The use of impedance pH measurements to determine the effect of gastro-oesophageal reflux in patients with idiopathic pulmonary fibrosis and cystic fibrosis

MD Research Degree Programme

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Author's Statement

The author acknowledges that during the project a number of individuals provided assistance in order to complete some of the important stages. The author is personally responsible for the following steps of the study:

- Design of the study protocol
- Ethics and R&D application, amendments and final submission
- Grant applications
- Recruitment of patients and provision of appointments
- Confirmation of consent to the study
- Collecting the clinical information of the patients
- Organising and coordinating of the patient investigations
- Performing manometry and pH-impedance
- Interpretation of all the oesophageal physiology
- Provision of all patient information leaflets
- Completion of all the reflux questionnaires before and after physiology
- Processing of the bronchoalveolar lavage (BAL) and production of cytospins
- Staining of the IPF cells
- Counting of all the IPF cell preparations
- 30% of the ELISA
- 20% of sputum processing
- Co-ordinating referrals for fundoplication in the lung transplant population and completion of this study (Aug 2009-Dec 2010)
- Performing analysis of the results

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Abstract

Introduction

For many decades gastrooesophageal reflux has been implicated in patients suffering from lung disease and in lung allograft injury. From the early 1970s studies have taken place investigating reflux in idiopathic pulmonary fibrosis (IPF) and cystic fibrosis (CF). However, these early studies were small and used primitive techniques to assess reflux. In addition, the role of microaspiration secondary to reflux has often been postulated as a cause of deteriorating lung function in these patients but has been under studied. It is also known that many of these patients require a lung transplant due to end-stage lung disease. Asymptomatic reflux and aspiration may be associated with allograft dysfunction post lung transplant. Early anti-reflux surgery has been suggested to improve long-term survival by treating reflux. This thesis reports a prospective assessment of reflux/aspiration in patients with IPF and CF. In addition, the study reports the largest European series of fundoplication in lung transplant patients.

Methods

Over a 2 year period patients with IPF and CF were recruited from specialist clinics. All patients completed objective assessment of oesophageal physiology using manometry and impedance-pH. Symptom and quality of life assessment using RSI, Demeester and GIQLI questionnaires were performed on all patients at the time of recruitment. For those patients taking proton pump inhibitor, questionnaires were done ‘on’ and ‘off’ their medication. IPF patients then had a bronchoscopy and lavage (BAL) whilst CF patients produced sputum. Cytospins of the BAL and sputum were produced and differential cell counts were performed and the cells were stained with Oil Red O and Prussian Blue (Perls). ELISA and mass spectrometry assays were also performed on the samples for pepsin and bile salts respectively. Lung transplant patients attended for impedance-pH studies over 3 years and those with symptomatic reflux or reflux and deteriorating lung function were referred for a laparoscopic fundoplication. Lung function assessment, symptom and quality of life questionnaires were performed before surgery and at 6 weeks and 6 months after surgery

Results

IPF Patients:

Thirty eight patients with IPF were initially approached and 29 consented to be studied. Nine patients dropped out from the study after consent. Twenty patients with IPF completed both the oesophageal physiology and BAL aspects of the investigation. In 12 patients there was objective evidence of reflux including 6 patients with proximal reflux. 60% of patients had an abnormal RSI score whilst taking a PPI and scores for the other questionnaires were not significantly different 'on' and 'off' PPI. Lung function was not related to the degree of reflux. The principal cell type identified was macrophages and both Oil Red O and haemosiderin scores were well above the normal range. Bile salts were detectable in 17/20 IPF patients but the levels were not higher than the normal range. 11/20 patients had higher than normal levels of pepsin in the BAL.

CF Patients:

Twenty-six patient with CF consented to the study but 15 dropped out. Eleven CF patients attended for oesophageal investigation and each provided 2 samples of sputum. 9/11 had reflux, including five with proximal reflux. All patients were taking acid-suppression medication and questionnaire assessments were abnormal whilst on their medication with 82% still having a GIQLI score below 121 despite medication for reflux. Twenty one samples of sputum were processed altogether. The principal cell type was neutrophils. Bile salts were detectible in all samples but these were at very low concentrations. Elevated pepsin was seen in 7/11 sputum samples with the median concentration ten times above the normal level.

Lung Transplant Patients

16 lung transplant patients with symptomatic reflux or deteriorating lung function and reflux on impedance-pH had a laparoscopic fundoplication. Symptom questionnaire and quality of life assessment was significantly improved in all patients. Half the patients had presented with declining lung function and all showed an improvement in respiratory function after surgery.

Summary

We have demonstrated that reflux is present in patients with IPF, CF and in patients after lung transplant. Using impedance-pH we have identified patients with proximal reflux. The presence of reflux appears to affect the patients' quality of life and despite PPI therapy the majority still had symptoms. High levels of haemosiderin stained macrophages in IPF indicate oxidative stress which may or may not be secondary to reflux. Pepsin levels are elevated in both IPF and CF patients, possibly indicating microaspiration.

Conclusion

Despite PPI therapy there is significant reflux in IPF and CF identifying a clinical gap in patient treatment that should be considered in management. Our results in the post lung-transplant group indicate there is a role for surgery in treating reflux and potentially reducing microaspiration. This has been shown to stabilise lung function in this cohort and may have implications for the treatment of reflux in patients with lung disease before transplantation.

Publications:

1. Identical Biofilm Forming Strains of Pseudomonas aeruginosa Occur in Lung Allograft BAL and Gastric Juice from CF Patients with Gastro Oesophageal Reflux. **A. Krishnan**, A. Perry, A. Robertson, M. Brodlie, J. Perry, P. Corris, M. Griffin, K. Gould, I. Forrest, J. Pearson, C. Ward. The Journal of Heart and Lung Transplantation Vol. 32, Issue 4, Supplement, Page S28
2. Anti-reflux surgery in lung transplant recipients: Outcomes and effects on quality of life. Robertson AG, **Krishnan A**, Ward C, Pearson JP, Small T, Lordan J, Corris PA, Dark JH, Karat D, Shenfine J, Griffin SM. Eur Respir J. 2012 Mar;39(3):691-7
3. Metformin as a cause of high stomal output. Rao VS, Sugunendran S, Issa E, **Krishnan A**, Pearson HJ. Colorectal Dis. 2012 Feb;14(2):e77.
4. Tomorrow's Doctors: students' satisfaction with a prosection-based undergraduate anatomy course and its implication for their future clinical practice. D Overbeck-Zubrzycka, **A Krishnan**, DW Hamilton, RF Searle, G Stansby. Bulletin of The Royal College of Surgeons of England, Jan 2012
5. Giant Colonic Mucocele following palliative surgery for metastatic adenocarcinoma. A Ali, **A Krishnan**, S Rehman, VSR Rao & H J Pearson. JSCR. 2011. 3:9
6. Stage-for-stage comparison of definitive chemoradiotherapy, surgery alone and neoadjuvant chemotherapy for oesophageal carcinoma (Br J Surg 2009; 96: 1300-1307).Clark E, **Krishnan A**, Dunn LJ, Robertson AG, Griffin SM. Br J Surg. 2010 May;97(5):792-3
7. Treatment of oesophageal anastomotic leaks by temporary stenting with self-expanding plastic stents (Br J Surg 2009; 96: 887-891).**Krishnan A**, Robertson AG, Dunn LJ, Robinson S, Hayes N, Griffin SM. Br J Surg. 2010 Feb;97(2):294

Presentations:

1. Initial experience of Anti-reflux Surgery in Lung Transplant Recipients in a European Centre. Poster Presentation at the Association of Upper GI Surgeons (AUGIS 2010)
2. The Safety and Feasibility of Anti-Reflux Surgery In Lung Transplant Recipients; The Initial Experience In A European Centre. Poster Presentation at the United European Gastroenterology Week (UEGW 2010)
3. Laparoscopic Fundoplication slows deterioration of lung function post-lung Transplant. Presentation at the Association of Surgeons of Great Britain at Ireland (ASGBI 2011). **First Prize in session**
4. Laparoscopic Fundoplication slows deterioration of lung function post-lung Transplant. Presentation at the North East Surgical Society (NESS 2011). **Runner-up award**
5. Ultrasonography of the Neck with FNA is a Useful Adjunct in the Pre-Operative Assessment of Patients with Oesophageal Cancer. Presentation at the North East Surgical Society (NESS 2011). **Runner-up award**
6. Can endoscopic stent insertion substitute traditional management strategies for mediastinal leaks after oesophagectomy. Poster Presentation at the Association of Upper GI Surgeons (AUGIS 2011)
7. Ultrasonography of the Neck with FNA is a Useful Adjunct in the Pre-Operative Assessment of Patients with Oesophageal Cancer. Poster Presentation at the Association of Upper GI Surgeons (AUGIS 2011)
8. Laparoscopic Fundoplication slows deterioration of lung function post-lung Transplant. **Prize Presentation.** European Society of Esophagology (ESE 2011)
9. Ultrasonography of the Neck with FNA is a Useful Adjunct in the Pre-Operative Assessment of Patients with Oesophageal Cancer. European Society of Esophagology (ESE 2011)
10. Laparoscopic Fundoplication slows deterioration of lung function post-lung Transplant. Presentation at the Royal College of surgeons of Edinburgh. (Lister Centenary Ceremony 2012)

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1st Prize in Session

Abbreviations

ASA	American Society of Anaesthesiology
ATS	American Thoracic Society
BAL	Bronchoalveolar Lavage
BCT	Bolus Clearance Time
BMI	Body Mass Index
BOS	Bronchiolitis Obliterans Syndrome
BSA	Bovine Serum Albumin
cAMP	Cyclic Adenosine Monophospate
CF	Cystic Fibrosis
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
ELISA	Enzyme linked Immunosorbent Assay
ERS	European Respiratory Society
FEF_{25-75}	Expiratory flow rate from 25-75% exhalation
FEV_1	Forced Expiratory Volume in 1 second
FVC	Forced Vital Capacity
GIQLI	Gastro-Intestinal quality of life index
GOR	Gastro-Oesophageal Reflux
HRCT	High Resolution Computed Tomography
ILD	Interstitial Lung Disease
IPF	Idiopathic Pulmonary Fibrosis
ISHLT	International Society for Heart and Lung Transplant
LOS	Lower Oesophageal Spincter
LLAM	Lipid laden Alveolar Macrophage
LPR	Laryngo-pharyngeal reflux
LSLT	Left Single Lung Transplant
mg/ml	milligrams/millilitre

µg/ml	micrograms per millilitre
MDT	Multi Disciplinary Team
MII	Multichannel Intraluminal Impedance
Ml	millilitres
mm	millimetres
mmHg	millimetres of mercury
µmol/l	micro moles per litre
NBT	nitrotetrazolium blue
ng/ml	nano grams per millilitre
^{o}C	Degrees Centigrade
PBS	Phosphate Buffer Solution
PEG	percutaneous endoscopic gastrostomy
PFT	Pulmonary function test
PPI	Proton Pump Inhibitor
r.p.m	revolutions per minute
RSI	Reflux Symptom Index
RSLT	Right Single Lung Transplant
SLT	Single Lung Transplant
SSLT	Single Sequential Lung Transplant

1 Introduction

1.1 Idiopathic Pulmonary Fibrosis

1.1.1 Definition

The term interstitial lung disease (ILD) encompasses a heterogeneous group of acute and chronic disorders characterised by diffuse pulmonary infiltrates with histologic features of pulmonary inflammation, exertional dyspnoea and restrictive lung patterns [1]. Under normal conditions the interstitium of the alveolar cells contain small quantities of macrophages and fibroblasts as well as collagen-related macromolecules. During injury an inflammatory process begins with an increase in permeability of the alveolar cell lining, enabling serum contents to enter the alveolar space. This results in an inflammatory cell response during which pro-inflammatory and pro-fibrotic cytokines are released. After this, fibroblastic proliferation and collagen deposition dominate leading to the histological hallmarks of interstitial lung disease. In ILD a number of different sources may be responsible for the injury of the lung parenchyma producing a disease with similar clinical, radiological and physiological features. The alveolar structures as well as the lumen and walls of the small airways can be affected in ILD[2].

Since the publication of the first ILD guidelines by the British Thoracic Society [3] almost 15 years ago the consensus on the definition of certain lung conditions within the spectrum of ILD has undergone considerable change; mainly brought about by a better understanding of the disease process. The term ‘interstitial lung disease’ is synonymous with ‘diffuse parenchymal lung disease’ and in the initial guidelines published in 1999 [3] it was this latter term that was commonly used. This was replaced only a few years later with ILD. However, a more difficult issue is the definition of the subgroups of diseases under the umbrella term of ILD. In the UK, the term ‘crytogenic fibrosing alveolitis’ (CFA) corresponded to a the characteristic clinical picture we now see as defined by idiopathic pulmonary fibrosis (IPF) but also encompassed other idiopathic interstitial pneumonias (IIP) as well as cases of hypersensitivity pneumonitis. This demonstrates that the use of the term CFA was unable to distinguish between IIP subsets as much as to say some patients had fibrotic lung disease and others had an inflammatory picture [4].

Two years after the first BTS guidelines the American Thoracic Society (ATS) in association with the European Respiratory Society (ERS) proposed a new classification system paying particular attention to developing a distinction between the diseases defined generally as CFA [5]. They compared the outcomes of subsets of patients with IIP and found that patients with 'fibrotic' non-specific interstitial pneumonia (NSIP) had a better prognosis than those with a histological pattern consistent with usual interstitial pneumonia (UIP). On this basis the core entity of idiopathic pulmonary fibrosis was redefined: characteristic clinical features were required in association with a histological pattern of UIP at surgical biopsy or a high resolution CT (HRCT) pattern typical of UIP. In addition, the absence of lymphocytosis on bronchoalveolar lavage (BAL) or the absence of features of an alternative diagnosis on trans-bronchial biopsy at bronchoscopy was required in patients not undergoing a surgical biopsy. Table 1-1 indicates the current internationally accepted standards by which a diagnosis of IPF can be made in the absence of a surgical lung biopsy [4]. In the immunocompetent adult the presence of all of the major criteria and three out of four of the minor criteria increase the likelihood of the diagnosis being IPF.

Major Criteria	Minor Criteria
Exclusion of other causes of ILD such as certain drug toxicities, environmental exposures and connective tissue diseases	Age > 50 years
Abnormal pulmonary function studies that include evidence of restriction (reduced VC, often with an increase FEV_1/FVC ratio) and impaired gas exchange (increased $P(A\text{-}a)o_2$ with rest of exercise or decreased T_{LCO})	Bibasilar inspiratory crackles (dry or 'Velcro' type in quality)
Bibasilar reticular abnormalities with minimal ground glass opacities on HRCT scans	Insidious onset of otherwise unexplained dyspnoea on exertion
Transbronchial lung biopsy or BAL showing no features to support an alternative diagnosis	Duration of illness > 3months

Table 1-1: ATS/ERS criteria for the diagnosis of idiopathic pulmonary fibrosis (IPF) in the absence of surgical lung biopsy [5]

Since the development of these definitions of IPF, a further set of guidelines have been published in 2011. This document is a joint consensus between the ATS, ERS, the Latin American Thoracic Association and the Japanese Respiratory Society (JRS) [6] . Their definition of IPF, in agreement with the BTS definition, states that IPF is a specific form of chronic, progressive fibrosing interstitial pneumonia of unknown cause, occurring

primarily in adults during the sixth and seventh decades of life, limited to the lungs and associated with the histopathological and/or radiological pattern of UIP.

In this document there is particular attention paid of the radiological diagnosis of UIP and the diagnostic criteria of IPF. UIP is characterised on HRCT by the presence of reticular opacities (Figure 1- 1), often associated with traction bronchiectasis. Commonly honeycombing is seen in UIP. The distribution of UIP on HRCT is characteristically basal and peripheral, but often can be patchy. The positive predictive value of a HRCT diagnosis of UIP is 90-100% [6].

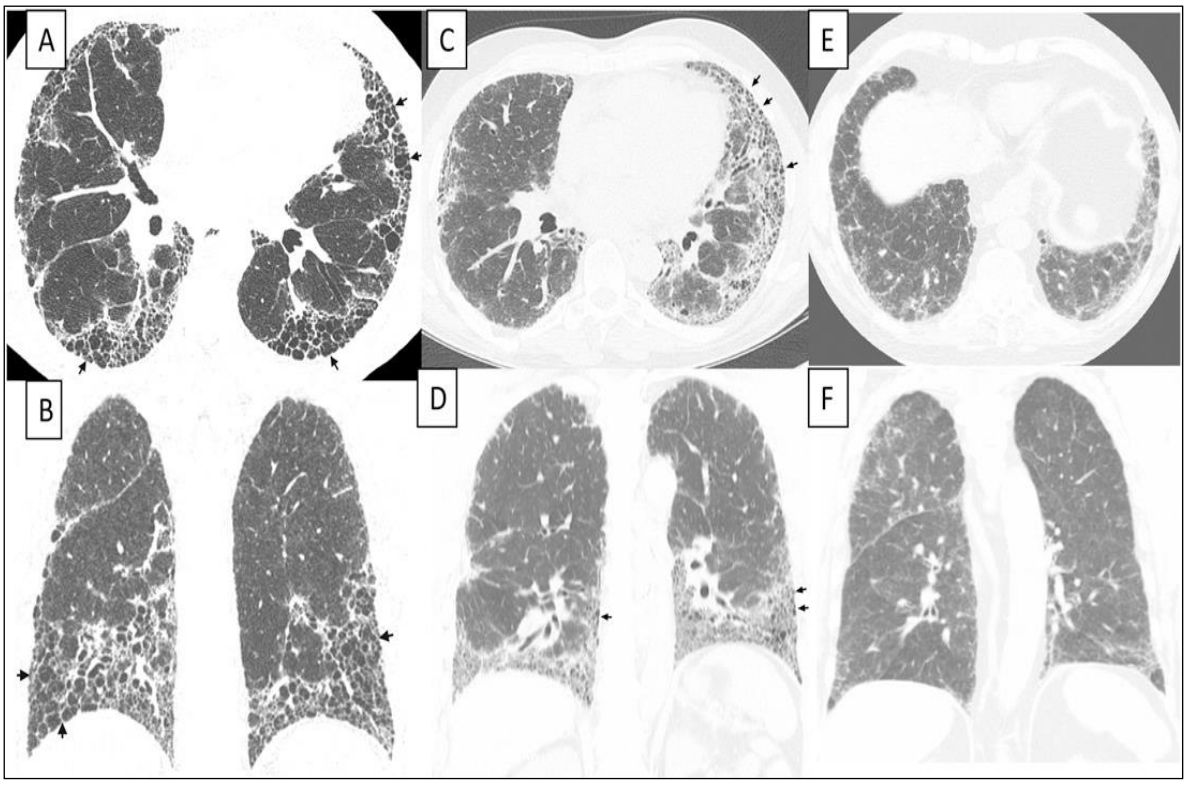


Figure 1- 1 : High-resolution computed tomography (HRCT) images demonstrating usual interstitial pneumonia (UIP) pattern and possible UIP pattern.

Legend: (A and B) UIP pattern, with extensive honeycombing: axial and coronal HRCT images show basal predominant, peripheral predominant reticular abnormality with multiple layers of honeycombing (arrows). (C and D) UIP pattern, with less severe honeycombing: axial and coronal CT images show basal predominant, peripheral predominant reticular abnormality with subpleural honeycombing (arrows). (E and F) Possible UIP pattern: axial and coronal images show peripheral predominant, basal predominant reticular abnormality with a moderate amount of ground glass abnormality, but without honeycombing [6].

The histological diagnosis of UIP is made at low magnification and is characterised by a heterogenous appearance in which areas of fibrosis and honeycombing alternate with areas of normal lung parenchyma. The areas of fibrosis are composed mainly of dense collagen and the honeycomb areas are cystic fibrotic airspaces lined with bronchial epithelium and often filled with mucus and inflammatory cells (Figure 1-2). When such strict criteria are used to make the histological diagnosis of UIP there are only several remaining possibilities for a differential diagnosis and these include some connective tissue diseases, chronic hypersensitivity pneumonitis and some pneumoconioses.

