered more likely if patients are taking specific drugs that omeprazole may interact with. These include some treatments such as warfarin, diazepam, phenytoin or some treatment for fungal infections. Our study protocol has strict rules which exclude patients taking such medicines, which further reduces the risks of the study.

Tests

Lung function tests. You are very likely to have had these before. Some patients find that the effort of blowing induces cough, which usually wears off after a few minutes.

6-minute walk test. The test is designed to find out how far you can walk in 6 minutes, or to find out if you have to stop within 6 minutes. The test is therefore quite likely to make you breathless. The test is carried out under supervision, and if

you are very breathless the test is stopped. Oxygen is available should you wish to use it because of breathlessness.

We are unaware of any side effects associated with the questionnaires or cough monitor.

The oesophageal physiology (stomach/gullet) tests are usually very well tolerated. Some patients have mild discomfort or a sense of wanting to sneeze when the tube is passed through the nose. Occasionally patients will retch when the tube is at the back of the throat, but this usually passes within seconds. Some patients get a degree of irritation in the throat from the tube.

The bronchoscopy procedure has some common, usually minor, side effects. The local anaesthetic sprayed in the mouth often tastes sour, and leaves the mouth feeling temporarily numb. The telescope usually induces some cough when it enters the lung initially – the coughing usually settles within a minute or so. The biopsies taken sometimes induce some coughing but should be painless, partly because the lung contains "cough nerves" rather than pain nerves, and secondly because we apply local anaesthetic to the area to be biopsied.

The telescope is passed through the mouth or nose. Going through the mouth is usually painless but can induce some some gagging. The nose tends to be more uncomfortable until the tube has passed (usually less than a minute).
However bronchoscopy is considered a safe, routine clinical procedure, which is generally very well tolerated. A collapsed lung (pneumothorax) is a very rare complication and occurs in <0.5% of patients. We select the segment of the lung to be washed carefully to minimise this risk still further. There is a small risk of low oxygen levels or an irregular heart beat (which is why we monitor oxygen levels and an ECG during the test, to pick up any suggestion of this), and a small risk of congestion of the lungs. Studies into bronchoscopy have estimated the risk of a serious complication to be less than 1 in 1000, and the risk of death to be less than 1 in 10,000.

After the procedure a proportion of patients describe a mild sore throat for around 24 hours, as the local anaesthetic wears off. Because of the biopsies, you may cough a small amount of blood after the test – this is usually seen as spots or streaks in the phlegm and very rarely lasts for more than 24 hours. If you have sedation you are likely to feel a little sleepy for the rest of the day. As well as relaxing you and making you sleepy, the sedation has a mild temporary effect on memory, and often the patient will not remember having had the procedure. For all these reasons, if you have sedation you must not drive, work, drink alcohol, operate moving machinery (including power tools etc at home), or sign legal documents until the next day. If

you have sedation you must be accompanied home by a responsible adult. The following day you can resume your normal activities.

In the unlikely event that we detect an abnormality at bronchoscopy we would let you know, and inform your GP of our findings. We would discuss with you and your GP the best way to investigate and manage any bronchoscopic abnormality.

What are the other possible disadvantages and risks of taking part?

We are unaware of other disadvantages and risks of taking part. We are conscious however that the study does take up quite a lot of your time on the study days.

What are the potential benefits of taking part?

There is no direct benefit.

Is there any reimbursement for taking part?

There is no payment for taking part in the study. We shall be happy to reimburse costs for travel to the RVI and parking at the RVI, but we shall need receipts before doing so please.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in part 2.

Will your taking part in this study be kept confidential?

Yes. All the information about your participation in this study will be kept confidential. The details are included in Part 2.

