Cough in Fibrotic Lung Disease

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for the degree of Doctor of Philosophy

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Abstract

Background: Idiopathic pulmonary fibrosis and other progressive fibrotic lung diseases are associated with significant mortality and morbidity. Cough has previously been described to be associated with worsening outcomes in IPF. The pathogenesis of cough in fibrotic interstitial lung disease (FILD) is unknown and as such few effective treatment options are available to treat this disabling symptom.

Methods: Two cohorts of patients were studied – the PROFILE cohort of 579 patients recruited between two major teaching hospitals. A second prospective cohort of 53 patients was collected for the purpose of measuring both subjective and objective cough. Patients in the latter cohort underwent fiberoptic bronchoscopy and bronchoalveolar lavage to allow measures of inflammation and microbiome to be taken. Blood was taken for genetic analysis to evaluate the *MUC5B* promoter polymorphism and its role in cough.

Results: The impact of cough on quality of life in the PROFILE cohort is significant with a median Leicester cough questionnaire score (LCQ) of 16.32. Objective cough was similarly elevated with a median cough count of 10.6/hr/24hrs. This burden is comparable to that seen in lung cancer and more than that seen in airways disease. Of various subjective measures of cough, cough visual analogue scale (VAS) best correlates with objective cough recording. The cough of FILD correlates only weakly with measures of lung function and not with the *MUC5B* promoter polymorphism or bacterial burden suggesting its independence

from disease process. Current treatments for FILD do not seem to reduce cough burden significantly.

Conclusion: Cough in fibrotic lung disease remains a therapeutic challenge with little change in cough burden through the duration of the illness. Targeting neuronal pathways seems a promising strategy, but more data is required to evaluate this. In particular cough challenge studies examining the heterogeneity of neuronal hypersensitivity would be prudent prior to embarking on trials of treatment.

Declaration of originality

I, Peter Saunders declare that the work presented in this thesis including the experiments, except where referenced, were performed by myself. Information derived from the published and unpublished work of others has been acknowledged in the text and full references are given.

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Abbreviations

6MWT	Six-minute walk test
ACE	Angiotensin converting enzyme
ATAQ-IPF	A tool to assess quality of life in IPF
BAL	Bronchoalveolar lavage
CHP	Chronic hypersensitivity pneumonitis
CPI	Composite physiological index
CTD-ILD	Connective tissue disease associated interstitial lung disease
DNA	Deoxyribonucleic acid
FEV1	Forced expiratory volume in 1 second
FILD	Fibrotic interstitial lung disease
FVC	Forced vital capacity
GAP	Gender, Age, Physiology index
GCP	Good Clinical Practice
GEE	Generalized estimating equation
HADS	Hospital anxiety and depression score
HRCT	High resolution computerized tomography
IPF	Idiopathic pulmonary fibrosis
LCQ	Leicester cough questionnaire
MPO	Myeloperoxidase antibodies
MUC5B	Mucin 5B
NSIP	Non-specific interstitial pneumonia
PFILD	Progressive fibrosing interstitial lung disease

PROFILE	he Prospective Study of Fibrosis in Lung Endpoint (PROFILE) study
PROM	Patient reported outcome measure
RARS	Rapidly adapting stretch receptors
RNA	Ribonucleic acid
SARS	Slowly adapting stretch receptors
SD	Standard deviation
SGRQ	St George's Respiratory Questionnaire
SNP	Single nucleotide polymorphism
TLCOc	Transfer factor of the lung for carbon monoxide corrected for haemoglobin
TRP	Transient receptor Potential
UIP	Usual interstitial pneumonia
VAS	Visual analogue scale

Chapter 1

INTRODUCTION

1.1.0

Idiopathic Pulmonary Fibrosis

Idiopathic pulmonary fibrosis (IPF) is a progressive and fatal disease of predominantly older men, carrying a median survival of 3-5 years (Gribbin et al., 2006). The incidence of IPF in the UK has been estimated to be 7.4 cases per 100000 and is increasing (Navaratnam et al., 2011). Whilst no causation has been demonstrated, there is association with having previously smoked (Baumgartner et al., 1997). Several genes have been described as increasing the risk of developing IPF, but the increase in absolute risk is usually small (Allen et al., 2017). As the disease progresses, there is progressive scarring of the alveolar: capillary barrier with a resulting reduction in gas exchange and respiratory failure.

The disease is also associated with an increased burden of cardiovascular disease (Kreuter et al., 2016a) and an increased risk of lung cancer (Li et al., 2014), which may contribute to premature mortality.

Treatments are available which can reduce the rate of progression of the disease but do not undo the damage to the lung, nor do they completely stop further progression (Noble et al., 2011, King et al., 2014, Richeldi et al., 2014).

1.1.1 Mechanisms of fibrosis

The alveolar membrane in health is made up of a single layer of type I and type II alveolar epithelial cells. The type II cells (AE2) are an important source of regenerative ability in the lung as well as secreting components of surfactant (Mason and Williams, 1977). Surfactant regulates surface tension within the alveolar space, preventing collapse of this delicate structure (Hills, 1999). Between the layer of alveolar epithelial cells and the capillary membrane lies a layer of extracellular matrix (ECM) containing fibroblasts.

The exact mechanisms of pulmonary fibrosis are yet to be fully understood, but it has been suggested that repetitive alveolar insults in a susceptible individual lead to an abhorrent wound healing response and the laying down of excessive collagen (Adamson et al., 1988). Injury of the alveolar epithelial cells leads to infiltration with fibroblasts which differentiate into myofibroblasts that secrete proteins to create a thickened extracellular matrix (ECM). This bridges the injury and with contraction of the ECM. In early electron microscopy studies of lung tissue from patients with idiopathic pulmonary fibrosis, excessive collagen and fibroblasts were seen in an expanded ECM (Gracey et al., 1968). Models using carbon nano-fibres to stimulate alveolar cell injury have demonstrated the recruitment of fibroblasts and their subsequent development into myofibroblasts (Dong and Ma, 2016). Myofibroblast differentiation can be induced by activation of transforming growth factor beta (TGF-β) in a lactate rich environment such as the IPF lung (Kottmann et al., 2012, Tuder et al., 2012).

TGF-β is an important cytokine in the development of lung fibrosis. In animal models, production of activated TGF-β through adenovirus mediated gene transfer led to the development of significant lung fibrosis (Sime et al., 1997). TGF-β can induce AE2 cells to become senescent which is associated with the expression of Interleukin 4 (IL-4) and interleukin 13 (IL-13) (Rana et al., 2020). IL-4 and IL-13 promote alveolar macrophage activation and proliferation (Jenkins et al., 2011).

Alveolar injury may cause AE2 cells through STAT-3 activation to promote a pro-inflammatory cytokine cascade which promotes lung fibrosis (Pedroza et al., 2016). Elevated levels of phosphorylated STAT-3 (activated) expression have been seen in macrophages, fibroblasts and AE2 cells in lung biopsy samples from patients with IPF (Pedroza et al., 2016). Inhibition of STAT-3 activation was shown to reduce fibroblast differentiation into myofibroblasts in a mouse model (Pedroza et al., 2016). IL-6 and TGF-β have both been shown to be able to cause STAT-3 phosphorylation.

Pulmonary macrophages are believed to be key mediators of alveolar injury (Bowden, 1976). Macrophages are the most abundant cell of the immune system found in the healthy lung (Shi et al., 2020) and are heterogeneous in their origin and function. They are divided into those cells found in the alveolar space (alveolar macrophages) and those found in the interstitium (interstitial macrophages) with the alveolar macrophages being more numerous in health (Hume et al., 2020). In an acute infection, monocyte derived circulating monocytes infiltrate the alveolar space and supplement the existing alveolar macrophage population. Long-term cigarette smoking increases the number of alveolar macrophages both by increasing in-situ proliferation, reducing apoptosis and by recruitment of monocyte derived

macrophages (Barnes, 2004). Macrophages play a key role in the phagocytosis of inhaled particulates (Karrer, 1960), bacteria and in repair after infection. They can secrete a variety of signalling proteins including galectin 3 which may then enhance the inflammatory response. Galectin 3 has been found to be expressed by alveolar macrophages in IPF patients in greater quantities compared to controls (Nishi et al., 2007). Galectin 3 has also been shown to cause migration of fibroblasts and stimulation of collagen production (Nishi et al., 2007). Monocyte derived macrophages can promote a fibrotic response (Misharin et al., 2017) which can be ameliorated by removing distinct populations of monocyte derived macrophages in experimental models (McCubbrey et al., 2018).

Finally, vascular remodelling appears to occur within the fibrotic lung with loss of alveolar capillaries in areas of significant fibrosis, but an increase in adjacent areas (Ebina et al., 2004). The expression of vascular endothelial growth factor (VEGF) messenger ribonucleic acid (mRNA) is reduced in the fibrotic areas of lung (Tachihara et al., 2006) which may explain the loss of alveolar capillaries.

1.1.2

Diagnosis of Idiopathic Pulmonary Fibrosis

The diagnosis of IPF is usually made radiologically, although guidelines suggest that this information is fitted to the patient's history, histology where available, and then discussed at a multi-disciplinary meeting (Raghu et al., 2011). Formal guidelines for the diagnosis of IPF were released in 2000 (King, 2000) and then updated in 2011 (Raghu et al., 2011) and further updates made in 2018 (Raghu et al., 2018b). IPF is usually associated with a distinct pattern

of computed tomography (CT) findings – lower zone predominance, peripheral reticular shadowing and honeycombing (Wright et al., 1984). This pattern is described as being that of usual interstitial pneumonia (UIP). The absence of honeycombing in an otherwise UIP pattern of radiology is labelled as probable UIP. Formal diagnostic criteria for UIP pattern imaging are shown in table 1.1. Whilst IPF is the most common cause of a UIP pattern on a CT scan, other diseases can produce this appearance including rheumatoid lung disease and microscopic polyangiitis (Myeloperoxidase (MPO) antibody positive) vasculitis.

In the absence of the classic radiology of idiopathic pulmonary fibrosis, the diagnosis may be made on lung biopsy – either surgical, cryobiopsy or less commonly with a transbronchial forceps biopsy. Surgical lung biopsies are not very popular in Europe because of a belief that they confer a risk of mortality which is unacceptable. In a large retrospective review of the open lung biopsies performed in the USA between 2000-2011, overall in-hospital mortality was estimated at 1.7%, but the risk was higher for men, those with a suspected diagnosis of IPF and the risk appeared to increase with age (Hutchinson et al., 2016). Cryobiopsy is a newer technique performed via bronchoscopic access to the lung. A large study of cryobiopsy in a high-throughput centre estimated the 30-day mortality of the procedure to be 0.4% (Ravaglia et al., 2019). Biopsies typically demonstrate a patchwork pattern of normal lung next to areas of scarring. Enlarged airspaces lined with bronchiolar epithelium are present and are termed honeycomb change. These areas may be filled with mucin and inflammatory infiltrate (Katzenstein et al., 2008). The criteria for diagnosing UIP pattern on histopathology are shown in table 1.2.

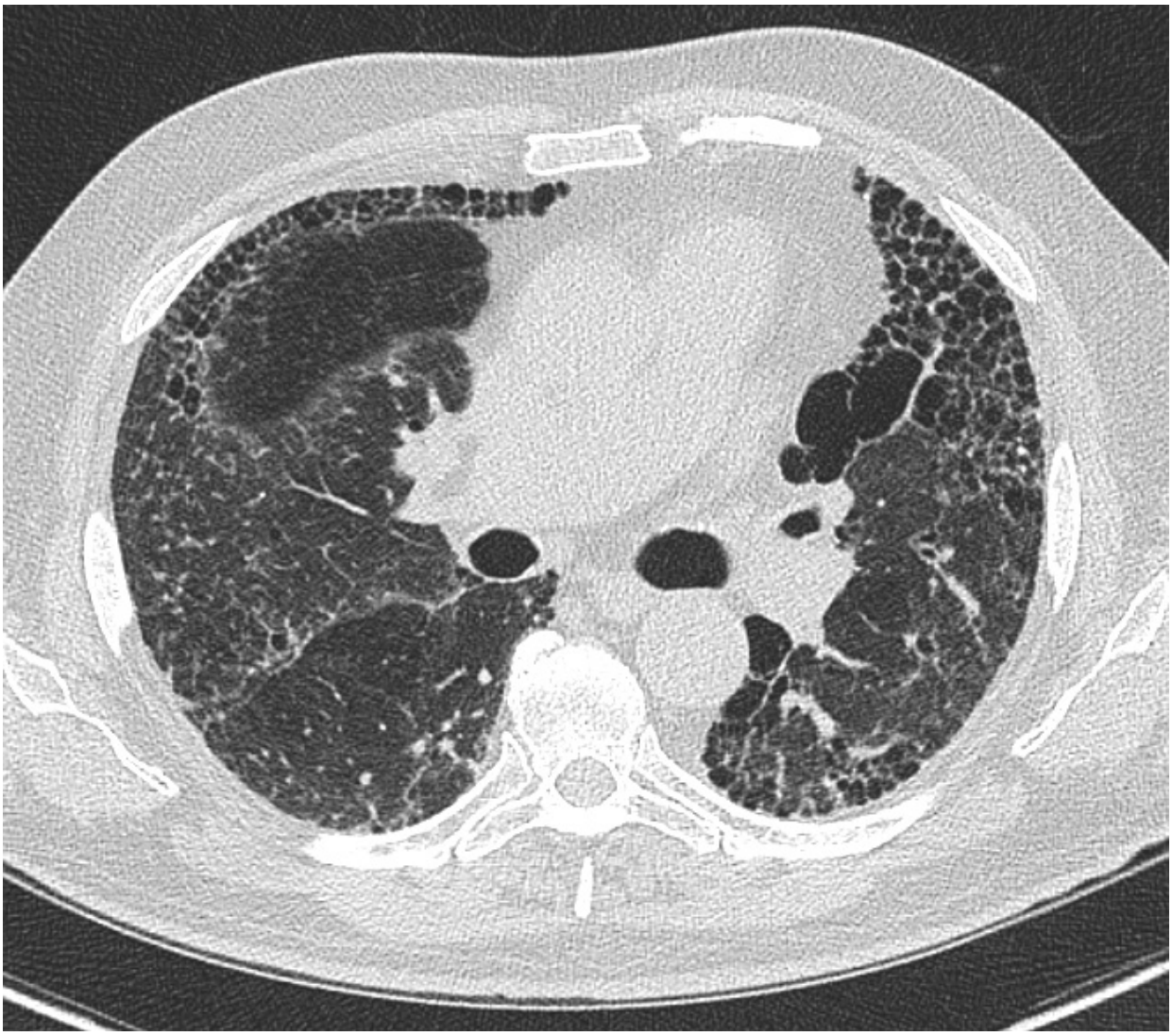


Figure 1.1 UIP pattern CT slice demonstrating peripheral reticulation and honeycombing

UIP Pattern (All 4 features)	**Possible UIP Pattern (All three features)**	**Inconsistent with UIP pattern (Any of the seven features)**
Subpleural, basal predominance	Subpleural, basal predominance	Upper or mid-lung predominance
Reticular abnormality	Reticular abnormality	Peribronchovascular predominance

Honeycombing with or without traction bronchiectasis	Absence of features listed as inconsistent with UIP pattern (see third column)	Extensive ground glass abnormality (extent > reticular abnormality)
Absence of features listed as inconsistent with UIP patent (see third column)		Profuse micronodules (bilateral, predominantly upper lobes)
		Discrete cysts (multiple bilateral, away from areas of honeycombing)
		Diffuse mosaic attenuation / air trapping (bilateral, in three of more lobes)
		Consolidation in bronchopulmonary segment(s) / lobe(s)

Table 1.1 ATS/ERS/JRA/ALAT 2011 Diagnostic criteria for CT imaging

UIP Pattern (All four criteria)	**Probable UIP Pattern**	**Possible UIP Pattern (All three criteria)**	**Not UIP Pattern (Any of the six criteria)**
Evidence of marked fibrosis /	Evidence of marked fibrosis /	Patchy or diffuse involvement of lung	Hyaline membranes

architectural distortion +/- honeycombing in a predominantly subpleural / paraseptal distribution	architectural distortion +/- honeycombing	parenchyma by fibrosis, with or without interstitial inflammation	
Presence of patchy involvement of lung parenchyma by fibrosis	Absence of either patchy involvement or fibroblastic foci, but not both	Absence of other criteria for UIP (See UIP Pattern column)	Organising pneumonia
Presence of fibroblast foci	Absence of features against a diagnosis of UIP suggesting an alternate diagnosis (see fourth column)	Absence of features against a diagnosis of UIP suggesting an alternate diagnosis (see fourth column)	Granulomas
Absence of features against a diagnosis of UIP suggesting an alternate diagnosis (see fourth column)	Honeycomb changes only		Marked interstitial inflammatory cell infiltrate away from honeycombing
			Predominant airway centred changes

			Other features suggestive of an alternate diagnosis

Table 1.2 ATS/ERS/JRA/ALAT 2011 Diagnostic criteria for lung biopsy

1.1.3

Symptoms associated with idiopathic pulmonary fibrosis

The most common symptoms at presentation are breathlessness on exertion, fatigue and cough. In a recent Scandinavian study of 253 patients with IPF, the frequency of common symptoms reported by patients were fatigue (95%), dyspnoea (88%) and cough (85%) (Rajala et al., 2017). There is often a substantial delay between the early onset of symptoms and subsequent diagnosis, partially because of the non-specific nature of the symptoms. The majority of patients will report cough at some point during their disease – usually dry in nature (Turner-Warwick et al., 1980). Previous studies have suggested an association with cough and severity of disease. It has also been suggested that cough may be a predictor of mortality (Ryerson et al., 2011). In this retrospective review of a prospective cohort, by Ryerson et al, the presence of cough recorded in patient records was associated with disease progression. 84% of the patients in this cohort had reported cough. Cough was more frequent in this group in those that showed desaturation on exertion, a marker of more severe disease (Ryerson et al., 2011). Further there is evidence to suggest that severity of cough predicts the amount of impairment to quality of life that patients report (Yount et al., 2016, Gries et al., 2013).

Disease progression in IPF has been described to follow one of four patterns. Some patients will slowly deteriorate, whilst others will have relative stability punctuated by periods of rapid progression (exacerbation). Finally, some patients will experience rapid progression from diagnosis to end stage respiratory failure. These patterns are demonstrated in figure 1.2, where A represents rapid progression, B represents the patient who has episodes of exacerbation with periods of relative stability between, C represents usual disease progression and D represents the slow progressors.

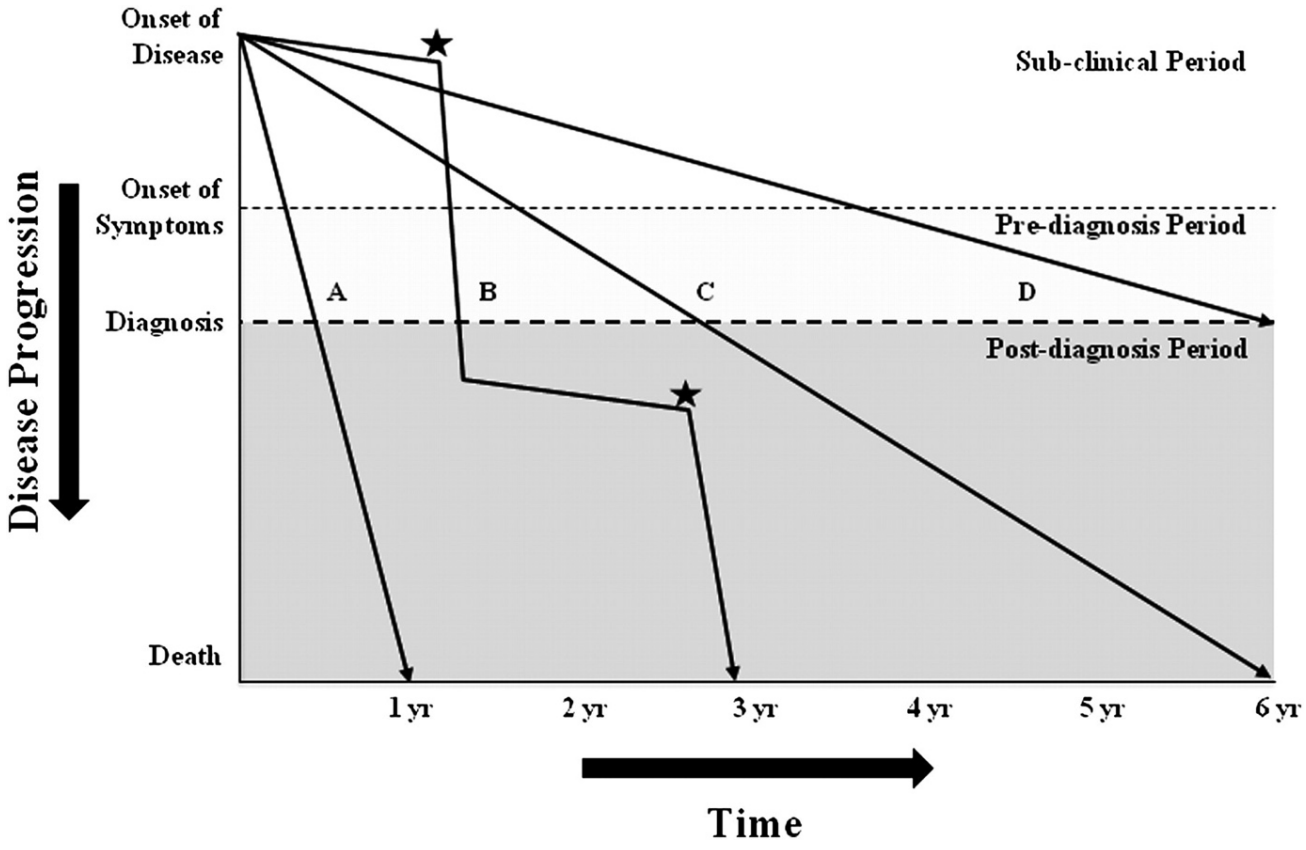


Figure 1.2 Patterns of disease progression in IPF (Ley et al., 2011)

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Currently there are no clinically available tests to determine the likely disease trajectory at the point of diagnosis. In trials however several promising imaging (Win et al., 2018) and serological tests (Maher et al., 2017) have shown potential towards helping to predict an individual patient's likely outcome. Exacerbations are difficult to predict and can be life changing. There are also no agreed therapies for the treatment of an IPF exacerbation.

Measuring severity of disease in idiopathic pulmonary fibrosis is difficult because no one single measure gives us the whole picture. The 2011 guidelines recognised the importance of staging for prognostication to allow earlier identification of younger patients who would benefit from early referral for lung transplantation (Raghu et al., 2011).

The variability of reporting in CT imaging of the lungs makes staging of imaging subjective, although recent machine algorithms for measuring fibrotic areas have tried to address this (Jacob et al., 2019, Jacob et al., 2018). Serum biomarkers such as Krebs von den Lungen-6 (KL-6) and Surfactant protein A and D have been shown to be useful for predicting the risk of mortality (Yokoyama et al., 2006, Takahashi et al., 2000) but are not recommended in the current guidelines (Raghu et al., 2018b) and are not widely available for laboratory testing.

Lung function has classically been the measure of severity and progression. A study using receiver operator curves to evaluate TLCO as a predictor of survival found that a TLCO of 39% predicted was optimal for differentiating survival. Based on this it was suggested that patients with a TLCO of <40% could be labelled as severe disease and that those with a TLCO of >40% predicted could be labelled as limited disease (Egan et al., 2005). Progression of fibrosis has been defined as an absolute decline in FVC of greater than or equal to 10% or an

decline in TLCO of greater than or equal to 15% in a six month period (King, 2000). Relative decline in the percent predicted FVC also appears to be a good predictor of mortality at the 10% threshold (King et al., 2005). However the minimal clinically important difference in IPF for FVC has been estimated to be between 2-6% and the risk of death increases by two-fold in those who have a 6 month decline in FVC of between 5-10% (du Bois et al., 2011). Prevention of FVC decline was also the measure by which successful therapies have been judged in clinical trials (King et al., 2014, Noble et al., 2011, Richeldi et al., 2014). FVC alone however has drawbacks as it is influenced by emphysema (which causes an increase in FVC) and chest wall deformities which can restrict FVC.

The composite physiological index was described in 2003 and combines spirometric and gas transfer data to give a single score of disease severity (Wells et al., 2003). The authors of this paper suggest that the CPI score fits better with CT appearances and with mortality than any single lung function parameter alone (Wells et al., 2003). GAP scoring is another model of staging IPF and predicting mortality. The GAP score is calculated from the patient's age, gender, FVC and TLCO – scores are then grouped into stages that correlate with mortality (Ley et al., 2012).

1.1.4

Genetics influencing the risk of developing idiopathic pulmonary fibrosis

Although IPF is not purely a genetic disease, it has been shown to be associated with several genetic polymorphisms; the most common being the *MUC5B* gene promoter polymorphism (rs35705950) (Stock et al., 2013, Seibold et al., 2011, Moore et al., 2019). This promotor

polymorphism may be found to be present in up to 9% of the general population based on the control group used by Seibold at al. in a study of the effects of *MUC5B* polymorphisms in IPF (Seibold et al., 2011). The MUC5B protein is a key constituent of airway mucous and is expressed by alveolar macrophages with increased expression in smokers (Sepper et al., 2012). MUC5B glycoprotein expression is increased in the bronchiolised distal airways and honeycomb cysts in patients with IPF compared with the distal airways of healthy patients (Plantier et al., 2011, Seibold et al., 2013). The presence of the rs35705950 variant (T allele) of the *MUC5B* gene promoter produces an odds ratio for developing IPF of 5.45 and if two variant copies are present, this increases to 18.68 (Moore et al., 2019). Whilst carriage of the variant allele of *MUC5B* caused over-expression of the *MUC5B* gene in non-IPF lung samples (Seibold et al., 2011), in IPF this finding is less clear. A 2011 study by showed no difference in *MUC5B* expression in IPF patients between differing *MUC5B* genotypes (Seibold et al., 2011), but a more recent study suggests MUC5B protein expression is greater in patients carrying the T allele of the *MUC5B* gene (Stock et al., 2020).

Other less common genetic variants which contribute to the risk of developing IPF include genes coding for the telomerase enzymes (Moore et al., 2019). These enzymes help to promote genetic integrity by maintaining a length of repeating sequence nucleotides at the end of chromosomes. Loss of telomere length is associated with ageing. Defects in the telomerase enzymes appear to cause premature telomere shortening. More recently similar genetic abnormalities to those found in IPF patients have been shown to exist in other chronic fibrotic lung conditions (Ley et al., 2017, Juge et al., 2018) suggesting perhaps that whilst the genes provided susceptibility to developing fibrotic lung disease, the disease phenotype is governed by something else.

1.1.5

Treatment of idiopathic pulmonary fibrosis

Prior to 2013, therapies for most fibrotic lung diseases were immunomodulatory. For IPF, the most common combination therapy was prednisolone, azathioprine and acetylcysteine used in combination. A change in treatment paradigm started in 2012 with the premature cessation of the PANTHER trial (Idiopathic Pulmonary Fibrosis Clinical Research et al., 2012) as a result of excessive death being found in the active treatment arm during a planned interim analysis. This trial had been testing whether triple therapy (Prednisolone, acetylcysteine, Azathioprine) was better than acetylcysteine alone or placebo. As a result of the trial, immunosuppressive therapies for IPF went out of favour, at a time when data to support antifibrotic therapy was beginning to become available (Noble et al., 2011).

Evidence based therapies to treat IPF have been available since 2013. Two therapies are currently licenced in the UK – Nintedanib and Pirfenidone. Both therapies reduce the rate of lung function decline (Richeldi et al., 2014, Noble et al., 2011, King et al., 2014) and are likely to increase life expectancy (Fisher et al., 2017). Treatment related side effects are common with 100% of patients experiencing at least one adverse event during the large RECAP study of Pirfenidone (Costabel et al., 2019). Interestingly this study showed that Pirfenidone was no more likely to cause side effects in those with severe disease compared to those with less severe disease. In England, the National Institute of Clinical Excellence has limited the availability of these medications to patients with a forced vital capacity of between 50-80% predicted based on the cost effectiveness rather than efficacy of treating patients outside of

these margins (NICE TA504 and TA379). Indeed in the RECAP study, the effectiveness of Pirfenidone in reducing the rate of FVC decline appeared similar in both severe and less severe disease groups (Costabel et al., 2019).

The goal of therapy with current therapeutics is to ideally stabilise, but more usually retard disease progression. The therapies do not appear to have a major impact on symptoms.

1.1.6

Other fibrotic lung conditions

A group of similar fibrotic lung diseases exist and more recently have been described as having a progressive fibrotic phenotype (Cottin et al., 2018). In this paper progression was defined as having a $\geq$10% fall in FVC over a 24-month period or relative decline of $\geq$15% in TLCO over the same time period or worsening symptoms or worsening radiological appearances with an FVC decline of between 5-10% (Cottin et al., 2018). This group of patients may include those with lung fibrosis secondary to chronic hypersensitivity pneumonitis, connective tissue diseases such as rheumatoid arthritis, sarcoidosis and those patients with genuinely unclassifiable disease. This group of patients is important because they often have similar presentations and clinical outcomes to patients with idiopathic pulmonary fibrosis. Some of these patients may have similar genetic predisposition to developing lung disease as patients with idiopathic pulmonary fibrosis (Ley et al., 2017). Radiology can be helpful in determining the risk of progressive fibrotic disease, with the presence of honeycombing increasing the risk of mortality regardless of the underlying disease process (Adegunsoye et al., 2019). Pre-clinical data has suggested that platelet derived growth factor (PDGF), vascular endothelial

growth factor (VEGF), fibroblast growth factor (FGF) and macrophage colony stimulating factor (M-CSF) are all elevated in quantity in the lungs of patients with the progressive fibrotic phenotype, regardless of their diagnosis (Hoffmann-Vold et al., 2019). These pro-fibrotic mediators are targets for the tyrosine kinase inhibitor Nintedanib (Wollin et al., 2015). More recently it has been shown that these patients may respond to the same anti-fibrotic therapies that have been shown to influence disease progression in IPF (Maher et al., 2019, Flaherty et al., 2019).

1.2.0

Cough and its pathophysiology

Cough is broadly divided into acute cough (that which lasts under 3 weeks) and chronic cough (lasting generally over 8 weeks). Despite cough being one of the most common reasons for patients seeking primary care assistance, there are few highly effective anti-tussive therapies. The over the counter market for anti-tussive therapy in the UK in 2017 was valued at 101.9 million pounds for cough syrups alone with a further 120 million pounds being spent on medicated confectionary (Connelly, 2018); perhaps highlighting the need for more effective therapies.

Each cough consists of three phases – initially a rapid intake of air, the closure of the glottis, resulting in increased intrathoracic pressure and finally the opening of the glottis with the release of air at high speed. This results in the characteristic sound of coughing.

In health the function of the cough reflex is to protect the airway from inhaled irritants and to clear airway debris. Ineffective cough leads to accumulation of debris in the airway resulting in atelectasis, bronchiectasis and infection. Patients who have recurrent pneumonia have been shown to have an impaired cough reflex to capsaicin (Niimi et al., 2003), highlighting the importance of the cough reflex to host defence. These complications can be seen in neuromuscular disease affected patients where cough sensory neurones are intact, but the respiratory muscles are not strong enough to produce cough (Dicpinigaitis et al., 1999). Similar problems may be seen in the elderly, where the sensitivity of the cough response appears diminished (Newnham and Hamilton, 1997) and there is an increasing incidence of aspiration (Miles et al., 2013). Interestingly by stimulating the cough reflex with capsaicin pastilles, it was possible to heighten the response (Ebihara et al., 2005).

1.2.1

Anatomy of cough

The respiratory tract is lined with goblet cells that secrete respiratory mucins – glycoproteins which form the bulk constituent of mucous. Mucous traps inhaled particulates and bacteria. There is a muco-ciliary clearance system which transports mucous from the distal airways to the larger airways. Coughing helps to clear mucous out of the respiratory tract.

The cough reflex is mediated by the vagal nerve which connects to sensory nerve receptors through the entire airway. Two major branches of the vagus (recurrent and superior laryngeal) supply sensory innervation to the trachea and major bronchi. The smaller airways derive sensory innervation from smaller branches of the vagus nerve. Transection of the

vagal nerve, as occurs in a lung transplant causes loss of the cough reflex (Hathaway et al., 1993), although this is regained with time after surgery, presumably due to regrowth of neural pathways (Duarte et al., 2008). Anaesthetic agents that block the transmission of sensory neuronal impulses such as lignocaine have been used to decrease the cough reflex in patients under general anaesthesia, further pointing to a neuronal basis for cough (Steinhaus and Gaskin, 1963).

Cough can originate from the larynx, where light touch can stimulate exhalation and closure of the glottis or from stimulation of small airway nerves. Obliteration of sensory supply to the larynx does not reduce cough sensitivity to inhaled citric acid for example (Stockwell et al., 1993). The alveoli and terminal bronchioles do not have the sensory supply to be able to trigger cough.

The airways are well innervated with sensory nerves which respond to changes in the environment around us. These nerves can respond to changes in temperature, humidity, pH and mechanical stretch.

Three distinct phenotypes of sensory nerve endings in the lung have been described – Slowly adapting stretch receptors (SARS), rapidly adapting stretch receptors (RARS) and c-fibres. C-fibres generally are sensitive to chemical insults such as cigarette smoke. C-fibres are unmyelinated and are present in both the lung parenchyma and airways. Stimulation of airway c-fibres can provoke cough and mucous hypersecretion. Interestingly however, stimulation of the intrapulmonary c-fibres can abolish the cough response in guinea pigs (Chou et al., 2017).

RARS respond to mechanical forces within the lung and to intraluminal pressure changes within the airways. RARS are myelinated small fibre nerves that rapidly adapt to sustained changes in lung inflation or deflation (Widdicombe, 2003). RARS can be activated by many of the substances that can provoke the cough reflex including capsaicin. The role of RARS can be demonstrated by cooling the vagal nerve to 7 degrees Celsius, which blocks RAR activity, but c-fibre activity is maintained. This blocks capsaicin induced cough in an animal model (Tatar et al., 1994)]. SARS also respond to mechanical forces, activating during inspiration to regulate the Hering-Breuer reflex which causes expiration. SAR activity does not initiate cough, but their activity can attenuate or potentiate the cough reflex.

C-fibres express transient receptor potential (TRP) pores. These are calcium activated cation channels which can be chemosensitive, mechanosensitive and thermosensitive. There are multiple subtypes of TRP, with many being implicated in cough.

The transient receptor potential vanilloid 1 (TRPV1) channel is expressed on airway C fibres and is activated by capsaicin, changes in temperature and drops in pH (Tominaga et al., 1998). Activation of TRPVI produces release of neuropeptides, tachykinins, neurokinins and substance P (all of which are pro-inflammatory). Previous clinical attempts to block TRPV1 however did not result in a clinical improvement in chronic cough, despite a reduction in sensitivity to capsaicin being shown, suggesting target engagement (Khalid et al., 2014). A side effect of blocking TRPVI is hyperthermia, suggesting that TRPV1 has a role in maintaining body temperature. There is some evidence that single nucleotide polymorphisms within the

gene coding for TRPV1 may be responsible for an increased burden of cough in those who are exposed to tobacco smoke or other environmental irritants (Smit et al., 2012).

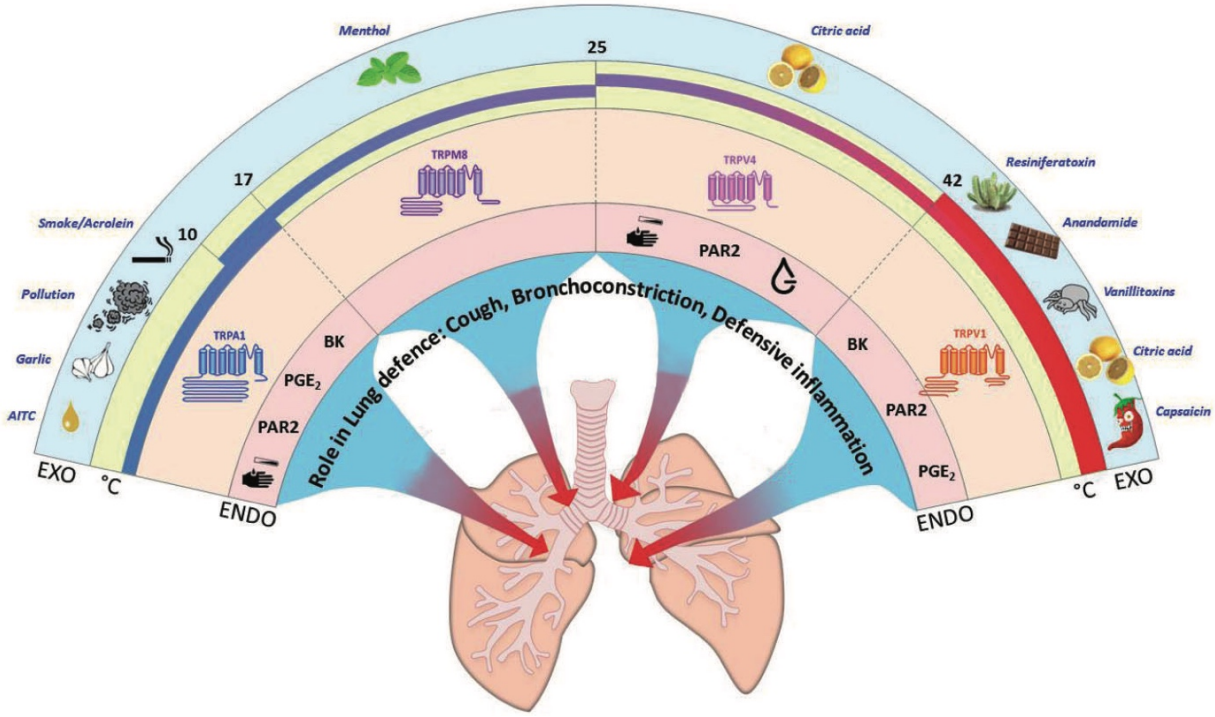


Figure 1.3 TRP receptors and their agonists / antagonists (Grace et al., 2014)
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TRPA1 is another calcium flux gated ion channel found on sensory nerves. Endogenous products of oxidative stress are able to activate TRPA1, as well as many noxious substances such as acrolein (Birrell et al., 2009).

TRPM8 is a temperature sensitive TRP channel. Clinically agonism of TRPM8 can be achieved with menthol, leading to a reduction in cigarette smoke induced cough in rodents (Willis et al., 2011). Interestingly it would seem that the mechanism for this may be antagonism of TRPA1, although menthol can also act as an agonist of TRPA1 in low concentrations (Karashima et al., 2007). Menthol was also shown to reduce citric acid induced cough in

human volunteers (Morice et al., 1994). Menthol is of course in use clinically, being almost ubiquitous in over-the-counter cough remedies.

TRPV4 is thought to sense changes in osmolality in the airways. It also responds to acidic pH. Citric acid and distilled water can both agonise TRPV4. Citric acid is often used in cough challenge testing. Blocking TRPV4 with novel compound GSK2193874 did lead to a reduction in citric acid induced cough, but not the cough produced by distilled water. This suggests that TRPV4 mediated signalling is not the only route by which hypo and hypertonic solutions can provoke cough (Buday et al., 2017). Importantly activation of p2X3 receptors is linked to activation of TRPV4. P2X3 has been shown to be an important target for potential novel anti-tussives (Abdulqawi et al., 2015).

Suppression of the cough reflex can be achieved by higher central nervous system processing. This can be demonstrated by the efficacy of therapies such as speech and language therapy (Gibson and Vertigan, 2009). Drugs that agonise 5HT-1 receptors in the brain can also exhibit anti-tussive effects, such as methysergide (Kamei et al., 1986).

1.3.0

Cough in idiopathic pulmonary fibrosis

The pathogenesis of cough in IPF is poorly understood. What is clear is that cough is incredibly common in IPF and is associated with a reduction in quality of life (Ryerson et al., 2011). Patients with IPF have been shown to have a more sensitive cough reflex compared to health control – both in response to inhaled challenge agents (Hope-Gill et al., 2003) and to

mechanical stimuli (Jones et al., 2011). Hope-Gill et al applied percussion of various frequencies to the chest walls of patients with IPF and to healthy controls. The patients with IPF showed enhanced cough sensitivity to this mechanical stimulation compared to the controls. Interestingly applying the stimulus to the areas of chest wall closest to the most fibrotic areas of lung stimulated more cough.

A study of the cough response to inhaled capsaicin in patients with fibrotic lung disease (then termed cryptogenic fibrosing alveolitis) demonstrated an enhanced cough response to capsaicin compared to controls (Doherty et al., 2000). In this study, patients and healthy controls were given nebulised capsaicin doses of differing concentrations via an air driven dosimeter. The number of coughs in the first 60 seconds post dose was recorded as well as the concentration of capsaicin taken to produce C5 (a concentration that produces five coughs). The study demonstrated cough was triggered by lower concentrations of capsaicin in the IPF patients and that the cumulative cough frequency was also significantly higher in patients. Interestingly there was no association between patient's perception of their cough as captured on a visual analogue scale and in a cough diary. This would suggest that either patients were not regularly exposed to substances that caused neuronal agonism like capsaicin or that there may be significant differences between subjective and objective cough measures in pulmonary fibrosis.

Given that cough sensitivity is likely to be a neuronal phenomenon, the transient receptor pore family have been investigated to understand their role in cough. Histological examination of tissue from IPF lungs demonstrated an increased presence of TRPV1 and TRPA1 on the airway nerves compared to healthy controls, but the difference was not

statistically significant. (Hutchinson et al., 2017). In the same study, mRNA expression of TRPV1 and TRPA1 were also shown to be raised in IPF patients compared to controls, but again the difference was not significant when other factors were taken into account. One flaw with this study was that it relied on endobronchial biopsies which represent central airways rather than the lung periphery where most of the structural damage is occurring in IPF. Studies of patients with chronic idiopathic cough have produced conflicting results with one study showing increased nerve expression of Transient receptor potential vanniloid-1 (TRPV1) (Groneberg et al., 2004) and yet the Hutchinson study was unable to reproduce this finding (Hutchinson et al., 2017). A study which evaluated cough challenge responses to TRPA1 and TRPV1 agonists found considerable variability of responses in patients with chronic cough suggesting that upregulation may be important in some but not all patients (Long et al., 2019). The guinea pig model of pulmonary fibrosis, in which the fibrotic process is initiated by intratracheal bleomycin instillation, also demonstrates increased expression of TRPV1 and TRPA1 (Guo et al., 2019). This difference between the human and guinea pig does highlight the problems of the animal models of IPF. Blockade of this neural pathway via P2X3 inhibition has been undertaken in chronic cough of unknown cause and has been therapeutic (Abdulqawi et al., 2015). Trials of the same agent in IPF have already taken place but remain unreported.