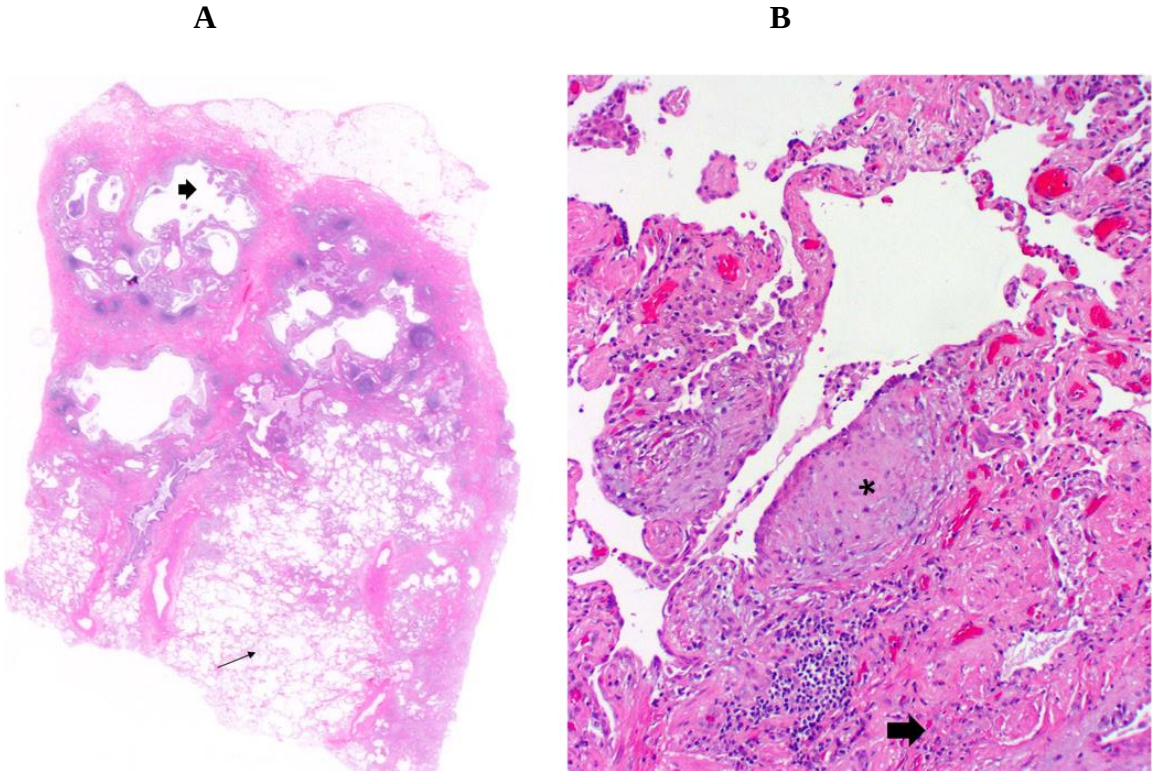


Figure 1- 2 : Surgical lung biopsy specimens demonstrating UIP pattern.

Legend: (A) Scanning power microscopy showing a patchy process with honey comb spaces (thick arrow), some preserved lung tissue regions (thin arrow), and fibrosis extending into the lung from the sub-pleural regions. (B) Adjacent to the regions of more chronic fibrosis (thick arrow) is a fibroblast focus (asterisk), recognized by its convex shape and composition of oedematous fibroblastic tissue, suggestive of recent lung injury [6].

1.1.2 Diagnosis of IPF

The diagnosis of IPF should be made in a multidisciplinary team discussion involving respiratory physicians specialising in ILD, radiologists and pathologists. Where this is not possible and a diagnosis of IPF maybe suspected, specialist referral should be made to an ILD centre. Although HRCT and a better understanding of the histological pattern of IPF have been useful adjuncts in the diagnosis of IPF, the ability to take a focused history and detailed examination remains paramount. A thorough medical, occupational, environmental and family history along with examination, lung function assessment and blood tests allow other diagnosis to be eliminated so that a diagnosis of IPF can be made.

The diagnosis of IPF requires the following [6]:

1. Exclusion of other known cause of ILD
2. The presence of UIP pattern on HRCT
3. The presence of UIP pattern on HRCT and confirmation of the diagnosis on lung biopsy when required.

The exclusion of other causes of ILD can be very subjective and therefore it is recommended that physicians use a standardised approach. It is most important to differentiate between chronic hypersensitivity pneumonitis and IPF as these two conditions have very similar patterns of presentation. The diagnosis of chronic hypersensitivity pneumonitis is more likely in the presence of a lymphocytosis (>40%). This demonstrates the importance of a multidisciplinary setting when making the diagnosis of IPF. The diagnostic criteria are presented in the flow chart below (Figure 1- 3).

The most recent recommendations for the diagnosis and management of IPF do not include the use of routine bronchoalveolar lavage (BAL) cellular analysis or trans-bronchial lung biopsy in making the diagnosis [6]. However, both maybe appropriate in a minority of cases. BAL can be useful in differentiating between IPF and chronic hypersensitivity pneumonitis. The sensitivity and specificity of trans-bronchial biopsy varies considerably between studies.

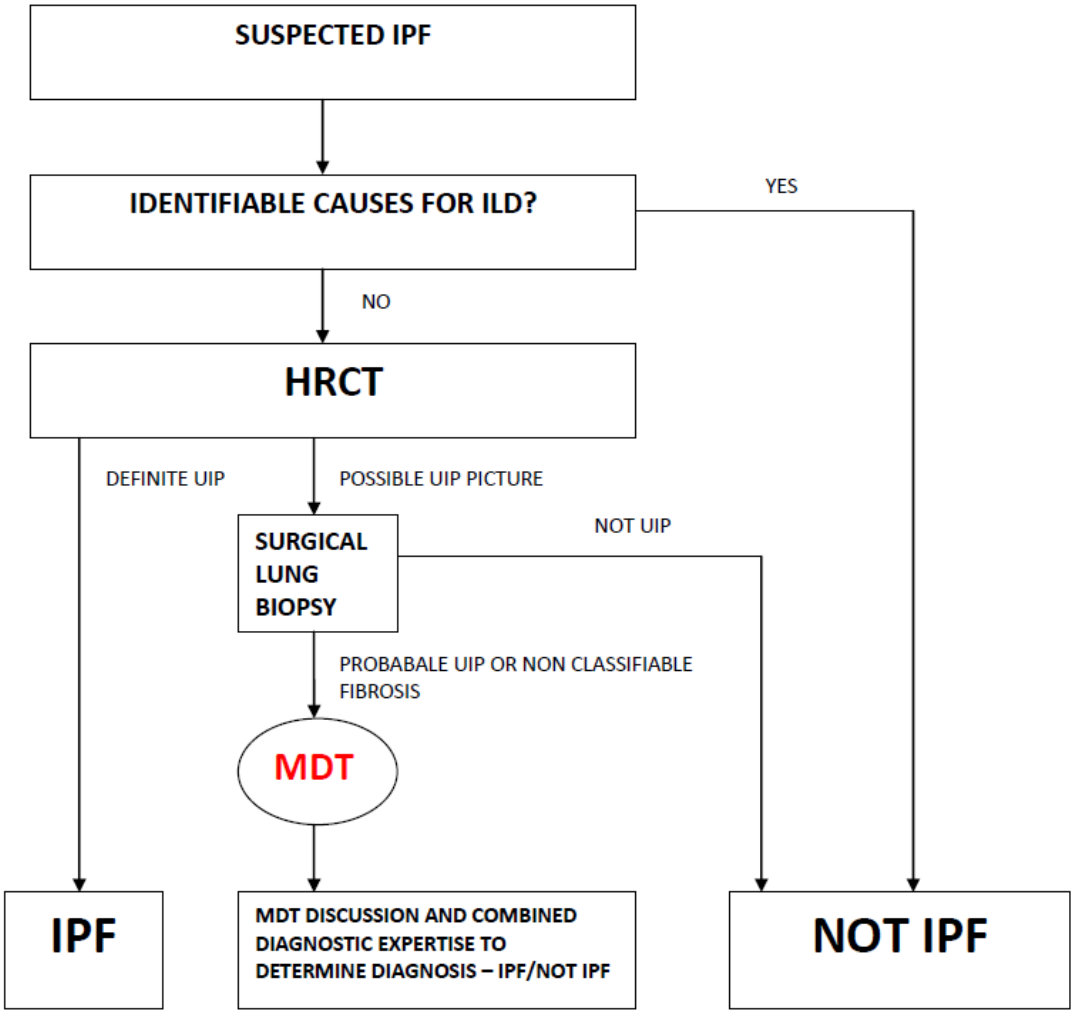


Figure 1- 3 : Diagnostic algorithm for Idiopathic Pulmonary Fibrosis (IPF) [6].

Legend: The patients with suspected IPF are those with evidence of interstitial lung disease (ILD), unexplained exertional dyspnoea and/or cough. If an identifiable cause is isolated then clearly this is not IPF. In the absence of an identifiable cause of ILD, HRCT is used. If this clearly demonstrates a pattern of usual interstitial fibrosis (UIP) then IPF can be diagnosed. When UIP is not clearly identified on HRCT, an MDT discussion using histology in combination with the radiology is important to identify those patients with IPF.

1.1.3 Incidence of IPF

There is considerable variability between studies of the incidence and prevalence of IPF. Studies from America estimate the incidence to be between 6.8 and 16.3 per 100,000 people [7]. In Mexico a population based study estimated the incidence of IPF in males to be 10.7 per 100000 and in females 7.4 per 100000 [8]. Studies from the UK have estimated a slightly lower overall incidence of IPF at 4.6 per 100000 people but claim that between 1991 and 2003 the incidence of IPF has increased by 11% annually with the current incidence of IPF in the UK 7.44 per 100000 [9] [10].

The large discrepancy in the estimates of the incidence of IPF are due to the fact that until recently there has been a lack of a uniform definition of IPF as well as the variation of study design and populations. It is also unclear the influence of geographic, racial and cultural differences on the incidence of the disease [6].

The incidence of deaths from IPF increases with advancing age. Studies from America suggest the mortality rate in men was 61.2 per 1 million and in women 54.5 per 1 million with the death rate being greatest over winter [11].

1.1.4 Aetiology of IPF

IPF is a disease normally found in older patients beyond their 5^{th} decade of life and typically at the ages of 70-80. It classically presents with unexplained chronic exertional dyspnoea, cough and on examination patients have bi-basal inspiratory crackles and often have finger clubbing. There are a number of risk factors that may be associated with the development of IPF including:

- Smoking – Patients smoking more than 20 pack-years maybe at a higher risk of developing IPF [6].
- Environment – Various environmental agents have been associated with the development of IPF, including metal dust particles and both animal and vegetable dust through farming. Supporting this has been the identification of these dust particulates in the lymph nodes of patients at autopsy who have been diagnosed with IPF [6].
- Microorganisms – There have been several studies that have shown a relationship between certain viruses and the development of IPF. In particular

the Epstein-Barr virus (EBV) has been shown to have the most common association with IPF with several studies demonstrating the presence of EBV in lung tissue of patients with IPF [12],[13]) However, the association must be taken judiciously as EBV is generally a common virus and can be found in lung tissue of patients with other disease. In addition, patients with IPF maybe on steroids predisposing them to the development of EBV and other viruses rather than the relationship to IPF being a causal one.

- Genetics – Genetic factors play a role in IPF where two or more members of the same family may be affected by the disease (familial pulmonary fibrosis) and in sporadic cases of IPF. Familial IPF tends to affect patients at a younger age and has been suggested in studies [14] to be associated with a gene controlling anti-viral responses called ELMOD2, located on chromosome 4q31[15]. The genetic transmission of the disease is in an autosomal dominant fashion with variable penetrance. The genetic association with sporadic cases of IPF has been through polymorphisms of genes encoding various factors associated with development of lung fibrosis including genes coding for various cytokines and profibrotic molecules [6].

  There has been more recent evidence suggesting the importance of genetic factors in the pathophysiology of IPF. MUC5B is the gene coding for Mucin 5 subtype B. A single nucleotide polymorphism (rs35705950) in this gene has been shown to be associated with interstitial lung disease in the general population [16]. More recently *Stock at al* [17] demonstrated in a prospective case controlled study consisting of 110 IPF patients and 416 healthy controls an association between the MUC5B polymorphism and IPF. The authors also investigated whether the MUC5B polymorphism increased the risk of lung fibrosis in systemic sclerosis and sarcoidosis; 440 patients with this subtype of fibrosis were studied but no association was discovered. Although MUC5B may indicate a predisposition to developing IPF, *Pelijto et al* [18] demonstrated that the IPF phenotype consists of at least two clinical subsets separable by the MUC5B genotype. In patients with the proven polymorphism of the MUC5B gene there appeared to be a survival advantage compared to those with IPF associated with other environmental or genetic factors.

  Many of the studies involved with the genetics behind the development of IPF are small and further work in necessary. However, recent advances in the

understanding of the genetic basis of IPF may help identify those individuals who are at risk of developing the disease and develop new targeted therapy

The majority of patients with IPF demonstrate a slow progressive decline in respiratory function over 2-3 years and may then succumb to the disease once they develop respiratory failure. However, in a minority of patients (5-10%) it can be rapidly progressive. These patients present with sudden unexplained worsening dysponea, hypoxia, and severe impairment of gas exchange with new alveolar infiltrates on chest imaging and the absence of other causative factors such as pulmonary embolism or infection.

1.1.5 Management of IPF

Patients who have been diagnosed with IPF have to have their disease staged appropriately into mild, moderate, severe and early or advanced disease to determine the prognosis and most appropriate therapy. Several important factors help the specialist appreciate the severity of the disease in an individual, these include:

- Baseline dyspnoea and the change of dyspnoea over time can correlate with the severity of IPF
- Pulmonary function tests can be very helpful and are part of the routine clinical assessment. The lung volumes (TLC, functional residual capacity and residual volume) are reduced. Early in the course of the disease the lung volumes maybe normal and can be higher in smokers with IPF compared to those who have never smoked. Expiratory flow rates (FEV_1 and FVC) maybe reduced but the overall ratio is maintained or increased. The diffusing capacity of the lung for carbon monoxide (TLco) is reduced due to the contraction of the pulmonary capillary volume and the presence of ventilation-perfusion mismatch.

  These tests are very valuable at rest but the magnitude of the abnormalities is accentuated by exercise. In particular the alveolar-arterial partial pressure oxygen gradient (A-a) PO2 widens by up to 20-30% causing severe desaturation. In an effort to compensate and maintain adequate oxygenation, patients with IPF increase their minute ventilation by increasing their respiratory frequency whereas individuals with healthy lungs tend to increase their tidal volume.

- Six-minute walk testing – this is often used in clinical practice where the shorter the distance walked and the longer the heart rate takes to recover may correlate to the severity and subsequent risk of mortality from IPF [6].
- Pulmonary Hypertension – Pulmonary hypertension rarely occurs at rest but can occur during exercise even in early IPF. The cause of the hypertension is due to the damage on the pulmonary vasculature as the fibrosis develops. The mean pulmonary artery pressure has to be greater than 25mmHg at rest. A mean artery pressure above 30mmHg is associated with a poor prognosis [6].

Once the diagnosis and clinical severity are established most patients require some form of medical management as without any intervention mortality from IPF is very high. The median survival after diagnosis is two to three years. Early diagnosis and management may have some control on the progression of the fibrosis and help maintain a degree of lung function compatible with a good quality of life. It is recommended that any therapeutic agent used in controlling a patient's IPF be trialled for at 3 months to ascertain the effect. The following agents may be useful in the management of certain patients with IPF:

- Corticosteroids – These have only achieved an improvement in symptoms in a minority of patients and the effects tend to be short-term. The recent consensus [6] does not recommended corticosteroids in the management of IPF.
- Azathioprine – This is an immunomodulatory agent that can be used in combination with a corticosteroid. Azathioprine is a purine analogue and acts as a prodrug for mercaptopurine inhibiting an enzyme that is required for the synthesis of DNA and affects the activity of lymphocytes. In addition, azathioprine supresses the activity of natural killer cells and some antibodies. However the drug needs to be used with caution particularly in the elderly due to its effects on the bone marrow, gastrointestinal tract and liver. The combination of azathioprine with a corticosteroid is not recommended in the management of IPF.
- Cyclophosphamide – This is also an immunomodulatory agent that is sometimes used in combination with a corticosteroid to manage some patients with IPF. It is an alkalyting agent of the nitrogen mustard group that is absorbed orally and metabolised in the liver into several cytotoxic products that target the activity of lymphocytes. The clinical response to treatment can take up to 9 months in some

patients and so a longer trial of these agents is required. Again the drug can have profound effects on the bone marrow, gastrointestinal tract and liver.

- Proton Pump inhibitors – *Lee et al* [19] reported that patients taking medication for gastro-oesophageal reflux had a lower fibrosis score on HRCT and the use of these medications was an independent predictor of longer survival time.
- Other treatments – Colchicine, Interferon Gamma, and Pirfenidone are some other agents that have been trialled but the data on their efficacy is limited. Consensus on the treatment of asymptomatic gastrooesophageal reflux is still very variable. Recent evidence has demonstrated clinical improvements of lung function with regular proton pump inhibitor use [19]. However, their benefit for patients with non-acid reflux is still questionable and anti-reflux surgery may have a more valuable role in the stabilisation of lung function but more research is required in this field [6].
- Long term oxygen therapy – there is very little evidence to support the value of long-term oxygen therapy in IPF, but recent guidelines [6] recommend its use in patients with significant resting hypoxaemia.
- For some patients, once they become unresponsive to medical management, lung transplantation may be offered. This is normally reserved for young patients with minimal comorbidities. The following objective parameters are used as part of the selection criteria for lung transplantation in end-stage IPF[6]:

    1. New York Heart association functional class III or IV; class III is physical activity limited severely enough that minimal exertion can result in dyspnoea, angina pain, fatigue and palpitations. Class IV is characterised by the inability to carry out physical activity often associated with discomfort at rest.
    2. Honeycombing or pulmonary hypertension on chest x-ray or HRCT respectively
    3. Physiological deterioration of TLC < 60%, (A-a)PO_2 at rest > 30, severe exercise desaturation

1.2 Cystic Fibrosis

1.2.1 Definition

Cystic fibrosis was formally known as mucoviscoidosis or fibrocystic disease of the pancreas. It is the commonest serious inherited metabolic disorder with the autosomal recessive gene being carried by 1 in 20 of the Caucasian population [20]. It affects about 1 in 2500 live births. The genetic defect is on the long-arm of chromosome 7 that codes for the 1480-amino acid protein, cystic fibrosis transmembrance conductance regulator (CFTR). The most common form of this mutation is the Δ508 in which three base pair deletions causes the loss of phenylalanine at position 508 of the protein. This mutation results in a dysfunctional CFTR protein which would normally function as a cyclic AMP-dependent chloride channel in the apical membrane of epithelial cells. The physiological result of this is reduced chloride conductance in all epithelial membranes and the most profound effects are seen in the gastrointestinal, respiratory, hepatobillary and reproductive systems.

The CFTR protein also regulates the activity of epithelial sodium channels (ENaC) and calcium activated chloride channels (CaCC), resulting in the inhibition of sodium transport through ENaC and an inhibition of CaCC (Figure 1-4). In addition, the CFTR affects the bicarbonate-chloride exchange. In sweat ducts, the failure of the reabsorption of chloride ions results in high levels of both chloride and sodium within the sweat which is the characteristic hall mark of cystic fibrosis [21].

There are numerous mutations of the CFTR gene and these are divided into five classes based on their effect on CFTR function:

- Class 1 – These defects affect protein synthesis of the CFTR
- Class II - These defects affect protein processing (this includes the Δ508 mutation)
- Class III – These defects affect activation of the CFTR protein
- Class IV – These defects lead to impaired chloride conductance
- Class V – These defects reduce the synthesis of normally functioning CFTR

Class I-III causes the more life threatening diseases whereas the other classes have less severe clinical manifestations.