Contact details

Should you wish further information please feel free to contact us at any time at the address or numbers below

Dr Ian Forrest
Consultant in Respiratory Medicine
Respiratory Medicine Unit

Royal Victoria Infirmary
Queen Victoria Road
Newcastle upon Tyne NE1 4LP
0191 282 0149

Prof John Simpson
Consultant in Respiratory Medicine
Institute of Cellular Medicine
Medical School
Newcastle University
Framlington Place
Newcastle upon Tyne NE2 4HH
0191 208 7770

Dr Wendy Funston
Clinical Research Associate
Institute of Cellular Medicine
Medical School
Newcastle University
Framlington Place
Newcastle upon Tyne NE2 4HH
0191 208 7770, 07765920130

If you would like to talk to an expert who is not involved in the project, we have an independent advisor for this specific purpose. This person is a fully qualified medical practitioner who is there to answer any questions or concerns you may have about the study. He is not in any way involved in the study, but understands all of the medical aspects of this particular project. The contact details are

Dr Graham Burns
Consultant in Respiratory Medicine
Respiratory Medicine Unit
Royal Victoria Infirmary
Queen Victoria Road
Newcastle upon Tyne NE1 4LP
0191 282 0149

This completes Part 1 of the Information Sheet.
If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making your decision.

PATIENT INFORMATION SHEET – PART 2

A pilot study in idiopathic pulmonary fibrosis.
Does switching off stomach acid (with omeprazole tablets) reduce cough, and can it improve other features of the disease?

Investigators: Dr Ian Forrest (Co-Investigator), Prof John Simpson (CI), Dr Wendy Funston, Dr Chris Ward, Prof Jeff Pearson, Dr Vicky Ryan, Prof Michael Griffin, Mr Rhys Jones, Prof Elaine McColl (all Newcastle), Dr Jacky Smith (Manchester)

What will happen if you change your mind and do not wish to carry on with the study?

You can withdraw completely from the study at any time without giving any reason.

However all the data collected is useful, therefore we would like to use the data you have already given us for our research even if you leave the study. We would also like to offer you the choice to come in for the final study visits and collect your cough monitor data although we would need your permission to do this if you do decide to withdraw.

Your withdrawal would not affect the standard of care that you can expect to receive in the future.

What if there is a problem?

If you have a concern about your treatment by members of staff during the study, you should ask to speak with the researchers who will do their best to answer your concerns (contact numbers are found at the end of Part 1). If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the hospital.

In the unlikely event that something goes wrong and you are harmed during the research study there are no special compensation arrangements. If you are harmed and this is due to someone's negligence then you may have grounds for a legal action for compensation against the Newcastle upon Tyne Hospitals NHS Foundation Trust but you may have to pay for your legal costs. The normal NHS complaints mechanisms will still be available to you.

Can you access the results of the research?

Yes. Should you wish to know the overall results of the study please get in contact with the team using the contacts at the end of Part 1. If that is the case we would

send you a summary of the results, including a lay summary, after analysis of the study data is complete.

Will your taking part in this study be kept confidential?
All information which is collected about you during the course of the research will be kept strictly confidential.

The bronchoscopy and lavage test will provide us with liquid and cells from your lung. The lung fluid samples you provide will be stored in our freezers, labelled with a number and the date (ie you can not be recognised from the stored fluid samples). The cells will be used immediately and either discarded or kept on glass slides for later analysis. The slides will be labelled with a number and the date (ie you can not be recognised from the stored fluid samples). Lung samples will be used principally to estimate the amount of inflammation in your lung, and whether there is any evidence that chemicals from the stomach have entered your lung at any point.

Because the understanding of lung biology is moving forward all the time, it is possible that we shall identify unanticipated future uses for the stored lung fluid samples. If we identify a proposed use for these samples that does not relate to the current study, we shall seek further approval from the Research Ethics Committee. In all instances, confidentiality will be maintained as above.

Tests done in the RVI, such as the bronchoscopy, comply with NHS standards, ie there will be clinical sheets with your details on them, as there would be for a routine NHS bronchoscopy. These sheets are subject to the usual NHS confidentiality rules.

Involvement of the GP

Your GP may be notified of your participation in this study, with your consent.

Will any genetic tests be done?