TRPV4 is activated by membrane stretch/stiffening and appears to mediate the transformation of fibroblasts into myofibroblasts in the animal model of fibrosis (Rahaman et al., 2014). Blocking TRPV4 in the bleomycin mouse model of fibrosis attenuated the fibrosis (Rahaman et al., 2014) and reduced inflammation following a chlorine inhalational exposure (Balakrishna et al., 2014). Blocking TRPV4 also seems to inhibit cough (Bonvini et al., 2016,

Buday et al., 2017). It seems entirely conceivable that alveolar injuries may lead to repetitive TRPV4 activation stimulating fibrosis and cough.

Genetic polymorphisms which contribute to the risk of developing IPF may also be implicated in IPF related cough. The *MUC5B* promotor single nucleotide polymorphism (SNP) has been shown to be associated with an increased risk of developing IPF (Stock et al., 2013). Mucin 5B is a key constituent of human mucous produced by goblet cells in the airway.

Previous cohort studies have demonstrated that the presence of a single nucleotide polymorphism in the *MUC5B* gene promotor region increases the risk of developing IPF (Seibold et al., 2011, Stock et al., 2013). Further-more presence of the variant *rs35705950* SNP in the *MUC5B* promotor appears to correlate with increased levels of the MUC5B protein detectable in the distal airways (Conti et al., 2016) and this appears to be unique to IPF (Stock et al., 2020). In the mouse model, the absence of MUC5B protein leads to inadequate mucocilliary clearance mechanisms (Roy et al., 2014). Carriage of this SNP is thought to functionally result in the production of excessive quantities of mucin B – a constituent of airway mucous. There is some data to suggest that carriage of this particular SNP affects the severity of cough in IPF (Scholand et al., 2014).

The activity of slow and rapid adapting stretch receptors has been shown to be altered in the bleomycin model of lung fibrosis (Schelegle et al., 2001). Potentially altered activity of these receptors could alter cough sensitivity.

Sleep disordered breathing is common in patients with IPF and sleep apnoea may be demonstrable in over 60% of patients (Lee et al., 2020, Bosi et al., 2017, Papadogiannis et al., 2020). CPAP therapy has been demonstrated to improve mortality in patients with both IPF and sleep apnoea (Papadogiannis et al., 2020). In the animal model of pulmonary fibrosis, intermittent hypoxia as would be seen in obstructive sleep apnoea lead to more collagen deposition and accelerated fibrosis (Kang et al., 2020). Sleep apnoea is recognised on its own to be associated with an increased burden of cough (Roy et al., 2020, Shi et al., 2019). In the study by Shi et al there were also differences in inflammatory mediators present in the induced sputum of those patients with OSA compared to healthy controls (Shi et al., 2019). A study of CPAP in patients with chronic cough and obstructive sleep apnoea found that there was a significant reduction in cough burden associated with the use of CPAP (Sundar et al., 2020). The higher prevalence of obstructive sleep apnoea found in patients with pulmonary fibrosis may therefore be partly causative in the increased cough burden experienced,

Gastro-oesophageal reflux (GORD) is another co-morbidity that is common in patients with IPF (Hoppo et al., 2014, Fahim et al., 2011). GORD appears to associate with increased disease progression in IPF and has been considered to be a possible cause of repetitive alveolar injury (Lee et al., 2011). Studies looking at pepsin in bronchoalveolar lavage fluid have found higher levels of pepsin (thought to signify increased GORD) are associated with worse outcomes (Kim et al., 2018). However, the data for beneficial effects of antacid therapy on IPF survival are conflicting (Kreuter et al., 2017, Lee et al., 2013a, Kreuter et al., 2016b). Experiments in patients with reflux have shown that exposure of the distal oesophagus to a low pH is associated with increased cough (Ing et al., 1994). Within the oesophagus of patients with GORD there is increased expression of TRPV1 on sensory neurones (Matthews et al., 2004),

although it is not known whether GORD could cause the same upregulation in airway neurones to enhance cough. A proof-of-concept study has suggested omeprazole may be associated with reduced cough burden in patients with IPF (Dutta et al., 2019).

Cigarette smoke is associated with the risk of developing idiopathic pulmonary fibrosis (Baumgartner et al., 1997). A smoking history is commonly identified in those with IPF – in the INSIGHTS IPF cohort 62% of patients had smoked (Kreuter et al., 2019). Cigarette smoke has been shown to trigger ATP release from airway cells (Baxter et al., 2014). ATP can activate P2X3 receptors on sensory nerves to trigger cough (Bonvini et al., 2016). Cigarette smoking may also alter the lung microbiome (Erb-Downward et al., 2011) and such changes may not revert after smoking cessation (Munck et al., 2016).

Proposed mechanisms for increased cough in IPF
High incidence of Gastro-oesophageal reflux disease
Increased incidence of obstructive sleep apnoea
History of chronic cigarette smoke exposure
Increased expression of respiratory Mucins via *MUC5B* polymorphisms
Direct stimulation of sensory neurones by gram negative bacteria present in higher quantities
Altered activity of SAR and RAR neurones in response to mechanical distortion caused by fibrosis

Table 1.3 – Proposed mechanisms for increased cough in IPF

1.4.0

The microbiome and its role in pulmonary fibrosis and related cough

The airways and alveolar spaces are home to a diverse range of organisms in health, termed the microbiome. Patients with IPF have been shown to have much higher bacterial burdens present within their lungs as well as a difference in the predominant bacterial species compared to healthy controls (Molyneaux et al., 2014, O'Dwyer et al., 2019, Dickson et al., 2019). Higher bacterial burdens within the IPF lung have been shown to associate with more rapid disease progression (O'Dwyer et al., 2019) and with exacerbations of disease (Molyneaux et al., 2017). In a recent study using explanted lungs, bacterial loads seemed to be highest in the major airways (Valenzi et al., 2020).

Mouse models of pulmonary fibrosis created with bleomycin show reduced fibrogenesis if the mouse is pre-treated with antibiotics instilled into the lung (Yang et al., 2019). Antibiotics targeting gram negative bacteria were more effective than those targeting gram positive bacteria in ameliorating the bleomycin induced fibrosis (Yang et al., 2019). In. the recently reported EME-Tipac study, in which patients with IPF took placebo or co-trimoxazole, no difference in either cough severity score of LCQ was demonstrated between the two groups (Wilson et al., 2020).

Bacteria cultured *in vitro* can be shown to produce extra-cellular ATP (Mempin et al., 2013). ATP is a potent stimulator of the cough reflex (Bonvini et al., 2016) and thus bacteria may be able to produce cough via exogenous ATP production.

1.5.0

Cough in other fibrotic lung diseases

There are other causes of lung fibrosis which also present with cough. Some of these forms of lung fibrosis behave clinically in a very similar manner to idiopathic pulmonary fibrosis (Maher et al., 2019). Patients with a diagnosis of chronic hypersensitivity pneumonitis (CHP) who have ongoing decline in FVC for example have increased mortality (Gimenez et al., 2017) and often have a radiological pattern of usual interstitial pneumonia (UIP) as seen in IPF. A study looking at cough in IPF, CHP and scleroderma associated lung fibrosis found that patients with CHP had very similar burden of cough to those with IPF (Cheng et al., 2017). Recent studies have suggested a lack of response of this condition to the traditional approach of combined immunosuppressive therapy (Gimenez et al., 2017). Owing to a lack of evidence-based therapies, increasing attention will be paid to these conditions and thus effective symptom relief will be an important factor in trials.

More recently it has been recognised that other fibrotic lung diseases can continue to progress through immunosuppressive therapies and behave in a fashion similar to idiopathic pulmonary fibrosis (Flaherty et al., 2017). This phenotype has been described as progressive lung fibrosis and may have multiple aetiologies including connective tissue diseases and hypersensitivity pneumonitis. Very little is known about cough in this group of patients although some studies exist looking at cough in other fibrotic diseases like scleroderma related NSIP (Cheng et al., 2017).

Symptom control is an unmet need in fibrotic lung disease at present. For breathlessness, ambulatory oxygen in those who are hypoxic may be of benefit (Visca et al., 2017). Low dose opiates such as morphine syrup are also commonly prescribed to reduce the sensation of breathlessness (Johnson et al., 1983, Allen et al., 2005). Pulmonary rehabilitation classes in which structured exercise programmes are undertaken have shown benefit (Tonelli et al., 2017).

Cough in IPF and the progressive fibrotic lung diseases is a challenging symptom to address, hampered in part by our lack of understanding as to its pathophysiology. There is randomised controlled study evidence for Thalidomide, however the cost of this therapy and its gastrointestinal side effects limit it usage (Horton et al., 2008). Pirfenidone has shown some promise in a recent trial (van Manen et al., 2017) as has a nebulised form of sodium cromoglycate (Birring et al., 2017). Interestingly the reduction in objective cough demonstrated by all three drugs is in the order of 30%. In acute cough in otherwise well individuals, the scale of reduction in objective cough required for a patient to feel better is estimated to be up to 50%. It seems therefore that for now treating cough in IPF remains an important area for research.

1.6.0

Measuring cough in patients

Multiple measures of cough for clinical trials exist. Some measurements will measure cough directly whilst others measure its impact. Measures may be subjective i.e. as reported by the patient, or objective i.e. cough counting from video or audio recordings.

1.6.1

Subjective measures of cough

Perhaps the simplest measure of cough is the cough visual analogue scale (VAS), a ten-centimetre line on which patients record a cross to show their perceived severity of their cough. Cough VAS can also be adapted to examine the urge to cough. The cough VAS has been shown to be reproducible (Brightling et al., 2001) and correlates well with more complex measures of cough (Fukuhara et al., 2019). In acute cough, the minimal difference of change thought to be clinically significant is 17mm (Lee et al., 2013b). An advantage of the cough VAS is its simplicity, which makes it ideal for use in the clinical setting. The interpretation of the cough VAS is cheap and does not require significant training.

The Leicester cough questionnaire (LCQ) was designed to demonstrate the impact cough has over a range of quality of life areas in patients with chronic idiopathic cough (Birring et al., 2003a). The questionnaire is made up of 19 questions which are divided into physical impact, psychological impact and social impact domains. In the original design study it was shown most patients were able to complete the questionnaire within 5 minutes (Birring et al., 2003b). One of the weaknesses of the LCQ is that it has not been validated in patients with a cause for cough such as airways disease or fibrotic lung disease. The questionnaire gives a lower score to patients with more severe quality of life problems arising from cough. In IPF, there is some evidence to support the validity of the LCQ with correlation between the LCQ and objective cough counting having been demonstrated in a small group (Key et al., 2010). Similar evidence exists to show moderate correlation between the LCQ and objective cough

counts in chronic cough (Decalmer et al., 2007). The LCQ captures multiple aspects of cough from the physical discomfort of coughing, the social embarrassment of cough and the anxiety and depression that can come about as a result of cough.

The cough quality of life questionnaire (CQLQ) is a similar tool to the LCQ in that it is aiming to record the impact of cough rather than quantity of cough (French et al., 2002) using 28 questions. The CQLQ was validated in IPF as part of a trial looking at the effects of thalidomide on cough in patients with IPF (Lechtzin et al., 2013). In a 16-week study, the repeatability of the CQLQ was shown to be good and it strongly associated with cough VAS and the SGRQ score. In a paper which compared CQLQ with the LCQ in chronic cough, bronchiectasis, asthma and COPD it was found the two scores correlated well and that the CQLQ offered some additional information about the psychosocial burden of cough (Polley et al., 2008). Previous work in COPD comparing objective cough counting to the CQLQ has shown only weak correlation between the CQLQ and the amount of time spent coughing (Smith et al., 2006a).

None of the previously described subjective measures of cough were developed in patients with pulmonary fibrosis. Descriptions of their use in IPF have so far been described in small studies only (Key et al., 2010, Horton et al., 2008, van Manen et al., 2017).

1.6.2

Measuring quality of life in fibrotic lung disease

Some more generic quality of life questionnaires exist for IPF patients and within these questionnaires there are usually domains specific to cough. The ATAQ (A tool to assess quality

of life)-IPF questionnaire was developed in 2010 at the National Jewish Health clinic (Swigris et al., 2010b). The questionnaire has 6 out of 74 questions dedicated to cough. In the original cohort in which the questionnaire was tested, there was a statistically significant but weak correlation between cough and lower lung function.

The St George's Respiratory questionnaire was designed in 1991 to measure impaired quality of life in patients with airways disease (Jones et al., 1991). The questionnaire has since been validated in a range of other respiratory conditions including IPF. The questionnaire is broken into three domains: symptoms (of which cough contributes significantly), activity and impacts on day-to-day life. In the original questionnaire paper, cough and wheeze were the strongest correlates to the symptom score. The minimal clinically important difference for this questionnaire has been calculate to be 4 units, however it is important to note that this was in COPD patients (Jones, 2005).

1.6.3

Objective measures of cough

Digital cough recorders have been used to demonstrate objective cough counts. Currently one small study has been performed in IPF to demonstrate objective cough counts in this condition (Key et al., 2010). The Vitalojak is a recording device used to perform 24-hour recordings of cough. The device has been validated against traditional video methods of recording cough (Smith et al., 2006b). The recording produced by the device is then reduced by software that removes silences and non-cough sounds prior to manual listening to produce a cough count (Sumner et al., 2010). Objective cough counts have been shown to only have

modest correlation with subjective measures of cough such as the LCQ in studies of patients with chronic cough (Decalmer et al., 2007). In asthmatic patients this relationship between objective cough counts and subjective measures of cough is even weaker (Marsden et al., 2008), suggesting that objective cough counts do offer valuable information in addition to that provided by subjective measures. A study in which the cough monitor was used to measure the change in cough with oesophageal manometry catheters put *in situ* found that a reduction in objective cough counts of 33% was clinically significant (Kelsall et al., 2011).

Measure	What is being measured?	Advantages	Limitations
Cough visual analogue scale	Patient perception of cough severity	Easy to perform with minimal instruction. Validated in IPF Easy to interpret	Responsiveness to change not well established. May be misinterpreted by patient – impact vs severity
Leicester cough questionnaire	Impact of cough on quality of life	Validated in IPF Familiarity – has been used across multiple diseases	Does not quantify cough.
Cough related quality of life questionnaire	Impact of cough on quality of life	Validated in IPF	Does not quantify cough. 28 items – longer to complete
Digital cough recorder	Quantification of cough – usually over 24-hour period	Well validated against video counting.	Training required to interpret result Interpretation is semi-automated and thus is time consuming Does not give evidence of the impact of cough
Video cough counts	Quantification of cough	Observation of video reduces the risk of non-cough	Patient not in own environment which may alter behaviour.

		sounds being mis-interpreted Provides accurate cough counts	Shorter monitoring periods.
Capsaicin cough challenge	Measure of cough sensitivity using markers such as C5 (the concentration required to produce 5 coughs)	Provides supportive evidence for a mechanism of cough	Unpleasant Time consuming Specialist equipment required

Table 1.4 – Methods for investigating cough

1.7.0

Drug treatment of cough associated with fibrotic lung disease

Since 2013 there have been NICE approved anti-fibrotic therapies available for the treatment of IPF. Initially the first approved therapeutic was Pirfenidone, a small molecule with pleiotropic effects and its true mechanism in IPF remains poorly understood. More recently, Nintedanib, a tyrosine kinase inhibitor previously used for non-small lung cancer, has also been licenced. Both drugs have been shown to reduce the rate of decline in forced vital capacity (FVC) by approximately 50% annually (Noble et al., 2011, King et al., 2014, Richeldi et al., 2014). Neither drug showed a benefit to patient reported symptoms at the time of licencing. There has been subsequent data to suggest that Pirfenidone may reduce cough frequency by around 30% at 12 weeks, in patients with IPF (van Manen et al., 2017). The trial showed smaller reductions in cough VAS and LCQ which were still none the less statistically significant, even though the reduction may not have been clinically significant. However, in a trial evaluating Pirfenidone in non-IPF progressive fibrosing lung disease, the drug appeared to have no benefit over placebo on cough VAS and the LCQ (Maher et al., 2019).

Thalidomide is one of the few therapies that has been proven to be efficacious against cough in IPF (Horton et al., 2008). This was a small study in which 23 patients received thalidomide or placebo in a cross-over fashion with each treatment period lasting 12 weeks and a 2-week washout period between treatments. The primary endpoint in this study was the Cough Quality of Life Questionnaire with cough VAS and SGRQ being secondary endpoints. Although the CQLQ and cough VAS were significantly improved with thalidomide versus placebo, there were significantly higher adverse events related to thalidomide therapy. In the NICE guidelines thalidomide was recommended for specialist initiation in those with intractable cough despite other therapies (National Clinical Guideline Centre for Acute and Chronic Conditions (Great Britain) and National Institute for Health and Care Excellence (Great Britain), 2013).

Cromolyn sodium was shown in a recent trial to reduce daytime cough frequency in IPF patients by 31.1% (Birring et al., 2017). The mechanism by which the drug acts is unknown. There is however, some evidence to suggest that cromolyn sodium can act on c-fibres via a G-protein coupled receptor GPR35 (Yang et al., 2010). Furthermore, cromolyn may act indirectly by stabilising mast cells which would otherwise release neural activating substances such as substance P, histamine and proteases. Mast cells have been shown to accumulate in the IPF lung (Hunt et al., 1992) and may stimulate fibroblasts and collagen synthesis via tryptase release (Akers et al., 2000).

Theophylline has been shown in human single nerve preparations and in the guinea pig to be able to inhibit bronchial c-fibre activation of the cough reflex by capsaicin (Dubuis et al.,

2014). There is no evidence to support using theophylline as an anti-tussive in fibrotic lung disease, however.

Tiotropium has been shown to antagonise TRPVI receptors (Birrell et al., 2014) which may explain why it can attenuate cough and capsaicin sensitivity in asthma patients (Fukumitsu et al., 2018). Similar clinical observations have been made of another antimuscarinic agent aclidinium in COPD (McGarvey et al., 2016). Both drugs appear to reduce cough induced by chemical and mechanical stimulation in an animal model where it is hypothesised the action is via not only TRPV1 but also via acid and mechano-receptor activity (Mutolo et al., 2016). As of yet neither of these drugs has been tested in patients with fibrotic lung disease.

Omeprazole belongs to the proton pump inhibitor class of medications. It reduces gastric acid production. Gastro-oesophageal reflux has long been speculated to be a potential causative agent in the development of idiopathic pulmonary fibrosis. Gastric acid's low pH may also be a potent stimulator of pH sensing TRP channels on the sensory nerves within the airway. A small proof of concept trial involving 45 patients being randomised in a double-blind fashion to receive either omeprazole or placebo was reported in 2019 (Dutta et al., 2019). This showed a 39.4% reduction in cough in the omeprazole arm as measured by objective cough counting. The study had a very high screen failure rate because of the number of patients already taking anti-GORD medication prior. Post-hoc analysis of the ASCEND and CAPACITY trials of pirfenidone looked at the impact of antacid therapy in a large group of patients taking Pirfenidone (Kreuter et al., 2017). The conclusion from this study was that there was no benefit from using antacid therapy to slow disease progression. This finding supported that found in analysis of Australian registry data where neither the presence of symptomatic GORD

or the use of proton pump inhibitors influenced the rate of FVC decline (Jo et al., 2019). Another small study has shown that using oesophageal probes it is possible to demonstrate significant abnormalities of oesophageal contractility and of subsequent reflux in IPF patients (Hoppo et al., 2014). The findings of the omeprazole trial (Dutta et al., 2019) and the analysis by Kreuter (Kreuter et al., 2017) could suggest that refluxed acidic GI contents stimulate cough but do not cause disease progression, hinting that cough itself may not be related to disease progression. Earlier retrospective analysis of cohort studies of patients with IPF however had suggested proton pump inhibitors were associated with an increased time to transplant survival (Ghebremariam et al., 2015). There have also been some concerns expressed regarding the safety of antacid therapy in IPF as it may be associated with an increased risk of infection (Kreuter et al., 2017).

Opiates have been used extensively for cough relief, particularly in the palliative care setting. The only IPF specific evidence however is for their management of breathlessness and not cough (Kohberg et al., 2016). Opiate drugs however have been reported for successful anti-tussive actions in other causes of cough since the 1950s (Snell and Armitage, 1957). Opiates have issues around tolerance, their addictive potential and side effects such as constipation, nausea and drowsiness. A recent expert opinion for the treatment in cough has recommended opiates for the treatment of cough in ILD patients where other therapies have failed (Birring et al., 2018).

Drug treatment of cough in idiopathic pulmonary fibrosis and other fibrotic lung disease is hampered by a lack of understanding of the mechanism underlying the cough. As such few

drugs are available that help this troublesome symptom and there is very poor quality evidence supporting their use (Birring et al., 2018).

1.8.0

Aims of research

Objective one – Describe the burden of cough and its impact on quality of life in fibrotic lung disease.

The burden of cough in patients with fibrotic lung disease is poorly understood and therefore this project aims to demonstrate this burden in terms of both the frequency of coughing and the impact it has on the quality of life.

Objective 2 – Describe the relationship between cough and disease progression

Previous work has suggested a link between cough and disease progression and mortality (Ryerson et al., 2011). This project will set out to see if such an association exists and will evaluate other potential mechanisms underlying cough in patients with fibrotic lung disease.

Objective 3 – Describe the relationship between cough and *MUC5B* genotype

Work by Scholand et al has suggested that the rs35705950 variant of the *MUC5B* promotor polymorphism is associated with worsening cough severity. (Scholand et al., 2014). I aim to describe both objective and subjective measures of cough across the different *MUC5B* genotypes.

Objective 4 – Describe the relationship between the microbiome in fibrotic lung disease and cough

IPF patients have been demonstrated to have alterations in the lung microbiome (O'Dwyer et al., 2019). Unpublished data from Professor Belvisi's group has shown some species of gram-negative bacteria can provoke cough through sensory nerves. I will examine possible relationships between changes in bacterial species in the lung and cough.

Project hypotheses:

Cough is a significant cause of morbidity in patients with fibrotic lung disease. In idiopathic cough, it has been shown there is hypersensitivity of sensory neuronal input into the cough reflex (Abdulqawi et al., 2015). There is some evidence from Professor Belvisi's group at Imperial College that gram negative bacteria can directly stimulate sensory neurones to elicit cough. Evaluation of the microbiome in patients with pulmonary fibrosis has shown increased levels of some species of gram-negative bacteria present compared to healthy controls (Molyneaux et al., 2014). In the same study it was also shown that *MUC5B* genotype affected bacterial burden.

It is hypothesised based on previous data (Key et al., 2010) that cough will associate with worsening quality of life in pulmonary fibrosis.

It is hypothesised that the burden of cough in fibrotic lung disease will be influenced by bacterial burden in the lung.

It is hypothesised that the correlation between cough burden and disease severity will be weak at best. Disease progression may influence sensory nerves given airway traction may influence stretch receptors.

Chapter 2

METHODS

The evaluation of cough in fibrotic lung disease involved the recruitment of patients from the Royal Brompton Hospital, Queens Medical Centre Nottingham and district general hospitals within the Nottingham referral catchment area. This was done as part of The Prospective Study of Fibrosis in Lung Endpoint (PROFILE) study. Ethics for this study were already in place at the time of starting my research (Royal Free Ethics Committee 10/H0720/12) and the studies were registered at clinicaltrials.gov (NCT01110694 and NCT01134822). A modification of the study ethics was granted in 2016 to allow the collection of digital cough recordings from patients.

The methods used to gather patient related data and process clinical samples are described below. The laboratory work was all performed by the Author, unless stated otherwise. All statistics were performed using SPSS version 26 for Mac and Prism version 8.4.0 for Mac.

2.1.0

Describing cough burden in patients with fibrotic lung disease

Previous pilot studies have demonstrated the burden of cough to be high in patients with fibrotic lung disease (Key et al., 2010). There has also been a suggestion that the severity of cough is likely to relate to disease progression and may predict mortality (Ryerson et al., 2011).

In exploring cough in a well-defined, large cohort of patients with fibrotic lung disease (IPF and idiopathic NSIP), I set out to determine the burden of cough and its impact on quality of life in fibrotic lung disease. With follow up data available I also wanted to better understand if there was a relationship between cough and disease progression. Such a relationship might help to suggest the mechanism of cough in fibrotic lung disease. The long duration of the PROFILE study meant that it was ideal to study mortality and its associations with cough.

2.1.1

The PROFILE Study

The Prospective Study of Fibrosis in Lung Endpoint (PROFILE) study is made of two prospective parallel observational cohorts of patients with fibrotic NSIP and idiopathic pulmonary fibrosis run simultaneously. Patients were recruited between the Royal Brompton Hospital (RBH), Queens Medical Centre (QMC) in Nottingham and district general hospitals around the Nottingham area. Both QMC and RBH are tertiary referral centres for interstitial lung disease and therefore have the multi-disciplinary teams required to make a confident diagnosis of idiopathic fibrotic lung disease. The original aim of the study was to explore potential biomarkers in fibrotic lung disease in addition to defining the natural history of the disease including in relation to exacerbations.

2.1.2

Recruitment to the PROFILE Study

The study aimed to recruit both patients with IPF and those with idiopathic NSIP. Patients received a copy of the patient information sheet (PIS) in advance of being consented for the study and were given adequate time to consider their entry. Informed written consent was provided prior to enrolment and the undertaking of study specific procedures as per Good Clinical Practice (GCP) guidelines (International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human, 2001). The study was approved by the London ethics committee (Ref 10/H0720/12).

2.1.3

PROFILE Study inclusion and exclusion criteria

The diagnosis of IPF for the purposes of PROFILE was based on the 2011 guideline for the diagnosis of IPF (Raghu et al., 2011). NSIP did not have a formal agreed diagnostic algorithm and so emphasis was put on MDT decision making at the individual sites.

Inclusion criteria for the study included:

Age of 18,

A diagnosis of probable or definite IPF or probable or definite fibrotic NSIP as based on the ATS/ERS 2010 criteria (Raghu et al., 2011).

Exclusion criteria for the study included:

Patient diagnosed earlier than 6 months prior to entry to the study.

The presence of co-existent conditions associated with the development of interstitial lung disease

Positive auto-immune profile suggestive of a possible connective tissue disease.

Participation in clinical trials of novel medications for fibrotic lung disease.

Diagnosis of a co-existent life limiting condition (defined as a predicted life expectancy under one year).

2.1.4

Study design and endpoints

The studies started recruiting in 2013 and by 2018, when recruitment closed, had 500 participants. The original purpose of the studies was to evaluate potential biomarkers in fibrotic lung disease and to better understand the natural history of the disease, including exacerbations. The size of the cohort was derived from a need to study exacerbations which were anticipated would occur in between 6-8% of the patients per year. The study was designed to provide 36 months of follow up to recruits. Premature discontinuation due to progression of disease and death was common. At the start of recruitment, therapies for anti-fibrotic therapy were only just starting to come into the clinical domain and as such many of the early patients were treated with therapies that are no longer commonly used such as cyclophosphamide and acetylcysteine. Later patients received Pirfenidone and/or Nintedanib.

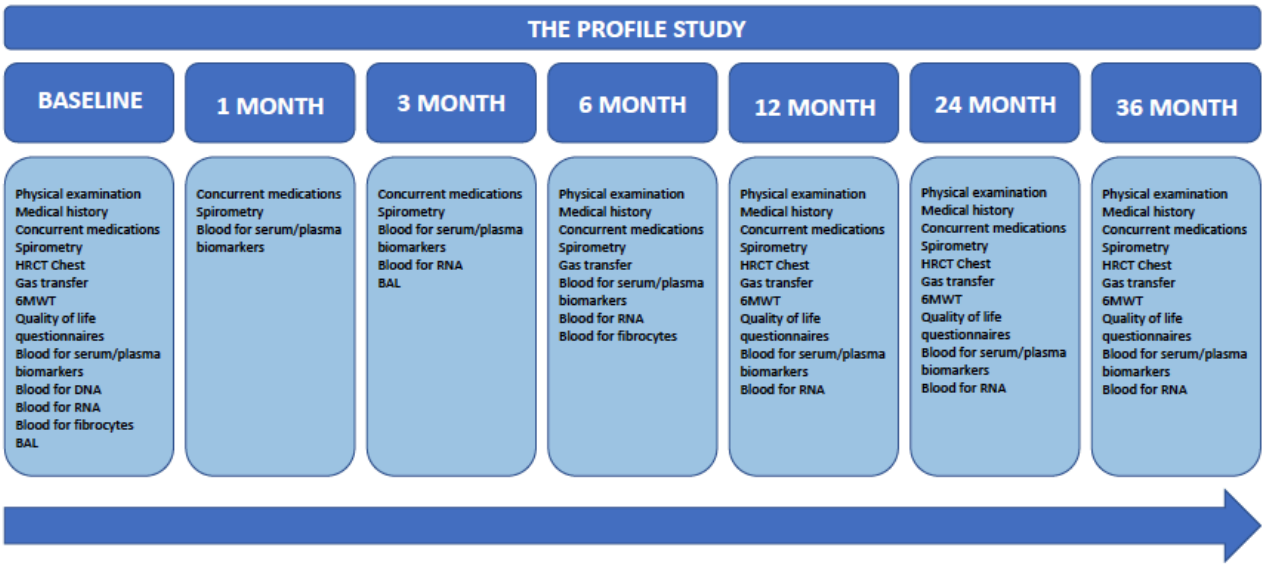


Figure 2.1 Schematic of the PROFILE study at Royal Brompton

At recruitment, many of the patients underwent a fibreoptic bronchoscopy with bronchoalveolar lavage to aid with clinical diagnosis. This procedure was more commonly performed at the time of recruitment than it is today. Essentially an absence of lymphocytosis on BAL cell differential helped to support a diagnosis of idiopathic pulmonary fibrosis in the context of the correct patient demographic, absence of exposures or autoimmune history and suitable imaging. It is worth noting that this is not a procedure that the 2011 guidelines recommended (Raghu et al., 2011). Patients had serial lung function testing and completed quality of life questionnaires at each visit – between Nottingham and RBH there were subtle differences in which measures were repeated at which timepoint. Smoking history was recorded at the time of enrolment. The Leicester Cough Questionnaire was completed at baseline for all patients.

2.1.5

Pulmonary function testing

Pulmonary function tests were recorded at baseline and then all subsequent visits. Gas transfer measurements were not recorded at 1 and 3 months and in some of the sites associated with Nottingham, were not recorded in other visits due to lack of availability. Tests were all done in approved clinical lung function laboratories by appropriately trained staff, although equipment was not standardised between recruiting sites. The sequential lung function data allowed progression to be estimated.

Both the GAP stage and CPI scores for patients in the PROFILE cohort were calculated.

2.1.6

Subjective measures of cough within the PROFILE study

The Leicester Cough Questionnaire (LCQ) contains nineteen questions crossing physical, psychological and social domains. It demonstrates quality of life in relation to cough and as such is a subjective measure of cough impact. A higher score indicates a better quality of life related to cough. The questionnaire was designed in an outpatient population of patients with chronic idiopathic cough (Birring et al., 2003a). The questionnaire was developed in three phases: the first generated 44 questions as a result of MDT discussion and structured interviews with cough patients. Each question was responded to using a 7-point Likert scale. In the second phase, patients completed the 44-item questionnaire and ranked each question

for its importance to them. This resulted in item reduction to 19 questions. Subsequently the questionnaire went through a third phase of testing in which the repeatability and responsiveness of the tool was assessed. After its initial design, the minimal important difference for the questionnaire has been developed (Raj et al., 2009) making it a useful tool for intervention studies. Sequential LCQs were performed at the Royal Brompton but not at Nottingham. There is already some data to suggest LCQ improves with a reduction in cough burden in IPF patients who were treated with Pirfenidone (van Manen et al., 2017) suggesting that the LCQ is responsive to changes in cough quantity. The LCQ and its relationship with cough frequency has already been evaluated in a small cohort and that suggested there was a modest correlation i.e. LCQ does inform us about cough frequency as well as impact (Key et al., 2010).

The mean LCQ across the group and its standard deviation will demonstrate cough's impact on quality of life in this large population. The Cronbach alpha statistic will be calculated for the LCQ to demonstrate the internal consistency of the LCQ within fibrotic lung disease. This will be the first time the reliability of the LCQ in IPF has been measured in a large cohort. The serial LCQ changes will help show the effect of time on cough, although no control can be made for prescription of medications which may have an anti-tussive action since this data was not recorded. Simple linear Pearson correlations examining LCQ and measures of lung function will be performed to see if there is correlation to suggest that LCQ changes with decline in lung function. This would be important as evidence of cough worsening with disease progression. The LCQ scores can be modelled against parameters of disease activity such as lung function tests in a mixed effects model to demonstrate the factors and variables that may predict LCQ.

2.1.7

Quality of life measures within the PROFILE Study

Patient's in the PROFILE study also completed the St George's Respiratory Questionnaire. This tool was developed in 1992 in patients with chronic obstructive pulmonary disease and was designed to demonstrate overall health status (Jones et al., 1991). The mean age of patients within the original cohort of patients used to develop the SGRQ is not dissimilar to that of the PROFILE patients (63 in SGRQ, 70 in PROFILE). The SGRQ has been shown to perform well in scleroderma ILD patients – with good concordance between the SGRQ and markers of physical activity such as a the 6MWT (Beretta et al., 2007). The mean SGRQ will be found from the PROFILE data and a Cronbach's alpha calculated. The relationships between SGRQ and LCQ will be examined to see if worsening cough impact on quality of life is related to overall quality of life – this will be done via the mixed effects model. A linear mixed effects model is likely to be a better fit for the data than a generalised estimated equation model due to the skew of missing data anticipated at the later time points.

To ensure that a more disease specific tool to monitor quality of life was used, the ATAQ-IPF questionnaire was also completed by patients. The tool was developed in 2010 and comprises 74 different questions (Swigris et al., 2010b). The questions were initially formulated in a sequence of patient interviews and focus groups, generating 207 possible items. 95 patients then completed the 207 questions to allow data reduction based on questions that did not generate strong responses and then using Rasch analysis. Cough is examined in 6 out of 74

questions in the final ATAQ-IPF. Interestingly in the original description of the ATAQ-IPF tool, cough (as measured by the tool) was shown to correlate with FVC, although the correlation was weak and other measures of function such as TLCO and 6MWT did not correlate with cough. This would suggest that ATAQ-IPF is not that useful in measuring severity of disease or indeed functional impairment. It perhaps better captures psychosocial consequences of having a chronic life limiting disease. Relationships between lung function and ATAQ-IPF scores will be examined using Pearson correlations to assess any possible relationship between disease severity and ATAQ-1PF score in this larger cohort. The relationship between ATAQ-IPF and LCQ can be measured as part of the mixed effects model.

2.1.8

Determining mortality within the PROFILE cohort

Kaplan Meir curves were planned from the PROFILE data. Separate curves would be determined for overall survival and then survival would be broken down into different levels of lung function impairment – in part based on the work of Egan et al(Egan et al., 2005). An exploratory survival analysis was based by dividing the cohort based on cough and quality of life data from LCQ and SGRQ scores.

2.2.0

Objective measures of cough in fibrotic lung disease

Subjective measures of cough such as its impact on quality of life as measured by LCQ do not tell us about the frequency of cough. A weakness of the PROFILE cohort is that there was no

attempt to capture objective markers of cough. To demonstrate cough frequency, cough must be directly measured. Ambulatory digital cough recorders worn for 24 hours can be analysed to count coughs. The Vitalojak is a digital, semi-automated device for counting coughs. Two microphones record sound, one attached to the patient's chest and the other to clothing or pillow at night. The chest wall microphone ensures that a cough sound is from the patient and not from the surrounding environment. The recorder records all sound over a 24-hour period. Two .WAV files are created on the internal SD memory card – one for each of the recording microphones. These recordings are 24 hours in length. The resulting sound files are processed digitally to remove all silences and non-cough sounds, resulting in a truncated sound file. This is then analysed manually to count all the cough sounds. This data can then be described in terms of mean coughs/hour/24 hours or mean coughs/hour/day or night.

The Vitalojak is the only validated ambulatory cough counting device (Smith et al., 2006b). In this small study, the digital recordings of cough made using the Vitalojak were shown to have excellent concordance with simultaneous video recordings. The video recordings required real time analysis and as such took significantly longer to analyse than the digital cough recordings.

At the time of embarking upon this research, objective cough recording had only been described in a very small group of IPF patients (Key et al., 2010, K McGuinness, 2012). In the study by Key et al (Key et al., 2010), reproducibility of objective count counts using the Vitalojak in idiopathic pulmonary fibrosis, 11 patients had repeated recordings (median 11 days apart). This reproducibility study showed that cough recordings were highly

reproducible with a mean difference in cough/hr/24hr of 0.3. No data existed as to whether cough frequency changed over time in patients with fibrotic lung disease.



Figure 2.2 The Vitalojak cough recorder

2.2.1

Collecting a new cohort of patients to measure cough objectively

In order to determine the burden of cough in fibrotic lung disease it was necessary to collect a second cohort of patients. These were recruited under a modification of the original PROFILE study ethics.

Inclusion criteria:

Age over 18 years old

Investigator determined IPF based on the 2011 ATS/ETS/JRA/ALAT guideline

Exclusion criteria:

Patient diagnosed within 12 months of entry to the study (later adapted to 12 months due to low recruitment).

The presence of co-existent conditions associated with the development of interstitial lung disease

Positive auto-immune profile suggestive of a possible connective tissue disease.

Participation in clinical trials of novel medications for fibrotic lung disease.

Diagnosis of a co-existent life limiting condition (defined as a predicted life expectancy under one year).

Patients were identified from a list of patients referred for management of IPF, who were coming for fibreoptic bronchoscopy as part of their clinical work-up. Computed tomography (CT) Imaging was reviewed prior to approaching the patient to ensure it was consistent with a diagnosis of IPF as per the 2011 guideline. Patients were sent a patient information sheet (PIS) for the study prior to coming for the procedure and were then consented and enrolled on the day of the procedure. The aim of using patients diagnosed on the basis of clinical history review and imaging prior to an MDT diagnosis being made was to capture patients prior to them undergoing fibreoptic bronchoscopy so that BAL could be utilised as part of the research.

52 patients were prospectively recruited from the Royal Brompton Hospital clinical ILD service with the specific aim of performing repeated objective cough recordings alongside subjective measures. This would allow the relationship between subjective and objective measures to be determined.

In this cohort it was anticipated that mortality was likely to be a rare event due to a planned follow up of 1 year. Progression would therefore be calculated using either decline in

absolute FVC of $\geq 10\%$ or a decline in absolute TLCOc of $\geq$15% as defined in the 2000 ATS Guideline for the diagnosis and management of IPF (King, 2000).

2.2.2

Determining the size of the cohort

The sample size of 50 was based on feasibility of recruitment within the time available as there was insufficient data on objective cough in fibrotic lung disease to perform a power calculation in advance of the study. Recruitment was extended slightly beyond 50 to allow for premature withdrawal of patients.

2.2.3

Design of the study

Upon recruitment patients performed a baseline visit. They were then seen every 3 months for one year of follow up. Recordings were performed at baseline and then three-monthly for one year.

The patient was sent away from each clinic attendance wearing the Vitalojak and was given instructions to remove the device at 24 hours and return it within a prepaid envelope. When the recorder was attached, a single use adhesive microphone was stuck to the anterior chest wall over the sternum. A second clip on microphone was attached to the patients collar with instructions that at night, this microphone should be clipped to the pillow. The Vitalojak was worn in a bag attached to the waist or that could be worn over the shoulder. Patients were

encouraged to partake in normal activities whilst wearing the recorder, although the necessity of coming to hospital to have the recorder attached clearly affected the activities they did on the day. Some patients had considerable journeys to the hospital and as such recordings may not have reflected typical behaviour.

At each time point quality of life questionnaires including those that subjectively measure cough (cough VAS, LCQ) were completed. This would allow comparison of objective and subjective measures of cough to better understand if they are measuring the same thing in this group of patients. The repeated measures would determine if cough was a continuous symptom or whether it varied in severity over time. This could potentially show if there is association between disease progression and cough.

As per the original PROFILE study, ATAQ-IPF. LCQ, HADS and St George's Respiratory Questionnaire were also completed by patients as a tool to demonstrate general quality of life and its change in. In addition the cough visual analogue scale (VAS) was completed because in COPD this measure has been shown to have moderate correlation with objective cough counts (Smith et al., 2006a). At the outset of the research, the assumption was that in these progressive conditions, quality of life would diminish over time. Some patients with fibrotic lung disease have a much slower disease trajectory and it was important that the group was not skewed towards slower progressors since this might affect detection of difference in symptoms over time. By documenting both lung function and quality of life over time it was possible to see whether slow progressors were over-represented.

2.2.4

Objective cough count data output and handling

Sound recordings from the Vitalojak were recorded as .wav files and stored on digital memory cards. These recordings were then uploaded locally and were transferred electronically to the University of Manchester, where staff from Professor Jacky Smith's laboratory truncated them using proprietary software and then performed the manual counts. The resulting counts were then returned to the Author for analysis.

To be able to compare the objective and subjective measures of cough it was necessary to log transform the objective cough counts. The rationale for this is that cough counting gives positively skewed data. Most of the statistical methods used assume normality of data and thus by log transforming the cough data, a more normal distribution is achieved.

The study was not designed to allow evaluation of response to therapeutics since this would have required much tighter control of prescribing over the year. Nearly all of the patients ended up either receiving antifibrotic therapies (Pirfenidone or Nintedanib) or anti-inflammatory therapies such as steroids. I did document whether drugs known to be anti-tussive in nature were being used such as the neuropathic agents or opiates. Drugs known to have cough as a side effect were also documented such as angiotensin converting enzyme (ACE) inhibitors, since these may artificially inflate cough burden.