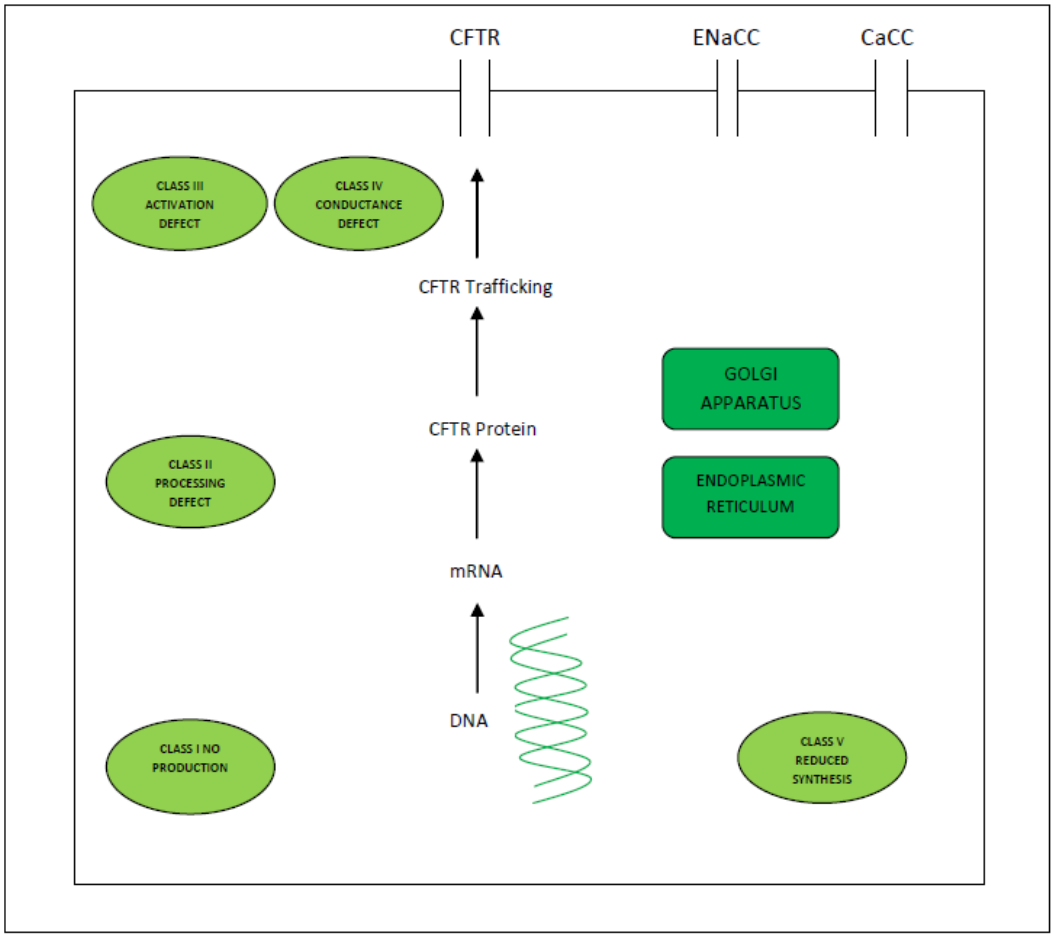


Figure 1- 4 : The CFTR protein and sites of the mutaations

Legend: The cystic fibrosis gene codes for a 1480 amino acid protein names Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) that is trafficked through the cell via the endoplasmic reticulum and Golgi apparatus and inserted into the apical membrane where it functions as a cAMP-dependent chloride channel. Class I mutations disrupt synthesis of CFTR and include mutations that lead to premature termination codons and no protein production. Class II mutations result in misfolded CFTR proteins that get degraded in the endoplasmic reticulum. Class III mutation CFTR protein reaches the apical membrane but fails to be activated and Class IV mutations produce CFTR protein with reduced conductance. In Class V mutations there is reduced synthesis of normal CFTR and thus reduced CFTR function at the cell membrane. CFTR also affects the regulation of sodium channels (ENaC) and calcium activated chloride channels (CaCC) [20].

1.2.2 Diagnosis of Cystic Fibrosis

The diagnosis of cystic fibrosis is made through a combination of sophisticated tests and clinical presentation. In children the presentation of meconium ileus at the time of birth is characteristic of cystic fibrosis. Other signs in the young are failure to thrive and recurrent chest infections.

The diagnosis of cystic fibrosis is confirmed by both DNA analysis and the sweat test. The sweat test identifies elevated levels of chloride ions caused by the effect of the defective CFTR gene on the chloride channels. Sweat chloride levels greater than 60mmol/L (normal<29mmol/L)after several tests are highly suggestive of CF [22]. In addition, the demonstration of two known CF mutations on DNA analysis is used to confirm the diagnosis. The DNA analysis can be made on chorionic villous samples allowing diagnosis to be made in the antenatal period. In some individuals DNA analysis may reveal only a single gene mutation and this may indicate that they are a carrier of the disease and these individuals may exhibit few or no symptoms.

As the ability to perform more sophisticated genetic tests becomes increasingly available a group of patients have been identified and diagnosed with 'atypical' cystic fibrosis. In the clinical setting they tend to be older patients with isolated manifestations of cystic fibrosis rather than the spectrum of clinical diseases seen in classical CF. In addition their sweat tests may reveal a chloride concentration of 30-59 mmol/L [22].

The early diagnosis of cystic fibrosis has also been also improved by newborn screening. A single dried blood spot is obtained using a Guthrie card; elevated levels of trypsin are seen in cystic fibrosis. The ability to identify individuals with CF early allows prompt management of the disease which may help slow the progression of the disease.

1.2.3 Clinical Manifestations of Cystic Fibrosis

Respiratory Manifestations:

The CFTR defect and subsequent effect on the chloride channel causes a reduction in chloride ion secretion and increase in sodium reabsorption from the bronchial mucosa epithelial cells resulting in viscous secretions. These secretions not only disrupt the mucocilliary clearance mechanisms but the elevated salt concentration of the secretion inactivates defensins on the epithelial membranes predisposing to bacterial infections. The sequeleae of infection and inflammation repeats itself eventually leading to bronchiectasis and respiratory failure (Figure 1- 5).

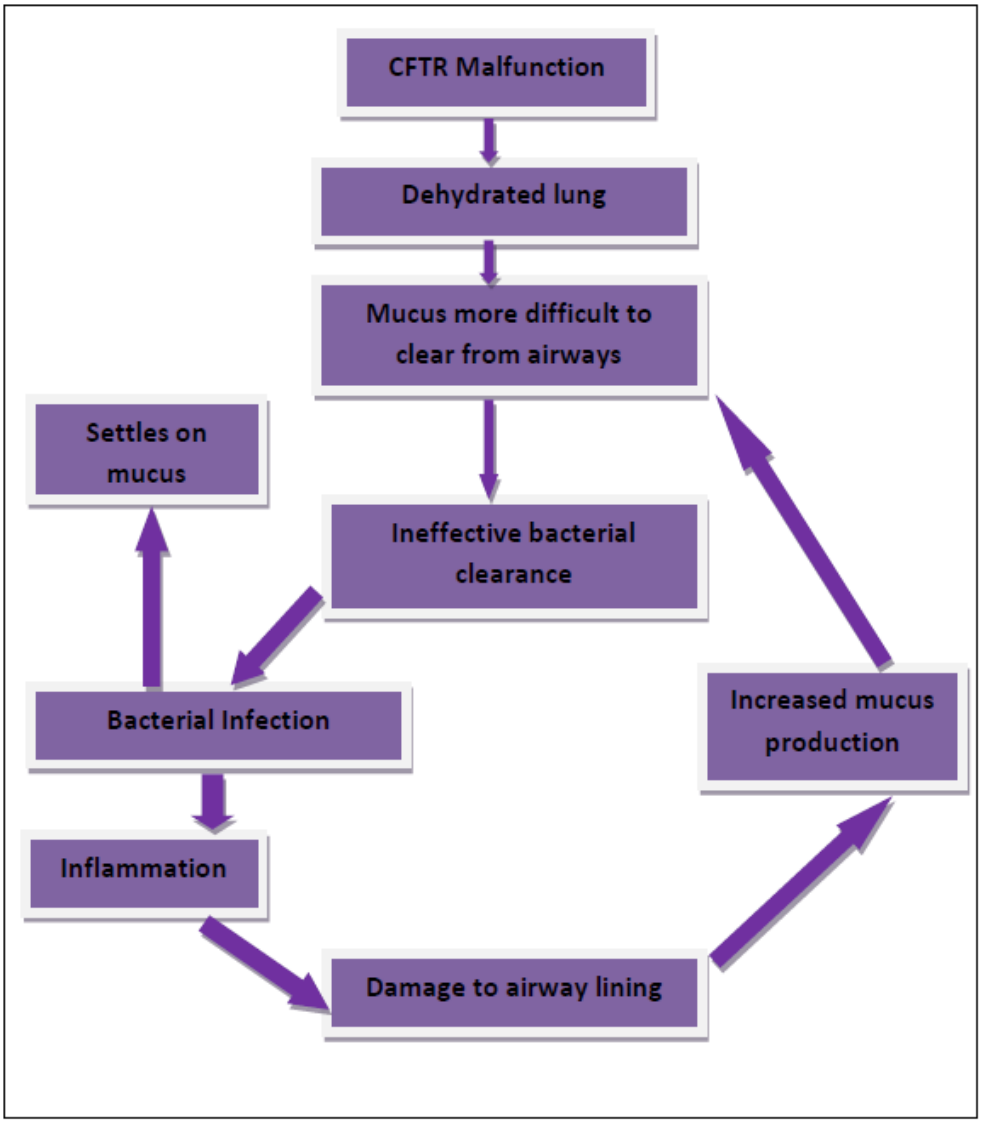


Figure 1- 5 : The cycle of infection, airway damage, increased mucous production and ineffective bacterial clearance [21].

The persistent chest infections and development of bronchiectasis leads to progressive airway obstruction. In younger patients with cystic fibrosis the common microorganisms causing infection are *Staphylococcus aureus*, *Haemophilus influenza* and *Streptococcus pneumoniae*. In adult patients the most common infection tends to be a mucoid strain of *Pseudomonas aeruginosa*. In addition to these infections a Gram-negative plant pathogen called *Burkholderia cepacia* complex is responsible for causing serious chest infections including a fulminant necrotising pneumonia known as 'cepacia syndrome'. This organism was thought not to be pathogenic to humans but has since been discovered to be an extremely aggressive infection amongst CF patients particularly those in close social circle. Over time with recurrent infection, irreversible lung injury with the destruction of lung parenchyma leads to severe life threatening complications including cor pulmonale, major haemoptysis, recurrent pneumothorax and progressive respiratory failure.

Gastrointestinal Manifestations

The main organ in the gastrointestinal tract affected by the defective ion transport is the pancreas. The blockage of the ducts by the thick mucus prevents the exocrine secretions being released into the duodenum resulting in irreversible damage to the pancreas often with inflammation (pancreatitis). The complete blockage of the pancreatic ducts by mucus often seen in young CF patients can lead to complete atrophy and fibrosis of the pancreas resulting in the complete loss of pancreatic function. This causes malabsorption and diabetes. In addition to the effects on the pancreas, abnormalities with the secretion of bile from the liver can cause biliary cirrhosis and gallstone formation.

In addition to pancreatic and hepatobiliary complications, from even early life the effects of the CFTR mutation on the functioning of the intestines can be very serious. Sludging of the intestinal contents in about 10% of neonates with cystic fibrosis can cause meconium ileus [20]. A similar condition can occur in the terminal ileum of adult cystic fibrosis patients and is described as distal intestinal obstruction syndrome. It is caused by semi solid faecal material obstructing the terminal ileum as a result of fluid malabsorption and disordered gut motility.

Other Manifestations

Both male and female patients with cystic fibrosis can be affected by infertility problems. The defective ion transport causes viscous secretions that can block the vas

deferens and affect cervical mucus. Another major complication of cystic fibrosis is the arthropathy caused by the deposition of antigen-antibody complexes in the joints. The joints can be also affected by the development of osteoporosis which is more common in cystic fibrosis.

1.2.4 Management of Cystic Fibrosis

The management of CF is through a multidisciplinary approach due to the multiple systems affected by the disease. The way this is best achieved is through specialist clinics with individual members of the multidisciplinary team managing the patients through these clinics. The following are the principal components of the management of CF patients:

1. Physiotherapy – The airways of CF patients become obstructed with thick viscous sputum which requires clearance with specialist physiotherapy employing a number of techniques including, postural drainage, chest percussion and devices using positive expiratory pressure to clear the peripheral airways.
2. Nutrition – The main supplementation is required due to destruction of the pancreas. Pancreatic enzymes are given to the patient in the form of Creon, taken after each meal. In addition, patients require fat-soluble vitamin supplementation, that is, vitamins A, D E and K. Due to the general malabsorption of nutrients and the need to combat recurrent chest infection, patients are encouraged to consume between 120 and 150% of their recommended daily calorie intake. In severe cases of nutrient deficiency patients may require admission and feeding via a nasogastric tube.
3. Medication – Antibiotics form a major part of the medical management of cystic fibrosis and begins in childhood. One of the major pathogens at this age is *Staphylococcus aureus* and patients are sometimes given long-term flucloxacillin to suppress the infection. Other antibiotics maybe required depending on the pathogens isolated from sputum. As patients advance in age the common pathogen is *Pseudomonas aeruginosa* and patients can become chronically colonised. Initial antibiotic therapy is usually in the form oral ciprofloxacin. However, nebulised colistin or tobramycin can also be used to combat the organism. Sometimes intravenous antibiotics are required and these can be given in hospital or at home. Infection caused by *Burkholderia cepacia* are often resistant to the more conventional antibiotics but can be sensitive to ceftazidime or meropenam.

Several strategies are employed to manage the thick mucus secretions in CF. Mucolytic agents include deoxyribonuclease, a genetically engineered enzyme used to cleave DNA from degrading neutrophils which contribute to the viscosity of the mucus. *Rubin et al [23]* suggested that in CF, the necrotic death of inflammatory and epithelial cells releases a large amount of F-actin which produces the thick viscous secretions. They used depolymerising agents such as thymosin Beta 4 (TBeta4) and gelsolin and demonstrated a dose-dependent decrease in Cf mucus cohesitivity. The use of nebulised hypertonic saline and mannitol as osmotic agents to increase the water content of the secretions remains controversial.

4. Lung Transplant – In patients with advance disease and deteriorating lung function a lung transplant maybe an option if a donor lung is available and the patient is suitable for surgery.

1.3 Gastro-Oesophageal Reflux Disease

1.3.1 The normal anatomy and physiology

The oesophagus is a muscular tube measuring approximately 25cm from the pharynx to the stomach and is situated in the posterior mediastinum. The distal 1-3cm is normally intra-abdominal having passed through the diaphragmatic hiatus. The oesophagus and stomach are united at the gastro-oesophageal junction (GOJ) or cardia (Figure 1- 6). The lining of the oesophagus is squamous epithelium up to the GOJ where it terminates at the Z-line and integrates with the columnar-lined epithelium of the stomach. The oesophagus is also divided into three histological layers, the mucosa, submucosa and muscularis layer. The muscularis layer of the proximal oesophagus is composed of striated muscle and the distal oesophagus is composed of smooth muscle. Between these sections the composition is a mixture of smooth and striated muscle fibres [24].

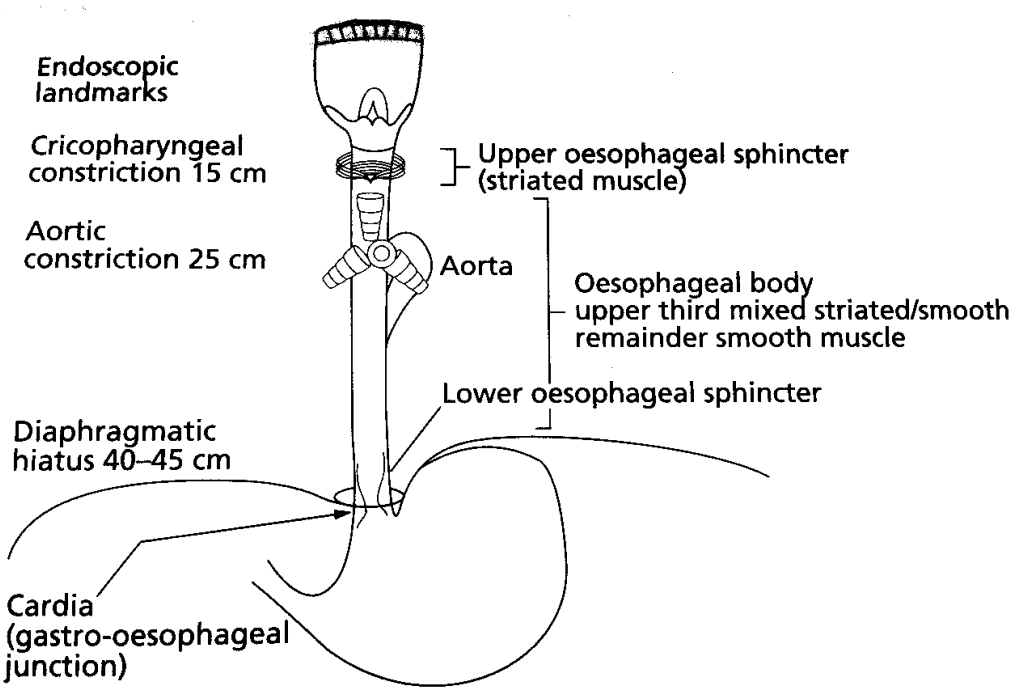


Figure 1- 6 : Important anatomical and functional structures in the human oesophagus [24]

The upper oesophageal sphincter (UOS) separates the pharynx and the oesophagus and functions to prevent aspiration of the gastrointestinal contents. It is formed by the cricopharyngeus muscle and is 3-4 cm long with a functional pressure of 50-100mmHg. The lower oesophageal sphincter (LOS) is found at the GOJ across the diaphragmatic hiatus. It is a high pressure zone measuring 2-5 cm and situated in the lower oesophagus. The pressure of the LOS demonstrates postural, diurnal and prandial variations in pressure.

The LOS is closed at rest and maintains a pressure of approximately 20mmHg. It is composed of two muscle components, a circular component which forms a semi-circular clasp and the gastric sling muscles form the most lateral aspect of the sphincter. Two main peripheral neurons mediate active contraction and relaxation of the LOS, acetylcholine is an excitatory neurotransmitter and nitric oxide acts mainly in an inhibitory way. In addition, relaxation of the LOS can part occur when tonic vagal cholinergic excitation to the LOS is switched off on the initiation of a swallow [25]. The sling muscles respond to cholinergic excitation and relaxation is predominately through turning off this excitation. The clasp component has a high intrinsic resting tone and relaxation is stimulated through the release of nitric oxide.

The diaphragm acts as an external sphincter as the distal oesophagus passes through the hiatus which is formed mainly by the right crus. The compression of the lower oesophagus from the hiatus varies with inspiration and the resulting LOS pressure can vary between 10-100mmHg. Increases in intra-abdominal pressure cause contraction of the diaphragm and thus an increase the LOS pressure. During swallowing, belching and vomiting the crural portion of the diaphragm relaxes. The phreno-oesophageal ligament is a distinct structure that runs between the diaphragm and the gastro-oesophageal junction. It is a two-layered structure, the upper layer attached to the oesophagus above the diaphragm and the lower layer runs caudally and is attached to the oesophagus just above the angle of His. It is composed of collagen and elastin fibres and so whilst the ligament is firmly attached to the oesophagus there is some flexibility as it functions to prevent migration of the oesophagus into the chest.

The oesophagus itself effectively delivers food to the stomach and this is only possible due to its histological composition. The oesophagus is normally collapsed but the lumen expands without mucosal injury when a bolus of food is swallowed. The mucosal lining is non-keratinised stratified squamous epithelium in humans. The underlying lamina propria consists of diffuse lymphatic tissue. The deep mucosa layer is the muscularis

mucosa consisting of longitudinal smooth muscle fibres, thicker at the proximal oesophagus to aid swallowing. The submucosal layer is dense connective tissue consisting of larger lymphatic and blood vessels as well as some nerve fibres (Meissner's Plexus). The muscularis externa is the final layer and is composed of an inner circular layer and an outer longitudinal layer. The fibres are different in these layers for each level of the oesophagus; the upper third is composed of striated muscle fibres, the middle third is smooth and striated fibres and the lower third is smooth muscle like the rest of the gastrointestinal tract. Between the inner and outer layers is the Auerbach's nerve plexus which helps coordinate peristalsis. Along the length of the oesophagus there are mucus secreting glands. They secrete slightly acidic mucus into the wall of the oesophagus except near the stomach where the secreted mucus is neutral.

1.3.2 Incidence of GORD

Gastro-oesophageal reflux (GOR) which is often short lived and may affect an individual on occasion can be regarded in these circumstances as a normal physiological phenomenon. It is often associated with completion of a meal or belching. It may sometimes affect an individual at night in particular after alcohol or spicy food. Pathological GOR is associated with symptoms and is usually caused by more frequent reflux episodes including some at night. This type of reflux may even lead to inflammation of the oesophagus called oesophagitis [24]. Gastro-oesophageal reflux disease (GORD), gastric reflux disease, or acid reflux disease are chronic diseases caused by mucosal damage due to gastrointestinal content coming up from the stomach into the oesophagus [26] .