No genetic tests will be performed and no genetic material will be stored.

What will happen to the results of the research study?

We intend for the results to be presented at scientific meetings and published in medical/scientific journals. You would not be identifiable from our data in any such meetings or publications.

Who is organising and funding the research?

The research is funded by the British Lung Foundation, an independent charity funding medical research, with support from the Newcastle Biomedical Research Centre.

Who has reviewed the study?
The study has been reviewed by the funding bodies above. It has been reviewed and approved by a Research Ethics Committee.

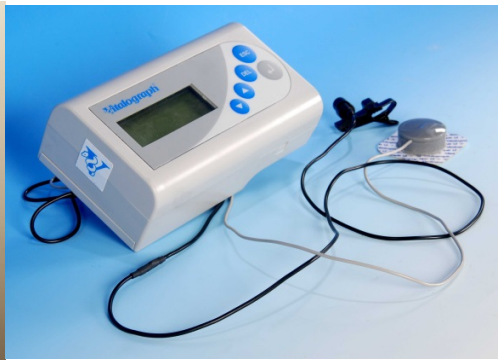
You will be given a copy of this Information Sheet and a signed consent form to keep. Thank you for taking time to read this sheet and for considering taking part.

Version 3.1, 05 August 2015

APPENDIX

1. COUGH MONITORING

You will be fitted with a cough monitor which will record the number of times you cough over a period of 24 hours during the day and night. The researcher will attach a sticky pad to your chest which contains a small microphone (see right hand picture, showing the apparatus). A clip with another small microphone will be attached to your clothing. The monitor itself will be kept in a `bumbag' around your waist (see the left hand picture). You must not get the monitor wet.



The monitor does not just record coughing sounds; it will also record your activities whilst you are wearing it. For example, it will record your conversations and in some instances may record the voices of people around you. We do however use computer software (algorithms) to remove parts of (cut down) the recording where there is no coughing, such as some speech, when you are reading or sleeping and distant noises, such as noise from your television. The cut down recordings are then listened to and analysed by a trained researcher who counts the number of times you have coughed during the day and night. Please be aware that if we should hear something on the recordings which might place either yourself or another person in danger we have procedures we are required to follow in order to report such instances; otherwise your recordings will be treated as confidential.

At the present time, cough recordings are cut down using software algorithms and then listened to by a trained researcher in order to calculate how often a person coughs. In some instances we may need to listen to the full 24 hour recording. We are working towards an automated system whereby our researchers will no longer need to listen to the recordings as the software algorithm will count the coughs automatically. In order to do this we need to use both 24 hour and cut down recordings to test and develop new algorithms and compare how well they work.

If you take part in this study you will be asked if you are happy for your cough recordings to be stored in our research database called RaDAR. RaDAR contains both 24 hour and cut down cough recordings which are used to test and develop software algorithms. When a 24 hour recording is stored in RaDAR any identifiable information (for example, your name) is blanked out before it is stored. All recordings are, therefore, anonymised. None of the researchers who may use your recordings would be able to identify you.

Recordings stored in RaDAR are categorised by gender, age and respiratory condition only. If your recordings are stored in RaDAR they will be used by researchers at The University of Manchester and University Hospital of South Manchester, and also by Vitalograph Ltd who developed the cough recording device. Other Universities or commercial companies can also apply to RaDAR to use the anonymous recordings for their own research. The RaDAR management team assess each application received for use of cough recordings rigorously and only allow the data to be used if the study is considered appropriate.

2. OESOPHAGEAL PHYSIOLOGY TESTS (tests of the gullet and stomach)

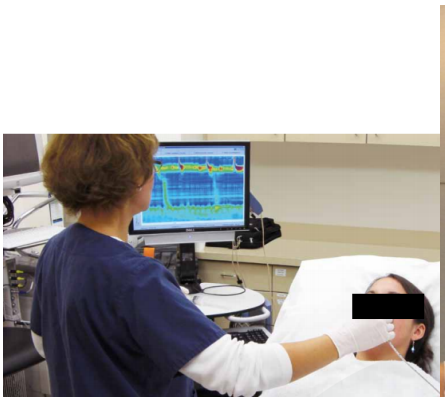
Oesophageal physiology testing provides information about the function of the gullet and stomach. The test has two parts, known as oesophageal manometry and ambulatory pH/impedance testing.