2.3.0

Evaluating the role of bacteria in the pathogenesis of cough in IPF

Changes in the lung microbiome have been demonstrated to occur in patients with fibrotic lung disease (Molyneaux et al., 2014). Furthermore, in exacerbations of IPF, the total bacterial burden is significantly increased. In unpublished data from Professor Belvisi's laboratory at Imperial College, some species of gram-negative bacteria have been shown to be able to stimulate sensory neurones and trigger cough by ATP release. Furthermore, a clinical study looking at organisms isolated in patients with acute cough demonstrated that finding bacteria or viruses on throat swab increased the severity of reported symptoms compared to those in which no organism was identified (Ordonez-Mena et al., 2019).

2.3.1

Fibreoptic bronchoscopy and BAL

Thirty eight of the 52 patients recruited to the PROFILE study for objective cough monitoring underwent a diagnostic flexible bronchoscopy as part of the evaluation of their fibrotic lung disease. The procedure was only performed in those where it was clinically indicated. The volume of BAL permitted to be used for research depended on the total return – sufficient BAL for microbiology and cytology assessment for clinical diagnosis had to be available prior to allocating any sample to research.

Bronchoscopy was performed at the Royal Brompton under midazolam sedation and via an oral intubation with mouthguard. Bronchioalveolar lavage samples were taken during the procedure (240mls of buffered saline 0.005% sodium bicarbonate divided into 60ml syringes and then slowly flushed into the right middle lobe 60mls at a time followed by maximal aspiration of sample). The samples were then put into 200ml Falcon (Fisher Scientific, Leicestershire, UK) tubes and stored on ice until they were taken to the laboratory. Samples were then stored at -80 degrees Celsius until analysis was performed. Patients were monitored for up to 4 hours post procedure.

2.3.2

Bacterial DNA extraction from whole BAL

I followed the same protocol described by Molyneaux et al for the recovery of bacterial DNA from the BAL sample and its subsequent quantification using 16S recognition (Molyneaux et al., 2014). This protocol is adapted from work done in 2000 on identifying bacterial species from soil samples by Griffiths (Griffiths et al., 2000).

Whole BAL (1.5mls) had been stored at minus 80 degrees Celsius post collection and was then thawed on ice at the time of extraction. The samples were then centrifuged at 21200 relative centrifugal force (rcf) for 30 minutes to create a bacterial pellet. Supernatant was removed and then the pellet vortexed in 100µl of supernatant. The resulting suspension was transferred into lysing matrix E tubes. These tubes contain ceramic, silica and glass spheres which when spun enhance cell lysis. The bacteria in the pellet were then inactivated by being incubated for fifteen minutes with 500µl CTAB (cetyltrimethylammonium bromide)

buffer. 500µl of Phenol: Chloroform: Isoamyl alcohol was then added to the tube and the tube was placed in a bead beater for 1 minute, resulting in cell lysis. The tube was then centrifuged at 16100rcf for 5 minutes. The liquid portion from the spun tube was transferred into a new locking Eppendorf (Eppendorf AG, Hamburg, Germany). This new tube was then centrifuged at 2080rcf for 2 minutes causing separation of the aqueous and alcohol sections. The aqueous section was pipetted into a fresh Eppendorf (Eppendorf AG, Hamburg, Germany) to which a further volume of Chloroform: Isoamyl alcohol was added, shaken and then centrifuged to cause separation. The aqueous section was then transferred again to a fresh tube which had 1µl of LPA (linear polyacrylamide) in it, which encourages precipitation of DNA. To this, 2 volumes of PEG/NaCl was added and the resulting solution was vortexed and then left in the refrigerator overnight.

The resulting purified DNA was sent away for bacterial burden quantification and species analysis. This is achieved by the identification of the 16S ribosomal RNA coding gene sequence which is conserved in almost all bacterial species.

Objective measures of cough will then be correlated with total bacterial burden and the counts of each phylogenetic group using Pearson correlations.

2.4.0

Evaluating the role of the *MUC5B* promoter polymorphism in cough in fibrotic lung diseases.

The *MUC5B* promoter polymorphism (rs35705950) is the most common genetic abnormality found to be present in patients with idiopathic pulmonary fibrosis (Moore et al., 2019, Seibold et al., 2011). Mechanistically, the presence of the abnormal allele causes overexpression of a key constituent of mucous. On the basis of a feasible mechanism for increasing cough and previous data to suggest this was the case (Scholand et al., 2014), I chose to evaluate this specific genetic polymorphism for its relationship with cough.

2.4.1

DNA Extraction from whole blood

Patients recruited to the original PROFILE cohort and to the subsequent cough cohort all had blood taken at baseline in EDTA tubes for genetic analysis. Patients were consented for the taking of blood for genetic analysis and were informed that the results of the analysis would not be fed back to them. The blood samples were then stored at minus 80 degrees Celsius to permit analysis to be performed in batches.

DNA was extracted from these EDTA blood samples using the commercially available Qiagen extraction kit (Gentra Puregene). Blood samples (6mls K2 EDTA tube) were thawed and then mixed with 18mls red cell lysis buffer in a 50ml Falcon tube (Fisher Scientific, Leicestershire, UK). The mixture was then inverted 10 times, before being incubated for 5 minutes at room

temperature and then centrifuged for 2 minutes at 2000xg to create a white cell pellet. Post centrifuge, the supernatant was discarded and the white cell pellet vortexed with the small amount of residual supernatant. This was then mixed with 6mls of cell lysis solution before being vortexed to mix. 30µl of RNAse solution was then added to ensure that the DNA was not contaminated with RNA. The mixture was then inverted 25 times before being incubated at 37 degrees Celsius for 15 minutes and then placed on ice for 3 minutes. Protein precipitation solution was then added to cause the precipitation of DNA. This was then vortexed and centrifuged at 2000xg for 5 minutes to produce a pellet. The resulting DNA pellet was washed with 6mls of isopropranolol and then 6mls of 70% ethanol. The DNA pellet was then dissolved in DNA buffer (600µl). The resulting solution was stored at room temperature for 24 hours and then was nanodropped to ensure adequate concentrations of DNA were present. The solution was then refrozen at -80 degrees Celsius and stored.

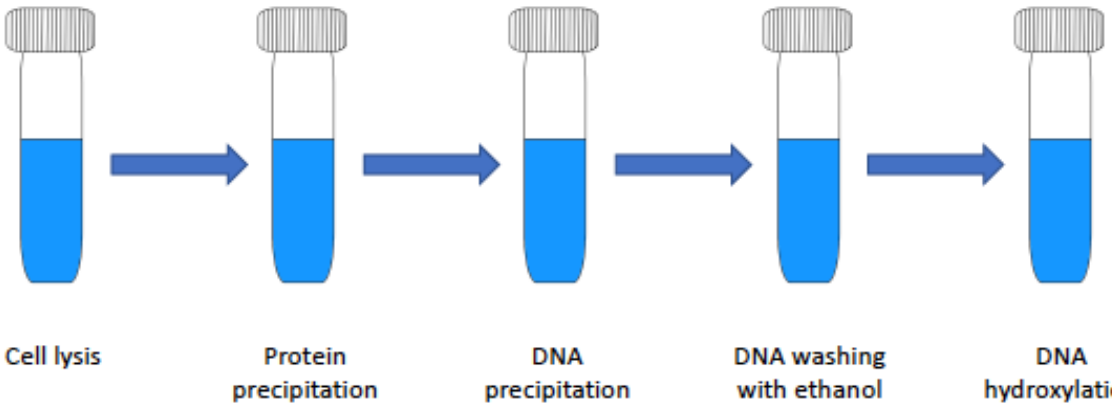


Figure 2.3 DNA extraction from EDTA blood samples

The DNA was sent to the University of Washington Northwest Genomics Centre, USA where full genomic sequencing was performed as part of a separately funded study (Moore et al., 2019). This laboratory has extensive experience of performing full gene sequencing in

idiopathic pulmonary fibrosis (Seibold et al., 2011) and processes large volumes of samples. This enabled better accuracy in the processing of samples than if they had been processed in house. The DNA sent to them was transferred into 96-well plates using the Freedom EVO liquid handling system. 500ng of DNA (50uL at 100ng/uL) was added to each well. Control samples were used on each plate. The initial quality control involved checking the sex of the patient (as determined by the DNA analysis) matched that on the clinical details along with checking the total amount, concentration and integrity of the DNA was adequate. Failed samples at this point were not processed further. Shotgun fragmentation of the DNA was performed using acoustic sonication (breaking DNA into smaller fragments) and the resulting fragments underwent end-polishing. The shotgun library DNA was then hybridised with biotinylated capture probes for 72 hours. The enriched fragments were then captured with streptavidin beads and PCR amplified. A HiSeq 4000 sequencer was then used to read the resulting enriched fragments.

From this the *MUC5B* promotor polymorphism was analysed and grouped as being either wild type (GG), heterogenous for the SNP (GT) or homozygous for the variant SNP (TT). Both objective cough counts and subjective cough measures were then compared between the different genotypes.

2.5.0

Statistical plan

Supplementing the statistical tools already mentioned in this chapter, I had planned to analyse data from the PROFILE study using SPSS version 26 and Prism 8.0.4. All tests will be considered significant if the two-sided p value is <0.05.

Demographic data from both cohorts will either be presented as percentages of the whole for categorical values or as mean values with standard deviations for continuous ones. For measures of lung function from the study, mean values with standard deviations will be presented. For the LCQ, mean and median values will be presented.

Kaplan Meir survival curves based on death as a primary outcome will be calculated for the original PROFILE study and then redrawn subdivided by both markers of lung function and LCQ. Mantle Cox log rank tests will evaluate the differences between the curves.

When dealing with quality of life questionnaires, domain or individual question results will be presented along with total scores. Alpha Cronbach statistics will be calculated for these questionnaires to show their internal reliability in fibrotic lung disease.

Where tests from the same patients are being compared over two different time points, a related samples Wilcoxon signed rank test will be used to compare differences between the two time points.

Objective cough counts will be presented in the form of coughs/hr or coughs/hr/24hrs. When used in correlations or statistical models, they will be log transformed to produce normally distributed data.

Simple Pearson correlations will be performed between markers of cough (LCQ, VAS, objective cough counts) and markers of lung function and quality of life to see if associations exist. These will then be further tested in either general estimating equation or a general linear mixed model depending which is more appropriate. The latter is more conservative in its assumptions but is able to cope with non-random missing data better.

2.6.0

Summary of plan

To demonstrate the burden of cough I will analyse subjective cough and quality of life data from the PROFILE cohort. I will collect a new cohort of patients to perform objective cough recordings and subjective measures of cough in. I will correlate the objective and subjective markers to determine if there is a relationship between the two.

To determine whether cough is related to the disease process I will correlate subjective and objective cough against the markers of lung function – FVC, TLCO and composite physiological index (CPI). This final marker has been previously shown to be better at predicting mortality in IPF compared to any single marker of lung function and is derived from the FEV1, FVC and TLCO (Wells et al., 2003). I will perform a survival analysis using Kaplan Meir curves to determine whether survival is influenced by lung function and by

cough as measured by LCQ. I will examine the relationship between *MUC5B* genotypes and cough.

To determine the role of bacteria in cough in fibrotic lung disease I will examine bronchoalveolar lavage fluid from patients recruited to the cough cohort and correlate bacterial burden and phyla burden with objective cough.

3.0
RESULTS FROM THE PROFILE STUDY – NATURAL HISTORY OF DISEASE AND CHANGES IN QUALITY OF LIFE

3.1.0
Description of recruitment

The PROFILE cohort was formed from two parallel prospective studies of patients with fibrotic lung disease collected between Nottingham University Hospital and Royal Brompton Hospital in London. The Nottingham cohort was collected from several district general hospitals feeding into the Nottingham referral centre and as such may have included patients who were earlier in the disease process than those collected in London. The study followed patients up over time with repeated measures of lung function, quality of life and biomarkers. First recruitment occurred in September 2010 with the final recruitment occurring in November 2015. Each site recruited over 250 patients with 579 patients recruited overall. The first patient death within study occurred in December 2010. Data taken from the study was censured on 1st October 2016.

Being an observational cohort designed to follow the natural history of IPF, The PROFILE study did not control for medication use in study. This is potentially important because the use of anti-tussive medication such as opiates, amitriptyline and gabapentin was not taken into account. Cough severity as reported in the study therefore may be biased by the free use of medications which may affect cough.

Similarly measures of reflux and PPI usage have not been recorded in this cohort. The use of proton pump inhibitors has been shown to be very common in other IPF registries (Jo et al.,

2019). Whilst symptoms of reflux appeared common in a prospective study of reflux in IPF performed by the Hull group, measured exhaled breath condensate did not appear to show increased markers of reflux in IPF compared to controls (Fahim et al., 2011). A study in 2019 looking at the effects of omeprazole in IPF showed it may potentially reduce cough – although 16/40 of the patients in this study had already been on a proton pump inhibitor prior to recruitment (Dutta et al., 2019).

Anti-fibrotic prescription was not common when the study first started recruiting, although became more common towards the end of recruitment. The use of anti-fibrotic medication was not controlled for in the analysis of study data.

The goals of studying this large patient cohort were to better understand cough's impact on quality of life in fibrotic lung disease. This is the largest cohort to report the use of LCQ in pulmonary fibrosis patients and as such helps to show the reliability of this tool and change in responses over time. The study allowed LCQ to be compared with both the St George's Respiratory Questionnaire and the ATAQ-IPF (IPF specific quality of life questionnaire) to better understand whether cough impact and overall quality of life are related. Finally, it also allowed exploration of the changes in quality of life and cough that occur with disease progression.

3.1.1

Patient characteristics in the PROFILE study

77.9% of patients in the study were male and 69.2% had a history of either being an active or past smoker. 177 patients had never smoked. In the smokers, the average smoking history was 26.9 pack years. This phenotype is typical for a cohort of patients with fibrotic lung disease. 90.2% patients recruited were suffering with idiopathic pulmonary fibrosis whereas the remainder had idiopathic non-specific interstitial pneumonia.

3.1.2

Patient dropout and death during study

Progressive drop out was seen during the study with 404 patients attending the 12-month follow up visit, 270 attending the 24-month visit and only 143 attending the final 36-month visit. This pattern of dropout was anticipated given that patients had a progressive disease with increasing symptom burden over time and the majority of dropout was due to patient death. Recruitment to the study started during a time in which no effective therapy for IPF was available and therefore the study is a good reflection of the natural history of IPF. Currently prescribed therapies for IPF require the prescribing centre to conduct periodic patient review, but the therapies available previously could be prescribed outside of specialist centres, making it harder to retain patients to the study.

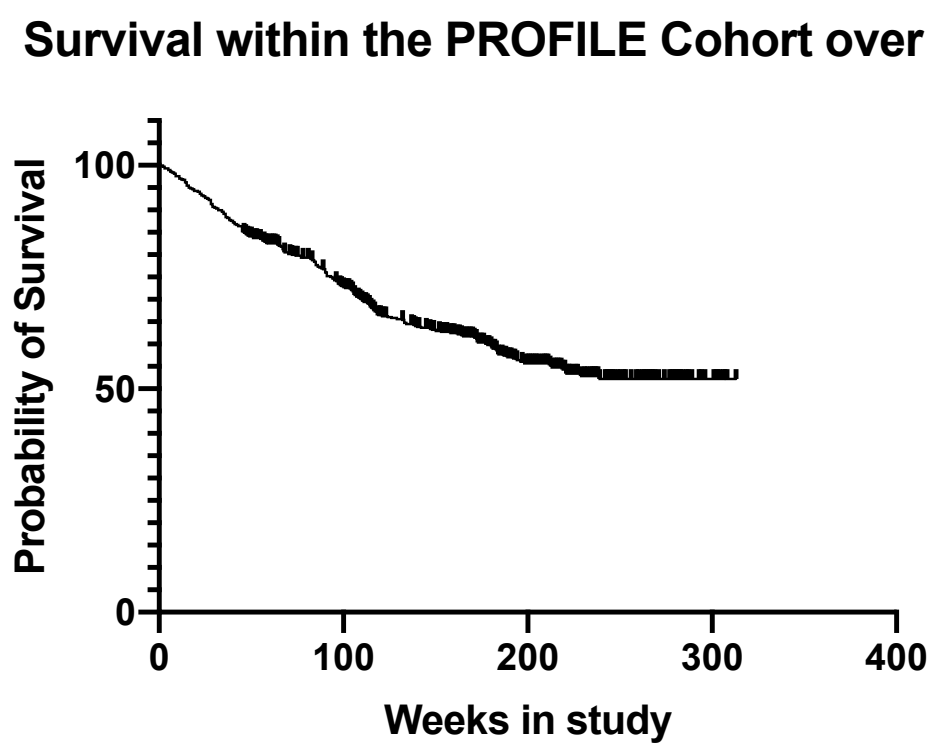


Figure 3.1 Kaplan Meir curve showing survival in the PROFILE cohort

By the censor date 40.1% of patients in the cohort had died (232 patients). Death reporting relied on either reporting by family or GP and was supplemented by checking on the National Health e-record service. It is anticipated therefore that all death was captured.

Baseline lung function affected the risk of dying during the study. This is demonstrated in the following graphs in which FVC, TLCO and the composite parameter CPI are assessed for their ability to predict outcome.

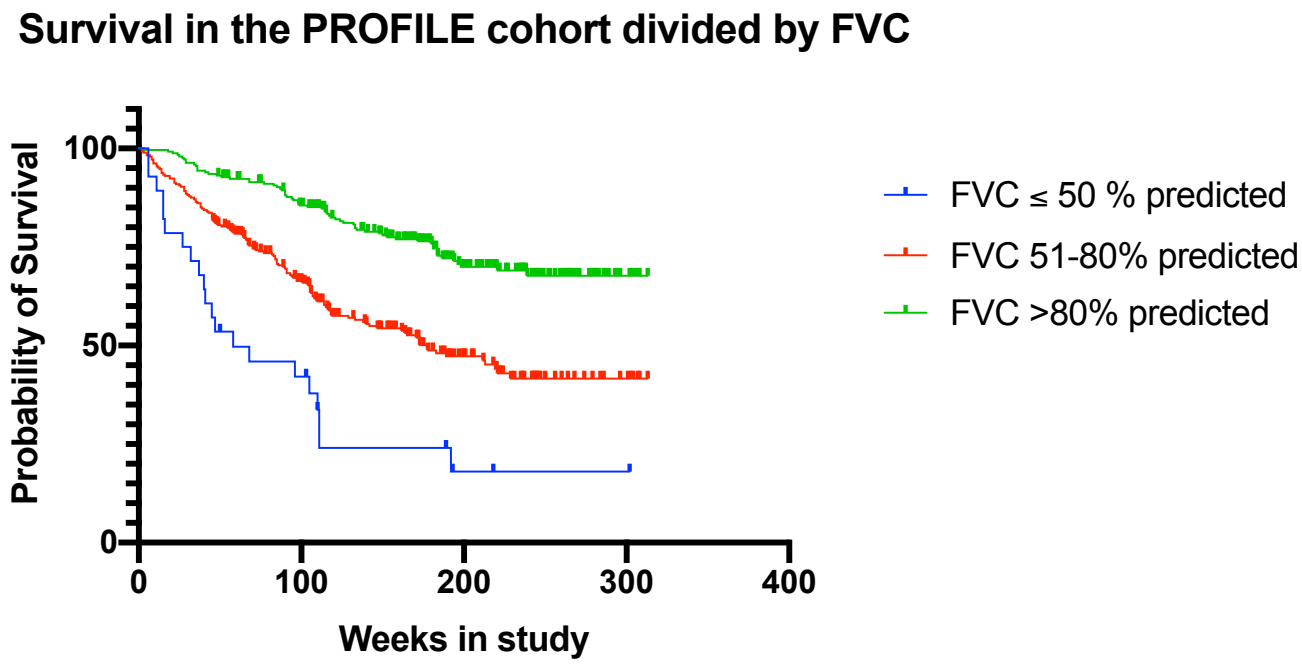


Figure 3.2 Survival curves for patients divided by baseline FVC

Patients with very low lung function as measured by FVC of 50 percent predicted or less appeared to have the worst survival. This group was a lot smaller than the other groups and therefore there is a risk that mortality within the group has been over estimated. Survival was highest in the patients with FVC greater than 80% predicted – the group that currently in the UK are unable to receive disease modifying antifibrotic therapy. Using a log-rank (Mantel-Cox) test the survival curves are significantly different with a chi-square statistic of 65.11 and a p value of <0.01.

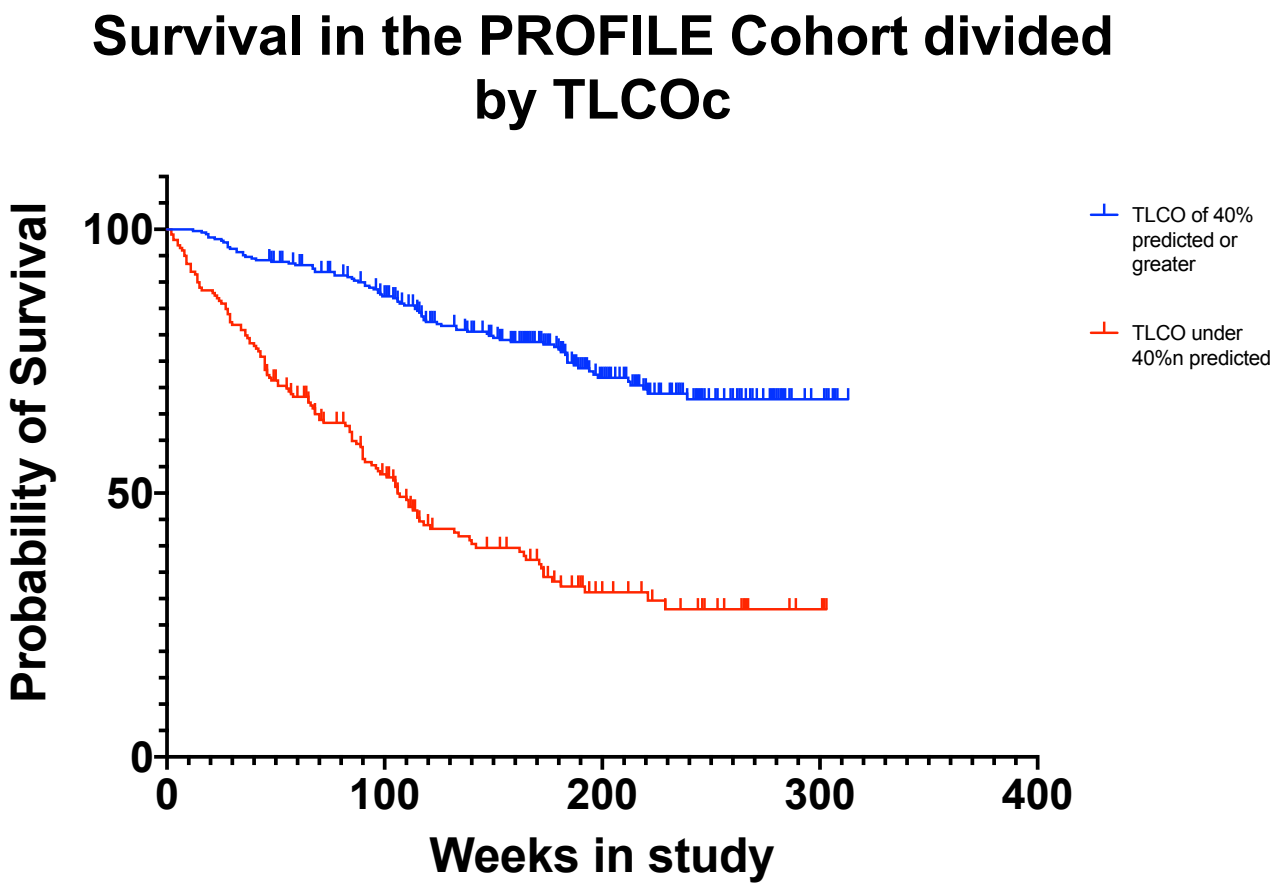


Figure 3.3 Survival curves for patients divided by baseline gas transfer

Gas transfer is potentially a more useful measure of disease severity because it tends to correlate better with functional markers such as desaturation on exertion. In figure 3.3, patients are divided into two groups – those with a TLCO or 40% of predicted or greater and those with a TLCO of under 40% predicted. These thresholds were chosen based on research from 2001 which showed this was an important threshold for predicting mortality (Mogulkoc et al., 2001). Patients with a lower gas transfer at enrolment to the cohort did significantly worse than those with a gas transfer of >40% predicted at baseline. The log-rank (Mantel-Cox) test of difference between the two groups (patients with a baseline TLCO of 40% predicted or greater vs patients with a baseline TLCO of under 40% predicted) showed a

significant difference between the survival curves with a p value of <0.01 and a Chi square statistic of 103.2.

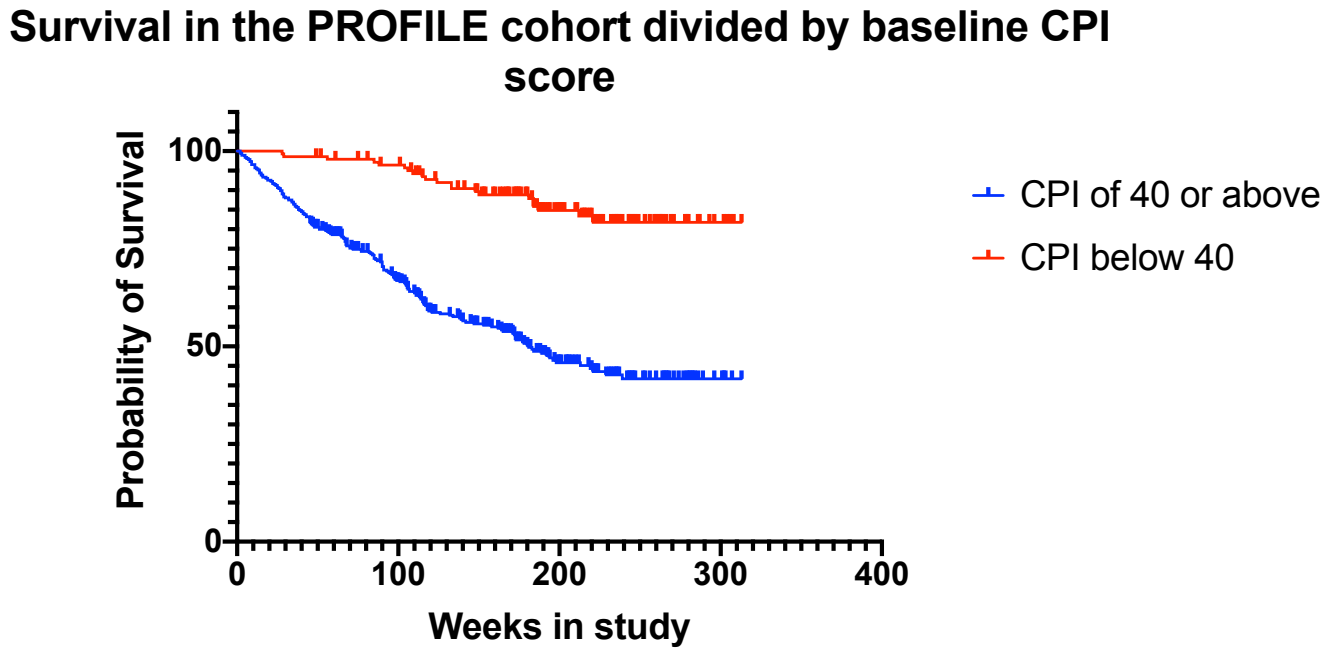


Figure 3.4 Survival curves for patients divided by baseline CPI score

The cardiopulmonary index (CPI) is a composite measurement of lung function derived from the FVC, FEV1 and TLCO (Wells et al., 2003). It has previously been shown to be a better predictor of mortality than any individual component of lung function in IPF (Wells et al., 2003). Figure 3.4 demonstrates the difference in survival for patients with a baseline CPI of 40 or above and those with a CPI of below 40 (more mild disease). Patients with a higher CPI score and therefore more severe disease at baseline had a much worse survival compared to those with a CPI score of less than 40 at baseline. The log-rank (Mantel-Cox) test of difference between the two groups (patients with a baseline CPI of 40 or greater vs patients with a baseline CPI score of under 40) showed a significant difference between the survival curves with a p value of <0.01 and a Chi square statistic of 56.98.

The death and progressive illness of patients within the study created a problem with missing data. The missing data was not random and was more likely to occur at the later study time points for reasons discussed above. In the subsequent analysis a complete case analysis method was used, i.e. cases with missing data were deleted. This does create an issue with under-representation of the effects of more severe illness on quality of life. Imputation methods of dealing with missing data such as using mean values for missing data, did not seem appropriate, given it was not known the effect time would have on quality of life and cough at the outset and therefore such a method may create overly optimistic estimates.

3.1.3

Baseline disease severity

Spirometry measurements at baseline were available for 564 of the 579 patients (97.4%) whilst 524 (90.5%) patients had gas transfer measurements made at the same time point. It was possible to calculate CPI scores for 519 patients. Lung function at the point of recruitment to the study showed moderate impairment with a mean FVC of 79.5% predicted (standard deviation 19.1) and TLCO 46.2% of predicted (standard deviation 15.4). The mean CPI was 46.5 (standard deviation 13.4).

Quality of life as demonstrated by the St George Respiratory Questionnaire was provided by 524 patients and was impaired with a mean score of 45.8 (standard deviation 20.2). The impact of cough on quality of life, as demonstrated by the LCQ was provided by 542 patients and was significant with a mean score of 15.5 (standard deviation 4.1).

3.2.0

Quality of life as measured by the SGRQ within PROFILE

The St George's Respiratory questionnaire has been used across many respiratory diseases although was developed in airways disease to highlight patient reported quality of life (Jones et al., 1991). Questions are divided into three groups – those describing symptoms, those describing physical health and those describing the impact of illness. In asthma, a 4 point change in the questionnaire result reflects a meaningful change (Jones, 2005). A study in US patients published in 2010 however suggested in IPF the minimally important difference for the SGRQ was between 5-8 points (Swigris et al., 2010a). A higher score represents a more significant reduction in quality of life, with a score of zero representing no issue with quality of life. A histogram of the baseline SGRQ scores from PROFILE is shown in figure 3.5 –524 patients had completed this at Baseline. The distribution of scores is approximating a normal distribution. The histogram was repeated for 6 -month values; 389 patients had completed a SGRQ at this point. Again, the distribution appeared normal. The median SGRQ score at baseline was 46.4. This compares to the quality of life seen in severe asthmatics with a history of recurrent exacerbation where scores of 42-50 have been seen (Nelsen et al., 2019).

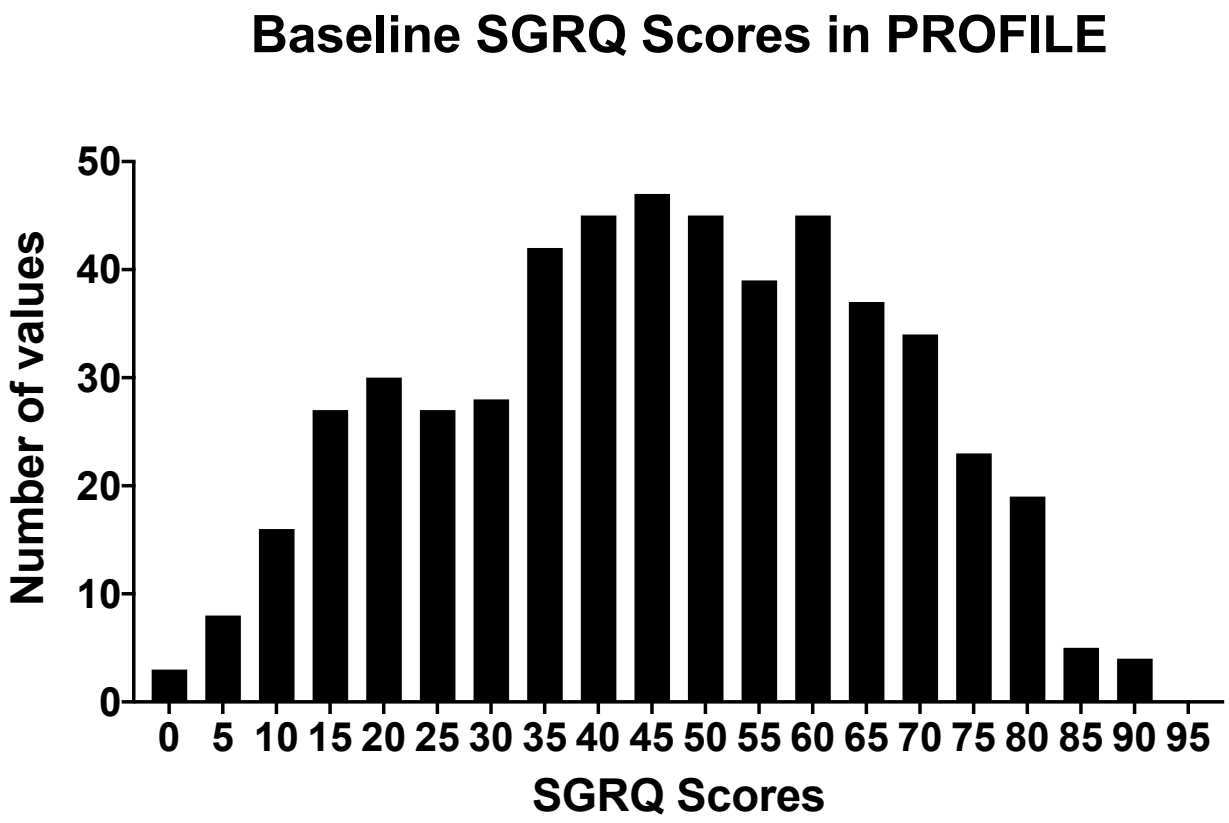


Figure 3.5 – Histogram showing the spread of baseline SGRQ scores

Within the PROFILE cohort, the symptom, activity and impact domain scores from the SGRQ were only available for 195 patients from the Royal Brompton Hospital. These scores are described in table 3.1. The Cronbach alpha for the SGRQ in PROFILE based on the baseline data symptom, activity and impact scores is 0.91, i.e. there is a high level of internal consistency.

SGRQ Baseline	Symptoms score	Activity score	Impact score	Total Score
Median	57.3	66.3	34.8	50.3
Mean	56.8	65.2	36.8	48.8

Std deviation	20.0	23.6	19.7	18.6

Table 3.1 – Domain scores at baseline for the SGRQ

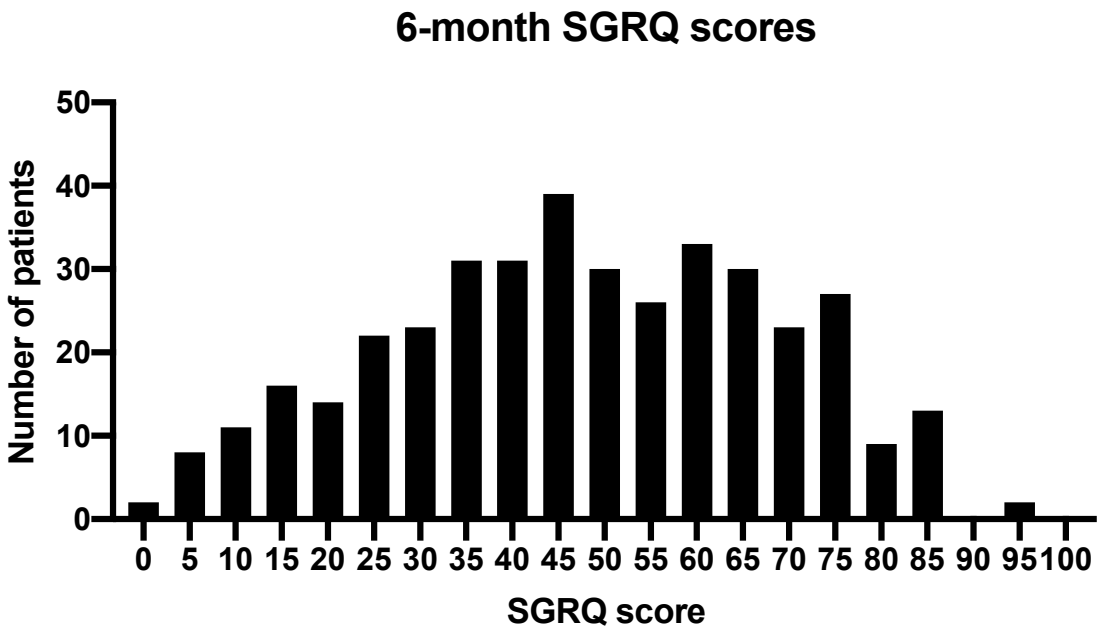


Figure 3.6 – Histogram showing SGRQ total scores at 6 months

3.2.1

Quality of life over time in the PROFILE cohort

Patients in the PROFILE cohort completed a SGRQ at baseline and then repeated the questionnaire at various time points in the study. Interestingly the mean SGRQ results at each time point were within 4 points of each other suggesting that on average quality of life was not significantly worsening during the study. A difficulty comes with bias from under-representation of more severely unwell patients in the later time points. Patients with more severe disease or more rapidly progressive disease died quicker and thus did not attend repeated follow ups. These patients would be expected to be more symptomatic and have

higher SGRQ scores. The healthy survivor effect potentially does make interpretation of the quality of life data over time difficult.

Timepoint	Mean SGRQ result	Standard deviation
0	48.8	18.6
6	45.6	22.5
12	46.6	21.2
24	48.0	21.1
36	50.3	21.4

Table 3.2 SGRQ total scores over time

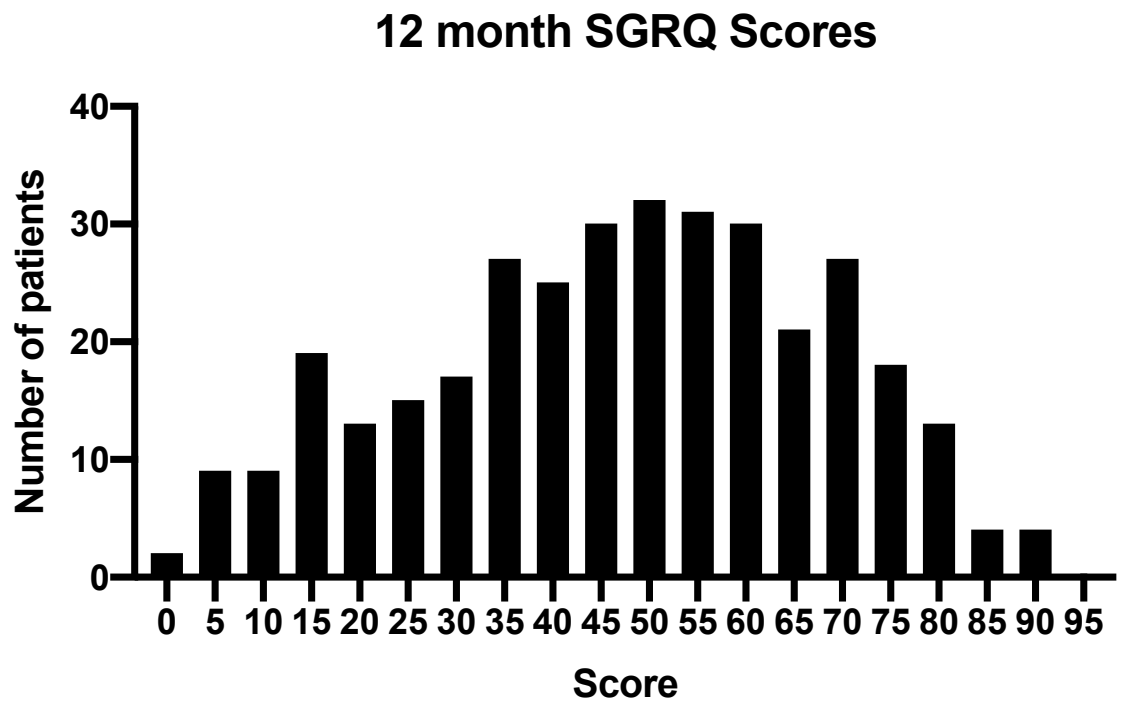


Figure 3.7 – Histogram of SGRQ total scores at 12-month timepoint

The histogram of SGRQ scores at 12 months is shown in figure 3.7. Similar to the baseline data, this follows a normal distribution. Since both timepoints follows a normal distribution a paired samples Students' T test was used to see if there is a significant difference between them. Only 100 patients from the Brompton cohort had SGRQ scores at baseline and at 12 months. In this group of patients, the mean SGRQ at baseline was 45.3 and 50.7 at 12 months. There was strong correlation between 0 and 12-month values with a correlation efficient of 0.715 and $p<0.001$ (figure 3.8). The t statistic was -3.85 with a p of <0.01. This suggests that within the 100 patients in whom both values were available, there was a significant difference in quality of life between the timepoints. The difference between means at the two time points is 5.4, just meeting the minimal important change (5-8) suggested by Swigris et al (Swigris et al., 2010a). The clinical meaningfulness however of a change that appears to only just meet the minimal important change is uncertain.