GOR is very common and it is believed up to 60% of the normal population may have symptoms of reflux at some point in their lives; 11% of Americans experience symptoms of daily reflux, and 33% experience these over a 72 hour period [27].

1.3.3 Aetiology of GORD

GOR occurs due to failure of one or more of the physiological protective mechanisms. The reflux of gastric contents in health is prevented through the combined action of the oesophageal musculature including the lower oesophageal sphincter (LOS) and the diaphragmatic crura providing an extrinsic pressure [28]. The majority of episodes of GOR occur during transient periods of LOS relaxation which is an abnormal phenomenon when it is not preceded by a corresponding primary peristaltic wave in the oesophageal body initiated by a voluntary swallow [24].

Disorders affecting the LOS maybe functional (transient LOS relaxation) or mechanical (reduced LOS tone) and may be caused by a number of factors including smoking, hiatus hernia, diet and drugs. Although the LOS in an important barrier to GOR it is only one of a number of factors that prevents reflux. The table below summarises the patho-physiological factors which contribute to reflux (Table 1-2).

Gastro-oesophageal reflux can occur in any period of life which suggests its aetiology is multifactorial. A multitude of anatomical and physiological defects caused by external factors such as smoking as well as other diseases may account for the development of reflux.

PRIMARY	SECONDARY
LOS hypotension	**Salivation production impairment**
LOS overall Length < 2cm	**Impaired oesophageal peristalsis**
LOS intra-abdominal length < 1.5cm	**Gastric acid hypersecretion**
Hiatus Hernia	**Gastric outlet impairment – gastroparesis**
Loss of angle of His (hiatus hernia)	**Small intestine outlet dysfunction (mechanical obstruction/visceral enteropathy)**
Crural diaphragm failure	
Loss of mucosal rosette (inflammation)	

Table 1-2: Possible Mechanisms of failure of the anti-reflux mechanisms[24]

The Anti-reflux Mechanism

Although the resting pressure of the LOS plays a major part in the barrier against reflux there are a number of factors that contribute to the anti-reflux mechanism. These are divided as follows:

Oesophageal Factors:

- LOS – The LOS acts as a two-way valve by using the flutter valve principle and is a weak sphincter with an intrinsic pressure of 10-25mmHg. It plays a major role in preventing the retrograde movement of gastric content back towards the oesophagus against the high variations of intra-abdominal (100mmHg) and intra-thoracic pressure (60mmHg). This discrepancy in pressure between these components and the basal tone of the LOS account for the high incidence of LOS dysfunction. The LOS overall length is important as it relates to valve competence. LOS length less than 2cm is associated with failure of the anti-reflux mechanism. Intra-abdominal LOS length is also a significant factor in the anti-reflux mechanism. There are

pressure differences across the diaphragm; positive pressure in the abdomen means negative pressure in the thorax. The greater the LOS length in the abdomen, the greater the augmentation of LOS pressure with any progressive rise in intra-abdominal pressure [24].

- Oesophageal Motility – Coordinated oesophageal contractions in the form of ordered peristalsis are required to propel the food bolus through the oesophagus and into the stomach. Any dysfunction in oesophageal motility can worsen gastro-oesophageal reflux due to inadequate clearance of refluxate [24]. Certain soft tissue disorders such as scleroderma can result in oesophageal dysmotility and a hypotensive LOS. However, inflammation of the oesophagus (oesophagitis), in itself is associated with reduced LOS pressure and oesophageal motility. In the body of the oesophagus, both reduced amplitude of the primary and secondary peristaltic waves and failed peristalsis are common. There is a vicious cycle between inflammation and dysmotility, with the disorder being most pronounced in patients with severe oesophagitis. It is unclear on the exact mechanism through which inflammation results in dysmotility but a decrease in cholinergic excitation and an increase in nitric oxide inhibitory mechanisms may have a role [25].

Anatomical Factors:

- Angle of His – This angle is formed at the cardia and creates a flap-valve mechanism which forms an effective anti-reflux mechanism. This angle is absent in a hiatus hernia and thus reflux is facilitated; as the hiatus hernia increases in size it can perpetuate the reflux symptoms. In patients with severe oesophagitis it is common to have a low pressure sphincter and hiatus hernia [25]. Oesophageal clearance of acid is reduced with a hiatus hernia [24]. The hernia acts as a reservoir, the diaphragm trapping acid resulting in repeated reflux predisposing to inflammation around the cardia.

- Mucosal Rosette – A mucosal rosette is formed by the convoluted folds of oesophageal mucosa and this forms a fluid and gas tight seal which acts as an anti-reflux barrier, but in oesophagitis the integrity of the rosette is disrupted and increases the frequency of reflux [24].

- LOS vagal reflex – A vasovagal reflex exists that responds to a rise in the intra-abdominal pressure and protects against reflux by causing a rise in the LOS pressure. Damage to the vagus nerve during some surgical procedures can abolish this reflex

- Salivary secretion – During mastication of food salivary secretions which contain bicarbonate ions increase and when swallowed neutralise stomach acid and protect against refluxed acid.

Gastric Factors:

- Gastric Motility – A loss of gastric motor function can cause gastric stasis. This can cause an increase in the intra-gastric pressure predisposing to reflux. Delayed gastric emptying is common in patients with GORD and present in 26% of patients on the basis of retention at 4 hours [29].
- Gastric acid secretion – The majority of studies do not demonstrate an increase in gastric acid secretion in patients with gastrooesophageal reflux disease (GORD)[24].
- Duodenogastric Reflux – the reflux of gastric content may include pepsin and substances such as bile and pancreatic and intestinal enzymes from the duodenum. Pepsin exhibits its maximum activity at pH2 and the combination of pepsin and gastric acid in reflux is responsible for inflammation of the oesophagus [26]. Exposure to the combination of bile acids and gastric acid appears to be more harmful than gastric acid alone and can damage the oesophageal epithelial layers, leading to oesophagitis, Barrett's oesophagus, and even oesophageal cancer [30]. There are marked differences in behavior of bile acids depending on the pH of the solution in which they reside. In strongly acidic conditions, conjugated bile acids enter mucosal cells in a non-ionized form, which occurs at a pH close to or below their acid dissociation constant (pKa)[30]. These refluxed bile acids can cause intracellular damage by the dissolution of cell membranes and tight junction proteins. Patients with both acid and duodenal reflux have a high incidence of oesophagitis and duodenogastric reflux is more common in GORD patients with strictures and Barrett's oesophagus [31].

1.3.4 Management of Gastro-Oesophageal Reflux Disease

The goal of treatment for gastrooesophageal reflux disease (GORD) has evolved over 30 years from short term symptom relief to long-term symptom control. Treatment is based on a step wise approach commencing with a single drug regime, progressing to multiple drug regimes and finally in some cases endoscopic and surgical treatment.

Before any type of medical treatment, lifestyle modifications are initially recommended. These measures include alterations in eating habit, dietary restrictions, postural changes

during sleep and eliminating exacerbating factors to reflux such as smoking and alcohol. However, whilst lifestyle changes are frequently requested the evidence suggests that these changes or attempts to carry out these modifications have limited effects [32]. Below is a summary of the treatment strategies employed for reflux disease (Figure 1- 7):

- Medical Treatments
  - Over the Counter - These include simple antacids and alginates such as *Gaviscon*. These are both effective at providing some symptom relief but in severe reflux or patients with oesophagitis they are less effective [32].
  - Acid Suppression – The first effective treatment for GORD were H2-receptor antagonists like cimetidine which worked by suppressing the production of stomach acid. These were replaced by proton pump inhibitors (PPIs) which were not only better at symptom control but were also effective at healing oesophagitis. In patients that respond well to a treatment dose of PPI, a maintenance dose is often prescribed long-term to prevent the relapse of oesophagitis [32] .
  - Motility agents - As well as improving peristalsis prokinetics often enhance gastric emptying and may reduce reflux of gastric contents.
  - Helicobacter Pylori Eradication – The treatment of H.Pylori infection has not been shown to effect GORD. However, many patients with reflux-like and dyspeptic symptoms often have ulceration from infection by the microorganism. These symptoms can be effectively managed by eradication therapy [32].

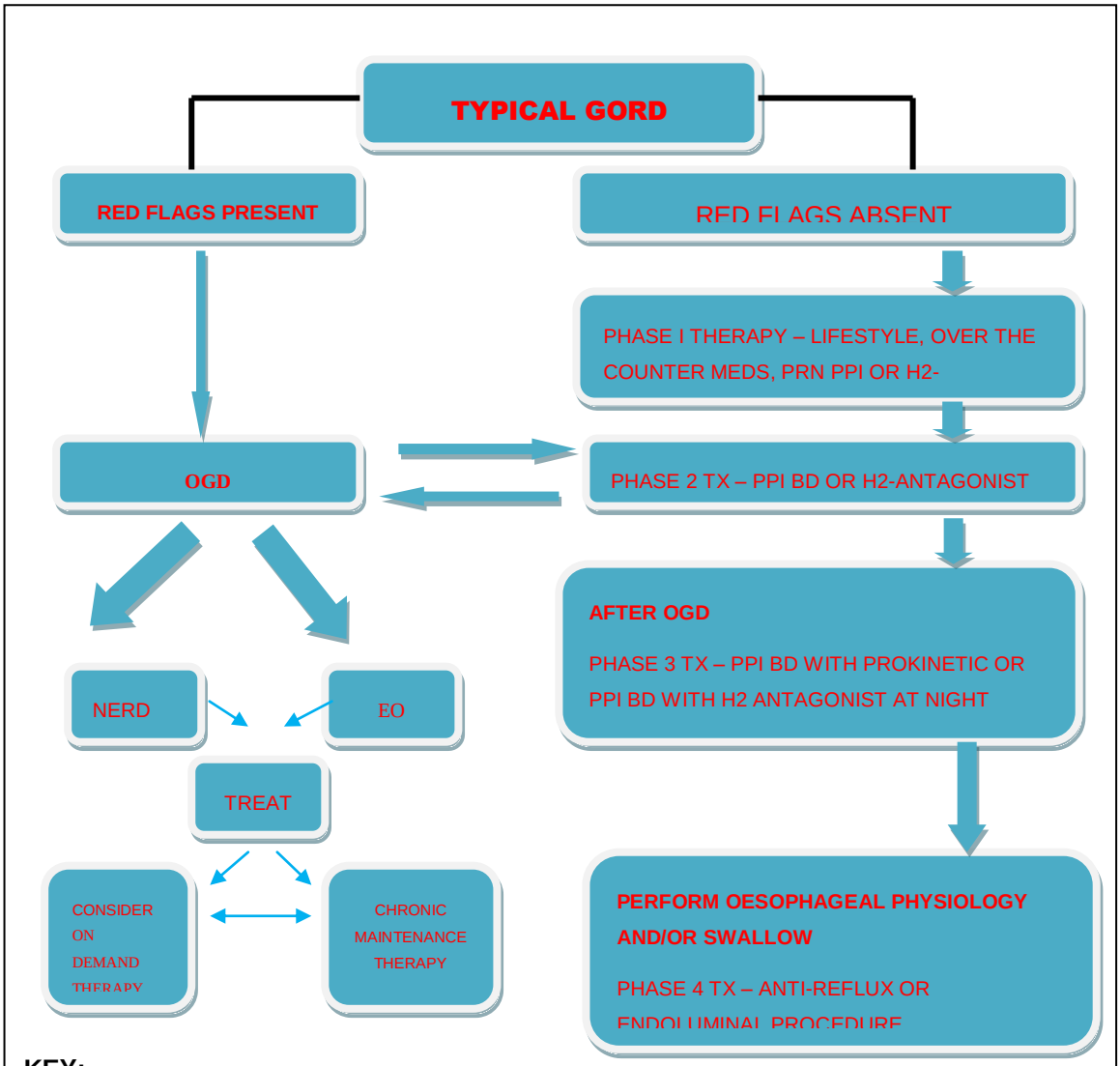


Figure 1- 7 : Algorithm for diagnosis and treatment of GORD[32]

❖ Surgical Management

➢ The primary indication for surgery is the failure of medical treatment which may be defined as persistent symptoms whilst on medical treatment or soon after stopping maximal medical treatment; the decision here being a balance between the reliance on medication and the suitability of an operation.

Anti-reflux surgery is effective in patients where reflux is secondary to a defective lower oesophageal sphincter (LOS) or where there is reflux in the presence of a normal LOS. There are a number of reasons that explain the success of anti-reflux surgery and these include the following [24]:

a. The floppy valve principle of the abdominal oesophagus is created by using the fundus to compress the lower oesophagus. As the intra-gastric pressure this acts as an antireflux mechanism.

b. A possible reduction in TLOSRs.

c. Exaggeration of the flap valve at the angle of His where the oesophagus joins the stomach.

d. An increase in mean LOS resting pressure as measured in post surgery manometry studies.

e. A reduction in the volume of the gastric fundus which may help gastric emptying and reduce acid secretions.

f. Prevention of shortening of the intra-abdominal oesophagus during gastric distention.

The principal behind any anti-reflux procedure is the creation of the barrier between the oesophagus and stomach. There are several ways of accomplishing this and surgery may be performed either as an open or laparoscopic technique. The most popular antireflux procedure is a Nissen 360º fundoplication. In this procedure, any hiatus hernia is reduced in to the abdominal cavity and the fundus of the stomach is brought behind the oesophagus and sutured anteriorly to the remainder of the fundus in a loose fashion hence the term ‘floppy’ Nissen.

Some surgeons choose to do a partial (180º) wrap such as the Toupet or Dor hemi-fundoplication (Figure 1- 8). The rationale behind this is that by partially wrapping the fundus around the oesophagus there is less likely to be complications of dysphagia sometimes associated with a complete fundoplication [24].

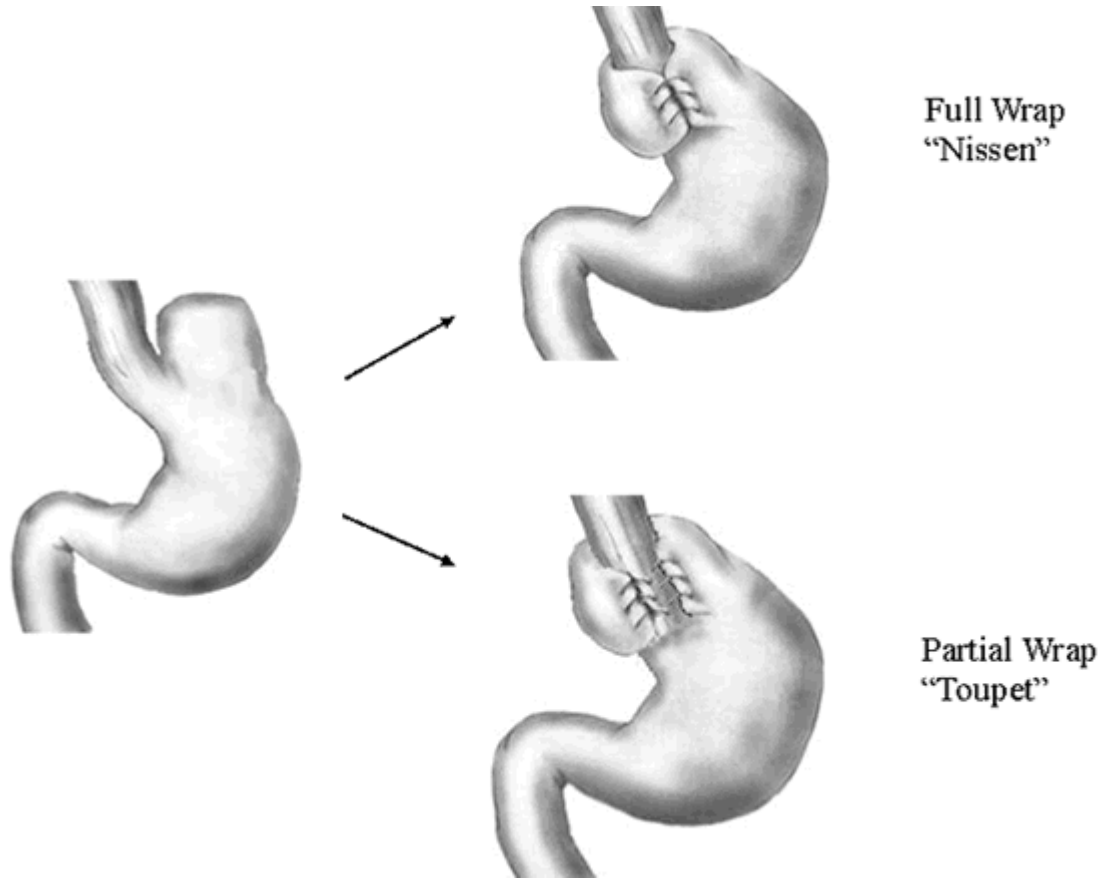


Figure 1- 8 : Diagrams of a floppy 'Nissen' fundoplication and a 'toupet' partial fundoplication.

Legend: The Nissen fundoplication is a full 360º loose wrap around the oesophagus and sutured anteriorly to the remainder of the fundus using non-absorbable sutures. The Toupet wrap is a posterior hemi-fundoplication with the fundoplicature sutures placed in the crural margins on both sides.[33]

1.4 Assessment of GORD

1.4.1 Endoscopy

Upper gastrointestinal endoscopy is the 'gold standard' for documenting the type and extent of mucosal injury to the oesophagus (Figure 1- 9)[32]. Only 40-60% of patients with positive pH tests for reflux have oesophagitis so the sensitivity of endoscopy for the detection of GORD is only around 60% at best but the specificity is between 90-95%. A third of patients with a normal oesophagus on endoscopy will have pathological reflux [34].

Beyond simply assessing the extent of mucosal damage secondary to reflux, endoscopy can be used to diagnose the other complication associated with GORD including Barrett's oesophagus and strictures secondary to inflammation. In addition, endoscopy can be used to exclude malignancies and some dysmotilty disorders such as achalasia.

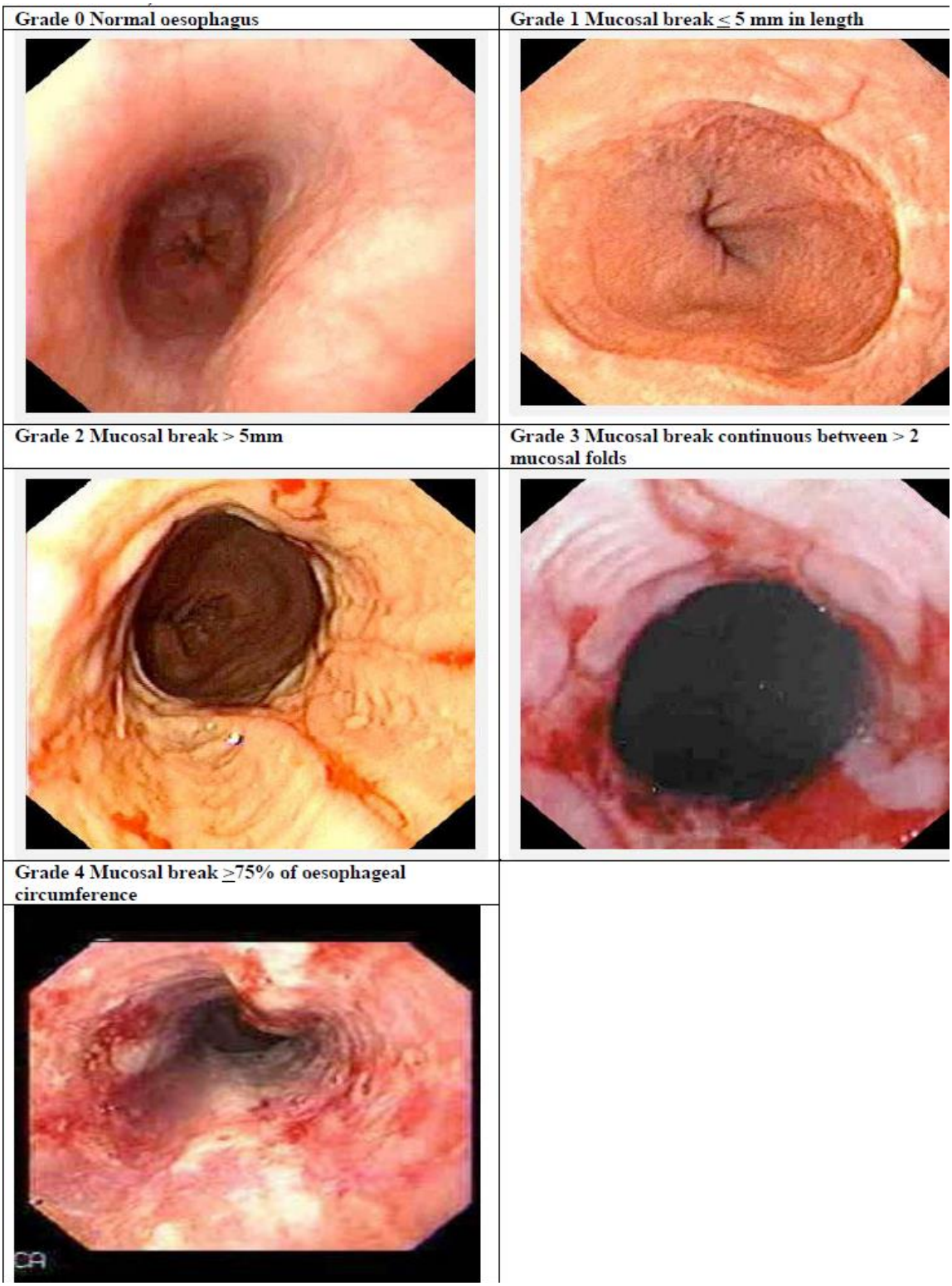


Figure 1- 9 : Endoscopic views of the oesophagus with the Los Angeles oesophagitis scoring system [34].