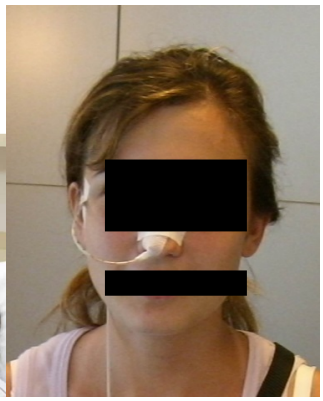
Oesophageal manometry uses pressure sensors to measure the waves of muscular contraction travelling down the gullet, and also the relaxation of the valves that help to keep food travelling in the right direction.

pH/impedance testing uses sensors of acidity and electrical resistance to measure "reflux" of material from the stomach back up into the gullet. As reflux can occur unpredictably throughout the day, the test runs over a 24-hour period but hospital admission is not required.

Patients come to the hospital fasted. In the endoscopy department, a fine tube is inserted via the nose into the stomach. The manometry test is then performed, which takes around twenty minutes and involves the patient performing a number of swallows (see the left hand picture below). This tube is then removed and the tube for the pH/impedance testing is inserted in the same way. This is much finer and is left in place for 24 hours, connected to a data-recording box. The test doesn't significantly restrict normal activities but some patients are self-conscious about going to work etc with the tube in place (see the right hand picture). Insertion of the tube can be slightly uncomfortable but isn't painful, and the test doesn't carry any risk.

The next day the removal of the tube takes a couple of minutes and the information from the test can then be interpreted.





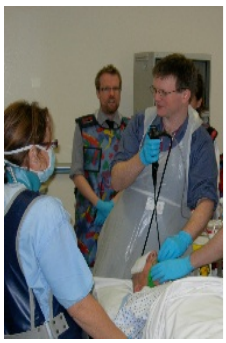
3. BRONCHOSCOPY AND BRONCHOALVEOLAR LAVAGE

Bronchoscopy is a telescope test of the lung, and lavage is a procedure in which a small segment of one lung is "washed" in order to retrieve a sample of cells and liquid from the deepest region of the lung. The telescope transmits light and allows pictures of the bronchial tubes to be passed to a television screen.

For this procedure a small plastic cannula would be placed in a vein in your arm or hand. A probe would be placed on your finger and ECG leads placed on your chest – this is entirely routine and simply allows us to continuously monitor your heart rate and the oxygen levels in your blood. You would be given oxygen through nasal "spectacles" or "plugs". You would then be advised to have local anaesthetic spray applied to your mouth (to numb the throat when the telescope is passed into the lung). We would also invite you to have a medicine called a sedative, so that you are relaxed and sleepy during the test. The sedative would be given intravenously, through the cannula in your arm.

When you are relaxed and comfortable, we would pass the telescope through your mouth (through a mouthguard which you hold gently between your teeth - this is to prevent the telescope being bitten), or through your nose (for the nose we offer you anaesthetic jelly for your nostril). The telescope is thin (less than a centimetre wide), soft and flexible with a light at the end. We pass this over the tongue, past the vocal cords (voice box) and into the lung. We then instill some saline (salty water) through the telescope then suck it back (in this way we sample cells from a very small area of your right lung). The bronchoscopy and lavage take approximately half an hour, which includes the time taken preparing you for the test. A picture of a patient having a bronchoscopy is shown below. You will see that the patient is lying comfortably in a well-lit room. The patient went home shortly afterwards.