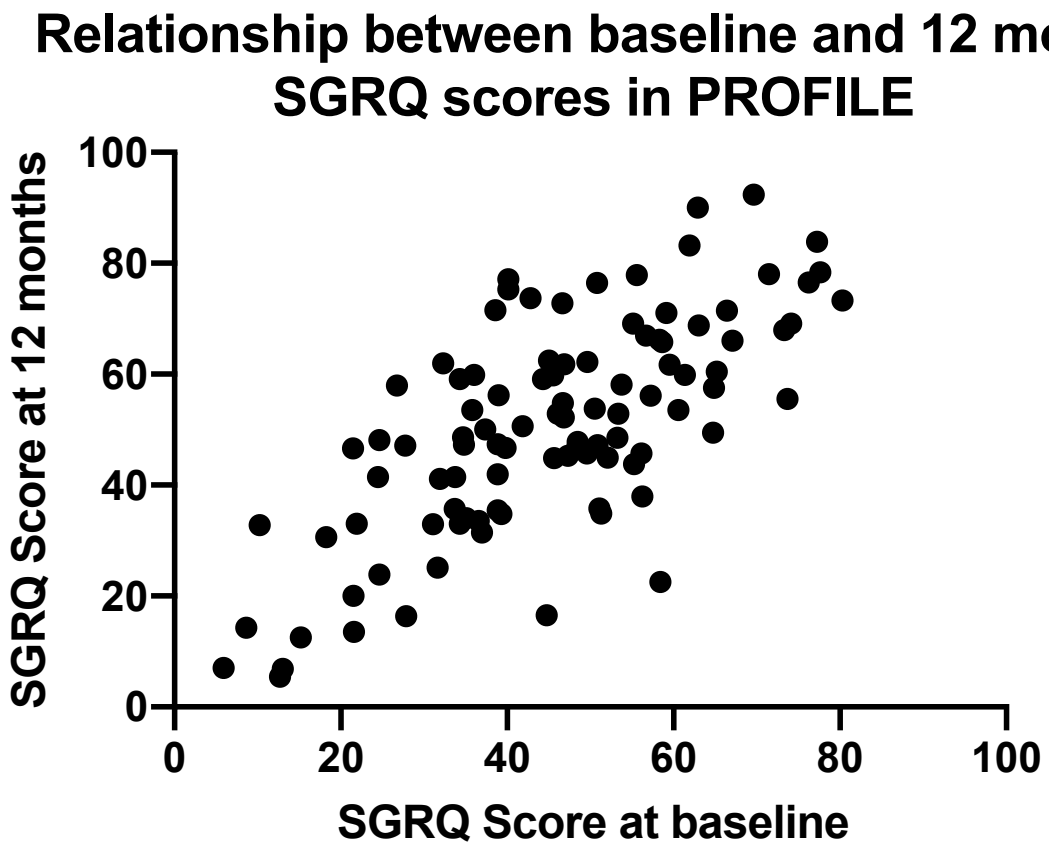


Figure 3.8 Correlation between baseline SGRQ scores and those at one year

In patients who experienced a ten percent or greater decline in FVC in the first year of follow up, there was a significantly higher baseline SGRQ than in those with slower progression. In the progressors the mean baseline SGRQ was 50.4 and in the slower progressors the mean SGRQ was 42.9. The Student's T test showed a significant difference between these means with a p value of <0.01 and the difference between the means meets the minimum important clinical difference for the SGRQ. This confirms the findings of Swigris et al that disease progression in IPF is associated with worsening quality of life (Swigris et al., 2010a).

Parameter	Wald-Chi Square	Significance
Gender	0.001	0.973
Diagnosis	1.359	0.244
Death in study	13.317	<0.01
FVC %predicted	6.662	0.01
TLCO %predicted	15.099	<0.01
CPI score	3.961	0.047
LCQ score	233.331	<0.01

Table 3.3 General Estimating Equation exploring factors and variables which predict SGRQ

Using the longitudinal data from the PROFILE study (2244 visits in total), it was possible to examine relationships of the SGRQ score with other factors in a generalised estimating equation. The rationale for this was to evaluate the strongest predictors of quality of life in IPF and to see if cough was one of those. Given that deterioration in lung function would likely lead to reduction in quality of life, markers of lung function were chosen along with death to assess in the model. Gender was chosen since women and men may perceive impairment of quality of life differently. Finally, LCQ was assessed in the model because we wanted to understand better how cough may impact overall quality of life. Using a general estimating equation (GEE) evaluating the St George Respiratory Questionnaire total score, the LCQ, FVC, gas transfer (TLCO% predicted), CPI and subsequent death were found to be predictors of score.

Interestingly gender and disease (IPF vs NSIP) were found to not predict differences in SGRQ. In a study of health-related quality of life in patients with COPD, women were found to have lower SGRQ scores compared to men of matched disease severity (Raherison et al., 2014). A study in IPF found a more complex dynamic however with similar SGRQ scores between men and women but differences in emotional wellbeing picked up by the Short Form Survey 12 (Han et al., 2010).

The same factors and variables were assessed using a generalised linear model. This model is more conservative in its assumptions and is better designed to deal with missing data that is not random. In PROFILE more of the missing data occurred in the later visits and so was not random across time points. Using this model death, FVC, TLCO, CPI and LCQ were all predictors of the SGRQ. Again, this model found that gender and diagnosis did not influence the SGRQ.

The SGRQ is seen as a useful tool in evaluating quality of life and its relationship with FVC is illustrated in chart 3.9. By using the same longitudinal data in a Pearson correlation between FVC% predicted and SGRQ, there was a significant correlation ($p<0.01$), although the correlation was very weak (Pearson statistic -0.351). This shows that disease severity is associated with quality of life as measured by the SGRQ, but that the relationship is not a strong one.

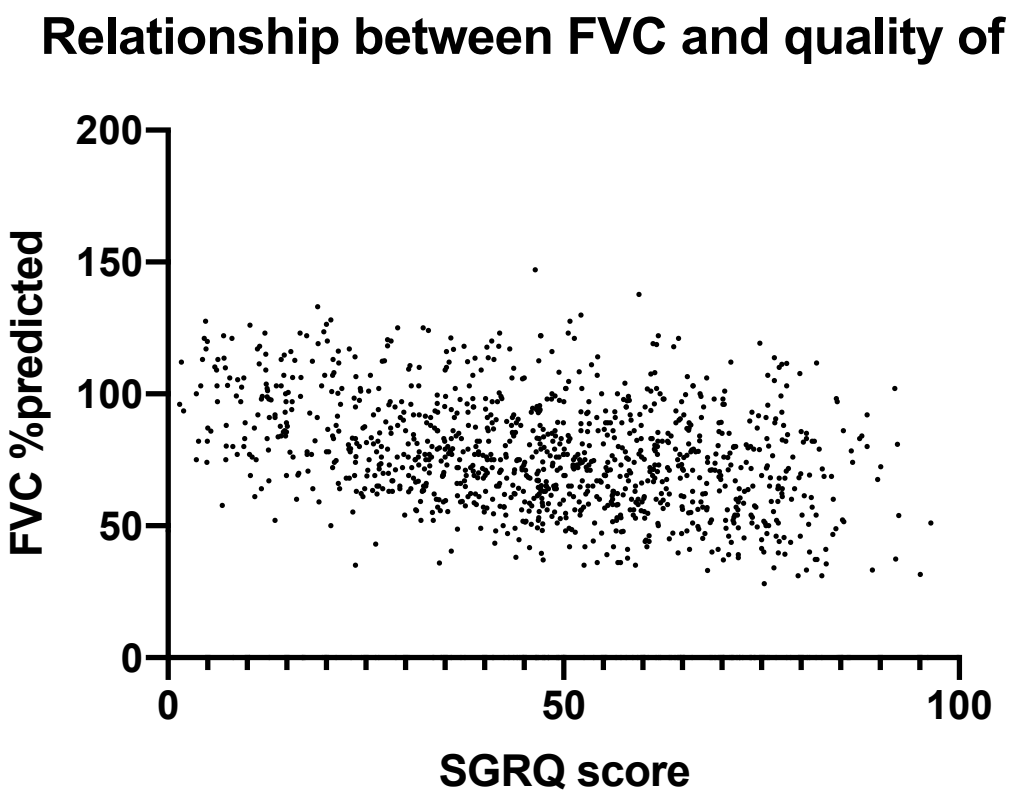


Figure 3.9 The relationship between lung function (FVC) and quality of life

The relationship with gas transfer is stronger with a Pearson statistic of -0.444 and significance of <0.05 (figure 3.10). The slightly stronger correlation here, with a lower TLCO associating with a worse quality of life is interesting. A previous study in IPF has suggested FVC better correlates with activity levels (Bahmer et al., 2016) when compared to TLCO. This highlights the complexity of measuring quality of life which may be a mix of functional impairment and psychosocial issues.

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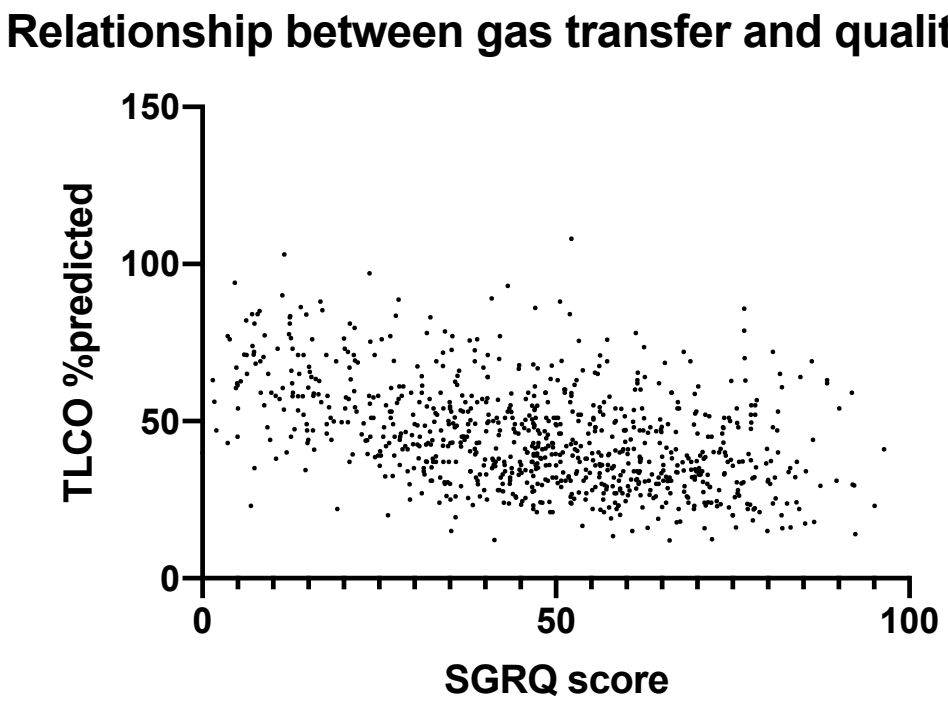


Figure 3.10 The relationship between lung function (TLCO) and quality of life

The lack of significant differentiation between IPF and NSIP in the model was tested with an independent sample T test. This showed that the mean SGRQ for IPF was 48.11 and 41.02 in NSIP. The difference was statistically significant with a p value of <0.01.

The relationship between SGRQ and death is suggestive of a decline in quality of life prior to death. This is supported by Kaplan-Meir curves drawn to show survival in groups of patients divided by their baseline SGRQ score. The patients with the highest scores and thus the worst quality of life would appear to have worse survival.

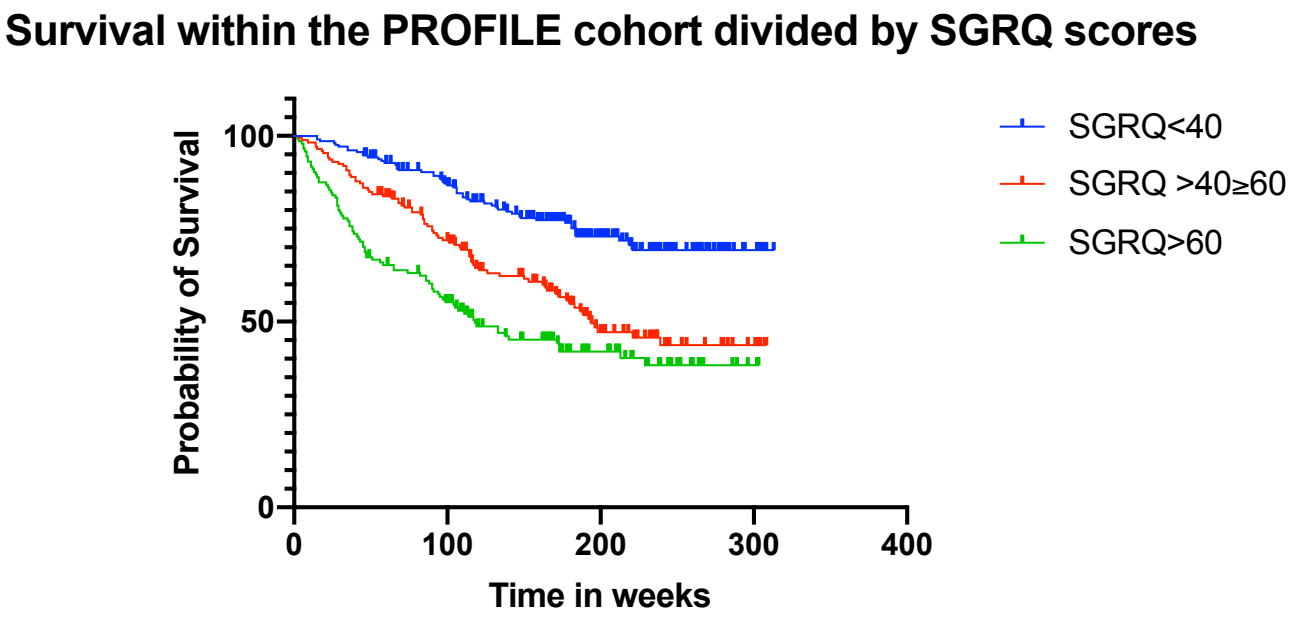


Figure 3.11 Survival in patients divided by SGRQ scores at baseline

A Log-rank (Mantel-Cox) test to assess whether the survival curves were significantly different from each other suggested a significant difference with a p value of <0.01.

3.3.0

Impact of cough on quality of life in the PROFILE cohort

The Leicester cough questionnaire is a 19-question tool which evaluates impact of cough on quality of life. The minimum difference of change for the LCQ is not known in IPF. The lower the LCQ score, the more significant the cough related impairment to quality of life is. Within the PROFILE study patients were asked to complete an LCQ at baseline and then at the Royal Brompton Hospital only, patients performed repeated LCQs over time. Patients in the Nottingham part of the cohort only completed an LCQ at baseline. The distribution of LCQ scores in the PROFILE cohort is positively skewed towards less impact from cough because

most patients would not be expected to have severe cough. The histogram of distribution is shown below.

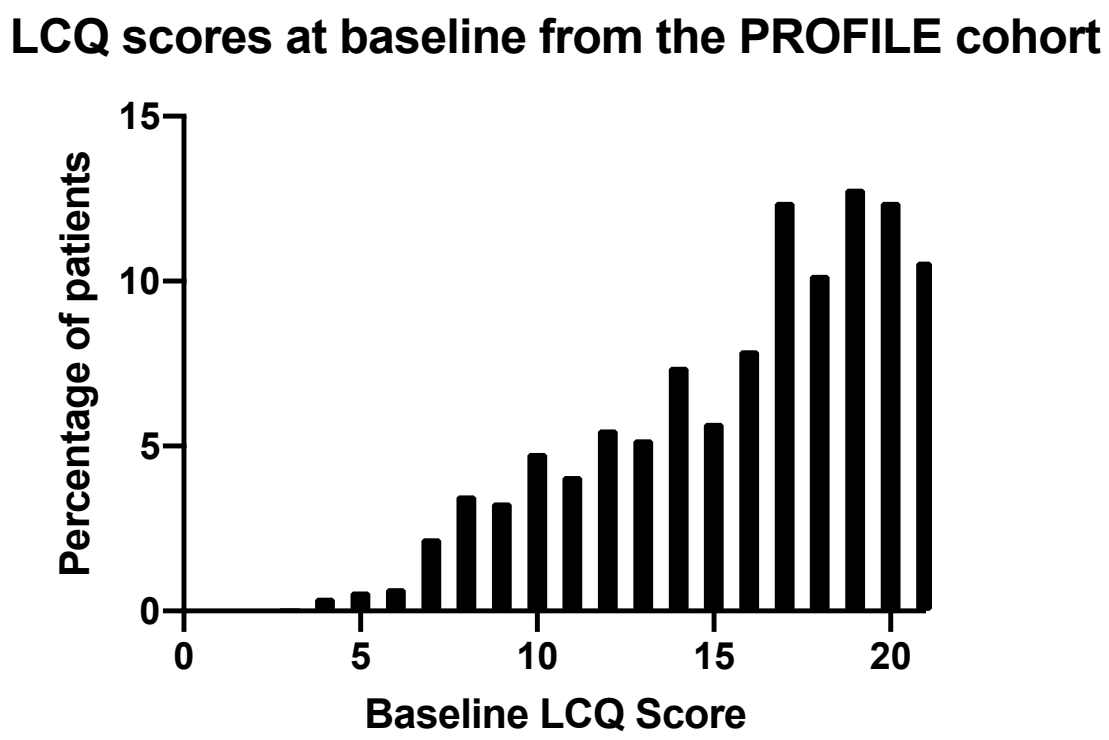
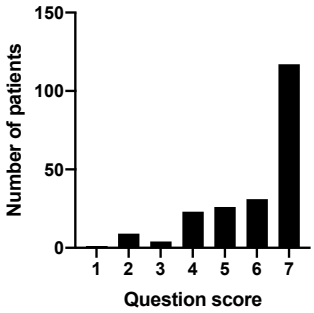


Figure 3.12 LCQ total scores within the PROFILE cohort at baseline

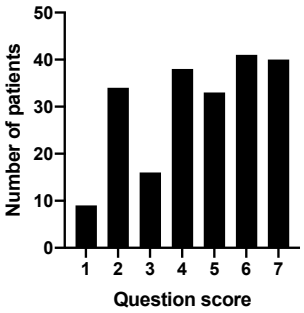
The median LCQ at baseline reflected impaired quality of life at 16.32. This compares to previously published data in airways disease where the median in LCQ in severe asthma is 15.48(Natarajan et al., 2017) and 17.8 (Fukuhara et al., 2019) in less severe asthma. In chronic cough however LCQ scores have been reported to be much lower (Zeiger et al., 2020). This may be in part due to differences in demographics with the patients in the Zieger study having a mean age of 64 and being 75% female compared to a mean age of 72 years in the PROFILE study and 73% being male. Age may be an important factor with relation to work life where cough may cause significant disruption to day-to-day activity.

Only within the Brompton patients from the PROFILE cohort was complete LCQ data available (responses to each of the 19 questions within the questionnaire). In the Nottingham patients, only the total score was available. Within the Brompton 250 patients, 216 had completed an LCQ at baseline study entry. Each individual question within the LCQ is scored from 1-7 with the lower value representing a more significant impact. The questions are sub-grouped as being within the physical, psychological or social domains. I evaluated the responses for each of the 19 questions from these 216 patients to see if any components of the LCQ were of less importance to patients with IPF (figure 3.13).

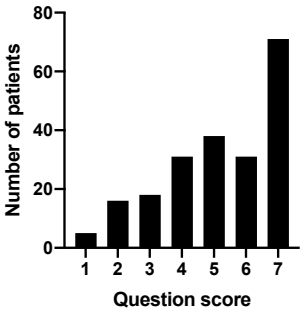
1.) Have you had chest or stomach pains as a result of your cough?



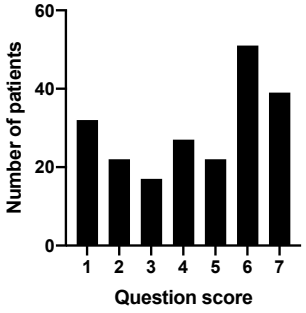
2.) Have you been bothered by sputum (phlegm) production when you cough?



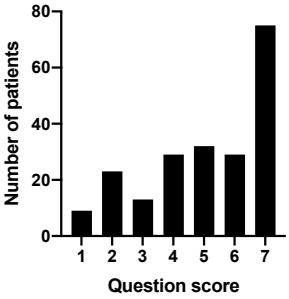
3.) Have you been tired because of your cough?



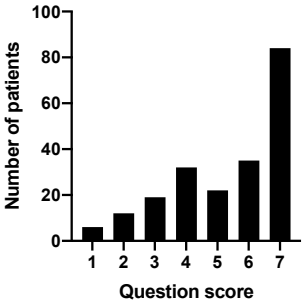
4.) Have you felt in control of your cough?



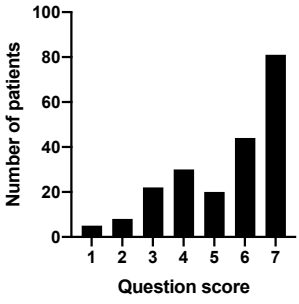
5.) How often have you felt embarrassed by your coughing?



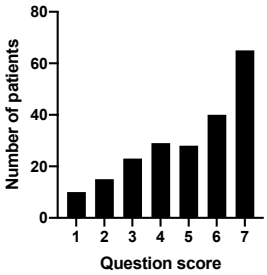
6.) My cough has made me feel anxious.



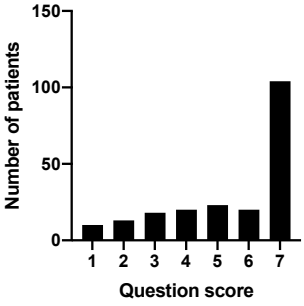
7.) My cough has interfered with my job, or other daily task



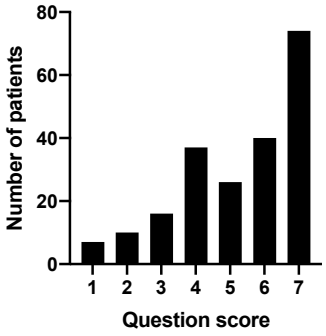
8.) I felt that my cough interfered with the overall enjoyment of my life.



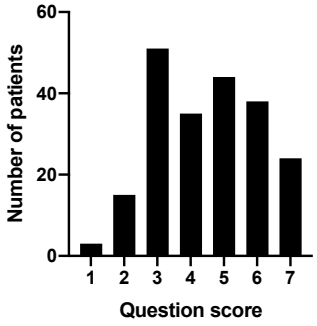
9.) Exposure to paints or fumes has made me cough.



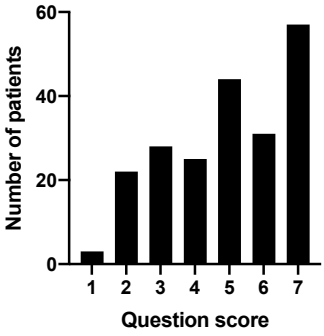
10.) Has your cough disturbed your sleep?



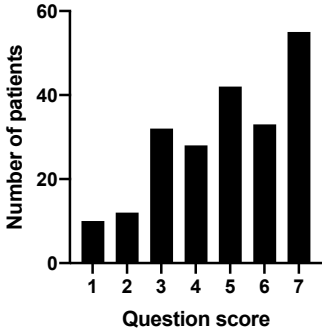
11.) How many times a day have you had coughing bouts?



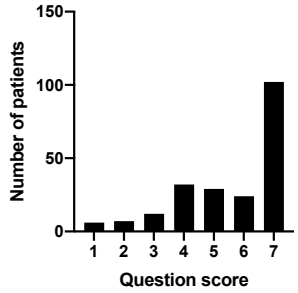
12.) My cough has made me feel frustrated.



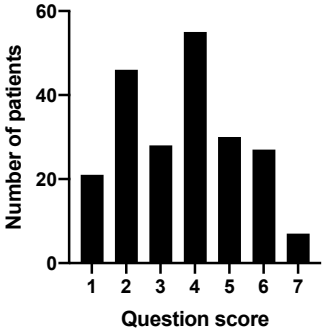
13.) My cough has made me feel fed up.



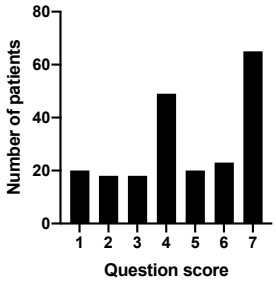
14.) Have you suffered from a hoarse voice as a result of your cough?



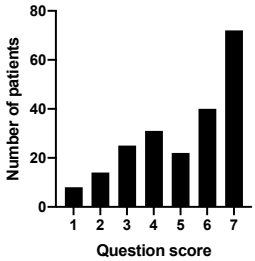
15.) Have you had a lot of energy?



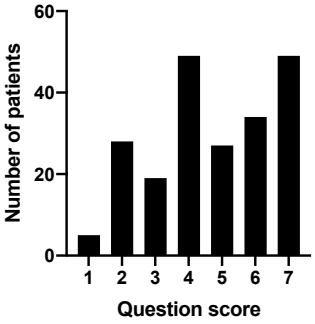
16.) Have you worried that your cough may indicate a serious illness?



17.) Have you been concerned that other people think something is wrong with you because of your cough?



18.) My cough interrupted conversation or telephone calls



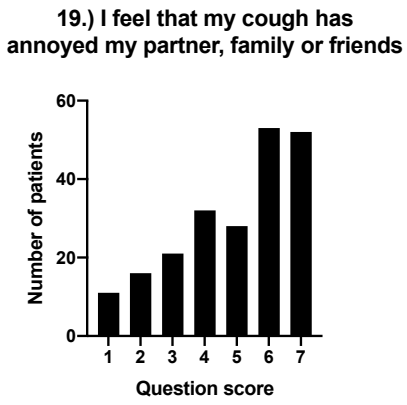


Figure 3.13 LCQ baseline question response histograms

Interestingly the questions asking about chest pain, hoarse voice and coughing in response to exposure to paint or fumes were all fairly skewed towards high scores suggesting they were not a significant issue. Questions about control over cough, coughing bouts and sputum production created a much more even spread of answers, suggesting that the conventional idea that IPF related cough is dry may be incorrect.

The relationship between cough as assessed by the LCQ and quality of life as assessed by the SGRQ was explored in 127 patients for whom a breakdown of the SGRQ into domain scores and an LCQ were available. The correlations for every domain and the total score were statistically significant, with the strongest correlation seen between the symptom score of the SGRQ and the LCQ. This is perhaps unsurprising since questions about cough and its impact are asked in this domain. The correlations are not particularly strong suggesting that cough is an important but not sole determinant of quality of life within patients with fibrotic lung disease. This is very similar to data seen in COPD where the Pearson coefficient for LCQ and SGRQ total scores is 0.6, although in this cohort it was the SGRQ impact score that had the best correlation with the LCQ (Berkhof et al., 2012).

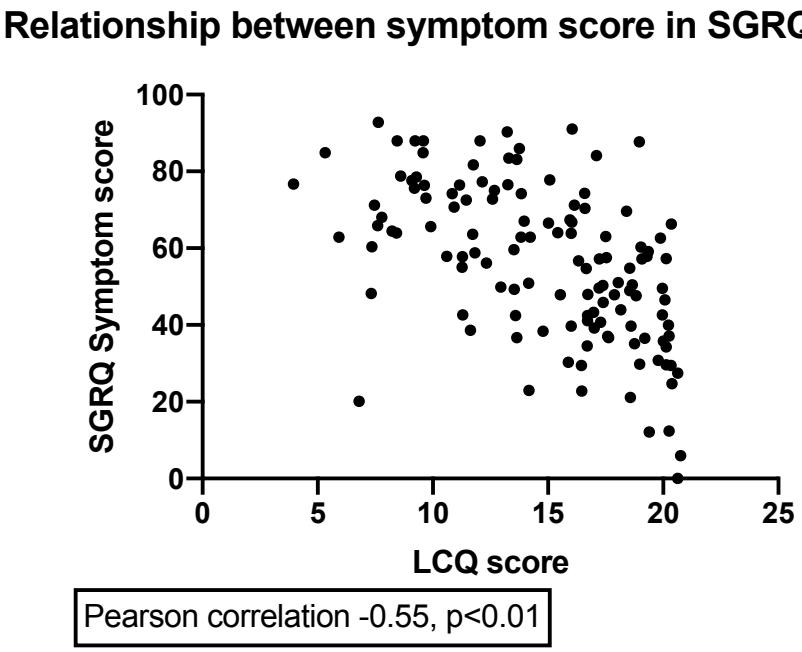


Figure 3.14 Correlations between the SGRQ symptom domain score and LCQ at baseline

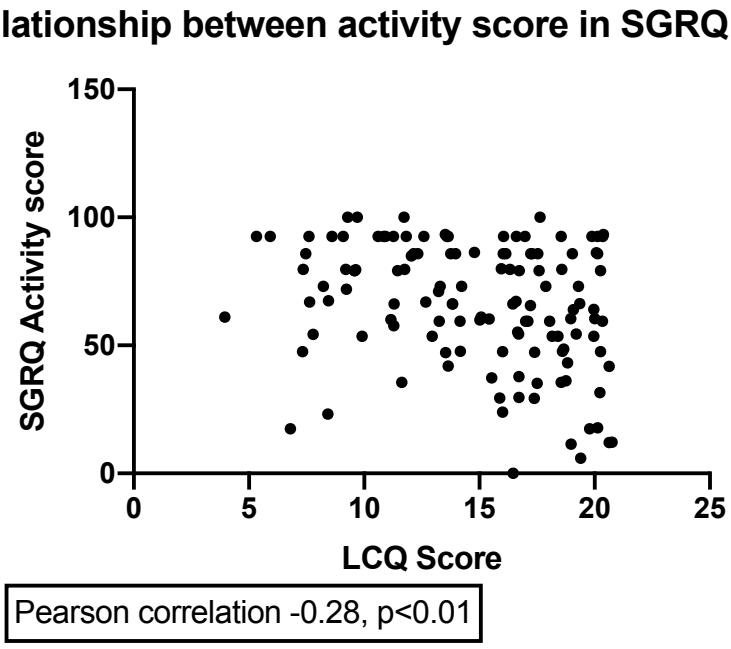


Figure 3.15 Correlations between the SGRQ activity domain score and LCQ at baseline

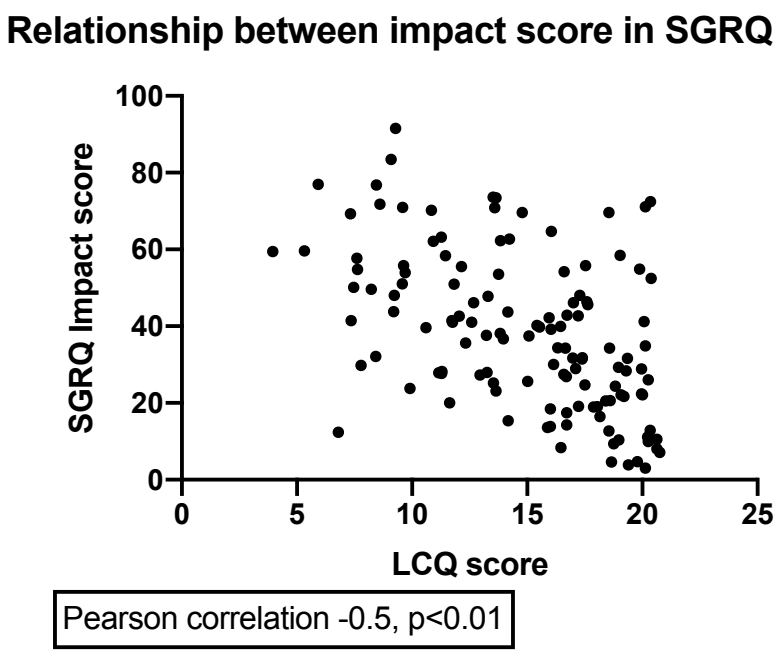


Figure 3.16 Correlations between the SGRQ impact domain score and LCQ at baseline

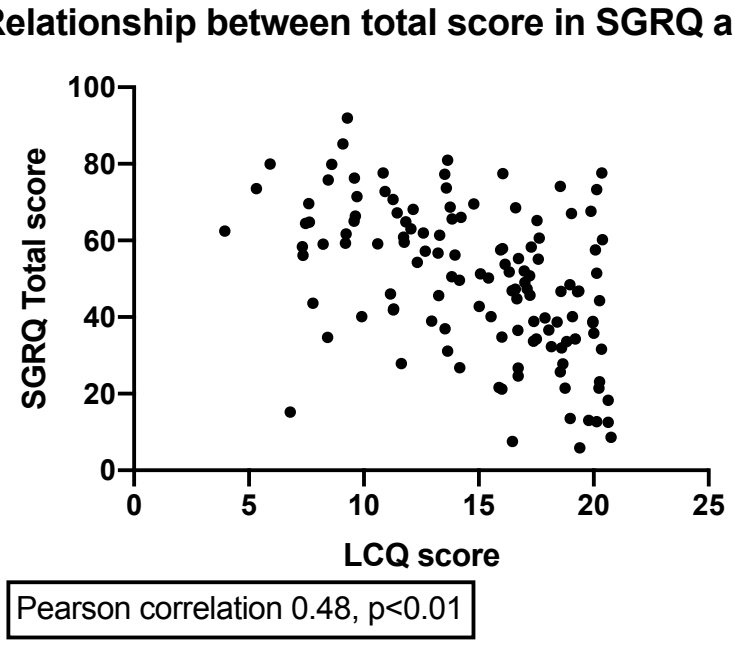
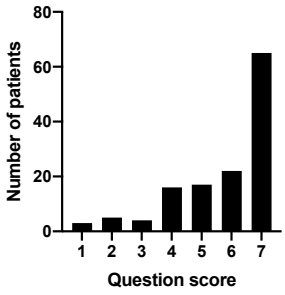


Figure 3.17 Correlations between the SGRQ total score and LCQ at baseline

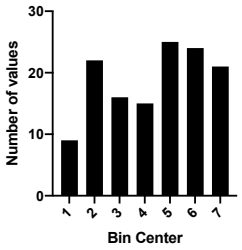
3.31

Assessing changes in cough over time

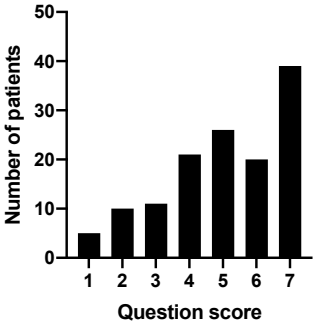
1.) Have you had chest or stomach] pains as a result of your cough?



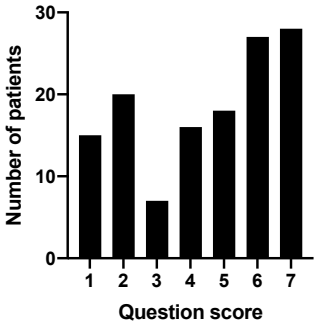
2.) Have you been bothered by sputum (phlegm) production when you cough?



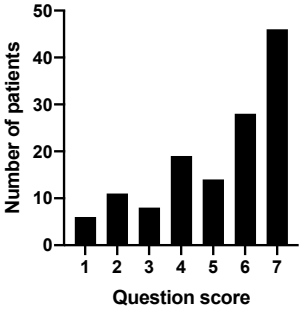
3.) Have you been tired because of your cough?



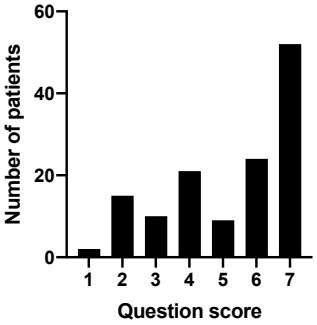
4.) Have you felt in control of your cough?



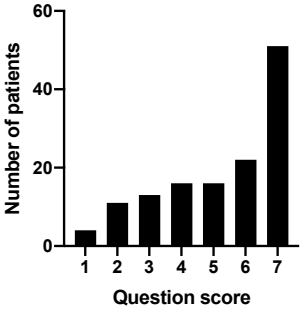
5.) How often have you felt embarrassed by your coughing?



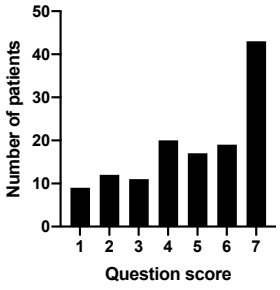
6.) My cough has made me feel anxious.



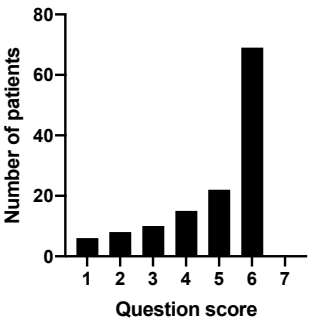
7.) My cough has interfered with my job, or other daily tasks.



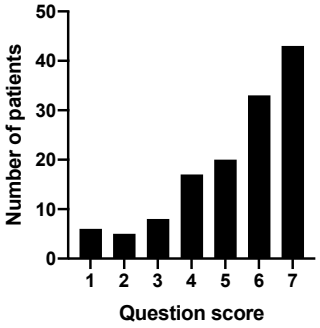
8.) I felt that my cough interfered with the overall enjoyment of my life.



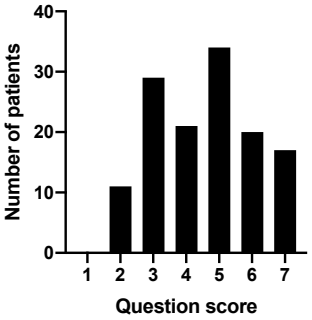
9.) Exposure to paints or fumes has made me cough.



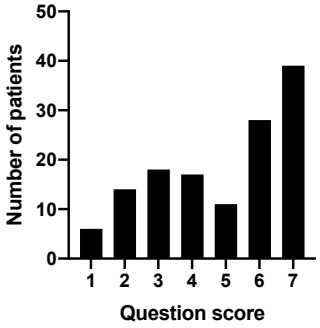
10.) Has your cough disturbed your sleep?



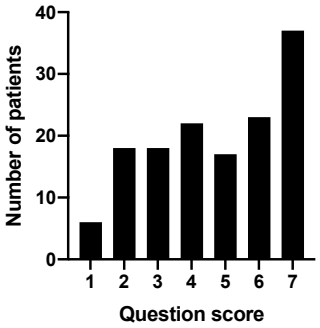
11.) How many times a day have you had coughing bouts?



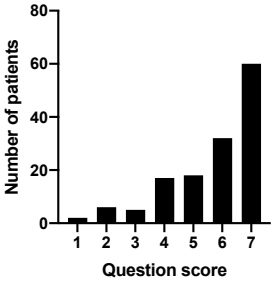
12.) My cough has made me feel frustrated.



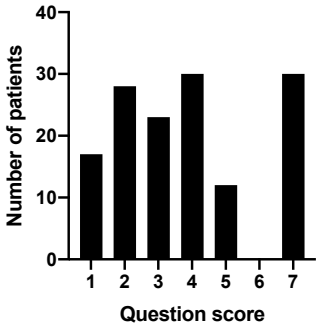
13.) My cough has made me feel fed up.



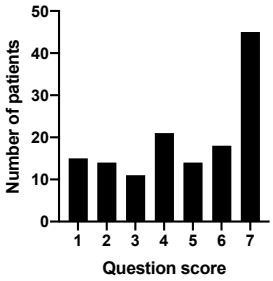
14.) Have you suffered from a hoarse voice as a result of your cough?



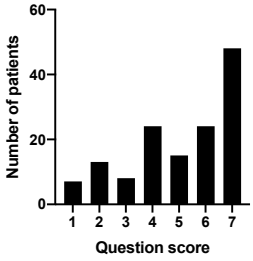
15.) Have you had a lot of energy?



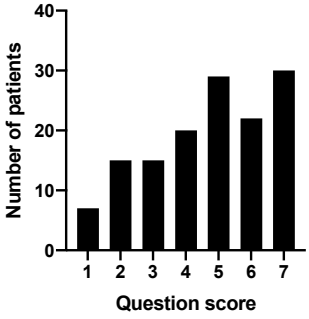
16.) Have you worried that your cough may indicate a serious illness?



17.) Have you been concerned that other people think something is wrong with your because of your cough?



18.) My cough interrupted conversation or telephone calls



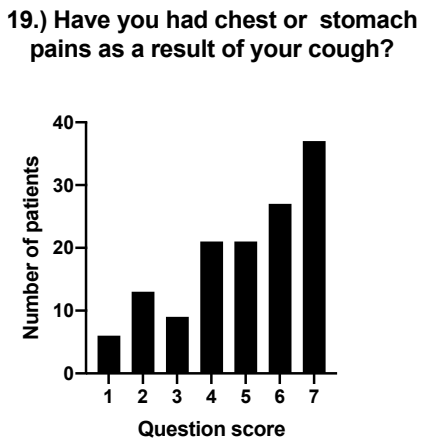


Figure 3.18 LCQ 12-month question response histograms

At the 12-month time point, 141 patients provided a completed LCQ. The results are presented in figure 3.18 and are very similar to the baseline values. The symptoms of chest pain, hoarse voice and coughing in response to exposure to fumes seem to remain of low importance to patients at 12-months. Change in LCQ question scores over the 12 months is shown in table 3.4. Interestingly the most improvement was seen in question 9 which deals with cough sensitivity to chemical smells. Question 19 which deals with physical pains brought on by coughing appeared to have the greatest worsening over time perhaps suggesting that cough severity may change over time.

LCQ Question	Δ change at 12 months	SD
1	0.15	1.46
2	0.22	1.68
3	0.19	1.78
4	0.04	2.46
5	-0.10	1.68
6	0.06	1.72
7	0.19	1.98

8	0.25	2.11
9	0.58	1.80
10	0.02	1.80
11	-0.02	1.72
12	0.06	1.81
13	0.13	1.79
14	0.01	1.62
15	-0.04	1.77
16	0.02	2.05
17	0.11	1.87
18	0.02	1.65
19	-0.18	1.79

Table 3.4 Delta change in LCQ scores over 12 months

Of the 250 Brompton patients, 139 patients had completed both a baseline questionnaire and a 6-month questionnaire. The difference between the mean of LCQ total score was evaluated using a Wilcoxon Signed Rank test. This was chosen over the T test because the distribution of LCQ scores is non-normal. This test showed a Z statistic of -0.171 with a p value of 0.864. This means that there is no significant difference between the median LCQ at the two time points. This finding is better illustrated by the histogram in figure 3.19 which shows the number of patients in which there was a positive and the number with a negative change in LCQ between the two timepoints. The distribution is normal, showing no difference between the two time points.