1.4.2 Manometry

Oesophageal manometry is the gold standard for the assessment of oesophageal motor function by providing information on contractile activities of the oesophageal body and lower oesophageal sphincter (LOS) [24]. In the assessment of GORD, oesophageal manometry is used to determine whether there is normal oesophageal motility and to identify the LOS to ensure accurate placement of the pH/impedance catheter 5cm above the sphincter.

The equipment necessary to perform manometric testing includes the catheter, pressure transducers and a recorder. Over recent years oesophageal manometry has become much more sophisticated to incorporate a variety of recording devices and approaches to manometric measurement and analysis [35]. The manometric systems can use either a water-perfused catheter system or one based on solid –state circuitry. The solid-state systems are more expensive and fragile but provide a better assessment of the proximal oesophagus and pharynx.

Oesophageal manometry is used to record the resting pressures of the lower and upper oesophageal sphincters as well as the timing and completeness of the relaxation. In the oesophageal body it provides an assessment of the peristalsis by measuring the velocity, amplitude and duration of the contraction in response to a swallow [32]. The number of readings obtained is dependent on the number of sensors, typically spaced 3 to 5 cm apart along the catheter. Traditional systems use an 8-channel catheter where each of the 8 sensors is connected to a pressure transducer which converts the physical changes in pressure into electrical signals. These signals are transmitted to a recorder which transforms the signal into a visual display by way of a polygraph (Figure 1-１０). Testing is performed by passing the catheter trans-nasally into the stomach and pulling it back across the LOS into the oesophagus

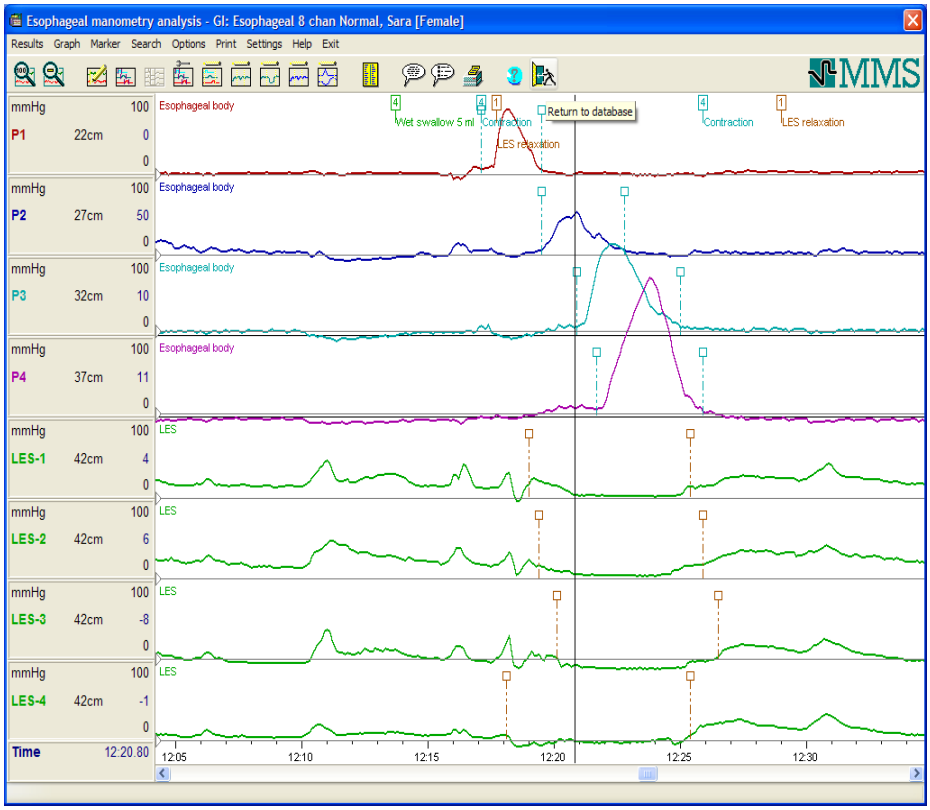


Figure 1-１０: 8 Channel Manometry trace for a single swallow [36].

Legend: The figure illustrates tracings obtained from the proximal four channels positioned in the oesophagus and distal four channels arranged radially in the LOS. A pull-through technique is used for sphincter assessment. At intubation the recording ports are passed beyond the LOS and withdrawn in small increment (usually 0.5-1cm). The LOS, oesophageal body and UOS can be identified by their responses to a 5ml bolus of water. The trace above illustrates the progression of a peristaltic wave through the body of the oesophagus. Detailed measurements of the LOS including assessing the degree of LOS relaxation and the resting pressure can also be assessed.

Technical advances in manometry have led to the development of a wide variety of recording equipment and approaches to manometric measurements and their analysis. The development of powerful computerised acquisition systems, along with high-fidelity multichannel perfusion pumps and manometric catheters has enabled measurement of oesophageal motility with high resolution manometry (HRM).

High Resolution Manometry (HRM) is simply an adaptation to the traditional 8-channel manometry basically incorporating an increased number of pressure sensors spaced closely together. However, a polygraph image using information from over 20 sensors can become very difficult to interpret. Clouse and Staiano [37] used a process of interpolation or averaging between sensors to display the information in the form of isobaric colour regions on oesophageal topography plots, or Clouse plots

(Figure 1-1 1).

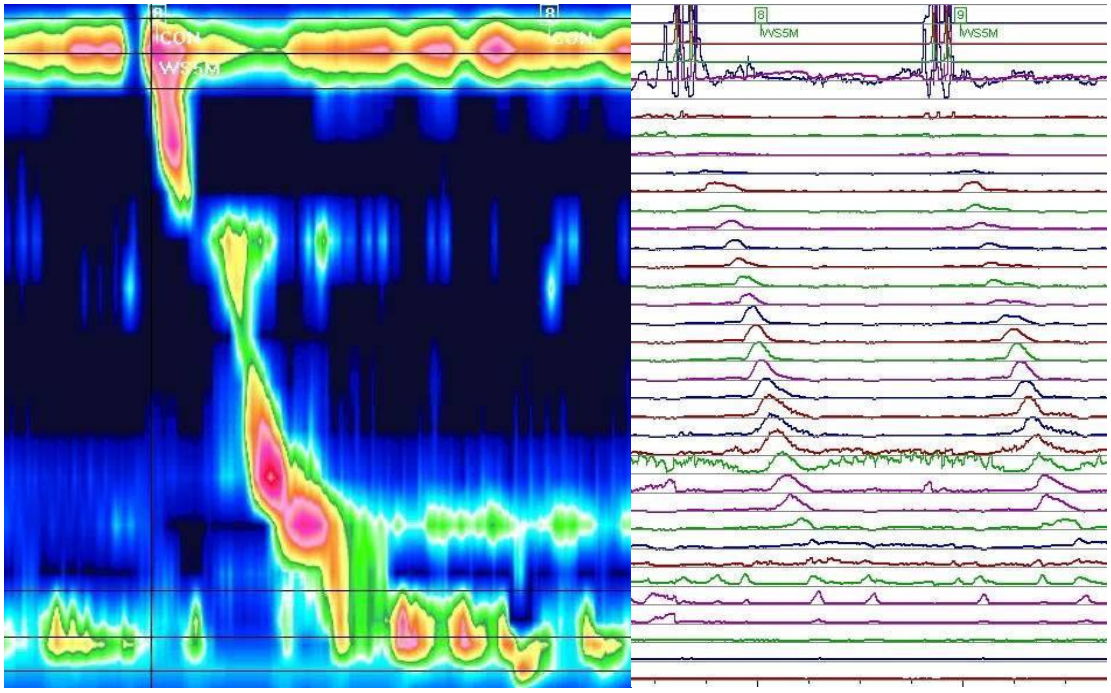


Figure 1- 1 1 : High Resolution manometry trace for a normal swallow

Legend: These topographic plots have the capacity to convert manometric information into distinct patterns that illustrate the physiology of contractile coordination and provide a better understanding of oesophageal body peristaltic function due to more detailed and accurate measurements [38].

1.4.3 Ambulatory pH Testing

Ambulatory pH monitoring has been used for many years to evaluate GORD. It has previously been called the "gold-standard" for detecting pathological reflux [39]. pH monitoring is very useful for assessing acid reflux and its function is through the measurement of H^+ ions [40]. The test is performed with a pH probe passed trans nasally and positioned 5cm above the lower oesophageal sphincter [39]. Measurements can be collected and logged into a data recorder as frequently as every 4 to 6 seconds and reflux episodes are considered significant when the pH of the oesophagus is less than 4. There was strong consensus agreement among specialists that acid reflux should be defined as reflux episodes that decrease oesophageal pH below 4, or reflux that occurs when oesophageal pH is already below 4 [41]. However there are several disadvantages of this definition. One important problem with pH monitoring is in the ability to correlate accurately with pathological evidence of GORD with studies producing a wide variation in sensitivity and specificity of 24-hour pH monitoring [32]. The sensitivity of pH monitoring to detect individual acid reflux episodes is determined by sampling frequency, duration threshold, pH threshold, and the recurrence of reflux prior to pH recovery [41]. Sampling frequency affects the number of reflux episodes detected. To be optimal this should be at a frequency of 1Hz but normal pH monitoring sampling frequency is considerably less at 0.25Hz which affects the sensitivity of the probe [41]. The specificity of pH monitoring is affected by the ingestion of acidic food substances as well as respiratory changes, movement and electrode drift. All these factors cause significant and frequent variations in the pH. Its other shortcoming is its inability to detect or acknowledge weakly acid and non-acid reflux. It is also unable to measure the proximal extent of reflux, although dual channel pH monitors have been designed to measure proximal and distal reflux [41].

1.4.4 Multichannel Intraluminal Impedance

Standard pH monitoring may underestimate the degree of reflux. In 1991 Sliny [42] was the first to describe multichannel intraluminal impedance (MII), a novel method of assessing intraoesophageal bolus movement . This was further developed to combine impedance with pH assessment in order to determine the nature of the reflux [40]. Through the improvements in catheter technology and the development of computer software in the last decade there has been a steady increase in the availability of Multichannel Intraluminal Impedance (MII) [36]. The direction and the proximal extent

of liquid and gas reflux events can be accurately measured by MII [40]. It is becoming the gold standard for assessment of reflux [43].

Theory, validation, intra-observer variability & reproducibility

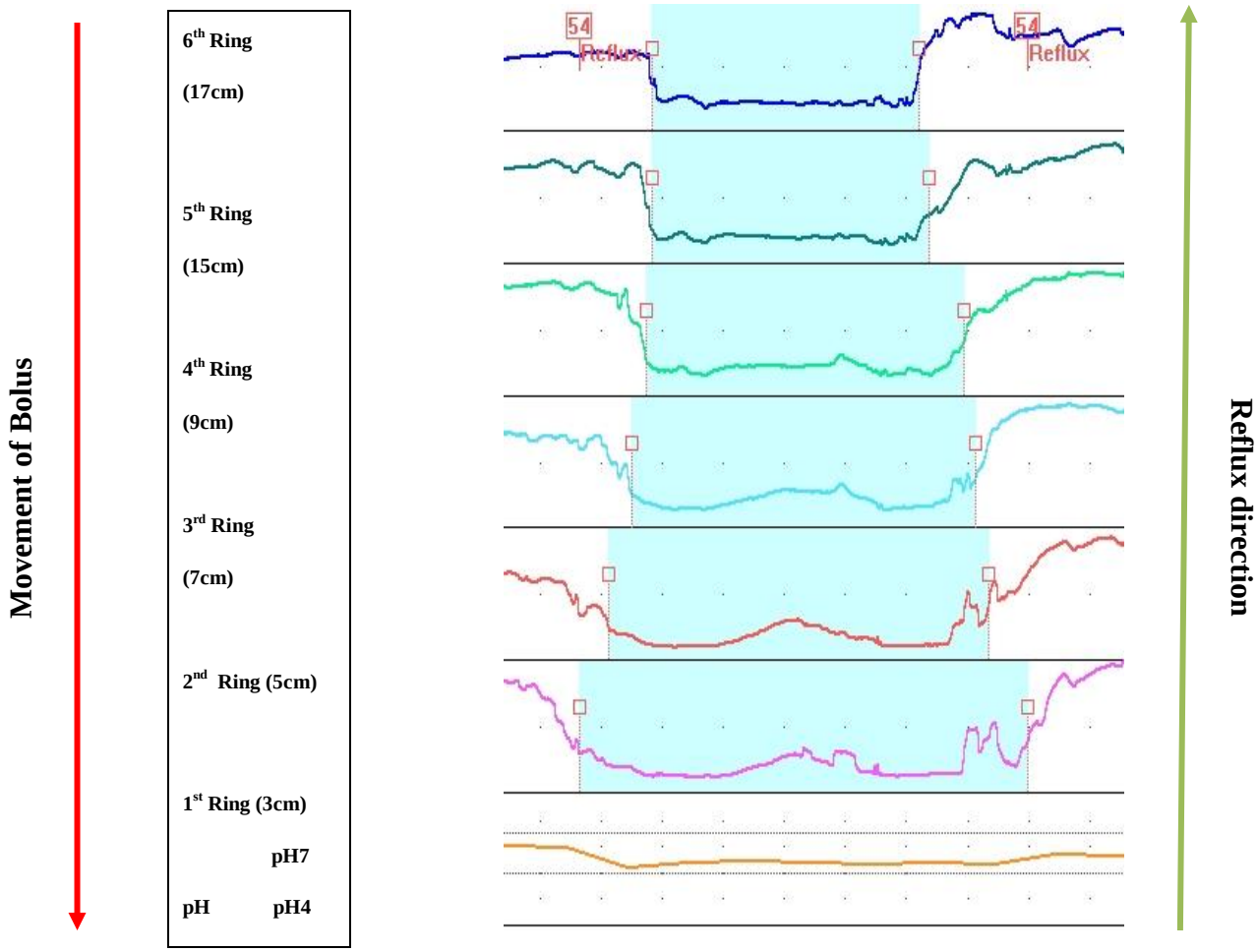
Impedance is inversely proportional to electrical conductivity and cross sectional area of the oesophageal lumen. It is studied using a catheter with multiple spaced, pairs of cylindrical metal rings connected in circuits to the lumen of the tubular organ [36]. Each paired ring circuit has a voltmeter outside the body. As boluses pass, there are changes in impedance recordings. Gases cause a sharp rise in impedance, with rapidly decreasing conductivity. Fluids (food, water and gastric contents) decrease impedance by connecting circuits between electrodes [40].

The empty oesophagus has an impedance value which is intermediate and reflects the conductivity of the oesophageal mucosa (approximately 2000Ohm). When a fluid bolus passes, impedance is low (e.g physiological saline solution = 100Ohm). After it has passed, impedance returns to the intermediate level of the oesophageal mucosa. (Figure 1-１２). These changes in impedance occur when the bolus is between a pair of electrodes. Liquid reflux will drop impedance by 50% in 2 consecutive sensors. Gas reflux is defined as a retrograde, simultaneous rise in impedance to >3,000 ohms [40]. Initially impedance was measured in the lumen of the gastrointestinal tract and has been validated by studies using barium radiographs in anaesthetised cats [44].

Multichannel Intraluminal Impedance collects data samples at high frequency rates, 50 Hz. This technique enables to determine the direction of the bolus[36]. This allows normal swallowing of liquids to be distinguished from reflux events and means swallowed air can be distinguished from "belched" air [40]. There is some intra- and inter-individual variability with impedance measurements. *Bredenoord et al* evaluated 20 healthy volunteers, 2 weeks apart and found that there was more variability between different subjects by >50%, than within the same subjects measured at different times [40, 45].

Refluxate can be acid (pH 4), weakly acid (pH 4-7) or weakly alkaline (pH>7) and can be composed of liquid, gas or a mixture of the two. Patients with pathological GORD, have more acid events and fewer non-acid and weakly acid reflux events when compared to normal subjects [36]. Pure gas reflux is a non-acidic event [40]. Gas reflux often occurs whilst in the left lateral decubitus position, and liquid reflux tends to occur in the right lateral decubitus position [40].

Figure 1-12 : A Weakly-acid Liquid Reflux Event [36]



Legend: This picture shows a combined pH/impedance trace. The bottom reading is of pH, from the pH sensor located 5cm above the lower oesophageal sphincter, and as this does not drop below pH 4, this shows this to be a weakly-acidic event. The traces above this (1st ring to 6th ring) represent the impedance values from 3,5,7,9,15 and 17cm above the lower oesophageal sphincter. The traces measure electrical impedance within the oesophagus and the sequential drop in impedance from the 1st to the 6th ring, demonstrates a reflux event reaching the proximal oesophagus.

It has been known for a long time that “some” reflux is physiological, with an oesophageal acid exposure of <4.5%/24 hours considered within normal limits [46]. In a “normal” population (72 healthy French and Belgian volunteers with a mean age of 35 years, with no known gastrointestinal disease or history of thoracic or abdominal surgery), a study showed that on average there will be 40 reflux events per 24 hours [47]. Using pH impedance monitoring, after a standardised liquid meal, most events were mixed gas and liquid, post-prandial reflux events [40]. In addition, Two thirds of reflux events are non-acidic or weakly-acidic events [40].

Impedance allows detailed evaluation of refluxate and also allows evaluation in patients on proton pump inhibitor (PPI) therapy [36, 40]. PPIs have been shown not to decrease the number or volume of reflux events, but render them non-acid or weakly acid events. Thus they do not prevent reflux [40]. Furthermore there is evidence to suggest that the volume of gastric secretions is not reduced with PPI [48]. A study of pH monitoring of 250 patients on PPI therapy, showed 3.8% to have an abnormal study. Impedance showed that weakly acid events were just as common after proton pump inhibitor therapy as acid events prior to acid suppression, i.e. acid levels detected were greatly reduced, but impedance showed that reflux events were just as common [40]. At least a third of reflux events are weakly alkaline or weakly acidic. These may elicit extra-oesophageal reflux symptoms such as cough, sore throat, hoarse voice and even pulmonary symptoms such as wheeze and dyspnoea [49, 50].

The association between atypical extra-oesophageal symptoms with reflux has been difficult to prove [36]. A study involving 10 subjects with extra-oesophageal symptoms used pH-impedance to study their episodes of reflux. Half of patients had a temporal association with reflux and their cough, though a causative link has yet to be proven [40].

Standard definitions have been created for acid reflux, superimposed acid reflux, weakly acid reflux and weakly alkaline reflux on the basis of combined pH/impedance measurements (Table 1-3). Oesophageal and extra-oesophageal symptoms can be related to less acid reflux [41, 47, 51, 52].

Acid reflux	**Refluxate of gastric juice which reduces the pH<4**
Superimposed acid reflux	**Further refluxate of gastric juice before the pH has recovered to >4.**
Weakly acid reflux	**Refluxate of gastric juice when the pH remains between 4-7.**
Weakly alkaline reflux	**Refluxate of gastric juice when the nadir pH is greater than 7**

Table 1-3: Standard Definitions for Reflux Events [41, 47, 51, 52]

Weakly acid reflux events often occur near meal times. In patients with prolonged gastric emptying, there may be an increase in weakly acid reflux and a decreased acid reflux [41]. Weakly acid refluxate causes less heartburn when compared to acid reflux, but patients may still suffer regurgitation or chronic cough. [41].