After the procedure you will be allowed to rest. You will not be allowed to eat or drink for at least 2 hours (because of the local anaesthetic). Your pulse, blood pressure and oxygen levels will be checked on the ward and you will be allowed home. If you have had sedation we insist that you either be picked up by a friend or relative, or we shall arrange for a taxi to take you home. Furthermore, if you have sedation you will be advised not to work, drive, drink alcohol operate moving machinery or sign legal documents on the remainder of that day. All of these measures are routine after bronchoscopy.



Appendix 5: PPIPF study: Patient Diary Card

PATIENT DIARY CARD

Date of Issue: _____ Study ID/Trial patient Number: _____

Pilot Trial Of Omeprazole in IPF (PPIPF Study)

Sponsor: The Newcastle Upon Tyne NHS Hospitals Foundation Trust
Funder: British Lung Foundation
Protocol Number: IAFIPF001
Eudra CT Number: 2013 – 003301 – 26

Chief Investigator: Prof John Simpson

Instruction for Patients

- This diary will help to assess the effect of medication and also its safety
- Please record any change to your health while you are on medication (more details inside)
- Please call the Investigator Team if you develop any symptom or illness that leads to hospital admission
  Contact Number: **0191 2087770**; Mobile Number: **07765706346**
- Please call the Investigator Team if you are Pregnant
  Contact Number: **01912087770**; Mobile Number: **07765706346**
- **REMINDER**: Please bring this diary with you on your last day of visit (Visit 6)
- **REMINDER:** Please **stop** taking trial medication 90 days after starting date. Please return trial medication pack with remaining drugs to Chest Clinic OPD (RVI) **within 7 days** of completion of study

medication. We apologize for inconvenience of extra clinic visit but it is essential to assess compliance and wellbeing of study participants, as soon as possible after completion of study treatment.

PATIENT DIARY CARD

Pilot Trial of Omeprazole in IPF (PPIPF Study)
CI: Prof John Simpson
Protocol Number: IAFIPF001
Eudra CT Number: 2013-003301-26

StudyID/Trial patient Number :_____

Start Date: _____ Stop Date:_____

Please record below if you experience any symptom or illness that are unusual or worse for you during the study.
You do not need to record your usual cough or breathlessness related to your lung disease
unless they are worse and/or you needed medical attention or new medication (i.e. antibiotics)
Please record each symptom on a separate line. Please complete the date and time at which
symptom started and stopped.
Please record appropriate severity (as shown) and any action/medication taken to help with
symptom.

Your Description (please record each symptom on separate line)	**Severity *** Mild = 1 Moderate = 2 Severe = 3	**Date/Time started** (dd/mm/yy hh:mm)	**Date/Time stopped** (dd/mm/yy hh:mm)	**Action Needed** (any medication, consultation with GP)

* **Mild -** awareness of symptoms or signs but easily tolerated (Tolerable) **Moderate –** symptoms interfere with usual activities (Disturbing) **Severe –** unable to perform usual/daily activities (Intolerable)				

PATIENT DIARY CARD

Pilot Trial of Omeprazole in IPF (PPIPF Study)
CI: Prof John Simpson
Protocol Number: IAFIPF001
Eudra CT Number: 2013-003301-26

StudyID/Trial patient Number :_____

Start Date: _____ Stop Date:_____

Please record below if you experience any symptom or illness that are unusual or worse for you during the study.
You do not need to record your usual cough or breathlessness related to your lung disease
unless they are worse and/or you needed medical attention or new medication (i.e. antibiotics)
Please record each symptom on a separate line. Please complete the date and time at which
symptom started and stopped.
Please record appropriate severity (as shown) and any action/medication taken to help with
symptom.