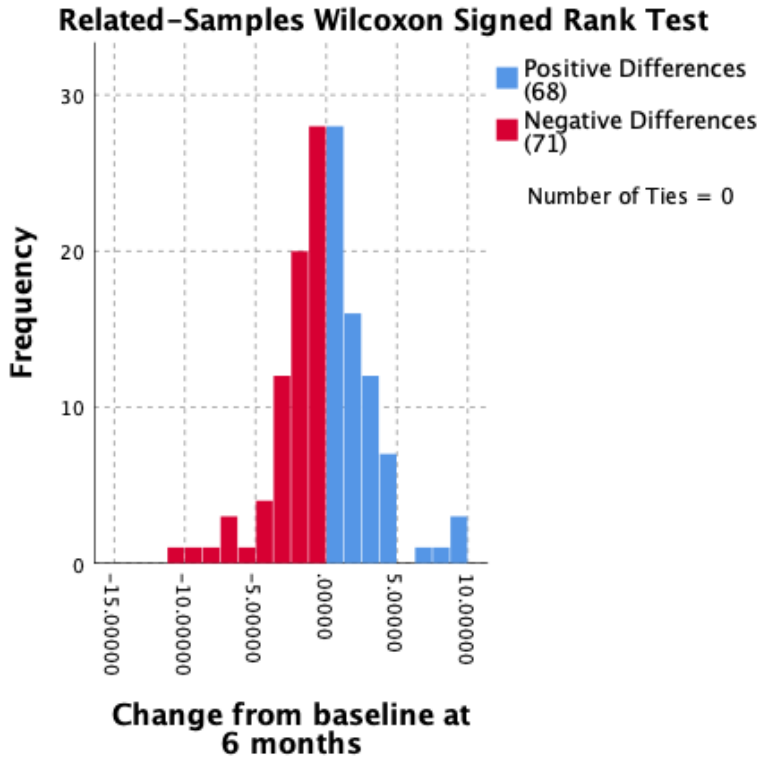


Figure 3.19 Histogram showing the individual differences in LCQ scores between baseline and 6 months

131 patients had completed both a baseline questionnaire and a 12-month questionnaire. The difference between the mean of LCQ total score was evaluated using a Wilcoxon Signed Rank test. This test showed a Z statistic of -0.966 with a p value of 0.33, meaning that there is no significant difference between the median LCQ at the two time points. Again, the histogram showing the differences in scores between the two timepoints is normally distributed, showing no difference between the two time points (figure 3.20).

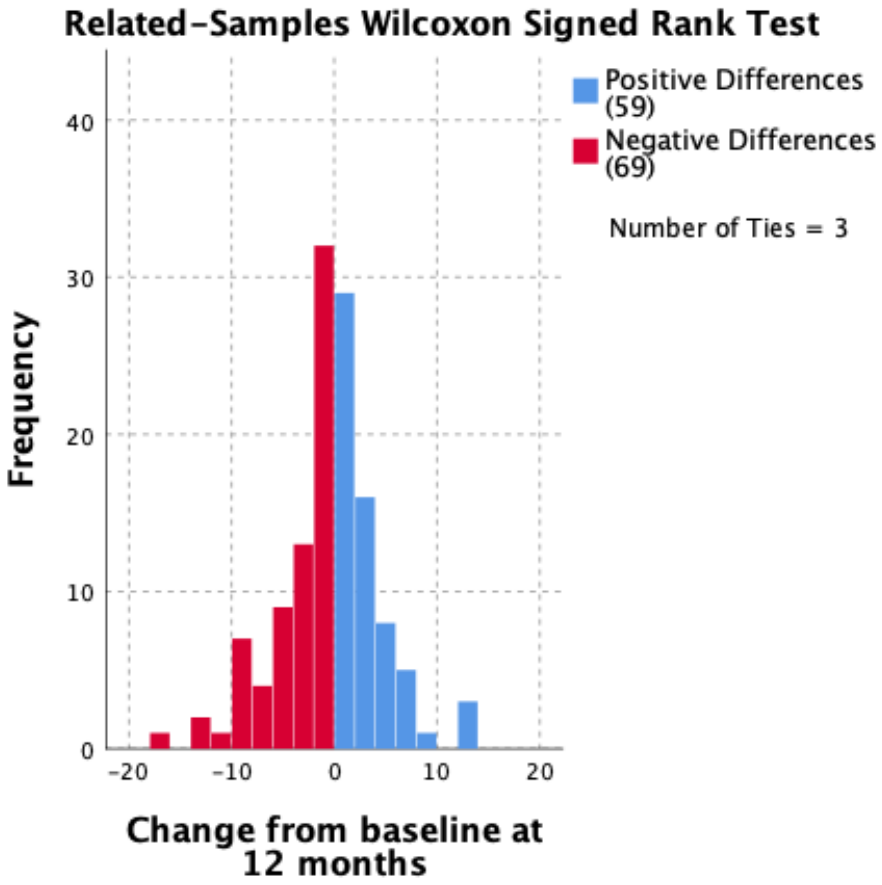


Figure 3.20 Histogram showing the individual differences in LCQ scores between baseline and 12 months

By 24-months, LCQ scores for those who had also completed a baseline LCQ were available in 92 patients. The difference between the median LCQ at baseline and at 24 months was evaluated using a Wilcoxon signed rank test. This gave a Z-statistic of -1.998 with a p value of 0.05. This suggests that cough related quality of life by 24 months is different from baseline, although the absolute difference is quite small as illustrated by the histogram of change in figure 3.21. Most of the changes in score are below the 4 units of clinical significance.

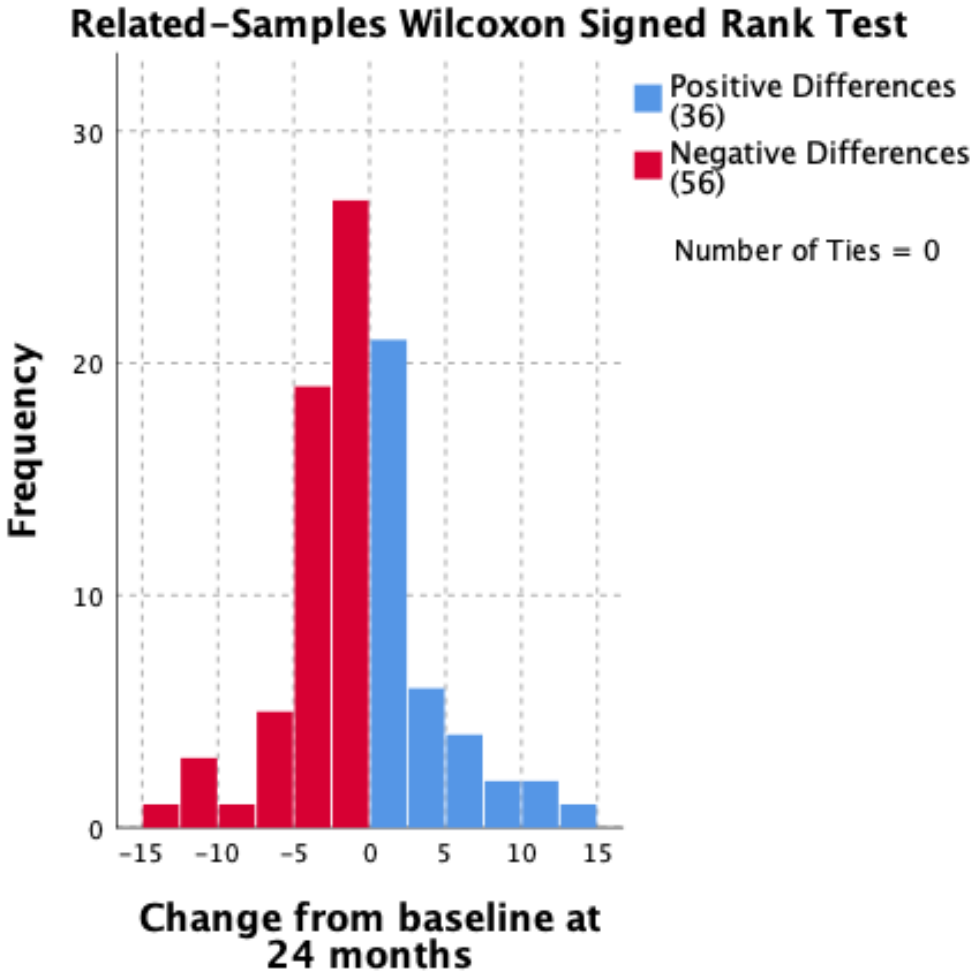


Figure 3.21 Histogram showing the individual differences in LCQ scores between baseline and 24 months

By 36-months, only 63 patients had completed an LCQ and had a baseline LCQ as a comparator. Interestingly the comparison of medians between the two timepoints showed no significant difference with a Z statistic of -1.164 and a p value of 0.24. Again, the histogram of difference between the timepoints followed a normal distribution (figure 3.22).

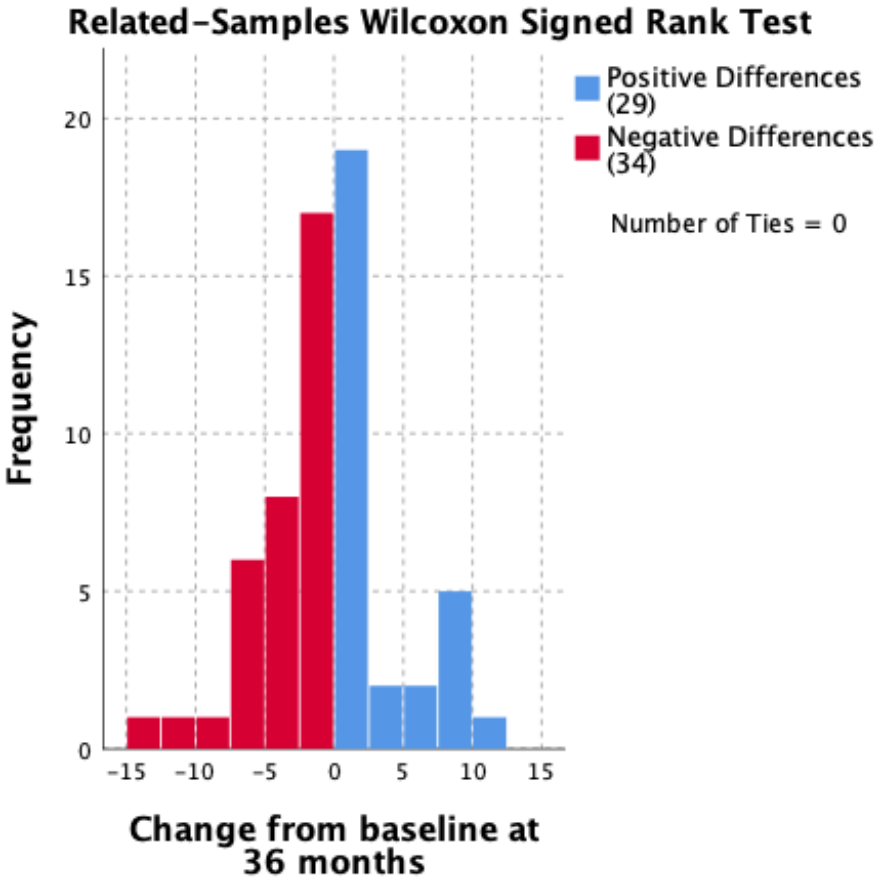


Figure 3.22 Histogram showing the individual differences in LCQ scores between baseline and 36 months

One final way of evaluating the change in LCQ over time was to assess the change over multiple timepoints using an Anova calculation. This model allows data that is unpaired to be used i.e. if a patient had only completed a baseline questionnaire, their data was still included in the model. Running an ANOVA on all the available cohorts to assess for change over time in LCQ showed an F statistic of 2.754 with a p value of 0.01. This suggests there is change over time in LCQ. A subsequent Tukey post hoc analysis however did not find a significant

difference between the various timepoints, although the difference between baseline and 24 months came close to significance with a p value of 0.07.

Overall the results show that whilst individual patients do experience change in their cough reported quality of life over time, the changes are small and are unlikely to be of clinical significance. As a cohort the change in cough reported quality of life over both short and longer periods of time is unlikely to be significant.

The various domains of the questionnaire baseline responses (216 patients from the Brompton) are analysed in table 3.5

LCQ Score	Mean	Standard deviation
Physical score	4.9287	1.2367
Psychological score	4.8442	1.5785
Social score	4.9504	1.5688
Total score	14.6551	4.2622

Table 3.5 LCQ domain scores at baseline

The internal consistency of the LCQ in IPF was able to be calculated using the Cronbach Alpha statistic. Table 3.6 shows the mean score for each question and its standard deviation.

LCQ Question	Number of valid responses	Mean response	Standard deviation
Q1	211	5.9621	1.44699
Q2	211	4.5877	1.84794
Q3	210	5.1810	1.74324
Q4	210	4.4000	2.11669
Q5	210	5.0905	1.90602
Q6	210	5.3476	1.77384
Q7	210	5.4190	1.69593
Q8	210	5.0476	1.85029
Q9	208	5.4471	1.92042
Q10	210	5.2905	1.72413
Q11	210	4.4857	1.54743
Q12	210	4.9333	1.75137
Q13	212	4.8821	1.78733
Q14	212	5.5991	1.67914
Q15	214	3.6355	1.64098
Q16	213	4.6901	2.02059
Q17	212	5.1368	1.83312
Q18	211	4.7204	1.78686
Q19	213	4.9577	1.81272

Table 3.6 Mean scores for each question within the LCQ

Using this data, the Cronbach alpha statistic was calculated to be 0.95 which demonstrates very good internal reliability of the questionnaire. The Cronbach alpha for the physical symptoms questions was 0.83, for the psychological symptom questions was 0.92 and for the social questions was 0.88.

Only 166 of the original 250 Brompton patients had a 12 month follow up visit. Of these patients, 25 did not complete a 12-month LCQ, leaving 141 respondents at the 12-month timepoint.

In chronic idiopathic cough, the minimal important difference in LCQ is 1.3 (Raj et al., 2009), although it is not known if this is the same in patients with fibrotic lung disease. The change in LCQ over time in the PROFILE cohort (table 3.7) is unlikely to represent a clinically significant change and thus it would appear not to support a worsening of cough related to worsening of disease status.

Timepoint	Mean LCQ result	Standard deviation
0	14.56	5.36
6	13.87	5.89
12	12.13	6.83
24	13.01	5.89
36	13.14	5.91

Table 3.7 LCQ scores at different time points

When looking at the LCQ scores in patients who experienced disease progression in the first year of follow up (FVC decline equal or greater than 10%) there was no significant difference compared to patients who did not progress (Mean LCQ 12.50 in progressors, 13.29 in non-progressors, p=0.09).

3.4.0

Cough and its relationship to disease process

Within PROFILE we were keen to evaluate the impact of disease severity on cough. To examine this, we initially performed a Pearson correlation between markers of lung function (FVC% predicted, TLCO% predicted and CPI). The composite pulmonary index score (CPI) was used because it has been shown to be the better predictor of mortality in fibrotic lung disease than any single lung function parameter (Wells et al., 2003). It is derived from the gas transfer, forced vital capacity and forced expiratory volume in one second (FEV1). In this way the effects of emphysema on volume are accounted for. For FVC, the Pearson correlation with LCQ was -0.17 with a significance of <0.01. For TLCO the Pearson correlation with LCQ was 0.03 with a significance of 0.34. Thus, there is only very weak correlation between FVC and the LCQ, with no correlation between gas transfer and LCQ. For CPI, the Pearson correlation with LCQ was -0.25 with a p value of <0.01. This can be visualised graphically in the two charts below in which the lung function parameters have been plotted against LCQ scores. These charts contain the data from both Nottingham and Brompton and in the case of the Brompton cohort, contain data from multiple timepoints.

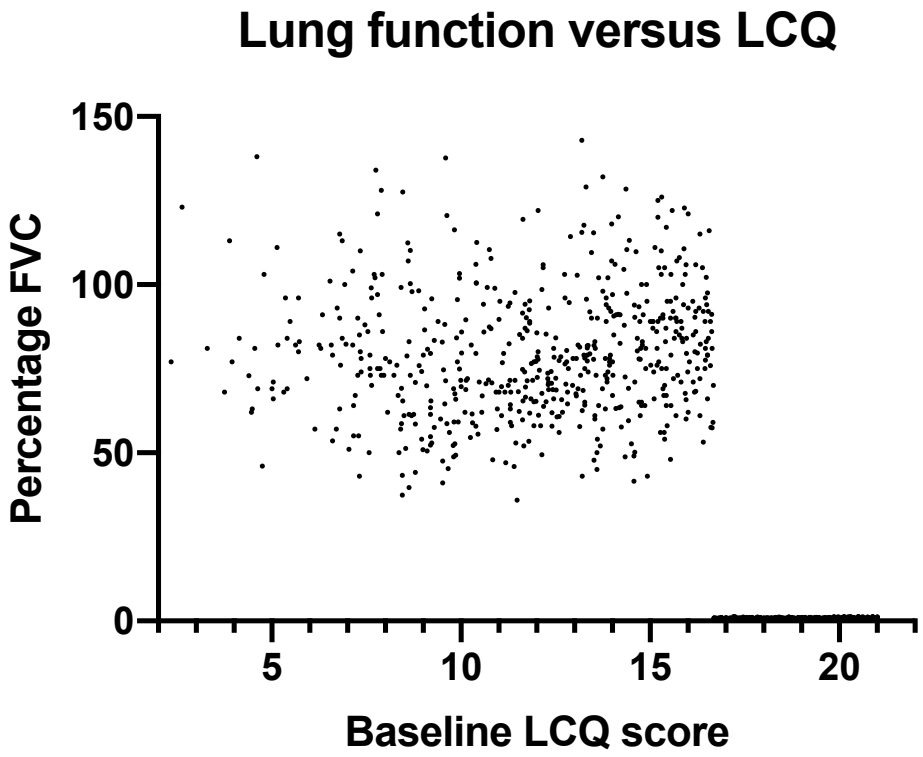


Figure 3.23 The association between LCQ and forced vital capacity

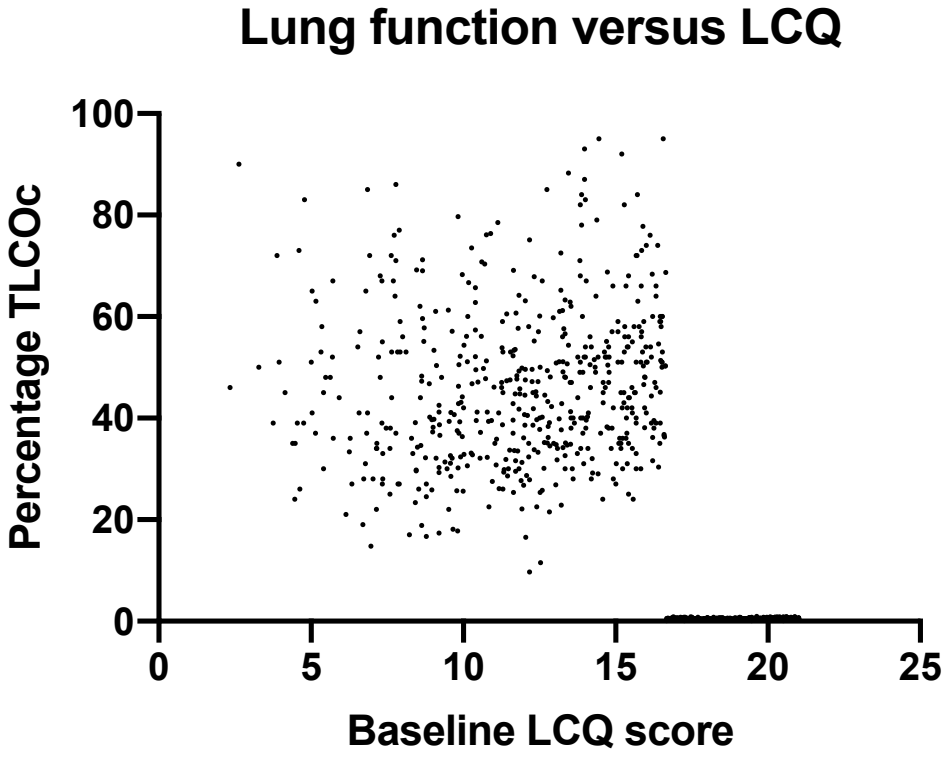


Figure 3.24 The association between LCQ and gas transfer

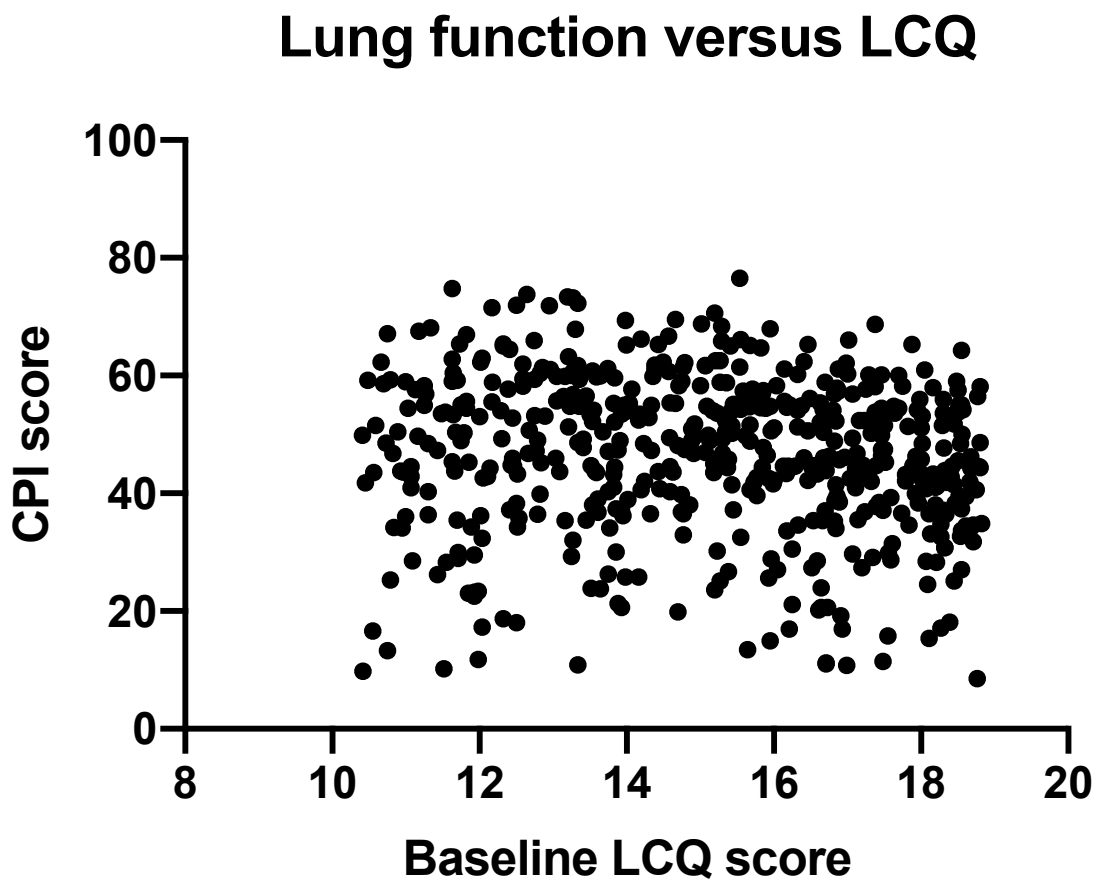


Figure 3.25 The association between LCQ and CPI score

Using the sequential PROFILE visit data in a general linear mixed model, the potential predictors of LCQ were evaluated. This model may be more appropriate because the missing data in the PROFILE study was skewed towards the later time points rather than being random. Three markers of lung function were included in the model; FVC, TLCO and CPI. The rationale for using all of these is not a single one is considered to be the best marker of disease progression or severity. FVC can be affected by co-existing emphysema, TLCO<40% predicted is often considered to be the marker of severe disease (Egan et al., 2005) and CPI has been shown to be a better marker of mortality (Wells et al., 2003). Disease progression and death occurring during follow up lead to premature discontinuation of follow up. The results of this model are shown in table 3.8:

Predictor	Wald Chi-square	P value
TLCOc %predicted	1.913	0.17
FVC %predicted	0.019	0.89
CPI score	0.380	0.54
Death during follow up	8.063	<0.01
Smoking history	3.010	0.22
Diagnosis – IPF vs NSIP	0.040	0.84
Gender	7.500	0.01

Table 3.8 General linear mixed model evaluating the predictors of LCQ

Interestingly gender and death were predictors of LCQ, but none of the lung function markers were. Lung function parameters were significant in the Pearson but with only very weak correlation. Gender's influence on LCQ is similar to that of chronic idiopathic cough in which women usually experience much more impairment to cough related quality of life(Kelsall et al., 2009). Cough correlating with mortality is difficult to explain because it does not correlate with markers of disease progression. This finding supports the findings of Ryerson et al. (Ryerson et al., 2011).

Kaplan-Meir survival curves were plotted for the PROFILE cohort looking at whether LCQ affected survival. The cohort was divided into those who had an LCQ score of 14 or less and those with a score above 14. The cut-off point was chosen at 14 based on the mean LCQ of 14.6 found within the PROFILE cohort. The patients with more severe cough related reduction

in quality of life (LCQ of 14 or less) appeared to have a slightly worse mortality, but this was not statistically significant using a log-rank (Mantel-Cox) test (p=0.28)

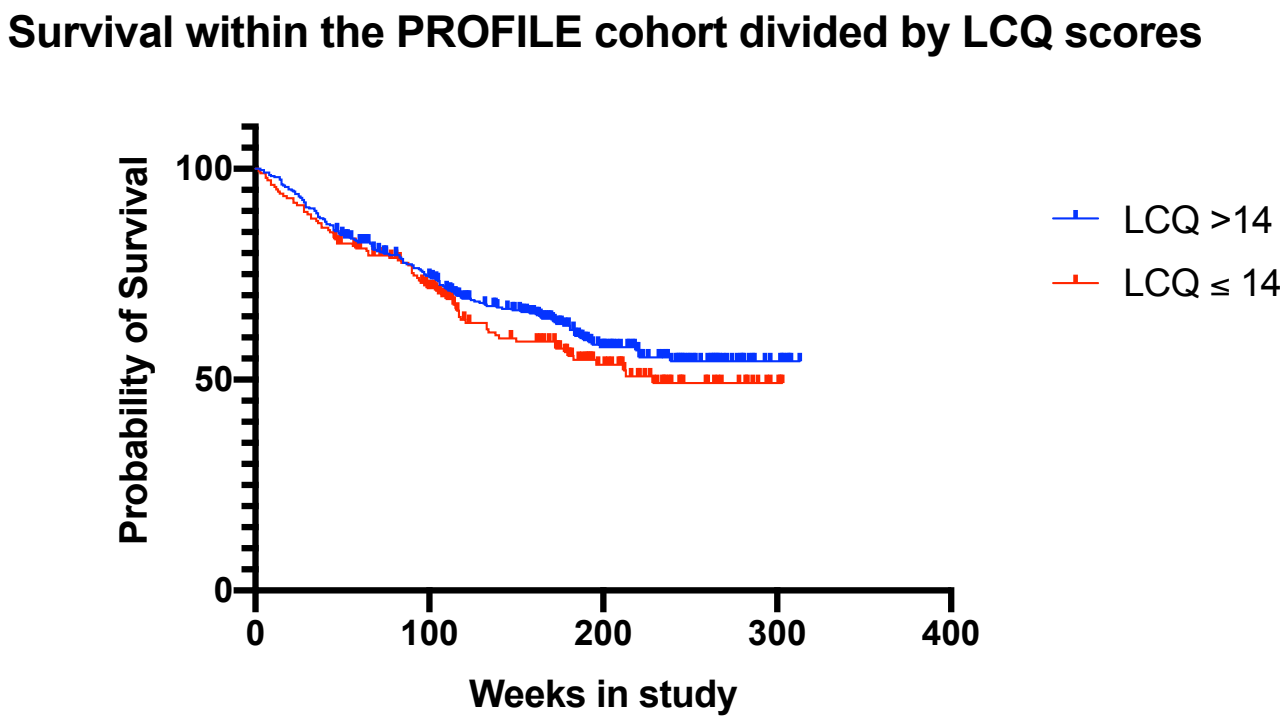


Figure 3.26 Survival in the PROFILE cohort divided by baseline LCQ score

3.5.0

Summary of results

1 Quality of life declines over time in fibrotic lung disease and is more severely impacted in those with more severe disease.

2 The quality of life as measured by SGRQ follows a normal distribution

3 Cough is a persistent symptom through the course of fibrotic lung disease with little clinically meaningful change over time

4 Cough related quality of life in fibrotic lung disease is significantly impacted with scores worse than those seen in asthma and COPD.

5 Cough bears little relationship to the severity of disease in fibrotic lung disease but it's relationship with mortality is more complex.

6 The impact of cough in fibrotic lung disease is worse in women.

4.0
OBJECTIVE COUGH MEASURES IN FIBROTIC LUNG DISEASE

4.0.0

Description of recruitment

In the original PROFILE cohort, subjective measures of cough and quality of life had been recorded. The Leicester cough questionnaire gave an idea of impact on quality of life related to cough. PROFILE has allowed validation of the LCQ in a large well characterised cohort of patients with IPF. The St George's Respiratory Questionnaire demonstrated overall quality of life and the symptom domain score appeared to relate to cough-related quality of life. Neither score actually asked patients to rate their cough in quantity of severity.

To date there has only been limited reporting of objective cough counts in patients with fibrotic lung disease and none of these studies have shown how cough frequency changes with time (Key et al., 2010, van Manen et al., 2017). The need to better understand cough burden in fibrotic lung disease is real if we are to understand how best to treat it.

This need to quantify cough and evaluate its behaviour over time led to the recruitment of a new cohort of patients under amended ethics of the PROFILE study. The purpose was to collect digital cough recordings at regular time points to understand how objective cough changed over time in relation to disease progression. The cough counts would also allow comparison to subjective measures of cough impact on quality of life such as the LCQ.

52 patients were recruited to the PROFILE extension study from the Royal Brompton ILD Clinical service. Recruitment began in June of 2016 and the final patient was recruited in August of 2017. Of these patients, 51 completed the initial visit including a 24-hour cough recording using the Vitalojak equipment. One patient who was recruited withdrew before completing the baseline visit and in a second patient the baseline cough recording failed to record. Thirty eight patients underwent diagnostic fibreoptic bronchoscopy with bronchoalveolar lavage as part of the clinical investigation of their lung disease. Where surplus sample was available, this was used to investigate the role of the microbiome in cough.

Recruitment is demonstrated in figure. 4.1.

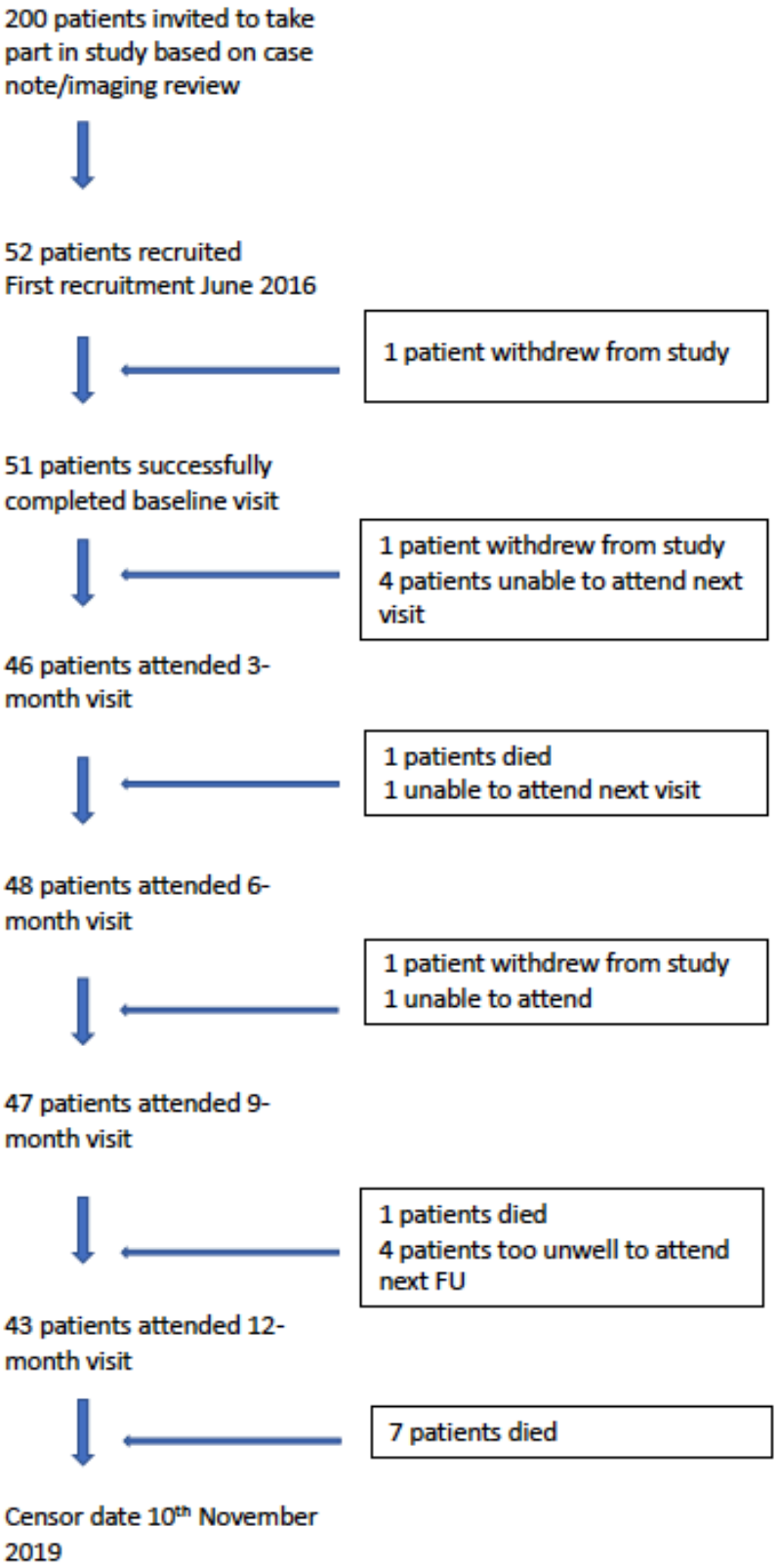


Figure 4.1 Recruitment to the cough cohort

4.1.0

Baseline clinical characteristics

The mean age of patients at recruitment was 70.2 years old (age range 50-84). As anticipated from the nature of fibrotic lung disease, the majority of patients recruited were male (73.1%) compared to 26.9% of patients being female. At the time of recruitment 44.2% of patients had never smoked, 53.8% of patients were ex-smokers and 1.9% were current smokers. These baseline characteristics are not dissimilar to those from the original PROFILE cohort and those of 2 large European IPF patient cohorts (Wuyts et al., 2018, Kreuter et al., 2019), suggesting that the patients recruited were representative (Table 4.1). The PROOF cohort was set up in 2013 and included patients collected from 8 centres in two countries. Patients with both definite and probable IPF were included with 8% of patients receiving corticosteroids at the time of enrolment (Wuyts et al., 2018). The INSIGHTS cohort was developed from 20 German hospitals with recruitment starting in 2012 and just over 25% of participants receiving corticosteroids (Behr et al., 2020). Changing guidelines for the diagnosis of IPF and diagnostic uncertainty make it very likely both cohorts contained other fibrotic lung diseases and therefore they are appropriate comparison groups for the PROFILE cohort.

What is noticeable however, is that the patients within this cohort experienced milder disease at baseline than the original PROFILE cohort and the INSIGHTS IPF cohort. This possibly reflects a pattern of earlier referral to specialist centres that had come about since the availability of anti-fibrotic medication.

	PROFILE Cough cohort	PROFILE cohort, UK	PROOF cohort (Wuyts et al., 2018), Belgium	INSIGHTS IPF (Kreuter et al., 2019), Germany
Number of patients	52	579	277	879, of which 424 reported
Gender	73.1% male	77.9% male	76.9% male	76.9% male
Ethnicity	94% white	Not reported	92.1% white	Not reported
Mean age (SD)	70.2yrs (7.6)	69.9yrs (8.4)	69.6yrs (8.6)	68.8yrs (9.1)
Smoking history	Current - 1.9% Ex – 53.8% Never – 44.2%	Current – 5.5% Ex – 63.8% Never – 30.6%	Current – 6.5% Ex – 66.8% Never – 26.7%	Current – 1.7% Ex – 60.4% Never – 38%
FVC% (SD)	82.4% (18.9)	79.5% (19.1)	80.6% (19.9)	75.9% (19.4)
Gas transfer% (SD)	45.1% (13.3)	46.2% (15.4)	46.9% (13.8)	36.1% (15.9)

Table 4.1 A comparison of the cough cohort with other IPF registries

Diagnostic uncertainty in idiopathic pulmonary fibrosis is a common problem as evidenced by a study by Walsh et al in which 60 cases of interstitial lung disease were evaluated by 404 respiratory physicians who were asked if idiopathic pulmonary fibrosis was their first diagnosis (Walsh et al., 2017). In this study even the expert physician group had a kappa intra-observer agreement of 0.65 whereas those working in a University based institution with access to an MDT had an intra-observer agreement of 0.56 (Walsh et al., 2017). A Canadian study looking at whether MDT discussion in an expert ILD centre showed in 20% of cases with

a provisional diagnosis of IPF, the diagnosis was altered by MDT discussion (Ageely et al., 2020). Although the original objective was to recruit patients with IPF, patients were recruited based on imaging (probable or definite UIP pattern) and clinical history, prior to MDM discussion of their final diagnosis. After bronchoscopy and occasionally biopsy, MDM discussion resulted in different diagnostic labels in some cases.

Ultimately the final cohort contained 69% of patients with a diagnosis of IPF, 12% with a diagnosis of chronic hypersensitivity pneumonitis and 19% with a diagnosis of progressive fibrotic ILD.

The first guideline for a systematic approach to diagnosing chronic hypersensitivity pneumonitis appeared in 2020 (Raghu et al., 2020), after this study had been completed. Previous studies of MDT evaluation of interstitial lung disease have shown that hypersensitivity pneumonitis has a low inter-MDT kappa agreement value at 0.24 between expert centres (Walsh et al., 2016). The BTS Guideline for interstitial Lung Disease puts weight on an index of suspicion based on occupation and other potential exposures along with MDT discussion (Bradley et al., 2008). The Royal Brompton MDT discussion for these cases included clinical and radiological findings along with bronchoalveolar lavage cell counts to help make a diagnosis of hypersensitivity pneumonitis in the presence of a BAL lymphocytosis (Wells, 2010). This has recently been shown to be a reasonable differentiator of IPF from hypersensitivity pneumonitis with a sensitivity of 69% and a specificity of 61% (Patolia et al., 2020). The diagnosis of progressive fibrotic ILD is by definition made on disease behaviour over time and is hinted at in the original BTS Guideline where a group of patients with NSIP who followed an IPF behaviour is described (Bradley et al., 2008). Criteria for the diagnosis

of this group were suggested in 2018 (Cottin et al., 2018) and were used for a subsequent trial of antifibrotic therapy in progressive fibrosis (Flaherty et al., 2019). These definitions were used by the MDT to assign the label of progressive fibrotic ILD in this study.

Further analysis of the cohort includes all patients because it is widely recognised that these other disease labels can behave in a similar way to IPF (Cottin et al., 2018). The characteristics of the patients in the cohort are presented below separated by disease type.

	IPF	Hypersensitivity pneumonitis	Progressive fibrosing ILD
Number of patients (% of cohort)	36 (69%)	6 (12%)	10 (19%)
%Male	72%	80%	73%
Smoking history	Current Ex Never	Current Ex Never	Current Ex Never
Mean FVC at recruitment	80.3%	86.7%	86.2%
Mean TLCO at recruitment	43.2%	44.4%	52.2%

Table 4.2 – Baseline characteristics of Cough cohort

4.1.1

Disease Severity

Disease severity is most usually measured using lung function markers such as the forced vital capacity. Composite parameters such as the CPI score (Wells et al., 2003) which uses spirometric and gas transfer values or the GAP score (Ley et al., 2012) which is composed of age, gender and two lung function parameters are also recognised ways of measuring severity. Both GAP and CPI may be better correlates of mortality than any single lung function parameter (Wells et al., 2003, Ley et al., 2012). The mean severity of the group was at least moderate with a gas transfer of 45% (standard deviation 13.3) and an FVC of 82% (standard deviation 18.9) at baseline. There was a gradual trend towards decline in lung function over the year of follow up, as would be expected in a group of patients with fibrotic lung disease, but the fall in mean FVC did not meet the 10% drop that is considered clinically significant. (Table 4.3).

	Baseline	6 months	12 months
Mean FVC percent predicted	82.4% (SD 18.9)	81.3% (SD 19.4)	79.9% (SD 19.4)
Mean TLCO percent predicted	45.1% (SD 13.3)	43.5% (SD 14.6)	43.4% (SD 14.4)
Mean CPI score	46.5 (SD 11.2)	48.0 (SD 12.7)	48.5(SD 12.3)
Mean GAP score	3.9 (SD 1.3)	3.8 (SD 1.4)	3.9(SD 1.4)

Table 4.3 – Baseline lung function results from the cough cohort

4.2.0

Progression of disease over time

Over the course of 12 months, clinical progression as evidenced by death from any cause, relative drop in FVC of 10 percent or greater or drop in TLCOc by 15 percent or greater occurred in 24 patients (46%). Inadequate follow up data was available for 2 patients, both of whom had withdrawn from the study, and they were not included in any subsequent analysis. Death within study was censored on 10th November 2019 and was a relatively rare event, reflecting the shorter follow up period for this cohort (Figure 4.2).