Comparison of pH monitoring to Impedance

Acid reflux events, detected by impedance appear to be shorter, as neutralisation of acid takes longer than the clearance of oesophageal volume. There is a higher detection rate of reflux events with impedance compared to pH monitoring [36]. In one study, Impedance detected 96% of reflux events compared to 28% detected by pH study using acid reflux event definition. Non-acid and weakly acid reflux events are common in both normal subjects and those with GORD [40, 53].

The Porto consensus devised in 2006 [35] and the British Society of Gastroenterology guidelines [54] on the detection of reflux both state that reflux is best evaluated by a combination of impedance and pH monitoring.

1.4.5 Other Techniques for Assessing Reflux

Barium Swallow

A barium swallow is an inexpensive, non invasive and widely available radiographic investigation using double-density barium as well as a gas forming agent. The test can delineate the oesophagus and oesophagogastric junction, revealing subtle strictures, rings and hiatus hernias. In addition, often with some specialised manoeuvres involving the patient, including coughing and rolling side-to-side, reflux can also be demonstrated [24]. The ability of the swallow to detect oesophagitis varies considerably. Sensitivities

of between 79% and 100% have been shown in the presence of severe oesophagitis, but the barium swallow is less accurate in the detection of mild inflammation [32]. The barium swallow is very useful for demonstrating peristalsis and disorders of oesophageal motility.

Bravo Capsule

To remove the technical difficulties of nasal catheterisation, the Bravo Capsule (Medtronic, Minneapolis, MN, USA) has been developed. This is a wireless pH probe which is attached to the lower oesophageal mucosa during endoscopy about 6cm above the normal z-line [36]. Its advantages are its tolerability; it is painless and does not interfere with normal daily activities or sleep and the fact that it allows recording for over 24 hours [39].

Bilitec

The Bilitec 2000 (Medtronic, Minneapolis, MN, USA) device only measures bile reflux [39]. It requires that patients adhere to a specific diet which can be difficult and affect compliance. In addition, refluxate can get stuck in the sensor opening, thereby causing an overestimation of bile exposure. The detection of bile refluxate is important, but a better understanding of bile reflux and aspiration may be achieved by the biomarker approach of assessing levels of bile salts in the bronchoalveolar lavage fluid [36].

1.4.6 Reflux Questionnaires

Questionnaires have been designed to detect symptoms suggestive of both oesophageal and extra-oesophageal reflux. Laryngopharyngeal reflux does not always cause classical heartburn or oesophagitis. Signs & symptoms of laryngopharyngeal reflux include hoarseness, vocal fatigue, excessive throat clearing, globus pharyngeus, chronic cough, post-nasal drip and dysphagia.

Several laryngopharyngeal reflux questionnaires have been designed. One such questionnaire, which has been validated is the Reflux Symptom Index (RSI) (Figure 1-1 3), which is 9 item questionnaire [55].

Within the **last Month** how did the following problems affect you	0 = No Problem				5 = Severe Problem	
Hoarseness or a problem with your voice	0	1	2	3	4	5
Clearing your throat	0	1	2	3	4	5
Excess throat or postnasal drip	0	1	2	3	4	5
Difficulty swallowing food, liquids or pills	0	1	2	3	4	5
Coughing after you eat or after lying down	0	1	2	3	4	5
Breathing difficulties or choking episodes	0	1	2	3	4	5
Troublesome or annoying cough	0	1	2	3	4	5
Sensation of something sticking in your throat or a lump in your throat	0	1	2	3	4	5
Heartburn, chest pain, indigestion or stomach acid coming up	0	1	2	3	4	5
	RSI					

Figure 1-13 : Reflux Symptom Index (RSI) questionnaire.

Legend: The patient is asked to assess the severity of their symptoms on a scale of 0 to 5 for each of the nine parameters tested by the questionnaire. The score is then added up to give a total RSI score. A score above 13 indicates an abnormal RSI score.

The RSI is easily administered and highly reproducible**.** It was validated on 25 laryngopharyngeal reflux patients and 25 controls [56]. The RSI correlates well with the Voice Handicap Index, another validated assessment of laryngopharyngeal reflux. A RSI score of greater than 13, is abnormal [55]. A limitation of this questionnaire is that 5/45 possible points can be attributed to heartburn. Thus, the RSI is not limited to extra-oesophageal reflux symptoms but can be elevated in patients with isolated oesophageal reflux.

The DeMeester Reflux Questionnaire is a validated assessment tool looking at basic reflux symptoms[57]. It is based on a score of 0-3 for symptoms of reflux, regurgitation and dysphagia. Sequential questionnaires are also useful in assessing the response to treatment.

1.5 Reflux and lung disease

1.5.1 Reflux Disease and advanced lung disease

The association between reflux, aspiration and lung disease is not well characterised. As early as 1927 it had been postulated that a dysfunctional gastrointestinal tract may lead to aspiration and lung disease. Following this several studies in the 1960s and 70s reported a high prevalence of pulmonary fibrosis in patients with clinical diagnosed GOR [58]. A landmark paper was written in 1979 addressing some of the key principles linking GORD and chronic lung disease [59]. In this study *Pellegrini et al* showed that patients were more likely to have respiratory disease if they had reflux associated with weak oesophageal peristalsis and slow oesophageal clearance. Some of the first studies describing the association between reflux and lung disease describe high incidence of impaired oesophageal motility in patients with parenchymal lung disease[60]. Since these small studies, many large epidemiological studies have been carried out which describe an association between GOR and respiratory disease. In 1999 *El-Serag et al* retrospectively studied 101,366 patients and showed that erosive oesophagitis and oesophageal disease was associated with a wide variety of upper and lower respiratory conditions including sinusitis, pharyngitis, COPD but were most strongly associated with bronchial asthma and pulmonary fibrosis [61]. More recently in 2006 *Ford et al* performed a questionnaire study of 4000 volunteers, the results of which show a strong correlation between chronic cough and GOR [62]. There is a high prevalence of foregut motility problems and GORD in patients with advanced lung disease [63]. In their study *D'Ovidio et al* [63] demonstrated that 72% of patients had decreased lower oesophageal sphincter pressure and 33-47% of patients had oesophageal body dysmotility and impaired peristalsis; in total almost 80% of these patients have oesophageal dysmotility and/or a hypotensive lower oesophageal sphincter [63]. *Sweet et al*, in their study of end-stage lung disease patients, suggest that 55% of patients with reflux had a hypotensive lower oesophageal sphincter compared with only 26% of patients without reflux. In addition, patients with GOR had impaired oesophageal peristalsis [64].

The prevalence of GORD in patients with advanced lung disease awaiting lung transplant has been reported to be in the range of 63-68% [65], though some studies [63] do report a lower prevalence of 38%; the latter figure may be as a result of cessation of acid suppression therapy for only 5 days as opposed to the recommended 10 days to ensure the effects of medication did not interfere with objective pH assessment.

The movement of stomach content into the upper airway is described as laryngopharngeal reflux (LPR) and can lead to extra-oesophageal reflux symptoms. There are three potential mechanisms causing the presence of extra-oesophageal symptoms associated with reflux [66]:

1. Direct irritation of the airway epithelium by reflux material
2. Afferent cough reflex hypersensitivity of the airway due to reflux
3. A neural reflex between the oesophagus and airway tract. Up to 50 episodes of reflux from the stomach to the oesophagus are within the physiological limits of normal but just one event of reflux that reaches the laryngopharynx could be enough to produce symptoms in the upper airway.

Characterisation of the reflux seen in patients with advanced lung disease was performed by *Patti et al* in 1993 [67]. They used a dual sensor pH monitor to correlate cough with proximal oesophageal reflux and extra-oesophageal reflux symptoms. They later suggested that micro-aspiration caused by proximal reflux was the likely aetiology of the cough [68]. There is some controversy as to whether proximal reflux occurs more commonly in the supine or upright position [63] [64], and people may suffer from proximal reflux despite having normal distal reflux [64]. A loss of laryngeal mechanosensitivity may contribute to microaspiration when associated with cough and significant respiratory disease [66].

Another theory that may account for the high prevalence of GORD in advanced lung disease is related to the exaggerated pressure fluctuations between the thorax and abdomen seen in pulmonary disease; these may challenge the normal gastro-oesophageal barrier and predispose to the movement of stomach contents up the oesophagus [69]. In their study *Ayazi et al* suggested that an inspiratory thoraco-abdomianl pressure gradient higher than the resting LOS pressure accounted for increased oesophageal acid exposure in 85.2% of patients. However, their study only used patients with manometrically normal lower oesophageal sphincters and no history of pulmonary disease, and though their conclusions imply that exaggerated ventilatory effort can result in GOR its application in patients with advanced lung disease may be limited [69].

1.5.2 GORD and Idiopathic Pulmonary Fibrosis (IPF)

Interstitial Lung Disease (ILD) comprises of a group of both acute and chronic disorders characterised by diffuse pulmonary infiltrates producing histological features of pulmonary inflammation as well as evidence of restrictive lung function [1] . Interstitial lung disease also encompasses the diagnosis of idiopathic pulmonary fibrosis when the aetiology of ILD is unknown. Despite IPF carrying a prognosis worse than most cancers it remains poorly understood with no effective disease modifying treatment [28]. It has been noted that IPF appears to be substantially more prevalent than previously reported. This could be as a result of changes in clinician diagnosis, but it is also likely that there has been a real increase in disease prevalence[70]. The pathophysiology of IPF is believed to be a result of fibroblast proliferation from chronic lung epithelial injury [58]. Understanding the source of the initial lung injury would provide a better understanding of IPF and may lead to more effective treatment strategies.

Since the early 1960s several studies have demonstrated an association between ILD and gastro-oesophageal reflux (GOR) [58]. Recently it has been demonstrated through 24-h pH monitoring that GOR is highly prevalent but often clinically occult in patients with ILD when compared to normal subjects; the use of standard dose proton pump inhibitors, appears to only affect the pH of the refluxate but the number and magnitude of reflux events remains unchanged [58, 71].

It has been postulated that the variations between the abdominal and thoracic pressure seen in ILD may account for increased GOR [1] but the exact mechanism behind GOR leading to the progression of ILD has never been elucidated. Until recently the assessment and treatment of GOR focused on using conventional pH monitoring. When performed in patients with interstitial lung disease, there has been limited benefit of acid suppressive therapy after pH assessment [72]. Conventional pH measurement is limited to detecting only acid refluxing from the stomach. The addition of oesophageal impedance measurements allows the detection of non-acid and weakly acid reflux events (refluxate pH >4) [27]. A recent study [72] demonstrated using oesophageal impedance in subjects with systemic sclerosis associated ILD, that increased non-acid reflux episodes could be associated with the progression of pulmonary disease. *Savarino et al* concluded that further studies should include reflux reducing measures to test whether the development and progression of ILD could be prevented by treating GOR.

A relationship between IPF and GOR was first postulated by *Mays et al* [73] when they noted that hiatus hernia is more common in IPF patients. *Tobin et al* [74] demonstrated in 17 patients with biopsy-confirmed IPF, that 94% had reflux confirmed with 24-hour manometry, 75% of these patients reported no reflux associated symptoms. Recently this has been confirmed in a larger cohort of 65 patients by *Raghu et al* [71]. This study demonstrated that GOR was present on 24-hour pH monitoring in 87% of their subjects. Interestingly *Raghu et al* showed abnormal oesophageal acid exposure in 63% of their patients who remained on a proton pump inhibitor during their pH studies. A recent case-control study [75] aimed to evaluate reflux in patients with IPF by analysing the scores from a validated cough questionnaire, the Hull airway reflux questionnaire (HARQ). The authors also used an exhaled breath condensate (EBC) to detect pepsin in suspected extraoesophageal reflux and *Helicobacter Pylori (H.Pylori)* serology to evaluate for the prevalence of this bacterium in the upper gastrointestinal tract of IPF patients. For the three aspects of the study the cases and control groups were not matched in numbers. For the HARQ component of the study, 40 IPF patientswere evaluated against 50 controls, EBC was collected from 17 IPF patients and 6 controls and *H.pylori* antibody detection was performed in 34 IPF patients and 23 controls. Significantly higher HARQ scores were recorded in patients with IPF compared with controls ($p<0.001$). This questionnaire is targeted towards non-acid reflux (larynopharyngeal reflux), but without objective impedance-pH monitoring it is not possible to be certain as to the nature of the refluxate in this patient group. The EBC measurements of pepsin showed no difference between the patients and controls. As the EBC was used in clinic at a set point in time it may have easily missed reflux episodes. The study did not show any significant difference in *H.Pylori* serology between patients and controls. The lack of correlation with the HARQ scored can be expected as *H.Pylori* colonisation is often associated with a reduction in acid reflux [25]. However, a further study from *Fahim et al* [75] clearly reinforces the hypothesis that reflux and IPF may have a causal relationship.

Idiopathic pulmonary Fibrosis patients with marked asymmetry of their lung disease on high-resolution CT (HRCT) have an increased prevalence of acute exacerbations, with increased reflux symptoms [76].The most recent guidelines *(BTS, 2008)* from the British Thoracic Society on ILD, recognises the potential of GOR to complicate IPF but since then the ATS/ERS/JRS/ALAT 2011 IPF [6] statement has also reiterated the lack of understanding of any link between GOR and IPF. It encourages further studies to

determine the exact nature of the reflux. This is not only important to improve our understanding but to ensure patients receive the correct therapy.

There is conflicting evidence of the role of proton pump inhibitor (PPI) therapy in IPF patients, with some studies claiming inadequate acid suppression with standard doses of PPI e.g. omeprazole 20mg once daily [71]. A single case study of a 60 year old patient with IPF demonstrated symptomatic improvement of IPF with treatment (high dose) PPI e.g. 20mg omeprazole twice daily. However, in this study they also made dietary and behavioural changes including abstinence from alcohol as well as sleeping in a slightly elevated position; it is therefore difficult to conclude from a single case report that the improvements are as a result of the PPI therapy alone [77]. More recently from the Mayo clinic, PPI therapy has been shown to improve survival and lower radiological evidence of fibrosis [78]. Interestingly 5% of patients still received anti-reflux surgery despite these findings.

1.5.3 GORD and Cystic Fibrosis (CF)

Cystic fibrosis is a multisystem disease which can have profound effects on the functioning of the digestive, endocrine, reproductive and respiratory systems. Cystic fibrosis transmembrane conductance regulator (CFTR) is a 1480 amino-acid glycoprotein that in humans is encoded by the CFTR gene expressed on chromosome 7. CFTR is a member of the ATP-binding cassette (ABC) transporter superfamily. All ABC transporters bind to ATP and use its energy to transport molecules across the cell membrane. Mutations in ABC genes have been linked to many diseases; one of the most common in the West is Cystic Fibrosis. Approximately 1 in 20 Caucasians are carriers for mutations in CFTR and the disease affects 60000 individuals worldwide [79]. This disease can present as exocrine pancreatic insufficiency, an increase in sweat sodium chloride concentration, male infertility and most commonly airway disease. The CFTR plasma-membrane cyclic AMP-activated chloride channels is found in the epithelial cells of many organs including the lung, liver, pancreas, digestive tract, reproductive tract, and skin. In addition to mediating the secretion of chloride ions, CFTR also regulates several transport proteins including the epithelial sodium channel (ENaC). Mutations of the CFTR gene affect the number of CFTR channels in the membrane, channel activity and intracellular trafficking of CFTR. This reduces the functional levels of CFTR in the plasma membrane resulting in a defect of chloride ion secretion, hyperabsorption of sodium and other changes affecting a number of organs, leading to cystic fibrosis. It is the effect on the respiratory system and the reduced capacity of the

cilia to clear bacteria from the airway which accounts for the morbidity and mortality associated with the disease. The major respiratory manifestations include chronic bacterial colonisation with *Pseudomonas aeruginosa*, cough and emphysema [80].

GOR has been reported as early as the 1970s in patients with CF and currently the prevalence is estimated to be between 35-81% [81]. Over the last 30 years advances in the care of patients with CF have results in a growing adult population with CF. There is a higher incidence of GOR in children with CF than in the general population [82], about 1 in 5 newly diagnosed CF infants have pathological reflux, [22] but there are very few comparable studies in the adult CF population. It is unclear whether GOR is increased in CF as a primary effect of the disease, or is prompted by non-GORD manifestations of CF and its treatments [83].

Several mechanisms have been suggested for the GOR seen in patients with CF including a reduced pressure of the lower oesophageal sphincter, the presence of increased number of transient LOS relaxations, delayed gastric emptying and the increased abdominal-thoracic pressure gradient often secondary to cough and postural drainage physiotherapy [81]. Although the role of physiotherapy exacerbating reflux in patients with CF is unclear as several studies have demonstrated no change in the number of reflux episodes, including proximal events when assessed in the 20º head down position [84]. A recent study [85] attempted to determine the relationship between the type of reflux, (GOR or duodeno-gastroesophageal reflux) with gastric emptying and demonstrated a positive correlation between the rate of gastric emptying and severity of duodenal reflux (n=5). However, the study used very small subgroups to determine the above relationship and so its application to a CF population in general is limited.

Most of the studies performed so far in this population use 24 hour pH monitoring which only allows the detection of acidic GOR. However, the nature of the refluxate in CF, that is, the volume and acidity maybe altered and simple pH monitoring may not effectively characterise the GOR [81]. GOR is thought to be highly prevalent in CF but has not been systematically studied with up to date methods such as impedance pH monitoring. This method of assessment allows the detection of acidic, weakly acidic and non-acid reflux which will provide better characterisation of GOR in CF [41]. There have been limited studies performed using pH impedance in CF patients [81] with interesting results. *Blondeau et al* performed pH impedance studies on 23 CF patients and demonstrated that up to 80% had acid GOR with subgroup having increased weakly

acid reflux. However, it must be noted that in this study the patients had been on long term PPI which was only stopped for 7 days prior to the assessment. This may have lead to under-detecting some patients with acid reflux, as depending on the PPI, acid suppression effects can last up to 7 days from stopping the treatment [86]. Another hypothesis suggested for the presence of acid reflux in CF patients is due to delays in acid clearance. Reduced bicarbonate secretion from the stomach, duodenum and pancreas may delay neutralisation and this could account for the acidic refluxate [83].

The studies above illustrate the importance of understanding the nature of the reflux as well as determining the underlying mechanism. Although weakly acid GOR is uncommon in CF, acid GOR can be prevalent as early as infancy [87], and this highlights the importance of early management of GOR in CF. *Fathi et al* [80] demonstrated that laparoscopic fundoplication was highly effective in controlling reflux in a small selection of CF patients, where medical treatment had failed. Further open studies which indicate the potential for anti reflux treatments to impact on the natural history of lung disease come from studies of lung allograft. This includes evidence from Davis et al, in 2003 [88], demonstrating that anti-reflux surgery may lead to increased survival post lung transplantation by preventing lung damage through reflux and aspiration.

1.6 Aspiration and Gastrooesophageal Reflux

1.6.1 Background to Microaspiration

The term aspiration is defined as the inhalation of oropharyngeal or gastric content into the larynx and lower respiratory tract[89]. When aspiration occurs at a sub-clinical level and the aspirate consists of tiny droplets it is termed microaspiration[90]. Depending on the frequency of these microaspiration episodes and the underlying medical condition patients may manifest with cough, wheeze or a decline in pulmonary function.

Lung transplant survival is reduced when compared to heart, liver and kidney transplant [65]. Death post lung transplant is commonly due to chronic allograft dysfunction otherwise known as bronchiolitis obliterans syndrome (BOS)[65]. Brochiolitis obliterans syndrome generally begins to develop between 6 months and 2 years following lung transplant [91] and affects 50-60% of patients at 5 years post-transplantation. Reflux and microaspiration have been shown to be risk factors for BOS following lung transplant [58].