Your Description (please record each symptom on separate line)	**Severity *** Mild = 1 Moderate = 2 Severe = 3	**Date/Time started** (dd/mm/yy hh:mm)	**Date/Time stopped** (dd/mm/yy hh:mm)	**Action Needed** (any medication, consultation with GP)

* **Mild -** awareness of symptoms or signs but easily tolerated (Tolerable) **Moderate –** symptoms interfere with usual activities (Disturbing) **Severe –** unable to perform usual/daily activities (Intolerable)				

Appendix 6: BTS poster presentation: Cyclophosphamide study

Efficacy of pulsed cyclophosphamide and methyl-prednisolone therapy in patients with progressive interstitial lung disease

P Dutta[1], P Avery[2], L Mansell[1], S Worthy[3], A Beattie[3], B Griffiths[3], I Forrest[3], A J Simpson[1]

1 Institute of Cellular Medicine, Newcastle University, 2 School of Mathematics & Statistics, Newcastle University, 3 The Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne.

INTRODUCTION

Therapeutic options for progressive interstitial lung disease (ILD) are limited, as no pharmacological intervention has been shown to improve mortality significantly. Intravenous (IV) pulsed cyclophosphamide and methyl-prednisolone are administered in some centers for rapidly progressive ILD, based on small clinical improvements in patients with ILD secondary to systemic sclerosis.

We studied the outcome of patients with progressive ILD who received IV cyclophosphamide and methyl-prednisolone therapy in our center.

METHODS

Patients were identified from a database of those receiving IV cyclophosphamide. We reviewed the case notes of patients receiving therapy between January 2010 and August 2014, **comparing the rate of change in forced vital capacity (FVC) and transfer factor for carbon monoxide (TLco) before and after therapy. Adverse effects were also noted.**

RESULTS

Results from 53 patients with mean age of 60 yrs. (range 39 – 81 yrs.) were reviewed; 29 (55%) were male.

Diagnosis included Connective Tissue Disease-ILD (21), Idiopathic NSIP (12), Chronic Hypersensitivity Pneumonia (8), IPF (6) and Undifferentiated-ILD (6).

Median number of cyclophosphamide pulses received was 6 (range 1 – 23).

41 (77%) patients received follow on immunosuppressive therapy in the form of mycophenolate mofetil (37 patients), azathioprine or rituximab.

The average rate of change of lung function was significantly less after cyclophosphamide therapy, both for FVC (Figure 1) p=0.0004 and TLco (Figure 2) p=0.00015.

Whilst on therapy **8 (15%) patients had bone marrow suppression, 4 (7%) had elevated liver function tests, 3 (6%) had abnormal renal function** and 1 was investigated for microscopic haematuria.

32 episodes of infections reported; 21 were of respiratory origin.

25 (71%) out of 35 who suffered from an adverse events were able to complete therapy.

Figure 1 - showing data plots and average fitted quadratic curve for FVC of patients who received IV cyclophosphamide and methyl-prednisolone therapy; FVC (litre's); Time relative to treatment (months)

Figure 2 - showing data plots and average fitted quadratic curve for Tlco of patients who received IV cyclophosphamide and methyl-prednisolone therapy; TLco (mmol/min/ kPa¹); Time relative to treatment (months)

CONCLUSIONS

In our single center, retrospective study, **pulsed intravenous cyclophosphamide and methyl-prednisolone therapy was associated with stabilisation of lung function in a mixed cohort of patients with progressive ILD.**

Adverse events were common but transient and managed with dose reduction and/or delayed schedule.

REFERENCES

1. Griffiths B et al. 2002 Systemic sclerosis and interstitial lung disease: pilot study using pulsed intravenous methyl-prednisolone and cyclophosphamide to assess the effect on high resolution computed tomography scan and lung function. *J Rheumatol. 29(11)*: 2371-9
2. Poomoghim H et al 2012 Cyclophosphamide for scleroderma lung disease: a systemic review and meta-analysis. *Rheumatol Int. 32(8)*: 2431-44

ACKNOWLEDGEMENTS

We thank the outpatient staff, medical secretaries and the specialist nurses of the Respiratory and the Rheumatology department at the Royal Victoria Infirmary and the Freeman Hospital, Newcastle upon Tyne for their help and support for this study.

The Newcastle upon Tyne Hospitals NHS Foundation Trust