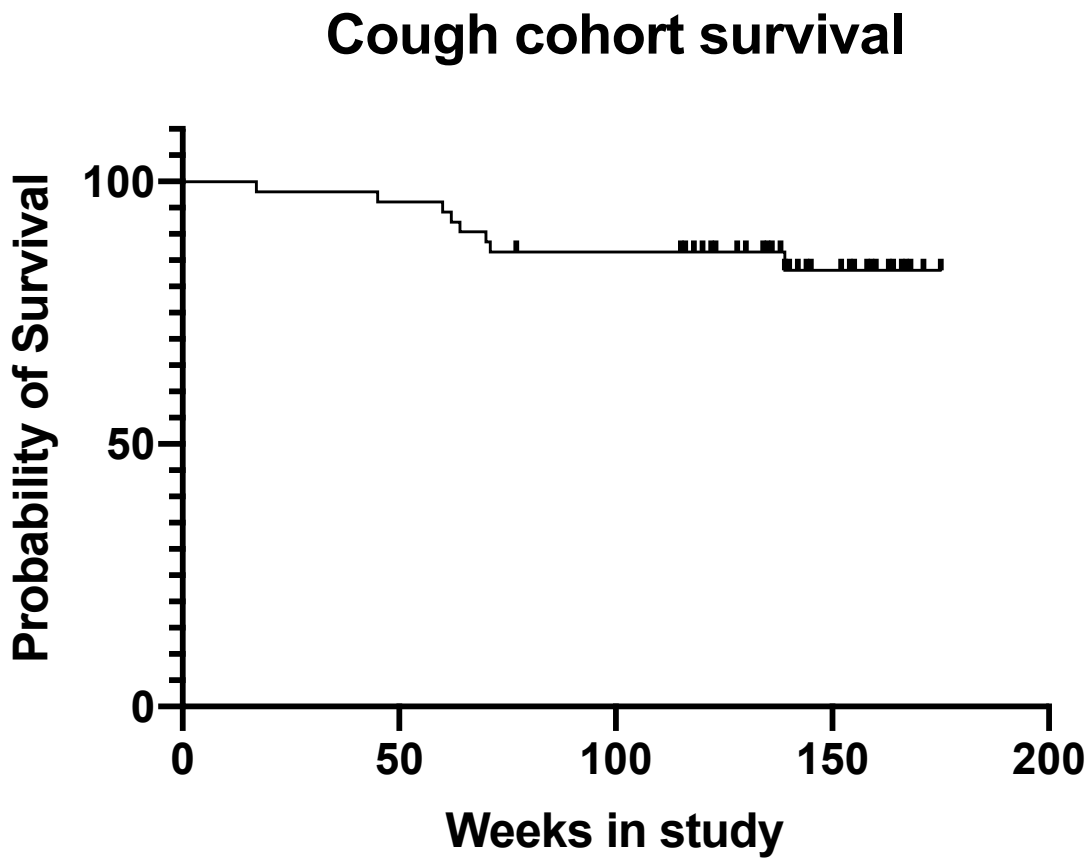


Figure 4.2 – Kaplan Meir curve demonstrating survival over time in the cough cohort

4.2.1

Treatment choices

At the time of recruitment, most patients had not started a therapy for their fibrotic ILD (Table 4.4). The proportion of patients subsequently receiving anti-fibrotic or immune-modulatory therapy increased progressively over time. The proportion of patients on recognised anti-tussive therapies such as opioids and bronchodilators also increased over time. Surprisingly the use of angiotensin converting enzyme (ACE) inhibitors was fairly consistent through the study. Given that ACE inhibitors are known to be associated with an increased cough sensitivity (Havelka et al., 1982), it is surprising to see their ongoing use in patients with cough.

	Baseline	6-months	12-months
Number of patients attending	52	48	43
Percent on oral corticosteroids	0	31.3%	34.9%
Percent on Pirfenidone	7.7%	20.8%	23.3%
Percent on Nintedanib	9.6%	37.5%	37.2%
Percent on Tiotropium	5.8%	14.6%	16.3%

Percent on antacid therapy	44.2%	66.7%	60.5%
Percent on neuropathic agents (Gabapentin, Pregabalin or amitriptyline)	3.7%	4.2%	7%
Percent on Oramorph	3.7%	6.3%	9.3%
Percent on ACE inhibitors	13.5%	10.4%	14%

Table 4.4 – Drug therapy changes over time in the cough cohort

4.3.0

Quality of life in the PROFILE Cough Cohort

In this cohort we used three key measures of quality of life. The first was the St George Respiratory Questionnaire, a generic quality of life assessment tool that was originally designed in airways disease patients(Jones et al., 1991). In addition, the disease specific ATAQ-IPF(Swigris et al., 2010b) quality of life score and the Hospital anxiety and depression scores(Zigmond and Snaith, 1983) were used. Each measure was repeated at three monthly intervals from baseline to one year.

4.3.1

Measuring Quality of life using ATAQ-IPF

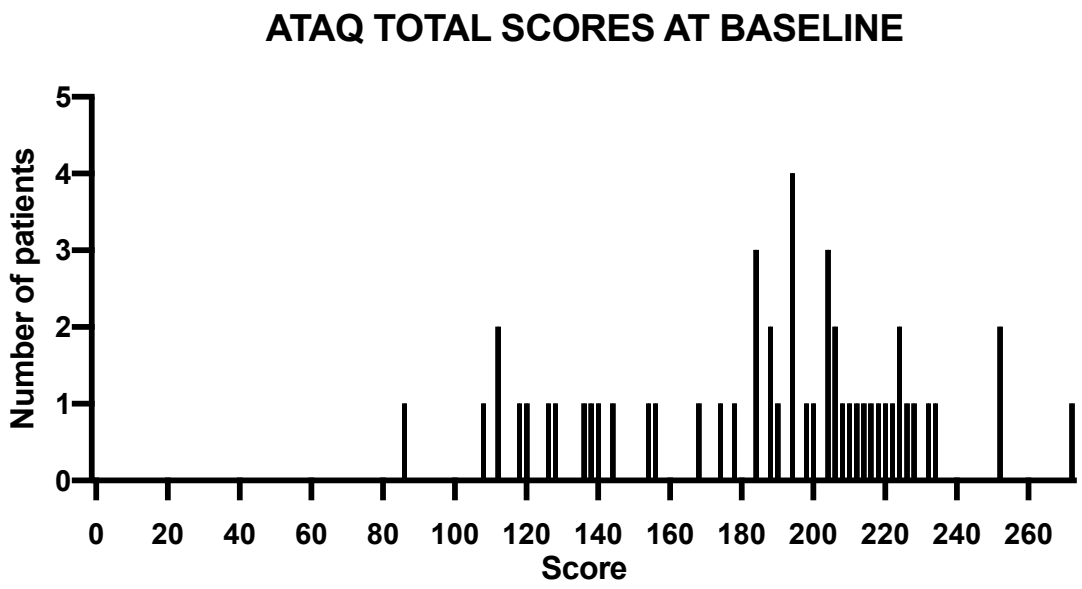
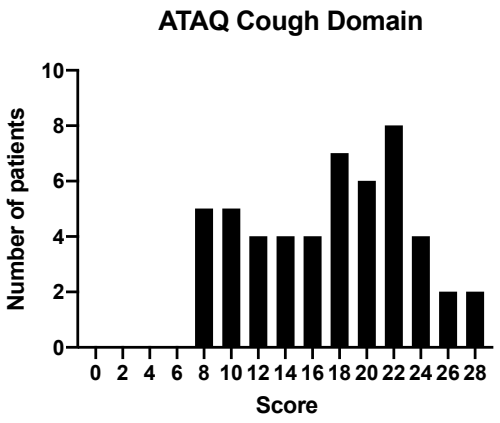


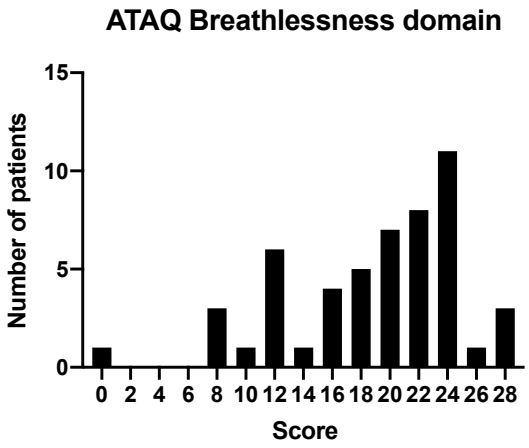
Figure 4.3 – Histogram showing total scores for the ATAQ-IPF Questionnaire at baseline

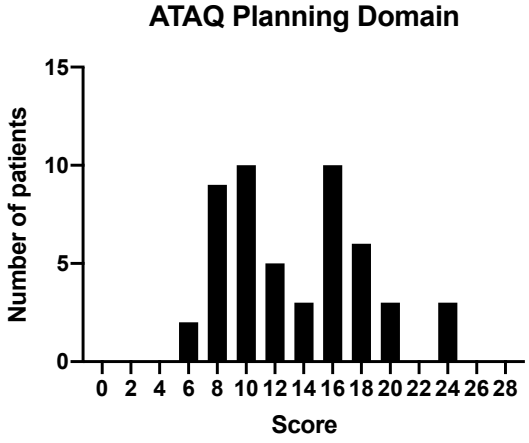
The ATAQ-IPF questionnaire was designed by Swigiris et al to measure quality of life in patients with fibrotic lung disease (Swigris et al., 2010b), 74 Questions are divided into 13 domains. Results are additive and the total score range is between 74-370 with a higher score representing worsening quality of life. The original reporting cohort for the tool had a mean time from diagnosis to completing the questionnaire of 2.9 years and had a mean total score of 210 (Swigris et al., 2010b).

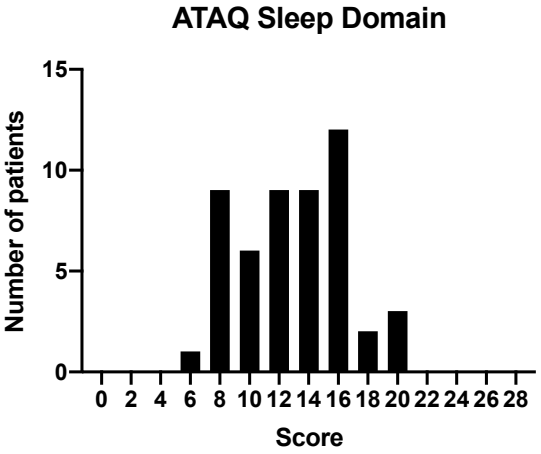
The total scores and nearly all of the components are normally distributed. The most obviously non-normal responses were to those questions regarding spirituality, sex and treatment (figure 4.4). In the case of spirituality, many of the patients identified as atheist and found the question irrelevant. The sex questions were not completely answered by a

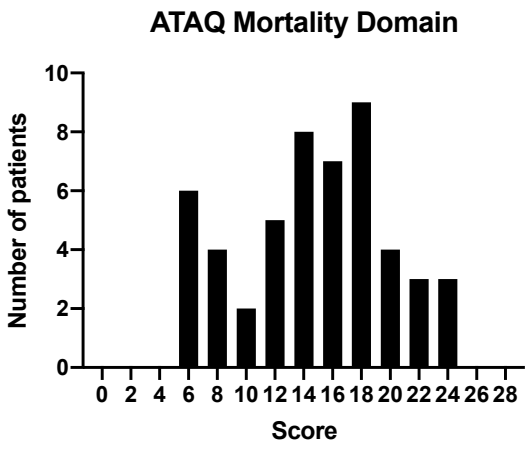
large number of the patients, resulting in a skew to a lower value. The treatment questions were unlikely to have a high relevance at baseline since the vast majority of patients were not on treatment.

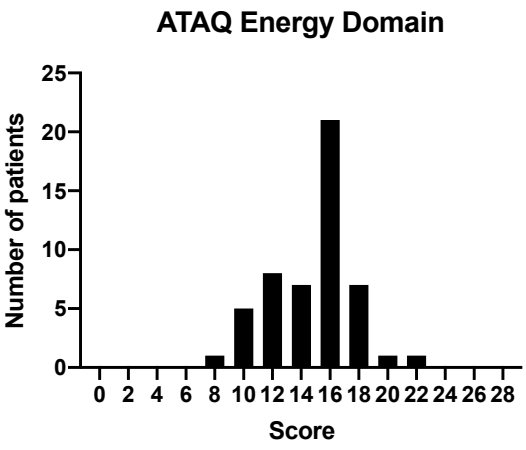


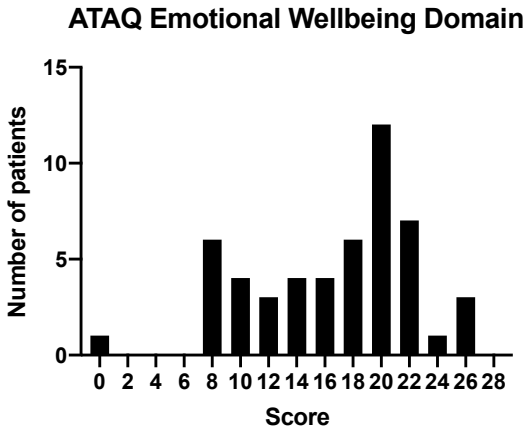


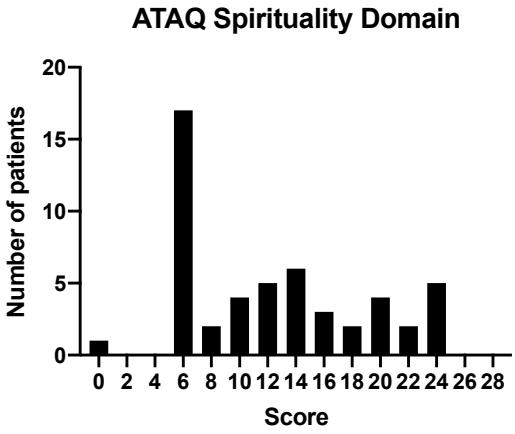


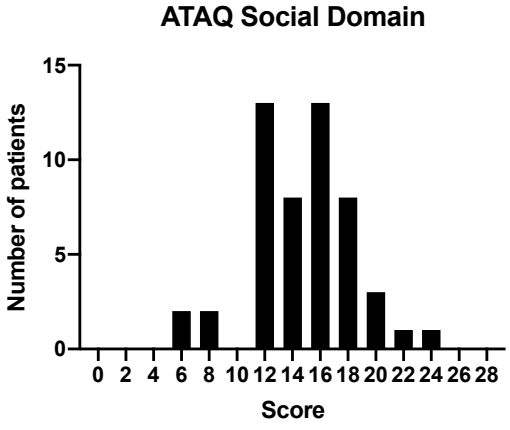


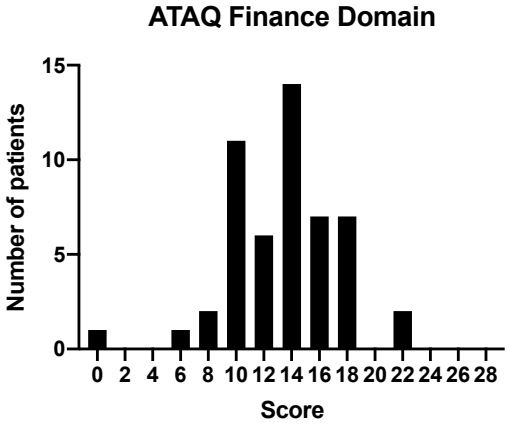

ATAQ Mortality Domain
Number of patients
Score
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28
0 2 4 6 8 10


ATAQ Energy Domain
Number of patients
Score
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28
0 5 10 15 20 25


ATAQ Emotional Wellbeing Domain
Number of patients
Score
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28
0 5 10 15


ATAQ Spirituality Domain
Number of patients
Score
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28
0 5 10 15 20


ATAQ Social Domain
Number of patients
Score
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28
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ATAQ Finance Domain
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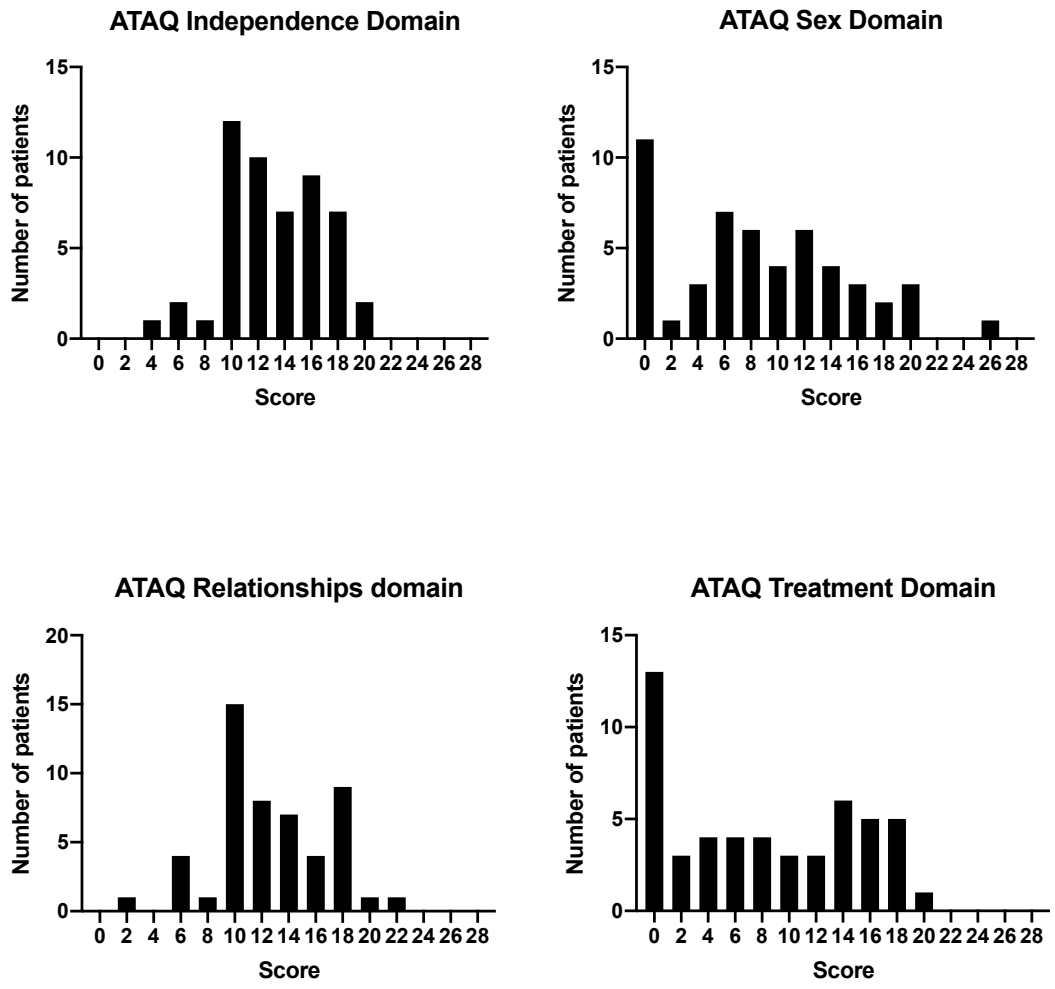


Figure 4.4 – Baseline domain scores for the ATAQ-IPF Questionnaire

The internal consistence of the ATAQ-IPF questionnaire was measured using the alpha Cronbach. In this cohort, the alpha Cronbach of the ATAQ-IPF in this cohort was 0.86 using the baseline values from each domain.

In order to better understand the relationship between subjective and objective measures of cough, a Pearson correlation between the cough domain scores of the ATAQ-IPF questionnaire and the objective cough counts was performed. To normalise the objective cough counts, these were log transformed (cough counts are otherwise positively skewed).

This showed a significant correlation of 0.32 with a p value of 0.03 (48 patients included in analysis; patients with missing data excluded). The total ATAQ scores did not correlate with objective cough counts (Pearson 0.13, p=0.38). There was no correlation with the objective cough counts and breathlessness or energy domains. The results seem to suggest that cough may not be the strongest determinant of quality of life in IPF, but that the cough questions in ATAQ-IPF help to tell us about cough frequency and impact.

There was very strong correlation between the St George's respiratory questionnaire score and the ATAQ IPF scores (Pearson 0.78, p<0.01). ATAQ also correlated well with the hospital anxiety (Pearson 0.48, p<0.01) and depression (Pearson 0.58, p<0.01) scores, highlighting the effects chronic lung disease has on mental health.

At 12-months, there had been significant change in responses in the treatment domain (figure 4.5), as would be expected given that more patients had now experienced treatment. The sex and religious themed questions however continued to elicit low scores.

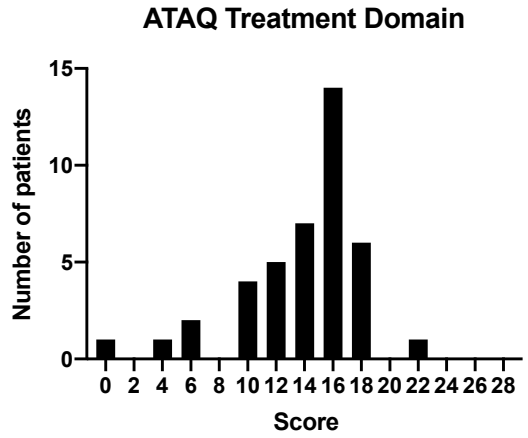


Figure 4.5 ATAQ Treatment domain scores at 12 months

The overall spread of scores however remained normally distributed. This allowed a Student's T test to be used to compare the quality of life between the two time periods. Of the 40 patients in which there was a completed ATAQ at both baseline and 12 months, there was significant correlation between the two time points (correlation 0.80, $p<0.01$) and no significant difference between the mean score at each time point ($t=-1.852$, $p=0.07$). This would suggest that quality of life in the group did not change significantly over one year. This is reflected in table 4.5 which shows that the median total scores changed little over the various timepoints. The only significant change occurred within the treatment domain.

		Baseline	3 month	6 month	9 month	12 month
Number of responses		52	46		44	41
Cough	Mean	16.7	16.7	16.3	16.6	15.3
	Std dev	5.8	5.6	6.0	6.4	6.5
	Median	17.0	17.0	16.5	17.5	16.0
Breathlessness	Mean	18.3	18.4	17.7	17.4	18.4
	Std dev	6.0	5.6	6.0	6.4	5.6
	Median	19.0	19.0	18.0	18.5	19.0
Planning	Mean	13.0	13.2	13.6	13.3	13.1
	Std dev	4.8	4.8	4.8	5.0	4.6
	Median	12.0	12.0	11.5	13.5	12.0

Sleep	Mean	12.4	12.2	12.4	13.1	12.7
	Std dev	3.5	3.1	2.8	3.6	3.8
	Median	13.0	12.5	12.0	12.5	13.0
Mortality	Mean	14.3	14.7	14.2	14.1	14.3
	Std dev	5.0	4.7	5.5	5.1	4.9
	Median	15.0	14.0	15.0	14.0	15.0
Energy	Mean	14.3	14.7	15.2	14.3	14.6
	Std dev	2.9	3.0	3.0	2.8	2.5
	Median	15.0	15.0	15.0	15.0	15.0
Emotional wellbeing	Mean	16.1	17.1	17.4	16.1	15.9
	Std dev	5.8	6.4	5.8	5.9	6.5
	Median	17.0	17.5	18.0	16.0	16.0
Spirituality	Mean	12.1	12.9	12.1	11.7	11.3
	Std dev	6.3	6.5	6.7	6.3	6.3
	Median	12.0	12.0	10.0	12.0	11.0
Social	Mean	14.4	15.0	14.8	14.9	15.1
	Std dev	3.6	3.3	2.4	3.2	3.0
	Median	15.0	16.0	15.0	15.0	15.0
Finance	Mean	12.8	13.2	13.0	12.9	13.0
	Std dev	4.0	3.9	4.0	3.6	3.7
	Median	13.0	14.0	13.0	13.0	12.0
Independence	Mean	12.7	13.5	14.0	13.2	13.6
	Std dev	3.7	3.5	4.1	3.6	3.8

	Median	12.0	13.5	14.0	13.0	14.0
Sex	Mean	8.2	8.2	8.4	9.4	8.3
	Std dev	6.5	6.0	5.8	6.3	6.5
	Median	8.0	8.0	8.0	10.0	8.0
Relationships	Mean	12.2	12.5	12.9	12.4	12.7
	Std dev	4.2	3.4	3.7	4.6	3.4
	Median	12.0	13.0	13.0	12.0	14.0
Treatment	Mean	7.9	10.9	12.7	12.5	13.5
	Std dev	6.7	5.8	5.1	5.1	4.1
	Median	7.0	12.0	14.0	14.0	15.0
Total	Mean	185.3	191.8	194.5	193.1	191.8
	Std dev	45.6	36.6	38.9	61.7	41.1
	Median	193.0	197.0	196.0	195.0	194.0

Table 4.5 Changes in ATAQ IPF domain scores over time

4.3.2

Measuring quality of life using the St George's Respiratory Questionnaire

The St George's Respiratory Questionnaire (SGRQ) is commonly used as a clinical trials endpoint for quality of life across a spectrum of respiratory disease. Its questions are divided into three domains. The score rises with worsening quality of life and the highest possible score is 100.

The baseline scores from the cohort are shown in figure 4.6. The distributions are normal in the symptoms domain, but slightly skewed in activity, impact and total scores.

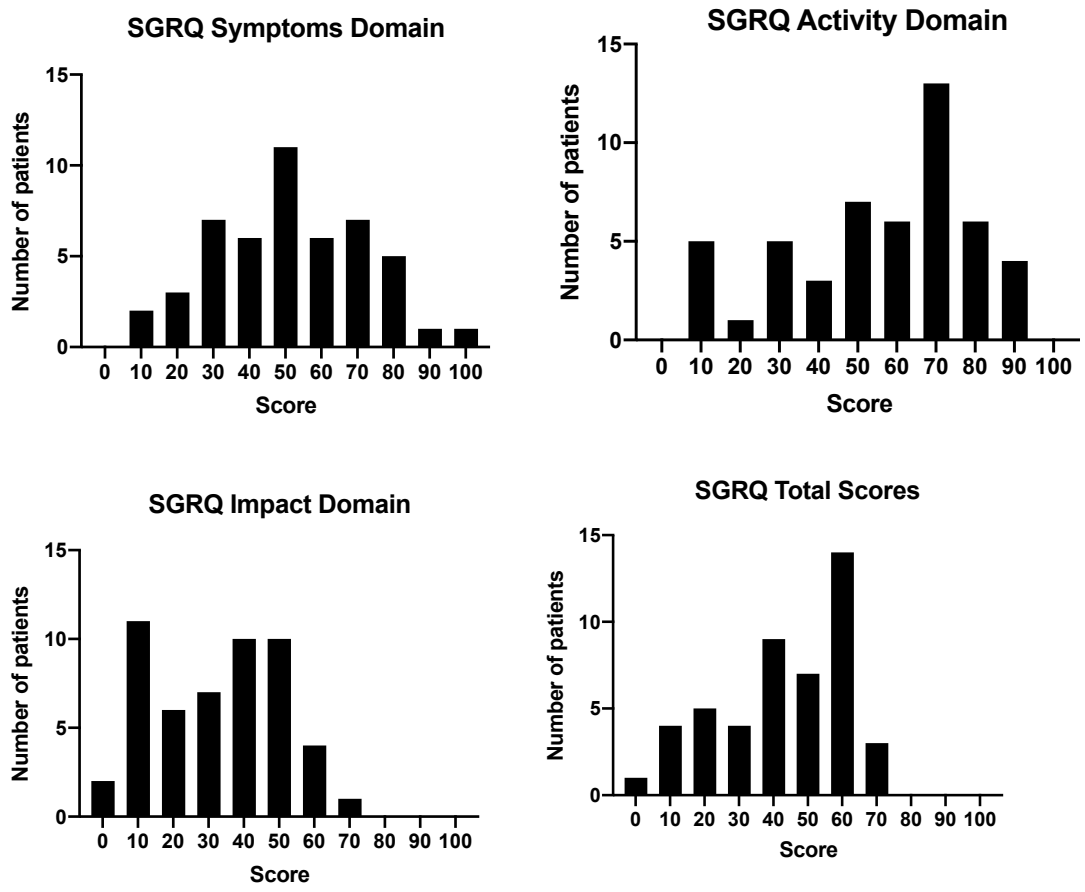


Figure 4.6 – Baseline St George's Respiratory Questionnaire domain scores

Compared to the original PROFILE cohort, the mean scores for all domains and the total scores were lower (table 4.6), suggesting that these individuals had better quality of life than the patients from PROFILE. This is probably related to the fact that the patients in the cough cohort had better lung function than the PROFILE cohort. This idea is supported by a strong correlation between the total SGRQ score and CPI (Pearson 0.46, p=0.01). The data again

back up the findings of Swigiris et al – quality of life deteriorates as disease worsens in fibrotic lung disease(Swigris et al., 2010a).

	Symptoms	Activity	Impact	Total score
Baseline mean (Cough cohort)	50.7 (SD 21.0)	55.4 (SD24.0)	32.2 (SD 17.8)	42.9 (SD 18.5)
Baseline mean (PROFILE cohort)	56.8 (SD 20.0)	65.2 (SD 23.6)	36.8 (SD 19.7)	50.9 (SD 18.6)

Table 4.6 Baseline St George's Respiratory Questionnaire domain scores

There was no correlation between either the SGRQ total score or the symptom specific score and the objective cough counts. Again, this suggests that cough is not the biggest determinant of quality of life in fibrotic lung disease.

Over the 12 months of the study there was no significant change in quality of life as measured by the SGRQ. This is true for the total score and all individual components (table 4.7). The difference was assessed using a Student's paired T test. There was significant correlation between the baseline and 12 months scores.

	Correlation	p value	Difference of means	t statistic	p value
Symptoms domain Baseline vs 12mo	0.57	<0.01	1.9885	0.601	0.55
Activity domain Baseline vs 12mo	0.77	<0.01	-1.13949	-0.390	0.70
Impact domain Baseline vs 12mo	0.62	<0.01	-0.51675	-0.203	0.84
Total score Baseline vs 12mo	0.71	<0.01	-0.31027	-0.123	0.90

Table 4.7 Changes in SGRQ scores over 12 months

4.3.3

Subjective cough severity – Cough Visual Analogue Scale

Patients in this cough cohort were asked to complete a visual analogue scale to assess how bad their cough had been over the last two weeks. This is a 10cm line on which the patient marks an x; the further to the right the x is, the worse they have rated their cough. A score of over 4cm is usually an entry criterion for any commercial clinical trial of cough therapeutics.

The cough VAS were non-normal in distribution (figure 4.7) and demonstrate the variability of cough as a symptom within the cohort.

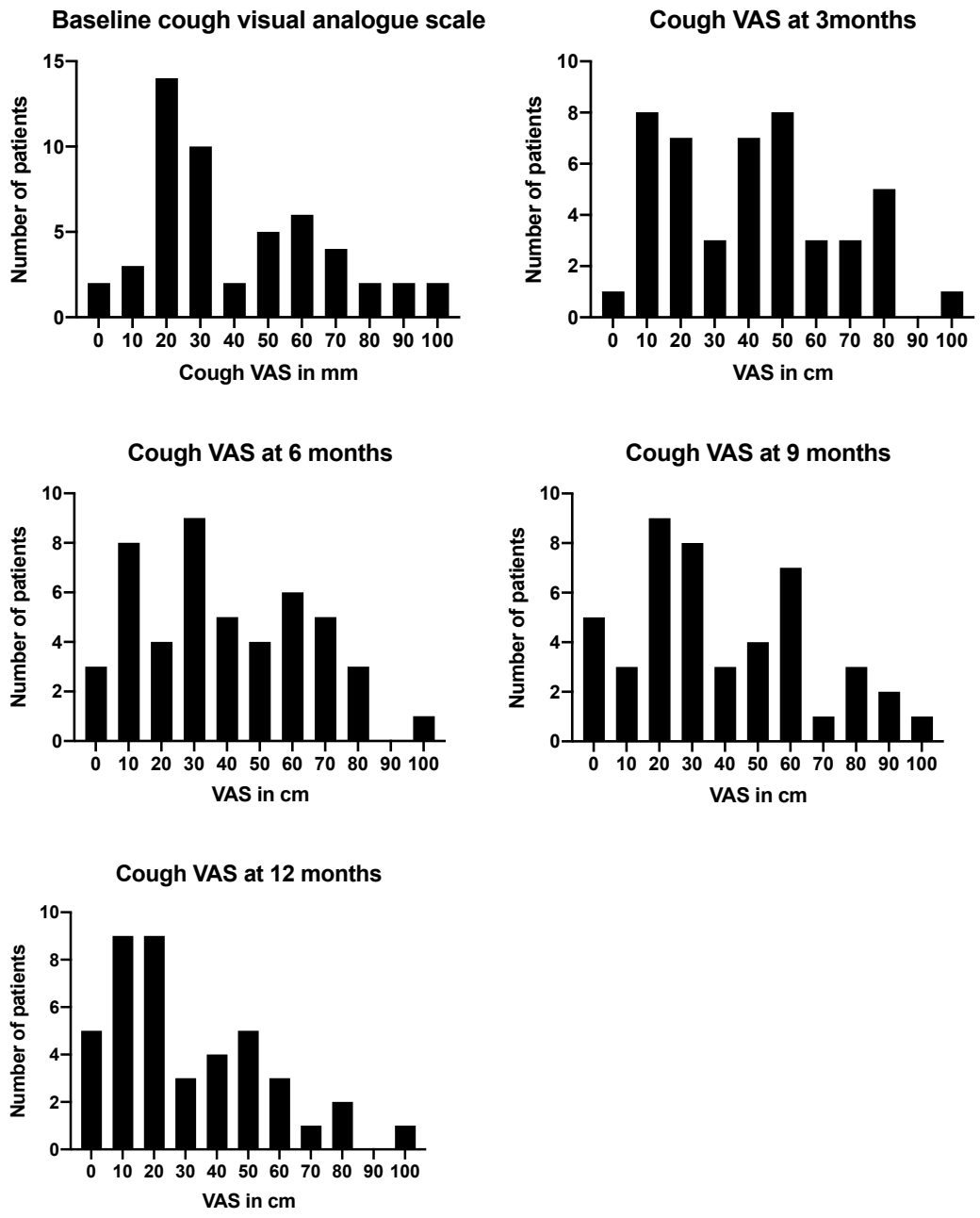


Figure 4.7 – Changes in Cough VAS scores over time

Using Wilcoxon Signed Rank tests to compare the differences between baseline and 12 months showed no significant difference in the median values (test statistic -1.536, p=0.13). The same was true when baseline was compared to each of the other time points. This would suggest that in the group cough did not change significantly over time. This is similar to the findings of the LCQ data from the original PROFILE cohort.

When examining all the data over the different time points, the cough VAS correlates strongly with objective cough counts using a simple Pearson correlation (Correlation 0.51, $p<0.01$). This correlation is shown in figure 4.8.

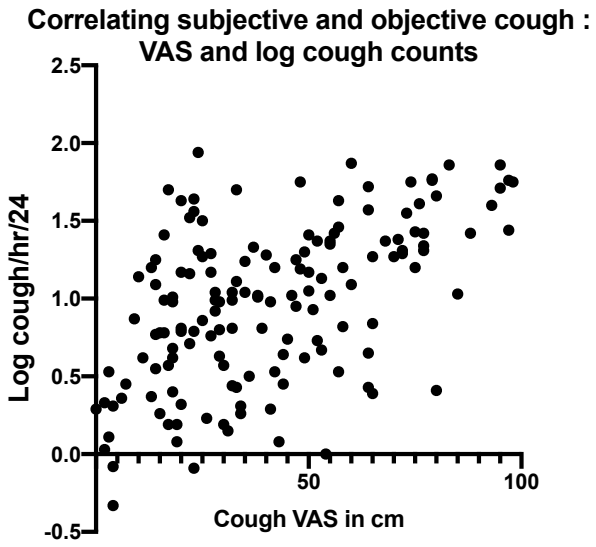


Figure 4.8 Correlating Cough VAS with objective cough

There was also very good correlation between objective cough counts and LCQ as shown in figure 4.9 (Correlation -0.74, $p<0.01$).

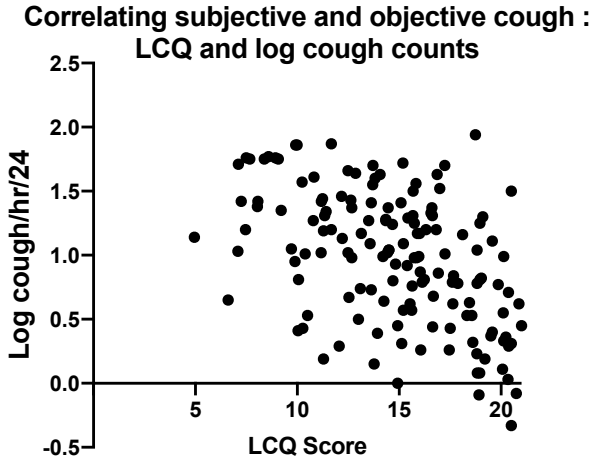


Figure 4.9 Correlating LCQ with objective cough

4.3.4

Subjective cough severity – Leicester cough questionnaire

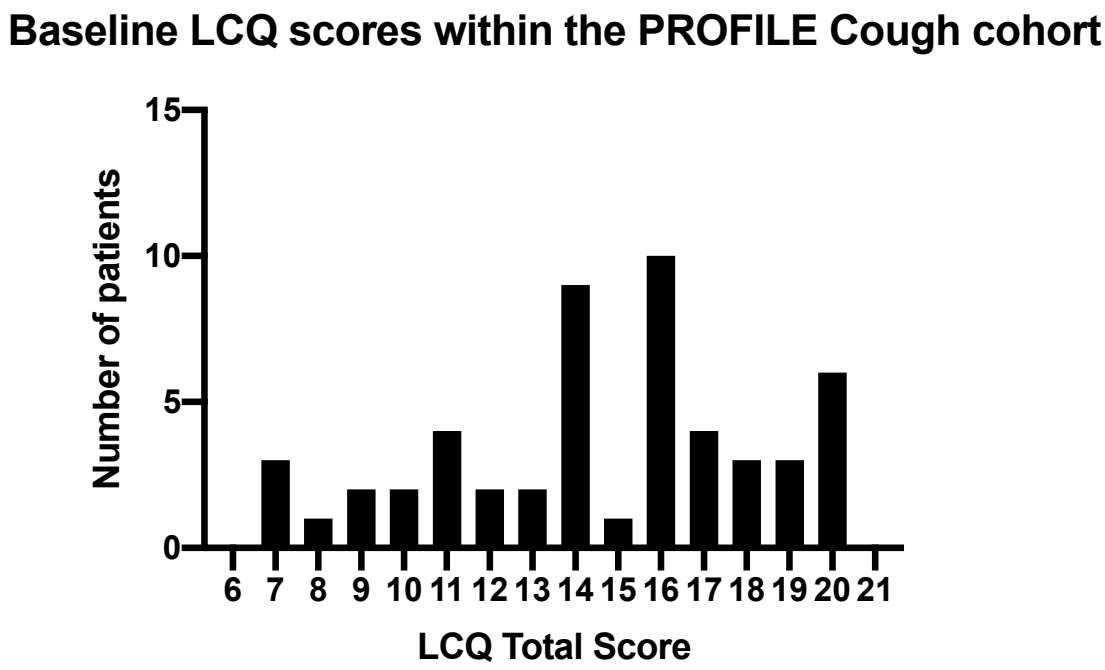


Figure 4.10 Histogram of baseline LCQ total scores

At baseline all patients completed a Leicester cough questionnaire (Figure 4.10). Whilst not as skewed as the results from the original PROFILE cohort, there is still a skew towards higher scores. The mean LCQ score in this cohort at baseline was 14.6 and the median was 14.9. This was lower than the median LCQ of the original PROFILE cohort (median 16.3), suggesting this group of patients had worse cough related quality of life.

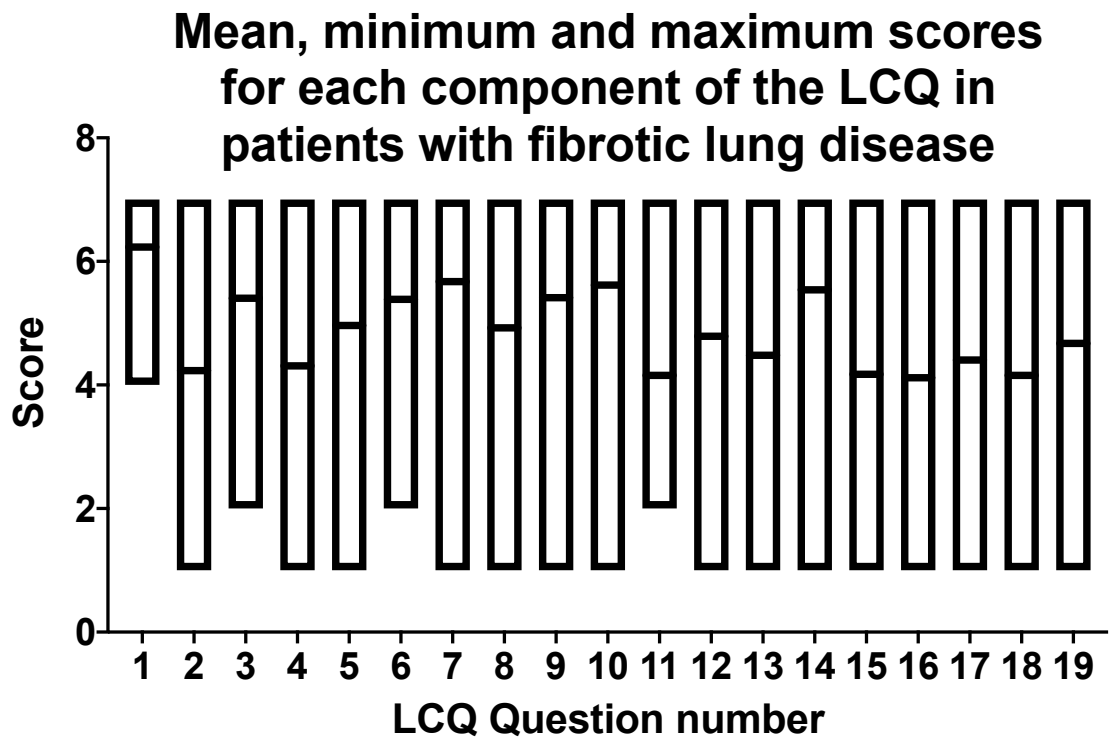
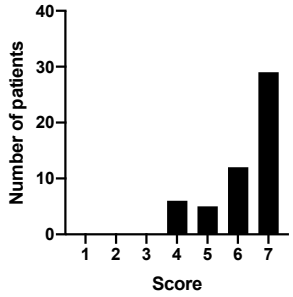


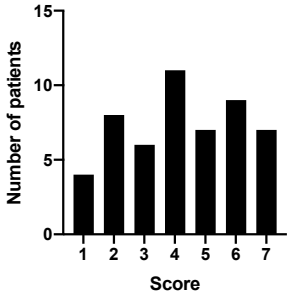
Figure 4.11 Baseline LCQ responses divided by component questions

The results of the baseline questionnaires were then examined to see which themes emerged as important to patients with fibrotic lung disease (figure 4.12). The results suggest that some questions from the Leicester cough questionnaire are less applicable to this population than the idiopathic cough population that the questionnaire was designed in. Very few patients reported chest pain or hoarse voice as part of the impact of their cough. Fatigue however seemed to be a common problem in the cohort. Very few of the questions appeared to generate a normal distribution of responses. The response histograms are very similar to those seen in the original PROFILE study.