Several studies have attempted to determine the incidence of microaspiration in lung transplant patients. Within our own research unit studies have shown that the lung epithelial lining fluid concentration of pepsin in lung allograft recipients was much higher than blood reference levels, with no detectable pepsin in controls [92]. *D'Ovidio et al* [93] examined the bronchoalveolar lavage (BAL) samples of 120 post-transplant patients and found elevated bile salts, normally found in gastro-duodenal tract in 17% of patients. The levels of these salts were higher in patients with more advanced BOS. A subsequent study [94] from the same authors concluded that the prevalence of microaspiration, as measured by bile salts, was as high as 43% at 3 months after lung transplant. *Blondeau et al* [95] used impedance-pH in order to characterise reflux in lung transplant patients. They also performed BAL analysis for pepsin and bile salts as markers of microaspiration. All lung transplant patients had increased levels of pepsin in BAL even those with normal impedance studies; bile acids were detected in 49% of samples. The authors concluded that reflux is detectible in lung transplant patients and that gastric aspiration occurs frequently as demonstrated by the elevated pepsin and bile salts in BAL. From this evidence several studies have also suggested that treatment with proton pump inhibitor does not protect from the aspiration of gastric contents while early anti-reflux surgery improves survival and decreases chronic allograft rejection

after lung transplant, by reducing microaspiration [96, 97]. Although the studies above have demonstrated the presence of pepsin and bile salts in BAL, the techniques used for the measurements of these markers, in particular the use of enzymatic kits for measuring bile salts have limited accuracy when compared to more recent spectrophotometric assays [98].

With regard to advanced lung disease and microaspiration there is limited information from human studies. Experimental models in animals and some descriptive studies in humans do support the concept of microaspiration as a potential cause of pulmonary fibrosis (

Figure 1-１４). Gastric juice has been detected in the lungs of dogs a short time after instillation into the main bronchus. In addition, when the lungs of rabbits and dogs are exposed to acid solution, they demonstrate histological manifestations consistent with fibrotic lung disease [90]. There is no direct data demonstrating that microaspiration leads to pulmonary fibrosis; much of the evidence to suggest it may be a causative factor comes from studies of gastro-oesophageal reflux in patients with IPF.

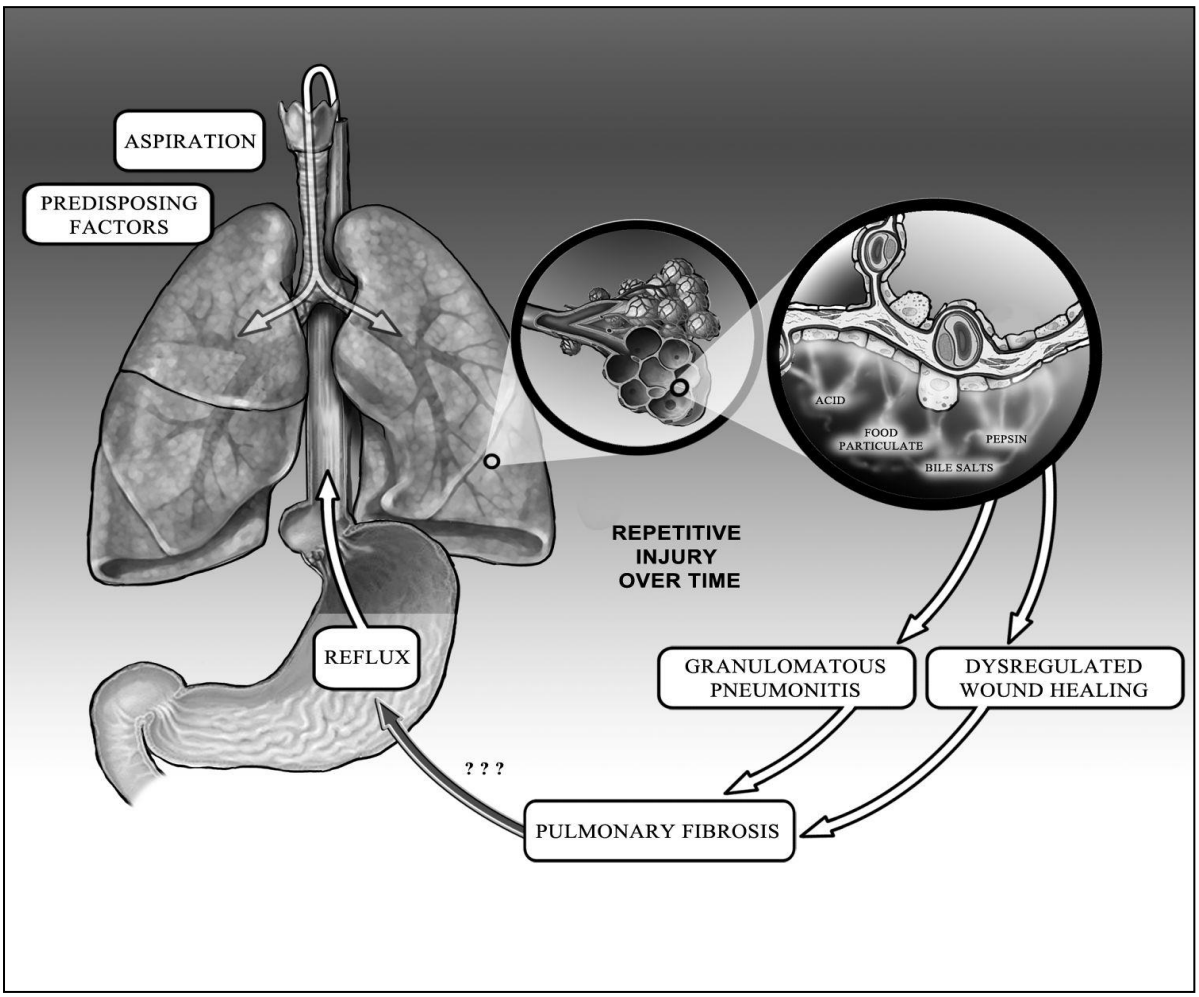


Figure 1-１４: Possible Pathogenetic Mechanism for Chronic Microaspiration in Idiopathic Pulmonary Fibrosis [90].

Legend: Gastric fluid can travel in a retrograde fashion through a weakened lower oesophageal sphincter(e.g. secondary to a hiatus hernia, traction from the diaphragm, or medications) up into the oesophagus. The gastric refluxate can travel as high up as the cricopharyngeal region and enter the airway. Normal host defences likely clear most gastric refluxate without clinical sequelae [90]. However, in some cases, components of the gastric refluxate (e.g. acid, bile, particulates) may directly injure the lung epithelium. In the genetically or otherwise predisposed patient, chronic microaspiration of gastric refluxate may cause repetitive injury over time leading to granulomatous pneumonitis, dysregulated wound healing, and eventual lung fibrosis. Additionally, progressive pulmonary fibrosis may lead to distortion of the mediastinal structures and traction on the oesophagus. This could cause additional weakening of the lower oesophageal sphincter, which could in turn lead to microaspiration, lung injury, and the accelerated decline and/or acute respiratory decompensation seen in some patients with idiopathic pulmonary fibrosis [90].

Lee at al [90] in their review highlight the difficulties in diagnosing microaspiration in IPF. Several approaches have been used but there are many limitations to each of these;

- Patient Symptoms – On their own, symptom screening for extra-oesophageal symptoms of reflux is a poor diagnostic tool for microaspiration. In a study of 65 patients with IPF over 50% had objective evidence of GOR but no symptoms due to clinically silent disease. [71]. Symptom screening for oesophageal reflux may only have sensitivity of 65% and specificity of 71% [99].

- Radiological studies – studies attempting to demonstrate microaspiration using barium swallows, computed tomography (CT) scans and radio-labelled nuclei scans are limited by poor sensitivities, inter-observer error and costs [90]

- Oesophageal studies – The use of pH impedance studies to allow the detection of acid and non-acid reflux as well as allowing an assessment of proximal reflux; these measures can assess only the risk of microaspiration.

Gastric microaspiration may be a common phenomenon in CF patients but the published evidence is scarce. *Ledson et al* [100] studied 24-hour ambulatory tracheal and oesophageal pH monitoring in 11 CF patients with symptoms of GOR and demonstrated tracheal acidification in those patients with significant GOR, suggesting a high proportion of these patients suffered from microaspiration. They showed that a longer period of tracheal acidification of 15-75 minutes correlated with longer periods of oesophageal reflux. This study was performed off PPI for 48 hours. Stopping PPI for such a short time after long-term use may result in rebound acid hypersecretion (RAHS) [101], accounting for the high levels of tracheal acidification in this study. The study focuses on acid reflux and takes no account of non-acid refluxate that is increasingly been detected on impedance-pH.

More recent studies have focused on demonstrating gastric microaspiration by analysing the presence of biochemical markers in both sputum and BAL. *Blondeau et al* [81] showed that a significant group of CF patients have evidence of microaspiration by showing elevated levels of bile salts detected in sputum and BAL. In this study they also demonstrated that half of the CF patients with increased GOR or microaspiration had no symptoms. They also showed a correlation between the CF genotype and levels of aspiration; bile aspiration was more prevalent in ΔF508 homozygotes. Although this study recruited 71 CF patients, 10 had received lung transplants, but this was the only group where oesophageal pH tests AND aspiration tests were performed allowing a

correlation to be made. However, methodological problems with bile salt assays illustrate the need for further studies and for consensus on the standardisation of bile salt measurements. Recent tests on the enzymatic bile salt kits have shown a failure to detect bile salts below 5µmol/L in BAL [102]. The majority of the studies above use these kits and suggest bile salt levels below this; but the evidence suggests the kits are simply not sensitive enough for detecting bile salts in BAL questioning the accuracy of the current consensus on markers of microaspiration. The process of transplantation involves denervating the donor lung thereby reducing the cough reflex and muco-cillary clearance; loss of these protective mechanisms may predispose to microaspiration [103]. Though this data supports a role for microaspiration in pathophysiological events in lung transplant recipients it has limited application in the pre-transplant CF population. The non-transplanted CF group were separated and a proportion had only oesophageal pH tests whilst the rest had aspiration testing. Therefore, correlation of the results cannot be made as there are two separate groups.

A more recent study [104] attempted to elucidate the link between aspiration of gastric content and lung inflammation in children with CF. The authors recruited 31 patients with CF nad 7 controls and demonstrated in over half of the CF patients there were high levels of 'pepsin', a biochemical marker of aspiration (see next section). High levels of pepsin appeared to correlate with higher levels of IL-8, a marker of inflammation, suggesting that chronic microaspiration may contribute to airways inflammation. Unfortunately, this study fails to objectively asses GOR in all the patients; only 6 patients had pH tests performed. In addition, 9 patients were still on PPI and it is unclear from the results how this may have affected the levels of pepsin in the BAL of these patients.

1.6.2 Biomarkers of Aspiration

Pepsin

Pepsin is a proteolytic enzyme (Figure 1-15) which is secreted by the chief cells in the stomach [24].

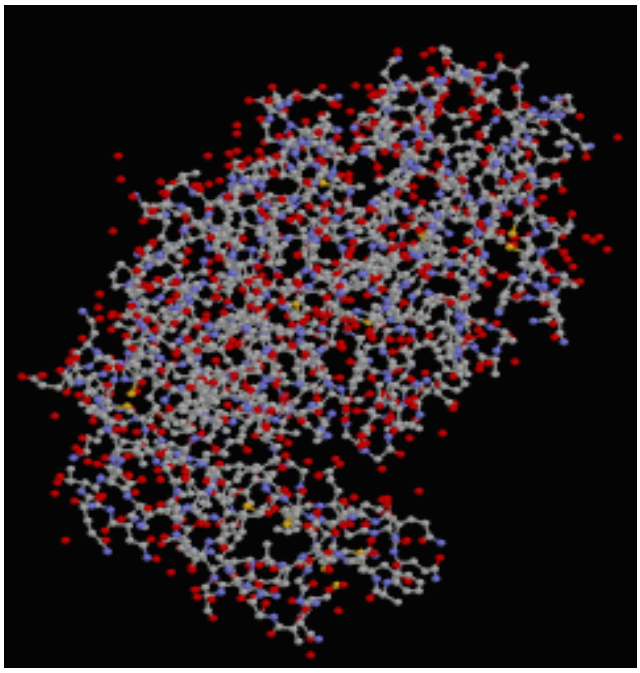


Figure 1-15: The chemical structure of the macromolecular Enzyme Pepsin

Pepsin can be detected at low levels in the lungs of healthy individuals as a small degree of aspiration may occur during sleep in healthy individuals [105]. In addition, individuals with GOR will not necessarily have elevated pepsin levels in their BAL. This suggests that simply identifying gastro-oesophageal reflux is not sufficient for diagnosing microaspiration [90] and a distinction between high and low levels of pepsin in BAL is important in identifying those patients at a significant risk of microaspiration [104]. Pepsin has been used as a marker of gastric aspiration, mainly through its detection in the BAL of lung transplant recipients [106]. Pepsin is measured using an ELISA, but assay variability between units can lead to marked variability in concentrations of pepsin detected (Table 1-4). Some papers suggest the lower limit of detection is 1ng/ml, but BAL can dilute the actual alveolar fluid by up to 200 fold reducing the concentration to as low as 0.5ng/ml potentially missing aspiration events [107]. Also with the process of performing a BAL further variability in the ability to detect pepsin is introduced by differences in the exact volume of fluid recovered and in the volumes of saline used for the lavage. This makes comparison of the various studies difficult.

Table 1-4: Variability in Pepsin levels detected in aspiration studies [36]:

Study	Instilled Volume	Pepsin levels
Ward, Forrest et al, 2005 [92]	**180ml**	**35-1375ng/ml**
Stovold, Forrest et al, 2007 [108]	**180ml**	**0-51.7ng/ml**
Blondeau, V. Mertens et al, 2008 [109]	**100ml**	**0-2000ng/ml**
Starosta, Kitz et al, 2007 [110]	**Unknown**	**0-2500ng/ml**

Bronchoalveolar lavage pepsin levels in clinically stable lung transplant patients were shown to be hundred times higher than control subjects (109ng/ml vs. <1ng/ml) suggesting gastric aspiration. Levels were 10-1,000 times higher than the serum reference ranges and pepsin was still detected in lung transplant patients taking high dose PPI; suggesting that aspiration can occur even when attempts are made to control acid secretion [92]. Detection of pepsin in BAL is a reliable method for diagnosing reflux associated pulmonary aspiration and can be highly specific (100%) and highly sensitive (80%) [111]. High levels of pepsin have also been shown to correlate with the number of proximal reflux events as detected with 24 hour pH monitoring [110]. In IPF elevated levels of pepsin in the BAL were seen in patients at the onset of an acute exacerbation of the disease [112]. This indicates that the contents of the gastrointestinal tract are capable of reaching the lung without an overt aspiration event and that microaspiration may even be a trigger to acute lung injury. Although detection of pepsin in BAL has been used as a biomarker of microaspiration, detection of pepsin in sputum would be a useful non-invasive tool for diagnosing reflux associated aspiration [113].

Bile Salts

Bile salts are steroids synthesised in the liver by hepatocytes during the metabolism of cholesterol. These are normally conjugated with glycine or taurine before secretion and release [114]. Their role is to aid digestion and absorption of lipids in the small intestine. The main bile acids present are the glycine and taurine conjugates

Table 1-5) [115]. Bile salts are later reabsorbed in the ileum and colon [114]. Bile acids exist as mixtures, and due to their detergent status, they will influence each other's solubility[36].

Table 1-5: Composition of bile and biochemical properties [115]:

Bile Acid	Solubility in water (µM/L)	pKa	% in Bile
Free Bile Acids			
Cholic Acid	**242**	**5.2**	**Trace**
Deoxycholic Acid	**100**	**5.02**	**Trace**
Chenodeoxycholic Acid	**142**	**4.98**	**Trace**
Glycine Conjugates			
Glycocholic Acid	**53**	**3.88**	**30**
Glycodeoxycholic Acid	**17.5**	**3.88**	**15**
Glycochenodeoxycholic Acid	**17.6**	**3.87**	**30**
Taurine Conjugates			
Taurocholic Acid	**$14x10^3$**	**<2**	**10**
Taurodeoxycholic Acid	**$82x10^3$**	**<2**	**10**
Taurochenodeoxycholic Acid	**n/a**	**<2**	**5**

As with the detection of pepsin, there is considerable variability in the levels of detection of bile salts in reflux studies. This is not only due to the different methods of detection but variability between individuals and the time of day samples were collected. A common assay is the 3α hydroxylase method described by Fausa & Skalhegg [116]. This assay is not affected by pH but the presence of food or colorants can interfere with results [117]. There is considerable variability in agreement about the lower limit of detection of mass spectrophotometric assays; *Collins et al* suggested 62.5µmol/L [118], *Klokkenburg et al* claims 5µmol/l [114], Biostat, who produce the commercially available assay claim a lower limit of detection 1µmol/L and the Leuven group have

claimed an accuracy of 0.2µmol/L [81, 109]. These levels are lower than serum bile salt levels (<8µmol/L) [119]. One group have found this type of assay to be unreliable [120]. Certain operations can affect the concentration of intra-gastric bile salt concentrations; 90% of the normal population will have intra-gastric bile salts concentrations of less than 250µmol/l [121]. Intra-gastric levels up to 34,260µmol/l have been reported after the formation of a gastro-jejunostomy [122].

Duodenogastric reflux is a physiological event that occurs most often after a meal and in the early mornings [114]. Levels of bile salts in the oesophagus are rarely over 1000µmol/L and are usually between 0 and 200 µmol/L even in Barrett's oesophagus. Approximately 25% of patients with reflux will have no detectable bile salts in the oesophagus [123]. Duodenal reflux events will combine with gastric refluxate by mixing with gastric contents and therefore bile reflux normally occurs on a background of weakly acid reflux (pH 4-7). Detection of bile salts above the level of the stomach signifies gastric as well as duodenal reflux.

Detection of bile salts in BAL as a marker of gastric aspiration has been used in the in patients post lung transplant and in those in whom BOS has started to develop. BOS has been shown to be associated with abnormal pH studies, the presence of bile salts in BAL and microaspiration. [94]. Bile acids have also been analysed in the sputum of patients in order to diagnose reflux associated aspiration [124]. In this study the authors induced sputum in patients with GOR and measured bile acid concentration and compared values to levels of TGF-beta 1. Patients with GOR had higher levels of bile salts in their sputum compared to controls ($p<0.005$) and this correlated with higher levels of TGF-beta 1 which has the potential to promote fibroblast proliferation. More recently *Blondeau et al, 2008* demonstrated the presence of bile acid in sputum of over 50% of CF patients they tested. They also showed that in these patients it was associated with exacerbations of respiratory infections and an increased requirement for intravenous antibiotics [125]. Other studies have analysed bile salt levels in the saliva of patients with CF and have shown that one-third of children with CF have bile salts in the saliva [87], which may indicate an increased risk of aspiration. However, as saliva is not a direct representation of lung aspirate like BAL and to some extent sputum, these measurements of bile salts maybe less clinically meaningful. Bile salts can predispose patients to lung injury due to disruption of the lung mucosa and also their effects on the lipids in surfactant. They also lead to down-regulation of the innate immunity

mechanisms in the lung hence predisposing patients to infection and further lung injury [94].

The major limitations to the measurements of biomarkers such as bile salts and pepsin in BAL, sputum or saliva is the lack of standardised methodology and unknown half-life clearance from the lower respiratory tract of these compunds [90]. However, based on currently available data the specificity of bile salts and pepsin to the gastrointestinal tract makes measurements from lung aspirates a useful diagnostic tool for microaspiration.