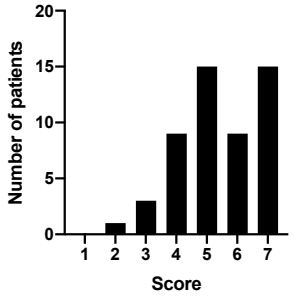
1.) Have you had chest or stomach pains as a result of your cough?



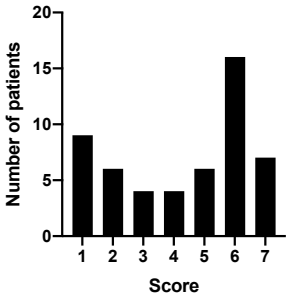
2.) Have you been bothered by sputum (phlegm) production when you cough?



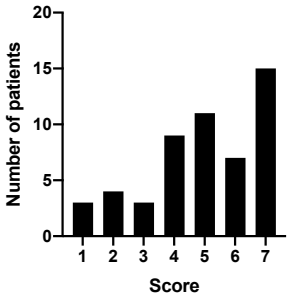
3.) Have you been tired because of your coughing?



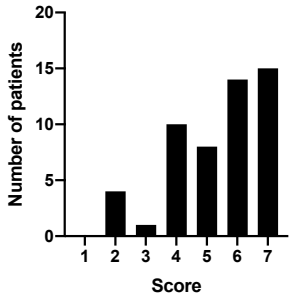
4.) Have you felt in control of your cough?



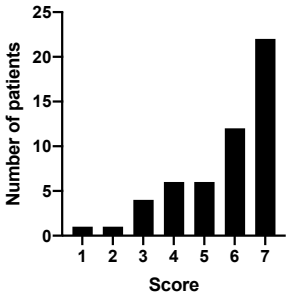
5.) How often have you felt embarrassed by your coughing?



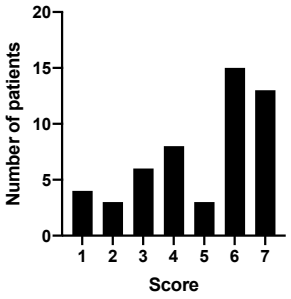
6.) My cough has made me feel anxious.



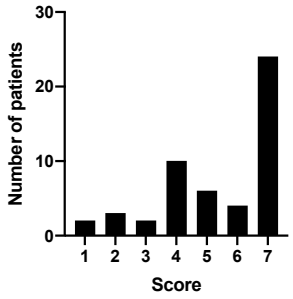
7.) My cough has interfered with my job, or other daily tasks.



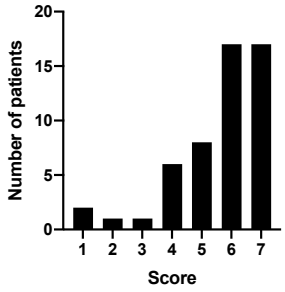
8.) I felt that my cough interfered with the overall enjoyment of my life.



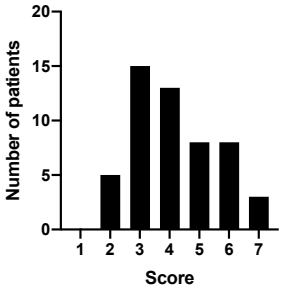
9.) Exposure to paints or fumes has made me cough.



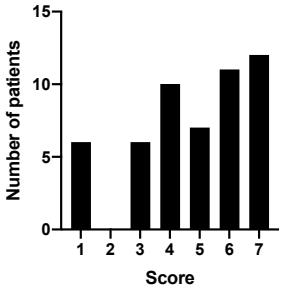
10.) Has your cough disturbed your sleep?



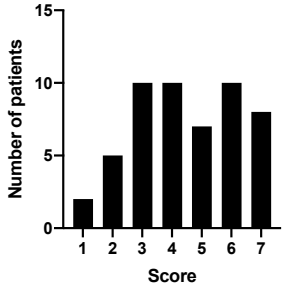
11.) How many times a day have you had coughing bouts?



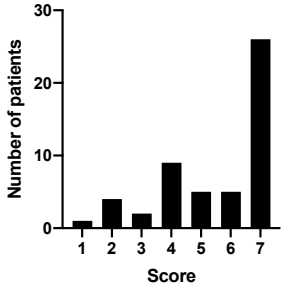
12.) My cough has made me feel frustrated



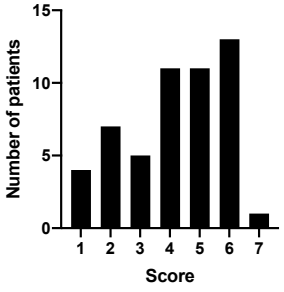
13.) My cough has made me feel fed up.



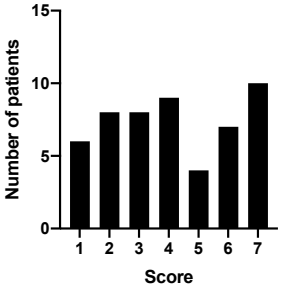
14.) Have you suffered from a hoarse voice as a result of your cough?



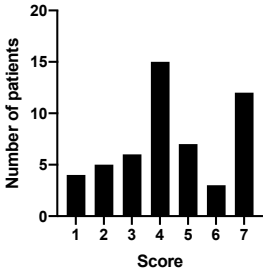
15.) Have you had a lot of energy?



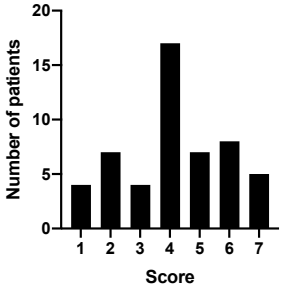
16.) Have you worried that your cough may indicate a serious illness?



17.) Have you been concerned that other people think something is wrong with you, because of your cough?



18.) My cough interrupted conversation or telephone calls



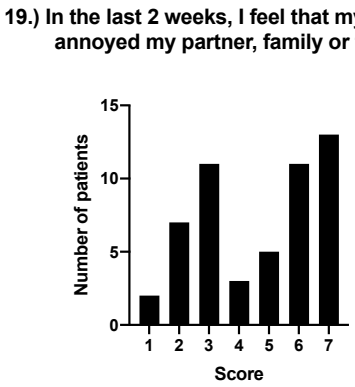


Figure 4.12 – Baseline responses to the LCQ divided by question

The Cronbach alpha statistic for all 19 questions is 0.94. This suggests a very high level of internal reliability. The Cronbach alpha for the physical symptoms score is 0.70, for psychological symptoms is 0.90 and for social symptoms is 0.85.

4.4.0

Objective cough counts

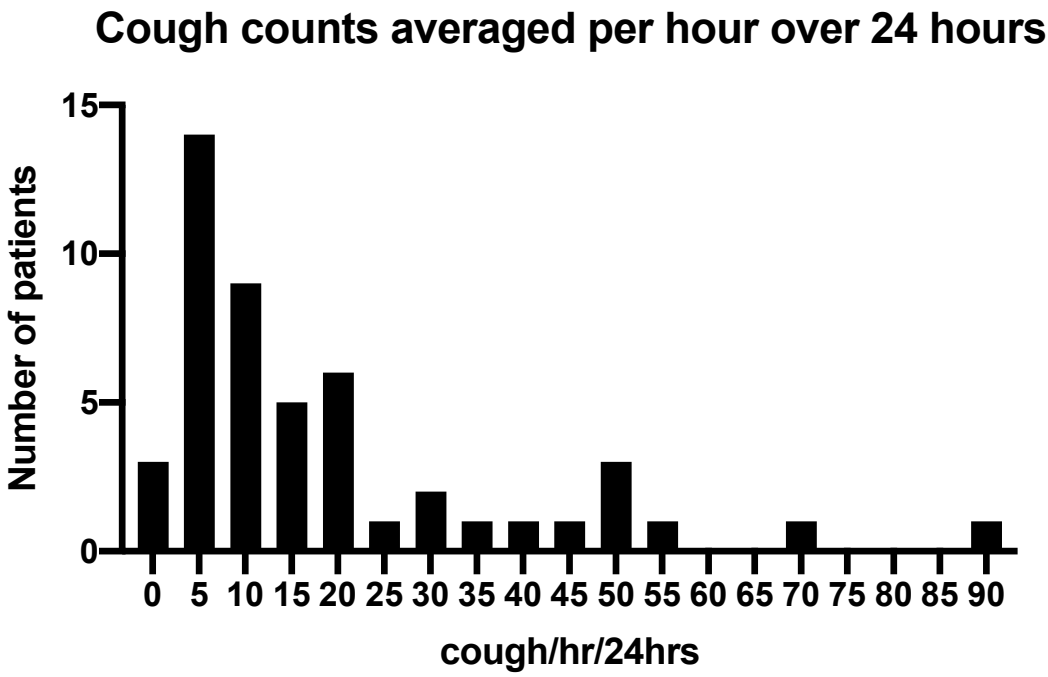


Figure 4.13 Histogram showing baseline cough frequency within the cough cohort

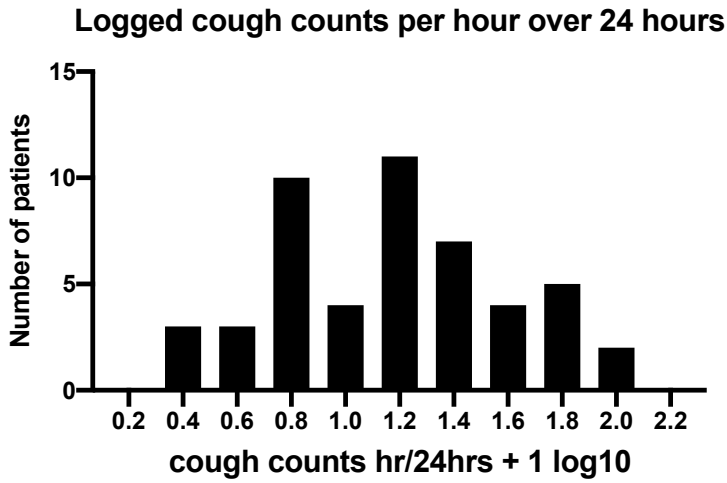


Figure 4.14 – Histogram showing the normal distribution of log transformed cough counts

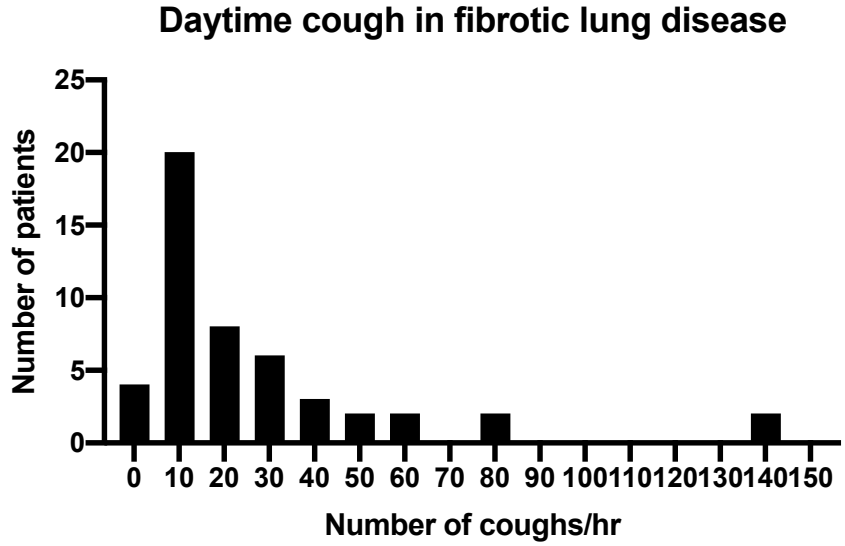


Figure 4.15 – Histogram showing coughs/hour in waking hours at baseline

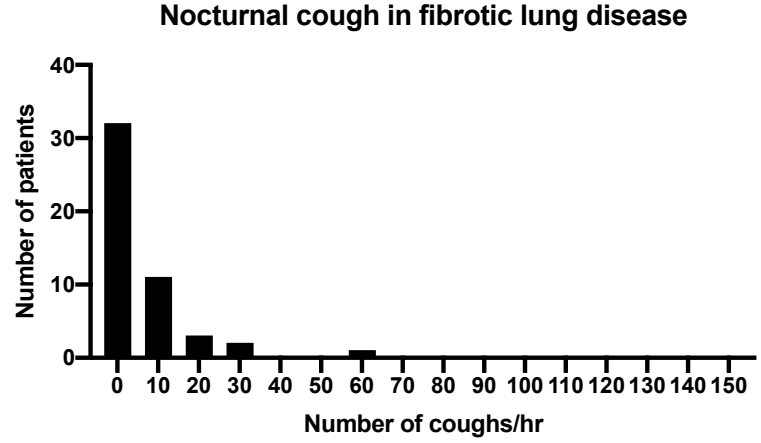


Figure 4.16 – Histogram showing coughs/hour in nocturnal hours at baseline

Figures 4.13 shows the results of 24-hour cough counts at baseline. These are displayed as the average number of coughs per hour in a 24-hour period. In figure 4.14 the same cough counts have been log transformed to normalise the data which was otherwise skewed. Figures 4.15 and 4.16 illustrate the difference in cough during awake hours and that occurring during nocturnal hours. It can be seen that nocturnal cough is much less common than cough in daytime hours. (median 2.7 coughs/hr vs 14.8 coughs/hr in awake hours).

In a generalised estimating equation evaluating the predictors of objective cough count, timepoint of the visit came out as a significant factor. ($P<0.01$). The baseline timepoint was the most different. There was not a consistent trend for improvement in objective cough over time, however, as is demonstrated in figure 4.17.

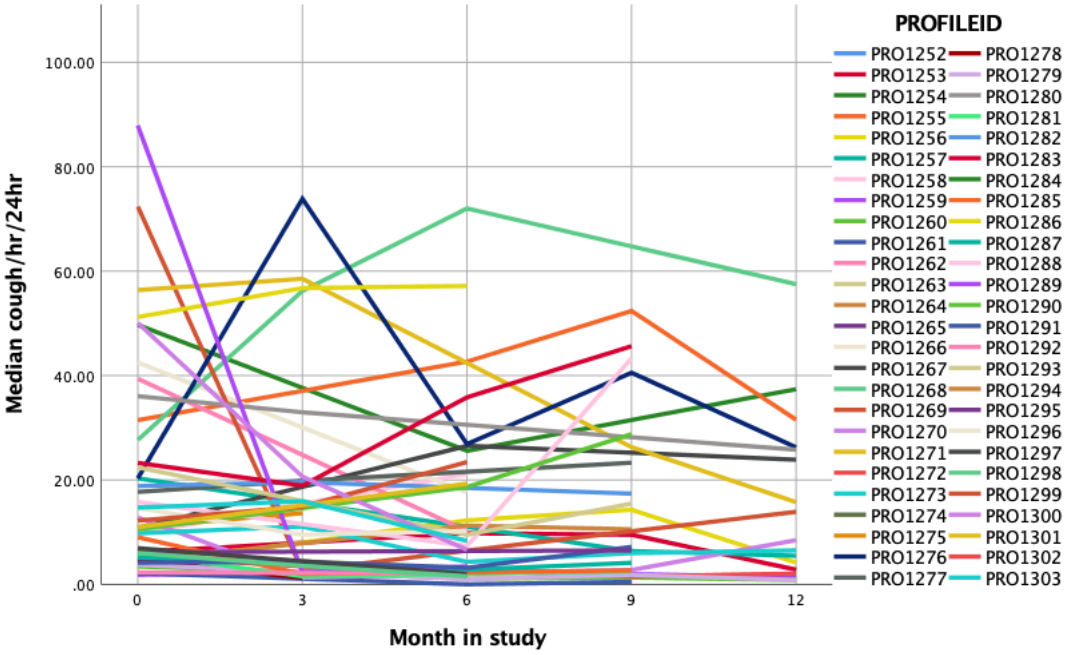


Figure 4.17 Change in cough over time for each individual patient in study

There was no significant difference in cough VAS over time, although at baseline there was a trend towards difference between the other time points (p=0.053). Table 4.8 demonstrates there is a trend towards reduction in cough VAS overtime. The difference in mean cough VAS over 12 months is 9.6mm which is unlikely to be clinically significant.

There was no significant difference in LCQ over time (p=0.33).

Quality of life as reported by ATAQ-IPF did significantly change over time (p=0.02). This relationship however was not straightforward with an initial worsening in quality of life that then seemed to level off.

The SGRQ symptom score was significantly different over time (p<0.01). All time points were significantly different from each other. Overall however the SGRQ did not significantly change over the year.

	Baseline	6 months	12 months
Mean cough VAS	40.3 (SD 23.2)	38.5 (SD 24.7)	30.8 (SD 23.9)
Mean LCQ Physical	5.1 (SD 0.9)	5.1 (SD 0.9)	5.1 (SD 1.2)
Mean LCQ Psychological	4.6 (SD 1.5)	4.9 (SD 1.4)	5.2 (SD 1.6)
Mean LCQ Social	4.9 (SD 1.5)	5.0 (SD 1.5)	5.3 (SD 1.6)
Mean LCQ Total score	14.6 (SD 3.7)	15.0 (SD 3.6)	15.6 (SD 4.2)

Mean SGRQ Symptom score	50.7 (SD 21.0)	53.1 (SD 24.2)	47.6 (SD 22.1)
Mean SGRQ Total	42.9 (SD 18.5)	42.4 (SD 21.1)	42.1 (SD 20.1)
Mean ATAQ Cough score	16.7 (SD 5.8)	16.3 (SD 6.0)	15.3 (SD 6.5)
Mean ATAQ Total	185.3 (SD 42.6)	194.5 (SD 38.9)	191.8 (SD 41.1)
Mean coughs in 24 hrs	457.9 (SD 462.1)	349.8 (SD 391.5)	373.2 (SD 385.5)
Median coughs per hr awake	14.8 (SD 30.4)	12.5 (SD 24.7)	11.6 (SD 26.1)
Median coughs per hr asleep	2.7 (SD 9.9)	1.3 (SD 4.5)	2.2 (SD 3.1)
Median coughs per hr / 24hr	11.0 (SD 19.3)	9.7 (SD 16.3)	8.5 (SD 16.1)

Table 4.8 Change in cough markers over time in fibrotic lung disease

Evaluation of which measures and factors influence objective cough counts was performed using a general estimating equation in which data from all time points was included (table 4.9). In this model although the total score from the St George's respiratory questionnaire was not a good predictor of objective cough counts, the symptom score was the strongest predictor of objective cough frequency in this generalised estimating equation. More surprisingly LCQ was not a good predictor of objective cough frequency in this model, whereas cough VAS was. This perhaps is because a cough VAS asks a patient to rate how bad their

cough is and therefore the patient is asked to estimate severity. The LCQ however evaluates the impact cough has on quality of life and this may not be related so well to severity.

			95% Wald confidence intervals		Hypothesis test		
Parameter	B	Std. Error	Lower	Upper	Wald-Chi square	df	Sig
(intercept)	0.993	0.4039	0.202	1.785	6.050	1	0.01
LCQ total score	-0.21	0.0135	-0.47	0.006	2.380	1	0.12
Cough VAS score	0.005	0.0018	0.002	0.009	7.914	1	0.01
ATAQ total score	-0.002	0.0014	-0.005	0.001	1.839	1	0.18
SGRQ symptom score	0.008	0.0026	0.003	0.013	10.166	1	<0.01
<0.1SGRQ total score	-0.003	0.0039	-0.011	0.004	0.697	1	0.40

Cough daytime score	0.070	0.0566	-0.41	0.181	1.530	1	0.22
Cough night-time score	0.15	0.0436	-0.70	0.101	0.126	1	0.72

Table 4.9 Generalised estimating equation evaluating predictors of objective cough counts

Interestingly the cough daytime and night-time scores that patients were asked to complete bore no relationship to cough frequency. This suggests perhaps this tool is inferior in estimating severity compared to cough VAS. Patients had a tendency to over-estimate their nocturnal cough, when the objective cough recordings suggested that nocturnal cough was not a significant problem. The finding of low frequency of cough at night is consistent with an earlier study of IPF patients undergoing sleep polysomnography (Schertel et al., 2017). In this study breathing pattern was found to be different between day and night with a significant reduction in respiratory rate. Given that higher respiratory rates are likely to promote airway drying, this may be one mechanism by which daytime cough is more frequent compared to nocturnal cough. The low rate of nocturnal cough in fibrotic lung disease is different from airways diseases such as asthma where nocturnal cough has been shown to be more common than in other causes of chronic cough (Fukuhara et al., 2020). Studies of nocturnal cough in COPD have shown whilst cough occurs in night hours, it is usually associated with EEG confirmed wakefulness and that cough occurring during sleep is rare (Power et al., 1984). Interestingly patients with chronic refractory cough appear to

demonstrate better correlation between subjective cough reporting using an identical scale to the one used here and objective cough counts (Decalmer et al., 2007). Perhaps cough at night is more disruptive because it prevents sleep and therefore leads to a higher estimation of impact by the patient.

A general estimating equation was used to evaluate factors that might impact objective cough (table 4.10). This model had a goodness of fit of 54.41. The patient's diagnosis (IPF, CHP, PFILD) was not significant overall, however in further analysis a diagnosis of CHP was significantly different in cough burden (p=0.04). This was confirmed using a Chi-square difference between means assessment for IPF and CHP in which there was a significant difference in objective cough (p=0.04). This would suggest that cough is very similar between the IPF and PFILD group. Gender did not make a significant impact, but smoking pack year history did. CPI score also had a significant effect on cough frequency.

Parameter	Wald-Chi square	df	Sig
Diagnosis	5.687	2	0.06
Gender	0.517	1	0.47
Smoking pack years	3.884	1	0.05
CPI Score	5.070	1	0.02

Table 4.10 Results of the general estimating equation examining factors affecting cough

Using Pearson correlations for the complete data set (all time-points), the association between objective cough and some lung function markers was confirmed (table 4.11). Interestingly FVC does not correlate with objective cough, but gas transfer does. Like in the original PROFILE cohort (in which the associations had been with a subjective measure of cough) these associations were statistically significant, but weak.

	Pearson correlation with logged objective cough counts	Significance (2 tailed)
%FVC predicted	-0.126	0.13
%TLCO predicted	-0.239	<0.01
CPI score	0.218	0.01

Tablet 4.11 Pearson correlations between markers of lung function and objective cough

A further general estimating equation was run looking at the effects of treatment on cough. Evaluating Prednisolone, Pirfenidone and Nintedanib, there appeared to be no benefit of therapy to cough – however this was an exploratory analysis using small numbers of patients (table 4.12). The goodness of fit of this model was 50.941. However, if treatment effect was stratified by diagnosis, Prednisolone seemed to produce a significant effect on cough in patients with hypersensitivity pneumonitis (p=0.02). This is an interesting finding because prednisolone appears to improve small airways dysfunction in hypersensitivity pneumonitis which may suggest the anti-tussive mechanism (Guerrero Zuniga et al., 2017).

The study was not set up to evaluate the effects of therapy on cough and therefore these results should be interpreted with caution.

Factor	Wald Chi-Sqaure	df	Significance
Pirfenidone use	1.004	1	0.32
Prednisolone use	1.327	1	0.25
Nintedanib use	0.841	1	0.36

Table 4.12– Effects of disease modifying therapy on cough

4.5.0

Summary of results

1.) Objective cough counting provides different information from that provided by subjective measures such as LCQ.

2.) Cough VAS appears to show strong correlation with cough counts and as such would be a useful clinical and research tool.

3.) Nocturnal cough is uncommon in fibrotic lung disease

4.) Chronic hypersensitivity pneumonitis appears to be associated with much higher amounts of cough than seen in IPF and the cough may be treatment sensitive

5.) In IPF and progressive fibrotic ILD cough persists over a year and response to therapy is less clear.

5.0

THE HOST DEFENCE AND ITS ROLE IN COUGH IN IPF

5.1

The *MUC5B* Polymorphism

There are several genetic mutations which increase an individual's lifetime risk of developing fibrotic lung disease (Moore et al., 2019). The most common genetic abnormality observed in IPF is a single nucleotide polymorphism in the promotor region of a mucin encoding gene, *MUC5B (Moore et al., 2019)*. The same genetic polymorphism has been found to be more common in patients with chronic hypersensitivity pneumonitis (CHP) (Ley et al., 2017) but not CTD-ILD (Stock et al., 2013).

Mucins are key components of the airway host defence system as they trap inhaled pathogens and debris. MUC5AC and MUC5B are the major gel-forming mucins that make up mucous. A mucociliary escalator system then leads to clearing of mucous with cough. In the distal airways, MUC5B is the predominant gel forming mucin (Seibold et al., 2013). The mutation of the *MUC5B* promotor is thought to lead to its gene being overexpressed(Yang et al., 2013) with subsequent accumulation of excessive quantities of MUC5B mucin and the formation of honeycomb cysts (Seibold et al., 2013). Surgical lung biopsies of patients with idiopathic pulmonary fibrosis there are almost twice as many MUC5B positive cells within the distal airways when compared to CTD-ILD biopsies (Conti et al., 2016). These cysts are the

hallmarks of a usual interstitial pneumonitis pathology that is the hallmark of idiopathic pulmonary fibrosis.

In a paper by Scholand et al (Scholand et al., 2014), cough severity as measured by the Leicester cough questionnaire (LCQ) was shown to differ between different *MUC5B* genotypes. The authors showed that carriage of the T allele was associated with more severe cough in a group of 68 patients with IPF. The study did not examine any objective markers of cough which as I have shown in the previous chapter, is important because objective cough counts do not strongly correlate with the LCQ.

The use of DNA samples extracted from whole blood from both the original PROFILE cohort and PROFILE cough cohort allowed us to examine the relationship between the *MUC5B* genetic polymorphism in both subjective and objective measures of cough.

The lungs are not a sterile environment and are home to a complex microbiome in health. There is increasing recognition that in respiratory disease changes occur to the make-up and quantity of bacteria within the lung (Molyneaux et al., 2014, Molyneaux et al., 2013, Huang et al., 2015). Previously published data in IPF has demonstrated that increased bacterial burdens are associated with faster disease progression and an increased risk of death (Molyneaux et al., 2014). In this study it was also noted that the *MUC5B* genotype was associated with bacterial burden. Patients carrying the t allele were shown to have lower bacterial burdens in the lung compared to those without the t allele. The study also demonstrated that certain species of bacteria are found in more abundance in the IPF lung

than the healthy lung. Such species include *Streptococcus*, *Haemophilus*, *Neisseria* and *Veillonella spp (Molyneaux et al., 2014)*.

Individual species of bacteria may be able to stimulate cough by their action on sensory nerves. This is likely to be an ATP dependent mechanism and a wide variety of bacteria have been shown to release extra-cellular ATP in their growth phase (Mempin et al., 2013). ATP acts on P2X3 receptors to cause firing of sensory nerves in the cough pathway (Bonvini et al., 2016).

Patients within the PROFILE cough cohort who required a bronchoscopy for clinical reasons were consented to allow residual bronchoalveolar lavage fluid to be analysed for research. Our intention was to explore whether bacterial burden or indeed changes to species within the lung microbiome would influence both objective and subjective measures of cough.

I hypothesized that patients carrying a mutation in the *MUC5B* promotor would have excessive cough, possibly as a result of the accumulation of excessive mucin or even secondary to changes in the respiratory microbiome.

5.11

***MUC5B* genotype of patients**

In the original PROFILE cohort 481 patients had a successful DNA extraction and analysis. 93 patients did not have a valid DNA sample.

GG homozygote – 40.7%, GT heterozygote – 52%, TT homozygote – 7.3%



Figure 5.1 Proportion of patients carrying the T allele in the PROFILE cohort

In the cough cohort 45 patients had a successful DNA extraction and analysis. This left 7 patients in which the process was unsuccessful.

GG homozygote – 28.9%, GT heterozygote – 60%, TT homozygote – 11.1%



Figure 5.2 Proportion of patients carrying the T allele in the PROFILE cough cohort

5.12

Other genetic polymorphisms in patients with fibrotic lung disease

Other described genetic mutations which may increase the likelihood of developing IPF are significantly rarer (Moore et al., 2019). Although it would have been interesting to assess relationships between other genetic mutations and cough, rarity of these mutations would have been problematic. Within the original PROFILE cohort, the abnormal T allele in the *MUC5B* promotor occurred in 59.3% of cases in which there was a valid DNA sample. Mutations in *SPDL1* (4.8%), *PARN* (2.5%), *TERT* (1.7%) and *RTEL1* (1.5%) were much rarer. To measure any impact these genes had on disease severity, cough or quality of life would have required a much larger sample size.

5.2.0

The relationship of the *MUC5B* promoter polymorphism with cough

Using data from the two patient cohorts (PROFILE and PROFILE cough), it was possible to explore the effects of T allele carriage for the *MUC5B* promotor polymorphism on objective and subjective markers of cough.

In the original PROFILE cohort, a one-way ANOVA statistic was performed to see if there were significant differences between LCQ scores in the different *MUC5B* genotype groupings. 454 patients were included in this analysis. There was no significant difference, with an F statistic of 0.062 and a p value of 0.94.

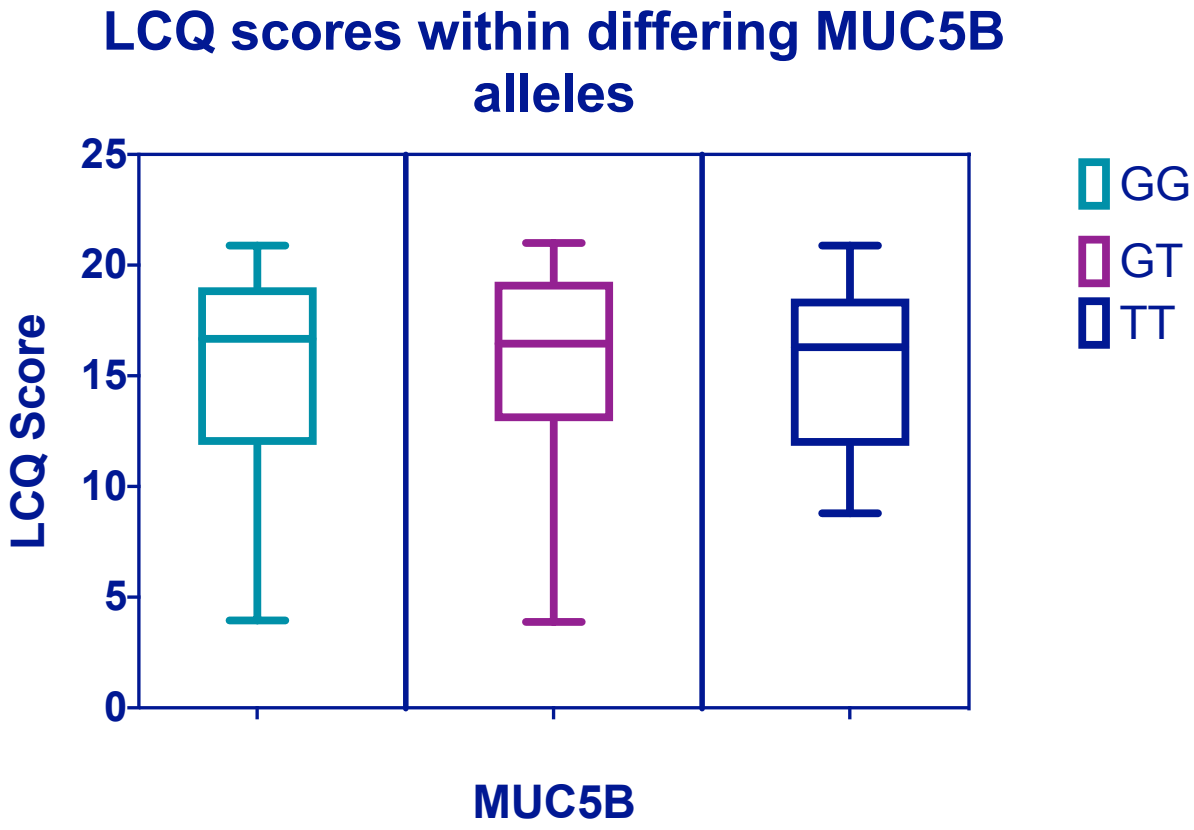


Figure 5.3 Differences in LCQ score between the different *MUC5B* genotypes

The patients from the cough cohort were then examined to see if objective cough varied between differing *MUC5B* genotypes. 42 patients had completed a successful baseline cough recording and been genotyped for their *MUC5B* status. A one-way ANOVA statistic was performed to see if there were significant differences between objective cough counts in the different *MUC5B* genotypes within the cough cohort. There was no significant difference in objective cough counts between the three *MUC5B* genotypes, with an F statistic of 0,754 and p value of 0.72 (Figure 5,4).

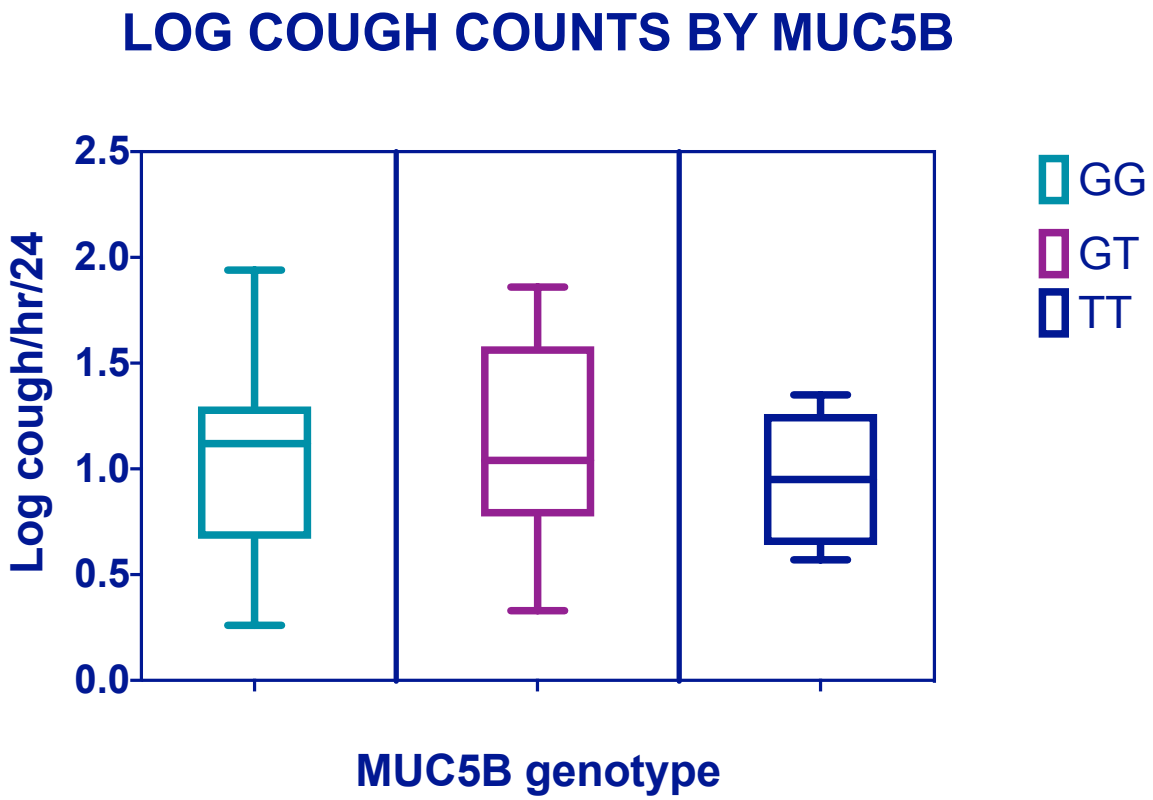


Figure 5.4 Differences in objective cough counts between the different *MUC5B* genotypes

Cough VAS and LCQ within the cough cohort similarly were not significantly different between the *MUC5B* genotypes (p=0.84 and p=0.81 respectively)

This apparent lack of difference in both objective and subjective markers of cough between *MUC5B* genotypes may possibly be explained by expression of the MUC5B protein. In experiments staining for expression of MUC5B protein, Seibold et al were able to show expression occurred in the distal airways and honeycomb cysts (Seibold et al., 2013). It is possible that over-expression is therefore a greater problem in more severe disease. This cohort did contain patients with other fibrotic lung diseases – MUC5B protein expression may not be increased in non-IPF fibrotic lung disease carriers of the rs3570950 variant (Stock et

al., 2020). Since it Is over-expression of the protein in distal airways that is the likely driver for cough, this difference between ILD subtypes and MUC5B protein expression is likely to be important.

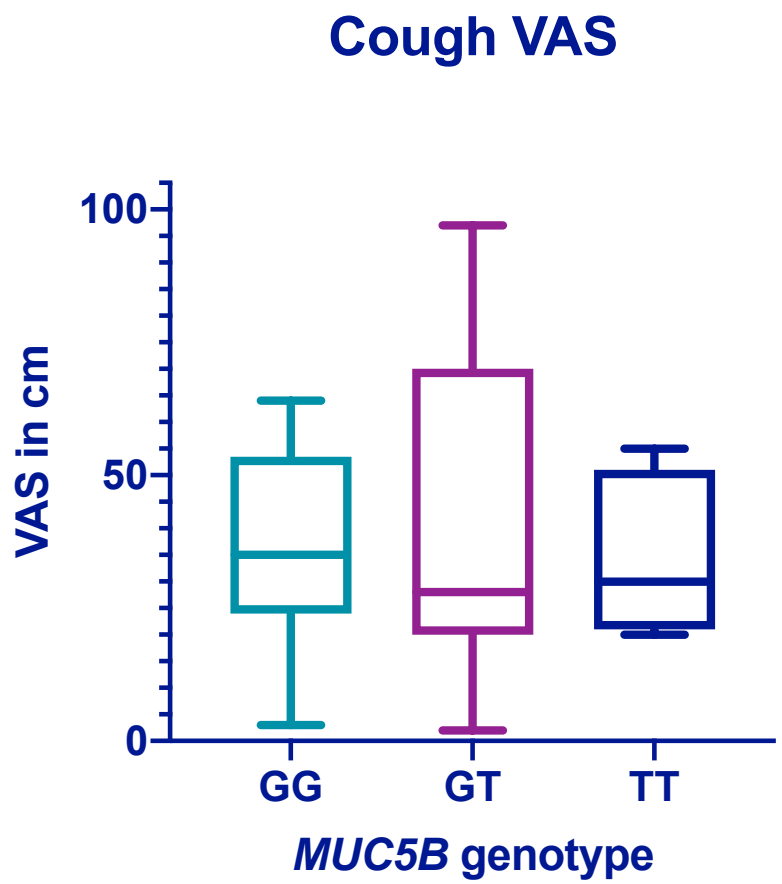


Figure 5.5 Differences in Cough VAS between the different *MUC5B* genotypes

5.3.0

The relationship between *MUC5B* promoter polymorphism and disease severity

An issue with assessing cough in patients of differing *MUC5B* genotypes is that disease severity may act as a confounder. *MUC5B* T allele carriage has been shown to be associated with milder disease progression (Stock et al., 2013). The relationship between lung function

and T allele carriage in the PROFILE cohort was tested using baseline lung function values (as measured by CPI) in a one-way ANOVA.

The result was that there was a significant difference in lung function between patients carrying different *MUC5B* genotypes. The carriage of the T allele was associated with more mild disease severity at baseline.

The survival advantage of carrying the T allele however was not born out in a Kaplan-Meier curve (figure 5.6), where it looked like more rapid progression was occurring in those who were homozygous for the T allele. This is most likely to be due to the small number of patients in this group. However the finding that heterozygous carries of the T allele had similar survival to those who do not carry the T allele is in conflict with previously reported studies (Stock et al., 2013). It is worth noting however that in this study by Stock et al that carriage of the T allele did not predict FVC decline in a univariate model, only in the multivariate model (Stock et al., 2013). In this model baseline lung function was not corrected for, although the patients carrying the T allele had milder disease severity at baseline. There are other possible confounders including age and smoking history. Mortality included all causes of death and perhaps a different result may have been produced by examining only respiratory causes of death. Finally it is worth noting that in a Chinese population, the MU55B promotor polymorphism was found to associated with more rapid disease progression and mortality (Jiang et al., 2015, Wang et al., 2019), whereas the population included in Stock's study were Caucasian.

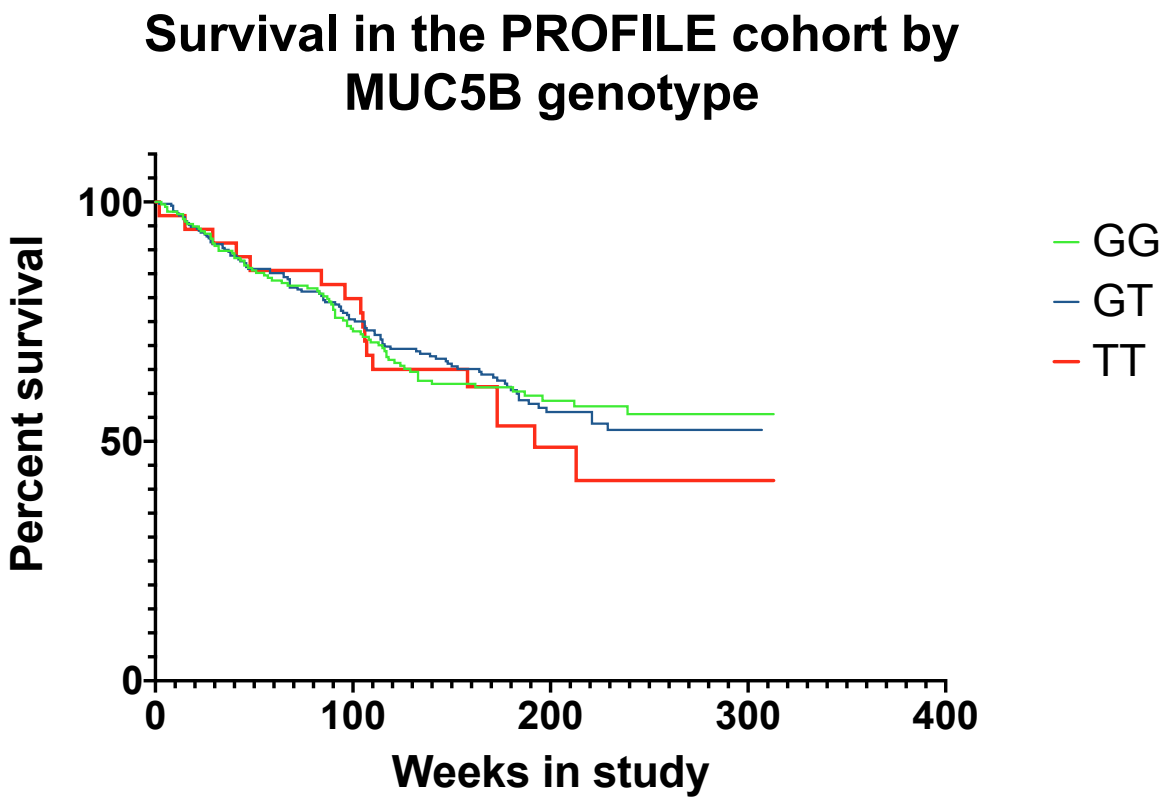


Figure 5.6 Kaplan-Meir survival curve for PROFILE cohort divided by *MUC5B* genotype.

5.4.0

Modelling cough in fibrotic lung disease to take account of lung function.

MUC5B	N of patients	Mean CPI	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
GG	175	77.2	20.8	1.6	74.1	80.3	19.9	90.8
GT	223	71.9	21.8	1.5	69.0	74.8	13.5	90.8
TT	33	69.4	20.2	3.5	62.3	76.6	36.5	90.8
All	431	73.9	21.4	1.0	71.8	75.9	13.5	90.8

Table 5.1 Baseline CPI scores in the PROFILE cohort

A mixed effects model was therefore employed to assess whether *MUC5B* status and / or lung function affected LCQ. Mixed effects modelling was chosen because it deals better with missing data that is not random. In the case of the PROFILE study (and most studies of progressive disease), missing data was skewed to the later time points as patients became too sick to come to visits. In this model the dependent variable was LCQ score with *MUC5B* genotype and CPI being a predicting factor and covariate respectively. The model looked at individual factor/variable effect and the interaction of both. The model had a goodness of fit of 6452.50, which suggests good fit of data to the model. The goodness of fit essentially is a measure of how far away the data is from fitting the statistical model. The higher the number, the worse the model fits the data.

In this model there was no significant effect of *MUC5B* status on LCQ (p=0.42), no significant effect of CPI on LCQ (p=0.06) and no interaction between *MUC5B* and CPI status affecting LCQ score (p=0.52). There is possibly therefore a weak effect of disease severity on LCQ, as was suggested by the original PROFILE cohort. *MUC5B* genotype did not affect cough, even when disease severity was accounted for.

5.5.0

Quality of life related to *MUC5B* genotype

Despite the *MUC5B* T allele being associated with better baseline lung function, there was no effect on patients' quality of life as measured by the St George's Respiratory Questionnaire.

This was tested in a one-way ANOVA statistic and gave an F statistic of 0.517 and a p value of 0.60.

5.6.0

Bacterial burden and cough

Bacterial burden in the airway was measured by DNA quantification from bronchoalveolar lavage fluid and is expressed as log copy number of the 16S rRNA gene.

Of the 52 patients in the cough cohort:

38 patients underwent bronchoscopy for clinical reasons

37 patients had a bronchoalveolar lavage

25 patients had BAL that was usable for the purposes of bacterial quantification (Twelve patients had insufficient sample to perform bacterial quantification). Patients who were included in the microbiome analysis are characterised in table 5.2.

	Whole group	IPF	CHP	Progressive fibrosing ILD
Number of patients	25	14	3	8
Age	69.2 years	69.2 years	73.3 years	67.7 years
Gender	72% male	64% male	100% male	75% male

Smoking history	60% ex-smokers 4% current 36% never	64% ex-smokers 7% current 29% never	67% ex-smokers 0% current 33% never	50% ex-smokers 0% current 50% never
PPI usage	52%	50%	67%	50%
Mean FVC	81.7%	78.1%	92.8%	84%
Mean TLCO	45.2%	42.6%	39.7%	52.8%

Table 5.2 Baseline characteristics of patients undergoing BAL and subsequent microbiome analysis

Patients with IPF have previously been demonstrated to have a higher burden of bacteria compared to people with healthy lungs (Molyneaux et al., 2014). Within this cohort, independent T-tests showed no difference in total bacterial burden between patients with IPF and those with non-IPF related diagnoses (p=0.43). Independent t-tests also showed in the entire group that there was no significant difference in the bacterial burden of patients on proton pump inhibitors compared to those who were no on such therapy (p=0.94).

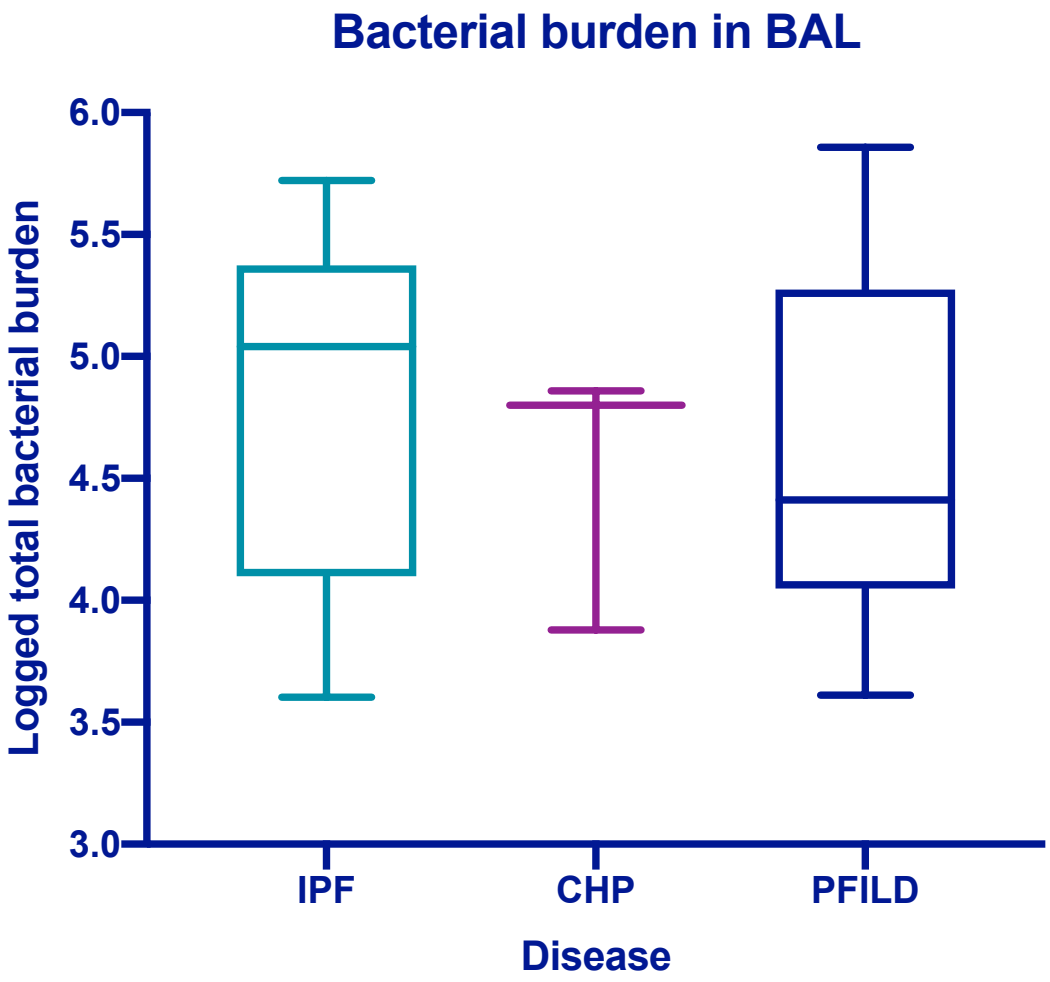


Figure 5.7 Differences in bacterial burden by disease

Evaluating the relationship between total bacterial burden and 24-hour cough rates, using a mixed model analysis, demonstrated no relationship. Looking at the same relationship with a mixed-effects generalised estimating equation produced a similar non-significant result (p=0.21).

My results suggests that the absolute bacterial burden is not a contributor to the pathogenesis of cough, however, individual bacterial species may have the ability to trigger/drive cough. The reporting of the BAL microbiome was divided into the following

phyla: actinobacteria, Bacteroidetes, firmicutes, fusobacteria, proteobacteria and others. Many of the species known to be present in a greater abundance in IPF compared to healthy belong to the proteobacteria phylum.

	Wald Chi-Square	df	Significance
Log of bacterial burden	1.541	1	0.21
Bacteroidetes	8.779	1	<0.01
Fusobacteria	0.012	1	0.91
Proteobacteria	0.284	1	0.59
Others	1.808	1	0.18

Table 5.3 Bacterial phyla associated with cough

Bacteroidetes are a large group of gram-negative organisms which play a vital role in the gut, but are one of the most common phyla of bacterial species found in the healthy lung (Hilty et al., 2010). Research in cystic fibrosis has shown that in patients with cystic fibrosis and gastro-oesophageal reflux, similar microbiota are found in the lung as in the gastric juices (Al-Momani et al., 2016). GORD has been postulated to be a causative factor in the development of IPF, although the association is felt to be weak at best (Bedard Methot et al., 2019). There did appear to be a possible relationship between the percentage of Bacteroidetes (gram negative organisms) in the BAL and cough rates in a general estimating equation ($p<0.01$) (table 5.3).

Interestingly there did appear to be a higher burden of Bacteroidetes present in the BAL of patients with IPF compared to those with non-IPF diagnoses although this was not statistically significant p=0.10. Usage of proton pump inhibitors was not associated with a statistical difference in either Bacteroidetes counts or %of the microbiome made up of Bacteroidetes in the group (p=0.10). The burden of Bacteroidetes in patients with differing *MUC5B* genotypes was not significantly different, as illustrated in figure 5.8

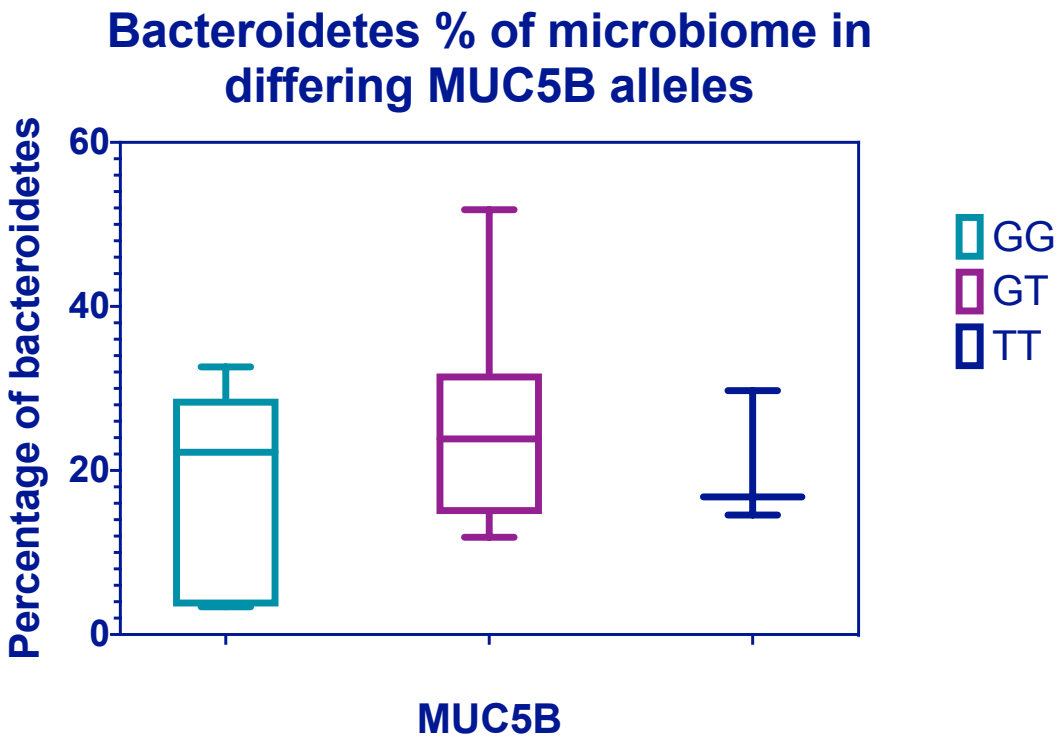


Figure 5.8 – The difference in percentage Bacteroidetes in BAL of patients with differing *MUC5B* genotypes.

There was no significant relationship between *MUC5B* genotype and bacterial burden although the mean bacterial burden was higher in the presence of the T allele. This contrasts with the findings of Molyneaux et al who had previously addressed this question in the original PROFILE cohort (Molyneaux et al., 2014). One possible explanation for this was the

presence of diseases other than IPF in this cohort. A recent paper by Stock et al identified that overexpression of the MUC5B glycoprotein was unique to IPF and not seen in other interstitial lung diseases where the rs35705950 promotor variant was present (Stock et al., 2020).

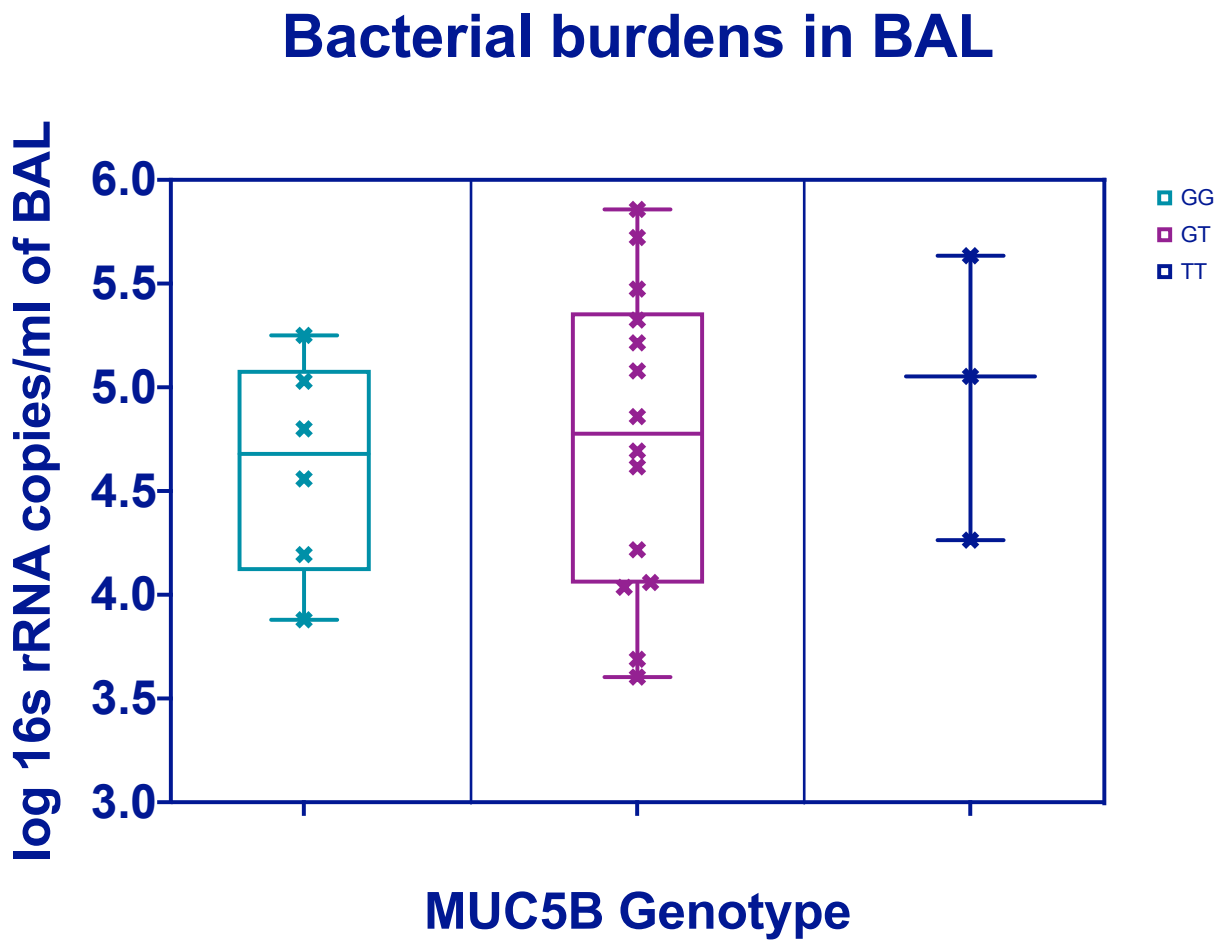


Figure 5.9 – The difference in bacterial burden in BAL from patients with different *MUC5B* genotypes.

The finding of higher levels of a gram-negative organism in patients with IPF is interesting; more so because it seemed to associate with cough. Previous studies in a mouse model showed that by removing gram negative bacteria with an appropriate antibiotic, it was

possible to lessen the fibrotic response to bleomycin (Yang et al., 2019). Repeating this set of experiments in the guinea pig model of pulmonary fibrosis may demonstrate the effect of depleting gram-negative bacteria on cough, although this would be hard to disentangle from the reduction in fibrosis. A clinical study could in theory be performed with inhaled antibiotic therapy targeting gram negatives (such as the aminoglycosides). A crossover design as is often used in cough studies would be difficult however because of the possibility of longer-term changes in microbiome induced by the antibiotic. Studies of nebulised gentamicin in bronchiectasis patients found the antibiotic was associated with a significant reduction in LCQ compared to placebo (Murray et al., 2011). A recently reported study of the use of co-trimoxazole in IPF has shown co-trimoxazole improved cough severity scores at 18 months versus placebo, although the difference in LCQ was non-significant (Wilson et al., 2020). Ideally to do such a study in IPF patients would require BAL microbiome analysis pre- and post-intervention to show that any cough reduction was due to depletion of gram negatives such as Bacteroidetes.

5.7.0

Summary of results

1.) Carriage of the *MUC5B* T allele associates with better baseline lung function, but in this cohort, provides no clear survival advantage.

2.) *MUC5B* T allele carriage does not predict cough in fibrotic lung disease or worsen cough related quality of life.

3.) *MUC5B* T allele carriage is not associated with increase bacterial burden in the lung.

Total bacterial burden does not predict cough.

4.) Bacteroides species bacteria may affect cough, although this requires further validation.

Chapter 6

DISCUSSION

6.1.0

Recruitment to the studies

The PROFILE study started recruiting in 2009 and the final recruitment occurred late in 2015. Follow up was designed to occur over three years from recruitment. Guidelines for the diagnosis of idiopathic pulmonary fibrosis changed in 2011 with the release of the ATS/ERS/JRS/ALAT clinical practice guideline (Raghu et al., 2011), which means that patients recruited over the course of the study may have had slight differences in how they were classified. This guideline has been subsequently updated in 2018, after recruitment was already completed. The diagnosis of Idiopathic NSIP is less common now as increasing auto-antibody testing (particularly for the myositis associated autoantibodies) may help to classify many of these as being related to a connective tissue disease.

Patients enrolled in the study prior to 2013 were diagnosed at a time where triple therapy (prednisolone, azathioprine and acetylcysteine) was the standard of care. This treatment was shown to be potentially harmful, when the triple therapy arm of the PANTHER-IPD study in IPF was stopped early because of excess mortality (Idiopathic Pulmonary Fibrosis Clinical Research et al., 2012). Antifibrotic therapies with the proven benefit of extending life(Fisher et al., 2017) did not become available until 2013. The Kaplan-Meier mortality curves from PROFILE may therefore represent a worse survival in fibrotic lung disease than would be seen

if the study were repeated today. The widespread use of antifibrotic medications reflected in the later cough cohort may be improving survival in these lung diseases. These drugs may soon be licenced outside of IPF to the progressive fibrosis phenotype, where trials are already showing benefit (Maher et al., 2019, Flaherty et al., 2019), further changing the landscape.

Recruitment to the cough cohort started in June 2016, at which time both anti-fibrotic therapies were available to patients. Originally the plan had been to recruit patients from lists of those undergoing bronchoscopy for diagnosis of their interstitial lung disease. Recruitment however was slower than predicted and this meant to ensure recruitment was completed in time, inclusion criteria were relaxed to include patients who had been diagnosed with a fibrotic lung disease in the last 12 months. These patients had often started therapy prior to their inclusion in the study and did not require a bronchoscopy to be performed for clinical reasons.

I had chosen to recruit patients at the point of bronchoscopy to ensure that BAL sample could be used for research purposes. The disadvantage of this approach was that a firm diagnostic label had not yet been decided by the MDT. Patients who had been recruited therefore were given a variety of diagnostic labels other than IPF, which is what I had set out to collect. In the case of the patients labelled as unclassifiable disease with a progressive fibrotic phenotype, this is probably not important since it has been shown they often behave in a manner similar to IPF (Cottin et al., 2018). Some patients however had diseases which we know tend to behave differently such as the CTD-ILDs and chronic hypersensitivity pneumonitis. In the case of the latter however, the genetic basis of the disease may be very similar to IPF (Ley et al., 2017). Small numbers of each of the alternative diagnoses meant

that although the study was able to suggest possible differences in cough behaviour between the diseases, the study was not powered sufficiently to be sure of these differences.

6.1.1

Bronchoscopy and lavage

Prior to recruitment it had been anticipated that a significant proportion of patients recruited to the PROFILE cough study would have a bronchoscopy as part of the diagnostic process. Bronchoscopy is not recommended in the 2011 guidelines (Raghu et al., 2011) for the diagnosis of idiopathic pulmonary fibrosis although an inflammatory lavage with lymphocytosis can help to suggest alternate diagnoses such as CHP. A combination of expanding the recruitment criteria to include patients who had been diagnosed and did not require a bronchoscopy and insufficient yield of bronchoalveolar lavage led to only half of the cohort having a usable bronchoalveolar lavage sample. Given the study was intended to be exploratory, the small numbers are acceptable, but the findings would need to be replicated in a larger cohort to be certain of their significance.

6.1.2

Digital cough recordings

The cough cohort study used the Vitalojak cough recorders with analysis of the recordings performed by Professor Smith's team at the University of Manchester. This was the first time cough had been recorded over time in fibrotic lung disease, with earlier studies having only reported cough in a single period of time (van Manen et al., 2017, Key et al., 2010).

6.2

Cough burden in fibrotic lung disease

Cough is an important problem in fibrotic lung disease which impacts quality of life significantly. The LCQ helps capture impairment of quality of life related to a patient's cough. A lower score indicates worsening cough related quality of life, with a top score of 21 and bottom score of 3. In the PROFILE cohort, the mean LCQ of 15.5 (median 16.3) was lower than that seen in asthma patients (Marsden et al., 2008) and similar to that of patients with bronchiectasis (Spinou et al., 2017). The median LCQ in the subsequent cough cohort was even lower at 14.9.

Patients perception of how bad their cough was using a cough visual analogue score correlated well with objective measures of cough with a Pearson correlation of 0.512. In the cough cohort the median baseline cough VAS was 32.5mm. A previous study of lung cancer by Sumner et al showed that the median cough VAS in lung cancer was not dissimilar at 40mm (Sumner et al., 2013). The cough VAS in asthmatics was shown to be lower by Marsden et al with a daytime cough VAS of 19mm (Marsden et al., 2008). Finally, a study of COPD patients by Sumner et al showed a median cough VAS of 45mm in COPD patients who smoke, 23mm in ex smoking COPD patients, 17.5mm in healthy smokers and 3.5mm in healthy non-smokers (Sumner et al., 2013). The cheapness and quickness to complete this test, along with its correlation with objective markers of cough make it the ideal test to use clinically for measuring cough and its response to interventions. In routine clinical practice it would be impractical to use objective cough recordings and other subjective measures take longer for

the patient to complete but might better capture other themes causing issues to the individual.

A key finding of cough severity in fibrotic lung disease was that there was very little change in median measures over time. In 250 patients from PROFILE, there was no difference in median LCQ counts between baseline and one year. Similar findings were demonstrated in the cough cohort where there was not a clinically meaningful change in LCQ scores over time. Mortality within the PROFILE cohort over time divided by baseline lung function demonstrates that worsening disease is associated with death (Figure 3.1).

Objective measures of cough have shown that daytime cough is far more common than nocturnal cough. The median number of coughs per hour at night in this cohort was 2.67 coughs per hour versus 14.8/hr in the daytime hours. In fibrotic lung disease the median number of coughs/hr/24 hours was 11.0 (IQR 17.0). This is significantly higher than the burden seen in healthy volunteers and similar to that seen in chronic idiopathic cough patients (Key et al., 2010). In a smaller study of idiopathic pulmonary fibrosis undertaken by Key et al, they found the median cough rate in coughs/hr/24hr to be 9.4 i.e. quite similar to that shown in the cough cohort.

Curiously the difference between objective cough day and night counts was not fully appreciated by patients. When asked to score the severity of their cough at day and then at night out of 5, patients in this cohort had a mean score of 2.6 for daytime cough and 1.3 for night-time cough. Patients therefore had rated night-time cough to be approximately half as severe at night as by day. The difference in objective counts however was that daytime cough

was almost 6x more frequent than at night. Perhaps cough is more disruptive at night and this might explain the difference in patient perception to the actual cough burden.

6.3.0

Cough's relationship to disease severity, progression and mortality

Lung function markers used in both studies were forced vital capacity, transfer factor (TLCO) and the composite marker CPI. CPI was chosen because in a study in which it was derived, it was found to be a better predictor of mortality than any single lung function marker on its own (Wells et al., 2003). LCQ in the PROFILE study was weakly correlated to all markers of baseline lung function. Correlation does not imply causation however and it is notable that the SGRQ also correlated with the same lung function markers. This might simply mean that the correlation reflects worse disease is associated with worse quality of life overall rather than reflecting a worsening of cough burden. Previous work by Swigris et al has demonstrated that quality of life declines with disease progression (Swigris et al., 2010a) and this was confirmed in PROFILE by the worse SGRQ scores seen in patients who experiences a 10% decline in FVC over the year.

Within the PROFILE study baseline lung function did appear to predict the risk of subsequent mortality. This was especially true for gas transfer, where using the 40% predicted cut off used by Egan et al (Egan et al., 2005) revealed striking differences in mortality (figure 3.3). Similar to Well et al, CPI and FVC at baseline were also shown able to predict mortality (Wells et al., 2003) (figure 3.2 and figure 3.4).

Prior retrospective studies of cough in IPF have shown that cough may be related to disease progression and the risk of mortality (Ryerson et al., 2011). In the study by Ryerson et al, case notes were reviewed retrospectively and cough was represented by a binary yes or no as to whether the patient had reported cough during their consultations. There was no attempt to quantify the severity of cough or its impact on quality of life. The potential for recall bias to have affected the documentation of cough was not taken into account. The presence or absence of cough was then fed into a multivariate analysis in which it was significant in its ability to predict decline in lung function but not significantly associated with mortality (p=0.08) (Ryerson et al., 2011). No attempt was made to validate the findings in a second cohort. As such whilst the findings of the paper whilst interesting may not be reproducible.

Mortality within the PROFILE cohort divided by baseline LCQ scores showed that cough did not influence the risk of death (figure 3.8). This would suggest that cough is a persistent, debilitating symptom for patients with fibrotic lung disease.

The problem with using a subjective measure of cough here is that it may be other factors impact the quality of life such as breathlessness which could influence the LCQ. The correlation between LCQ and SGRQ scores in PROFILE hints at this. The analysis of individual responses in the LCQ showed that fatigue was a problem for a significant number of patients – a symptom that is likely to be polyfactorial rather than solely relating to cough.

In the objective cough study, there was weak correlation between markers of lung function (gas transfer, CPI but not FVC) and objective cough counts. This could mean that worsening pulmonary fibrosis is related to more cough, however confounders again might explain the

very weak statistical association. We know that disease progression is associated with increased bacterial burden compared to stable disease(Molyneaux et al., 2014) and it may be that these changes in the microbiome are responsible for a change in cough frequency. In this cohort death occurring within the first year was a rare event and the patients who experienced significant worsening of disease dropped out of follow up. This might have led to a healthy survivor effect in the patients who continued to attend follow up and thus underestimate the effect that change in disease has on cough. Future studies may need to follow up patients for longer, or use a bigger sample size, or use home follow up to ensure that worsening disease is better represented.

An important avenue to consider, which has not been demonstrated in these experiments is the possibility that cough could contribute to progression of fibrosis. In chronic cough endobronchial biopsies show thickening of the sub-basement membrane, increased vascularity and goblet cell hyperplasia (Niimi et al., 2005). Repeated stimulation of cough in guinea pigs leads to neutrophilic inflammation in the lungs, increased substance P (a tachykinin) production and increased TRPV1 expression in the major airways and trachea (Xu et al., 2018). Substance P has been shown to be elevated in patients with IPF compared to healthy controls (Takeyama et al., 1996). Substance P has been shown to be able to stimulate fibroblast proliferation in vitro (Harrison et al., 1995). If cough itself could accelerate fibrosis, this might explain the observations of Ryerson et al that cough was associated with mortality in IPF (Ryerson et al., 2011).

Cough's overlap with gastroesophageal reflux (GORD) is hard to untangle when assessing the relationship between cough and disease progression in IPF. GORD is common in IPF

(Hoppo et al., 2014). GORD has been suggested to be a risk factor for mortality in IPF (Lee et al., 2011) Treatment of GORD has produced two different results with regards to cough reduction (Kilduff et al., 2014, Dutta et al., 2019). Cell culture studies have suggested that proton pump inhibitors may have a direct anti-fibrotic effect related to inflammatory cytokine downregulation as well as reducing reflux (Ghebremariam et al., 2015). Anti-reflux surgery has not proven to prevent progression of fibrosis although may reduce exacerbations of IPF(Raghu et al., 2018a). GORD may also affect the lung microbiome (Al-Momani et al., 2016) which in turn may influence cough. Contrary to this however PPI use did not appear to produce changes in the lung microbiome as described in chapter 5.

6.4.0

Differences in cough burden between different fibrotic lung diseases

One of the most interesting findings in the study of objective cough was the apparent difference in objective cough in patients with hypersensitivity pneumonitis compared to those with idiopathic pulmonary fibrosis. The study was not originally designed to include patients with chronic hypersensitivity pneumonitis and as such was not powered to detect this difference. With such small numbers it is entirely possible that the difference in CHP is being over-estimated. Other groups have found that when using subjective measures of cough, CHP has less cough when compared to idiopathic interstitial lung diseases (Sato et al., 2019). Again, this study suffered from small numbers in the CHP group and made no attempt to control for medication.

The apparent response of cough to prednisolone in CHP suggests that an inflammatory mediator is contributing to the cough. This should be the focus of further research. Prednisolone did not seem to be a useful treatment of cough in the other progressive fibrotic lung diseases.

The pathology of hypersensitivity pneumonitis is a mix of type III and type IV hypersensitivity, i.e. there is a mix of antibody mediated and T cell mediated response. There is an inflammatory T lymphocyte infiltrate within the alveolar space (Costabel et al., 1985). These lymphocytes appear to be stimulated by alveolar macrophages (Israel-Assayag et al., 1999). There are also raised levels of antigen specific immunoglobulins of all subclasses in the bronchiolar lavage of patients with hypersensitivity pneumonitis (Reynolds et al., 1991). Patients with hypersensitivity pneumonitis have higher levels of blood mononuclear cells which express extra cellular matrix markers and have the potential to migrate into tissues and develop into fibroblasts (Garcia de Alba et al., 2015). These fibrocytes appear to be present at high levels in areas of inflammation and fibrosis within the HP lung (Garcia de Alba et al., 2015). The pathological pattern of chronic hypersensitivity pneumonitis can vary and closely resemble that of usual interstitial pneumonia (UIP) (Garcia de Alba et al., 2015). Corticosteroids potentially can inhibit some of these pathways such as the proliferation of lymphocytes but may not be able to alter fibrotic pathways initiated by alveolar macrophages (Lacronique et al., 1984).

The benefits of prednisolone for the treatment of hypersensitivity pneumonitis are controversial with some evidence that whilst improvement in lung function occurs, the overall prognosis may be unchanged, especially in fibrotic disease (Kokkarinen et al., 1992, De

Sadeleer et al., 2018). In a rabbit model of hypersensitivity pneumonitis in which the animals were vaccinated with ovalbumin and then challenged with an aerosol of ovalbumin, steroids reduced but did not prevent blood vessel inflammation and alveolar septal thickening (Dill et al., 1977).

One mechanism by which steroids may improve cough in CHP is by improving small airways dysfunction. In a study which employed impulse oscillometry and ultrasonic pneumography measurements in 20 CHP patients, it was shown that abnormalities of small airway structure were partially improved by immunosuppressive therapy resulting in increased lung volumes (Guerrero Zuniga et al., 2017). Inflammation of the small airways may result in increased protease activated receptor-2 (PAR2) expression. PAR-2 levels are elevated in the bronchoalveolar lavage fluid of patients with hypersensitivity pneumonitis, but diminish with fibrotic disease progression (Matej et al., 2014, Sterclova et al., 2019). Activation of PAR-2 can enhance the activity of TRPV1 on sensory neurones (Gu and Lee, 2009), potentially upregulating cough (Gatti et al., 2006).

Studies of cough in hypersensitivity pneumonitis have been hampered to date by the lack of a single diagnostic pathway, although the new consensus guideline may help this (Raghu et al., 2020). Understanding better the cough of chronic hypersensitivity pneumonitis will require prospective cohort data with objective and subjective cough measures recorded. The change from an inflammatory predominant to fibrotic phenotype that seems to occur in some patients with hypersensitivity pneumonitis presumably will impact the burden of cough and its response to therapy. Animal models of hypersensitivity pneumonitis will be necessary to work out the effects of inflammatory mediators on sensory neurones.

6.5.0

MUC5B and cough

Previously published studies of the *MUC5B* promotor polymorphism have suggested that carriage of the T allele results in increased cough(Scholand et al., 2014). There is evidence that carriage of the T allele leads to increased build-up of the MUC5B mucin in the distal airways (Conti et al., 2016). In data from the original PROFILE study, I demonstrated no evidence of such a relationship. In the study by Scholand only 68 patients were included and no attempt was made to validate the findings in a second cohort (Scholand et al., 2014). In the original PROFILE study data was provided from 454 patients and then the findings were validated in the cough cohort. The Scholand study also only was able to evaluate LCQ and did not consider objective markers of cough which may have been important given that their hypothesis had been the minor T allele of the *MUC5B* genotype would be associated with more severe cough. The LCQ does not really provide information about cough severity, but instead gives information about the impact of quality of life on cough. The percentages of patients with each *MUC5B* genotype (GG, GT, TT) was similar in both studies.

I found that the LCQ scores in all three *MUC5B* genotypes were not significantly different from each other (figure 5.3). In the cough cohort, I found no relationship between *MUC5B* genotypes and objective count counts (figure 5.4). The proportion of patients carrying the T allele in both the homozygous and heterozygous states was similar between the Scholand study, PROFILE study and cough cohort.

6.6.0

Microbiome and cough

This study was the first attempt to examine the role of bacteria in cough in fibrotic lung disease. Previous studies of the microbiome in IPF have found that patients have an increased bacterial burden and increased burdens of certain species (Molyneaux et al., 2014). There was difficulty in recruiting patients with suspected IPF who required a bronchoscopy for clinical reasons. In part this is because patients with a typical background (male, over the age of 60, ex-smokers, lack of exposures to entertain a diagnosis of CHP/asbestosis and lack of clinical features of an autoimmune disease) with definite UIP imaging and a negative auto-immune screen do not need a bronchoscopy to diagnose IPF (Raghu et al., 2011). The patients who do get asked to have a bronchoscopy for diagnosis of their lung disease often have atypical features and this is why some patients in the study then had alternate diagnoses to IPF. 8 patients were labelled unclassifiable progressive fibrosis (some would meet the criteria for the IPAF classification), 6 had CHP and one had a CTD-ILD.

Only 38 patients underwent a bronchoscopy. Of these one did not tolerate the procedure and a lavage was not performed. Only 26 patients had enough BAL yield to produce excess sample for research. The small sample size does make it difficult to examine differences between the disease groups. Reporting from the original PROFILE study has shown that bronchoalveolar lavage is safe and that yield is sufficient to provide adequate sample for a differential cell count (Molyneaux et al., 2021). What was not analysed in this study was whether cough during the procedure affected yields of BAL. Coughing during a

bronchoalveolar lavage can cause the scope to dislodge from the airway it is wedged into and thus potentially reduce return. The patient who did not tolerate bronchoscopy had IPF with a mean cough count of only 2.13 coughs/hr/24hr. The 26 patients in whom sufficient BAL was available for research had an average 24-hour cough count of 20.45 coughs/hr, whereas the patients in who insufficient sample was obtained had an average 24-hour cough count of 27.39. Although the patients in whom inadequate sample was obtained appear to cough more, the difference was non-significant in an independent T test with a p value of 0.44. Patient anxiety, adequacy of topical anaesthesia to the larynx, cords and major airways, collapsibility of the airways as well as operator technique are likely to all play a part in yield of lavage.

There was no significant difference in bacterial load between the groups, although visually the mean appeared higher in IPF (figure 5.7). There would be value in further study of bacterial burdens between disease groups in fibrotic lung disease because it may be a marker of epithelial damage. Trials of chronic antibiotic use in idiopathic pulmonary fibrosis (Shulgina et al., 2013, Oda et al., 2016) have hinted at a mortality benefit from this approach. It may be that the benefit of antibiotics is more in those with higher bacterial burden in the airways. In particular the Japanese retrospective study showed in more rapidly advancing IPF that co-trimoxazole was associated with a survival benefit(Oda et al., 2016). The work by Molyneaux et al showed that a higher bacterial burden was associated with more rapid disease decline (Molyneaux et al., 2014) and that in exacerbations of disease, bacterial burden increases significantly (Molyneaux et al., 2017).

Bacteroidetes are a phyla of gram-negative species found in the gut and lung in health. My study suggests that whilst total bacterial burden did not influence cough, the quantity of this phyla was associated with increased cough. The possible confounder here would be if the increased burden of Bacteroidetes is due to gastroesophageal reflux allowing resulting in the lung microbiome becoming more like that of the gut. There has already been a small study in cystic fibrosis has shown that there is a relationship between gastric and lung microbiota (Al-Momani et al., 2016). Gastroesophageal reflux will include hydrochloric acid from the stomach and the lower pH may be responsible for increasing cough since TRP receptors on the sensory nerves in the airways will detect changes in pH. A study by Tobin et al looking at gastroesophageal reflux in a small group of patients with IPF demonstrated that these patients had much more reflux than in healthy controls and that the percentage of time spent with lower pH in the proximal oesophagus was higher compared to the controls (Tobin et al., 1998).

6.7.0

Key findings

The findings in this thesis have demonstrated the significant burden of cough in idiopathic pulmonary fibrosis and other fibrotic diseases. The PROFILE cohort allowed validation of the reliability of the LCQ in a large cohort of fibrotic lung disease and gave a better understanding of how cough related quality of life changes over time.

This is the largest study of objective cough counting in fibrotic lung disease and the first to evaluate changes in cough over multiple timepoints. One of the most interesting findings

was that cough in chronic hypersensitivity pneumonitis appears to be worse than that seen in IPF and is responsive to steroids, hinting at a different mechanism.

The mechanisms underlying cough have been explored and the finding that changes in the microbiome may affect cough frequency is novel. Polymorphism of the MUC5B promotor appears to not have a significant effect on cough – in contrary to previously published data (Scholand et al., 2014). The relationship between cough and disease progression is weak at best and this conflicts with previously published data (Ryerson et al., 2011).

6.8.0

Nerves as a further target for research

The PROFILE study and subsequent cough cohort have shown that disease progression is not the driver of cough in fibrotic lung disease. Patients appear to have significant cough burdens with resultant loss of quality of life throughout the course of their illness; from diagnosis to years after diagnosis. The variability of cough within patients makes the concept of hypersensitivity of sensory nerves in the cough pathway seem an attractive concept. This concept has already been well demonstrated in chronic idiopathic cough. There is evidence to support that there is no increase in the number of TRP receptors on sensory nerves (Hutchinson et al., 2017). Functionally however there seems to be increased activity of sensory nerves with IPF patients having been shown to have enhanced cough in response to capsaicin challenge and vibratory challenge (Doherty et al., 2000, Jones et al., 2011).

The findings of higher cough burden in hypersensitivity pneumonitis need to be validated in a larger cohort using both digital cough recorders and subjective measures. As our understanding of the progressive fibrosis phenotype grows this will be another important group to understand cough in better. Does a patient with rheumatoid lung fibrosis have a similar cough to the patient with an idiopathic fibrotic organising pneumonia? Given that so much of our disease classification revolves around radiology a future study should also evaluate if there is a relationship between cough burden and radiological pattern of disease.

GORD and microbiome manipulation seem the obvious targets for clinical trials and indeed the upcoming TIPAL trial (ISRCTN13526307) and the recently reported TIPAC trial (Wilson et al., 2020) have attempted to evaluate such therapies. These two paths may intertwine however and any study of gastro-oesophageal reflux will surely need to evaluate airway microbiome to see if therapy is inducing changes which may also alter cough. The findings that microbiome density may be highest in the major airways (Valenzi et al., 2020) may indicate that trials should focus on nebulised antibiotic therapy instead of oral antibiotics.

There is an urgent need to now perform a study looking at the neuronal profile of enhanced cough in fibrotic lung disease. This will involve cough challenges being performed in patients with lung fibrosis across a spectrum of different TRP agonists. Cough challenges with hypertonic / hypotonic solutions, capsaicin, citric acid and menthol would help to show whether the pattern of TRP overactivity is the same in all patients or whether there is variability. The latter would make drug treatment of fibrotic lung disease associated cough harder since there would unlikely be a one drug for all unless an upstream target of the TRP receptors could be blocked. Another possible way of testing this would be to do a trial of

treatments using drugs we already have that can block different pathways in the sensory stimulation of cough.

Chapter 7

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