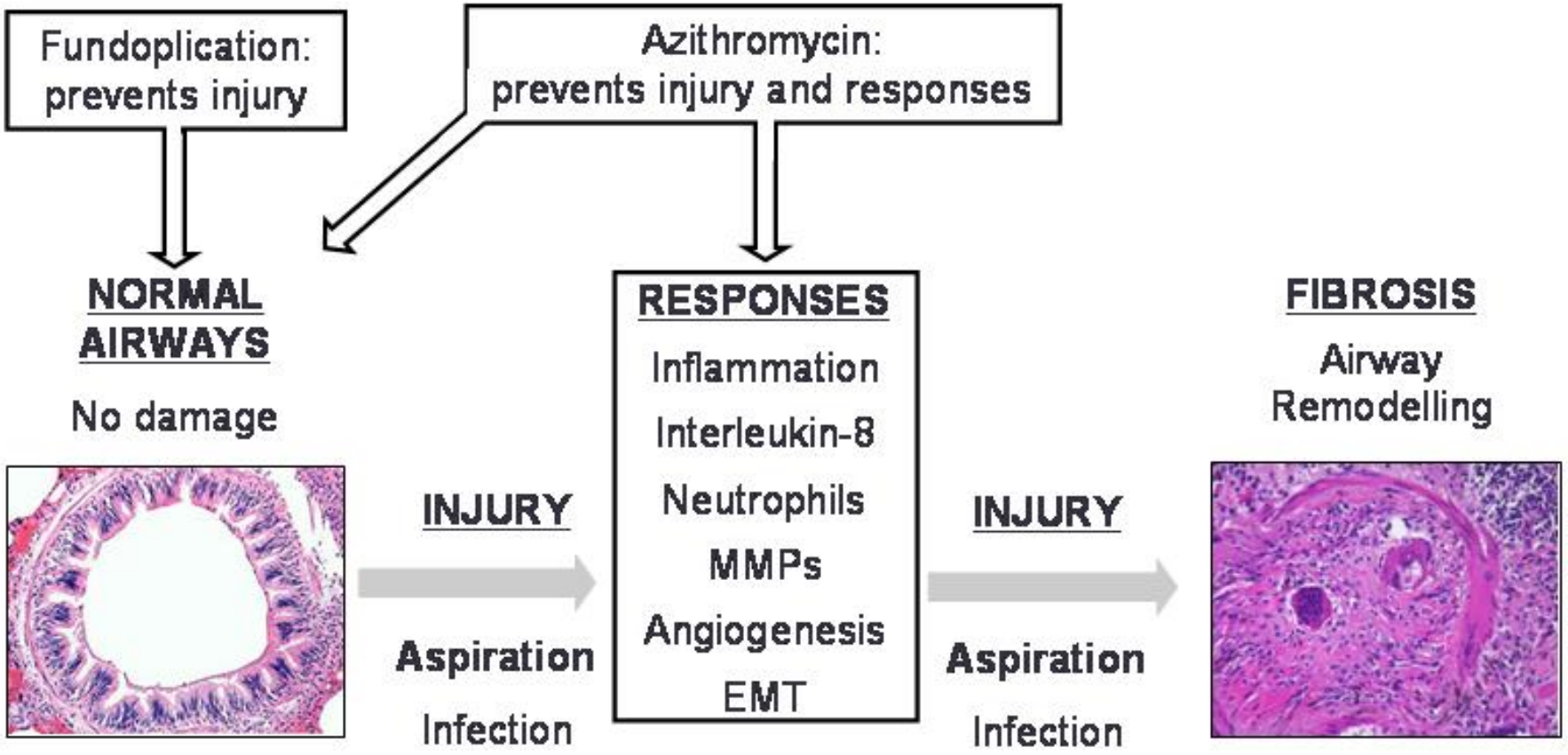
1.7 Lung Transplant and Reflux

1.7.1 Bronchiolitis Obliterans Syndrome (BOS)

Compared to other allograft transplantation, survival form lung transplantation is poor with only 60% of patients alive 5 years after their lung transplants [97]. One of the main reasons for this is the development of Bronchiolitis Obliterans which is believed to be the pathological process of chronic rejection [126]. Bronchiolitis Obliterans Syndrome generally develops between 6 months & 2 years post transplantation [91] and affects 50-60% of patients at 5 years post-transplantation. The 5 year post-transplantation survival is 20-40% lower than average in patients with BOS [119]. Bronchiolitis Obliterans Syndrome is a significant process which leads to decreased quality of life by causing graft failure and as a result leads to an increased mortality [65].

The pathology behind this process involves progressive fibrosis of the small airways, leading to complete obstruction with sclerosis of the airways, intimal thickening and destruction of the pulmonary vasculature (Figure 1-16)[126]. BOS is thought to be mediated by a number of risk factors including the process of acute allograft rejection, HLA mismatch, cytomegalovirus and more recently the development of GORD and microaspiration [36, 65].

Figure 1-16 : Model of Non-alloimmune Lung Allograft Injury and Inflammation in BOS pathogenesis from Robertson et al Am J Trans 2009 [126].


Fundoplication:
prevents injury
Azithromycin:
prevents injury and responses
NORMAL AIRWAYS
No damage
INJURY
Aspiration
Infection
RESPONSES
Inflammation
Interleukin-8
Neutrophils
MMPs
Angiogenesis
EMT
INJURY
Aspiration
Infection
FIBROSIS
Airway Remodelling

Clinically the ISHLT definition of BOS is a decrease in FEV_1 from the best post-operative value in the absence of anastomotic strictures, infection or other complication and is categorised by a simple scoring system (Table 1-6):

Table 1-6: Bronchiolitis Obliterans Syndrome (BOS) scores [36, 127]

1993 Classification		2002 Classification	
BOS 0	**FEV_1: 80% or more of baseline**	**FEV_1: >90% of baseline and FEF_{25-75} >75% of baseline**	**BOS 0**
		FEV_1: 81-90% of baseline and/or FEF_{25-75} ≤75% of baseline	**BOS 0p**
BOS 1	**FEV_1: 66-80% of baseline**	**FEV_1: 66-80% of baseline**	**BOS 1**
BOS 2	**FEV_1: 51-65% of baseline**	**FEV_1: 51-65% of baseline**	**BOS 2**
BOS 3	**FEV_1: ≤50% or more of baseline**	**FEV_1: ≤50% or more of baseline**	**BOS 3**

1.7.2 Reflux post Lung Transplant

Chronic microaspiration, secondary to extra-oesophageal reflux, may plausibly contribute to bronchiolitis obliterans syndrome (BOS) post-lung transplant. Up to 75% of lung transplant patients have demonstrable gastro-oesophageal reflux disease (GORD) [36, 128-132]. Elevated biomarkers, pepsin and bile salts, have been documented in the BAL fluid post-transplant, suggesting microaspiration[108, 109, 119]. A number of reasons for this have been suggested including damage to the Vagus nerve leading to delayed gastric emptying and dysmotility of the distal oesophagus promoting reflux after lung transplantation [133]. In addition, it has been suggested that a large proportion of patients (63-68%) with end-stage lung disease suffer from reflux prior to their transplantation [65].

Anti-Reflux surgery has been demonstrated to improve lung function as early as 2000 [91]. However in was not until 2003 that evidence from Duke University provided a better understanding of the possible role of fundoplication in lung transplant patients and hence the possible role of microaspiration. Their study involved 43 patients undergoing anti-reflux surgery after lung transplantation. An improvement of FEV_1 was demonstrated in 24% of patients with reversal of BOS in some patients [88]. From the same centre only one year later a study involving 76 lung transplant patient undergoing

fundoplication demonstrated a similar success of anti-reflux surgery particularly in the first 90 days post-transplant (Figure 1-17) [65].

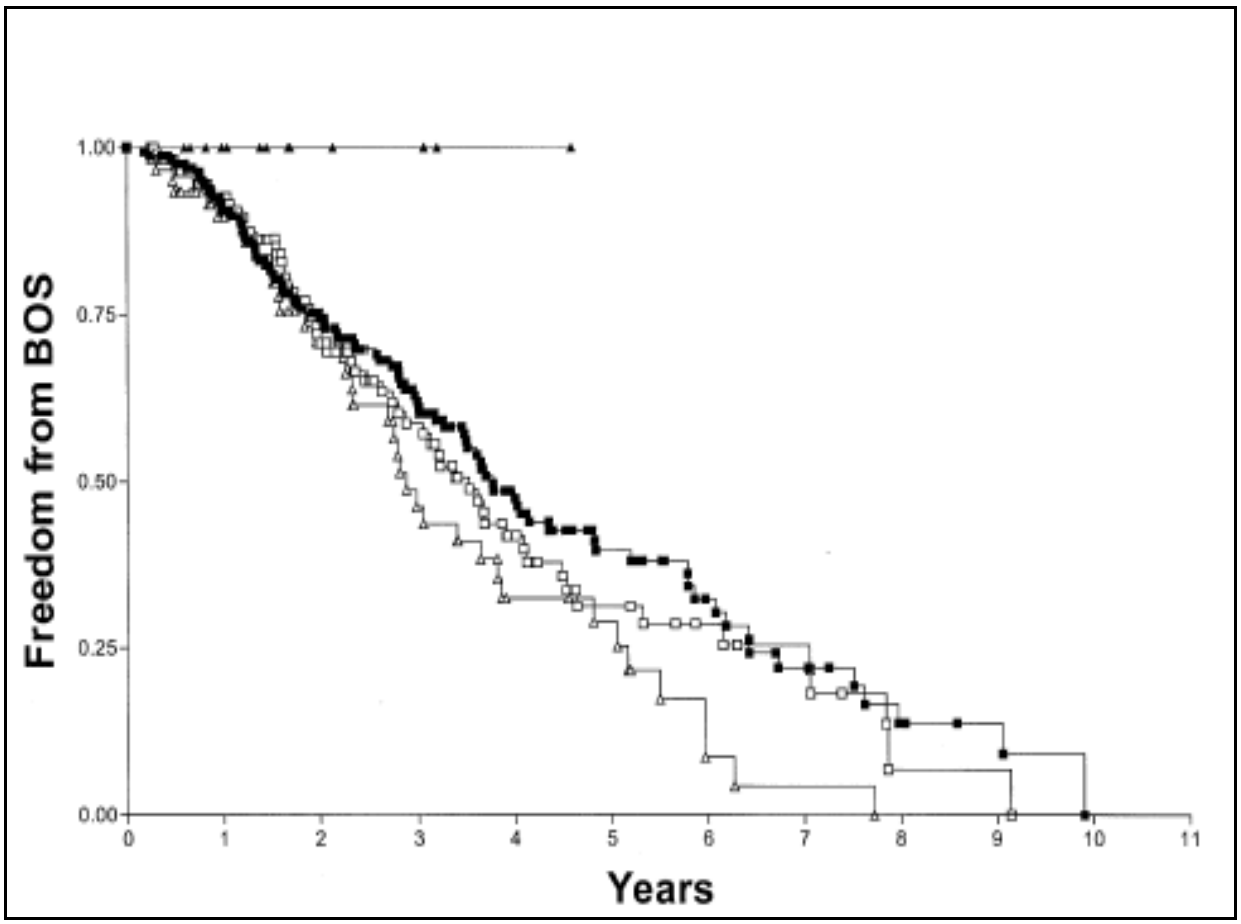


Figure 1-17 : Freedom from Bronchiolitis Obliterans Syndrome [65]

Legend: A Kaplan Meier survival graph showing freedom from BOS at 1 and 3 years. The horizontal plotted line at the top of the graph indicates the group with reflux who received early fundoplication. The other plotted lines represent 4 groups; those with normal pH studies, those with reflux that did not receive fundoplication, those with reflux who received late fundoplication and those with unknown reflux status. There is a significant difference between those that underwent early fundoplication and the other groups (p=0.01) [65].

The limitations of the studies performed so far examining the role of fundoplication after lung transplantation is that they are mainly from a single centre and there is a lack of basic information regarding the assessments of quality of life after anti-reflux surgery in these patients [97]. Such information is important because physiological post-operative complications are common following fundoplication, and may lead to a reduction in quality of life, despite resolution of reflux symptoms. Specific complications include temporary dysphagia, nausea[134], discomfort from gas bloat and increased flatulence [126]. There is very little evidence on the effects of fundoplication on quality of life in this population. Additional surgery may put these patients at risk of physiological dysfunction and reduced quality of life after surgery. To date no studies have been performed assessing the response of extra-oesophageal reflux symptoms to fundoplication and quality of life improvements of this intervention in the transplant population.

1.7.3 Fundoplication and lung Transplant – Work from the Unit

Between June 2006 and October 2009 lung transplant patients were referred to the Northern Gastro-Oesophageal Unit at Newcastle's Royal Victoria Infirmary [36]. A laparoscopic fundoplication was offered to those patients with symptomatic reflux and for those with reflux associated with a decline in lung function. Quality of life questionnaires including Gastrointestinal Quality of Life Index (GIQLI), Demeester and Reflux Symptom Index (RSI) were performed prior to surgery and then in the early and later post-operative period. Pulmonary function was monitored regularly in these patients throughout the study.

In total 9 patients had a laparoscopic fundoplication performed. There were no major complications secondary to the surgery. There were significant improvements in the quality of life score both at 6 weeks and 6 months after surgery and median FEV_1 increased from 2.35 litres to 2.68 litres at the latest follow-up. Although, the numbers in this study were very small, the work illustrates the importance of objective reflux assessment after lung transplant allowing the option of surgical management in this patient group. Further work and results will be presented in the later sections of this thesis.

2 Purpose and Theory behind the Study

2.1 Hypothesis

I propose that both symptomatic and asymptomatic reflux is a common feature in patients with advanced lung disease. I hypothesise that, in patients with idiopathic pulmonary fibrosis and Cystic fibrosis, this reflux together with the subsequent (micro) aspiration of stomach contents into the lungs can lead to long term deterioration of lung function. Detection of reflux using established techniques combined with laboratory measurements of biomarkers in refluxate will identify both the extent and severity of gastro-oesophageal reflux (GOR) in these patients. The translational significance of this is that there are both surgical and non surgical treatments available for reflux. The subsequent treatment of GOR identified patients could preserve long-term lung function and improve their quality of life.

In this study I will test the hypothesis that in IPF and CF there is objective evidence of GOR. Subsequent aspiration represents a potential mechanism through which GOR may lead to lung damage and may be denoted by increased lung levels of pepsin and bile salts. This will represent a potential explanation for an association between GOR, aspiration and impaired lung function.

2.2 Aims of the Study

2.2.1 Purpose & Theory

The overall aim of this study is to evaluate the prevalence of gastro-oesophageal reflux (GOR) in patients with idiopathic pulmonary fibrosis (IPF) and cystic fibrosis (CF) and its potential role in the development of chronic lung dysfunction. Many patients with advanced lung disease are considered to suffer from gastro-oesophageal reflux (GOR), but this has not been systematically characterised. This GOR may be symptomatic or asymptomatic and in some cases can lead to microaspiration which significantly injures the patient's lungs and affects their quality of life.

In order to determine potential associations between impaired lung function and gastro-oesophageal reflux, I will perform a range of specialised investigations for which the centre has international recognition. In order to determine the degree of reflux, patients will be invited to attend for both oesophageal manometry and impedance pH measurements. This will provide a detailed objective assessment of both acid and weakly acid reflux (refluxate pH >4) in these patients; Pulmonary function tests including spirometry will be used to identify impairment of lung function. This will be related to the patient's impedance pH test results, testing for association between GOR and reduced lung function.

Both groups of respiratory patients (CF and IPF) will have lung samples analysed in the lab for bile salts and pepsin; two biochemical markers of aspiration. The IPF group of patients will have provided samples through bronchoalveolar lavage (BAL). The CF group of patients will have daily physiotherapy where they would be encouraged to clear their airways. A small amount of this induced sputum will be taken at this stage and analysed for markers of aspiration (Figure 2-1).

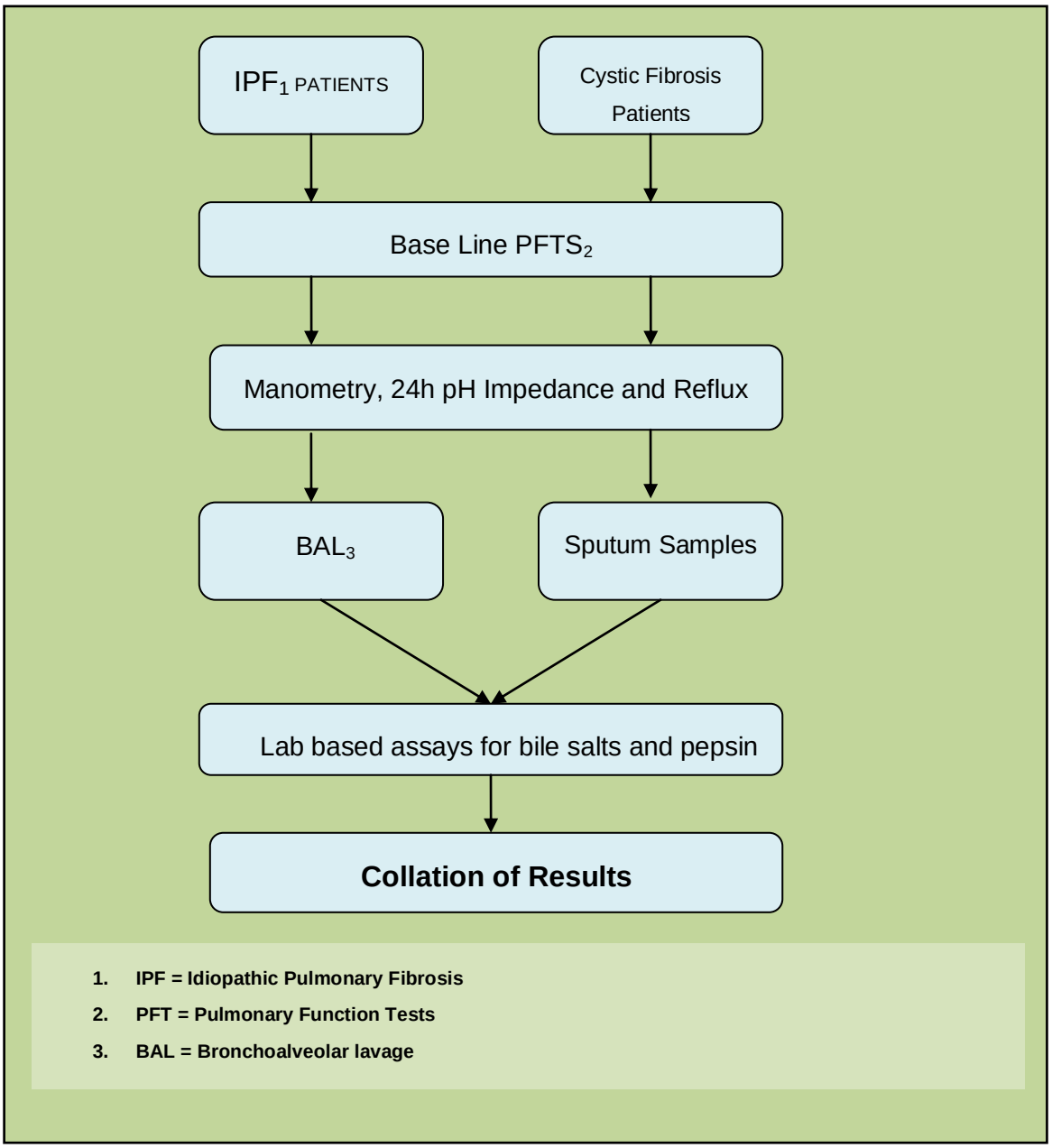
2.2.2 Aims

- To measure impedance pH in patients with IPF and CF to objectively assess reflux
- To measure patient symptoms of reflux disease, using validated questionnaires
- To compare objective assessment of reflux (impedance pH) with patient experience of symptoms (questionnaire)
- To compare objective and clinical assessments of reflux and symptoms with markers of aspiration(pepsin, bile salts); using BAL samples (IPF group) and sputum samples (CF group)
- To correlate the above investigations of reflux with lung function
- To identify patients suitable for specialist referral and subsequent management of reflux disease; and assess the effect of the intervention with regular lung function assessment

The study will provide a subjective assessment of symptoms, objective evidence of GOR physiology and laboratory based assessments of markers of aspiration in patients with IPF and CF. The information gathered from the studies above will be used to develop our understanding of the association between these lung diseases and gastro-oesophageal reflux.

Potential development from the study: Those patients with significant reflux that could warrant treatment may be offered referral to an upper GI specialist for the most appropriate management.

Figure 2- 1 : Summary of Study Protocol



3 Methods

3.1 Ethical Approval

Ethical approval was obtained from County Durham and Tees Valley 2 Research Ethics Committee (*Appendix 3*). Approval for the research to be carried out at the Newcastle upon Tyne Hospitals NHS foundation Trust was granted by the Research and Development department at the Royal Victoria Infirmary. (*Appendix 3*)

3.2 Patient Recruitment

Patients with IPF were recruited with the aid of a national interstitial lung disease specialist, already closely involved in the study. From the start of the study in the summer of 2010 until the current day a rapid expansion of the interstitial lung clinics took place. Initially at the Royal Victoria infirmary, ILD clinics were organised twice a month and recruitment of IPF patients was by the primary investigator, directly from these clinics. As the clinics expanded due to practicalities IPF patients who were suitable for the study were selected by the specialist and communication was made over the phone to recruit the patient.

CF patients were recruited directly from designated specialist clinics. There are currently two specialists at the Royal Victoria infirmary, and patients were approached directly by the primary investigator and provided with a patient information leaflet. The recruitment of CF patients was also through the help of the CF specialist nurse or the patient's clinician.

3.2.1 Inclusion & Exclusion Criteria

All IPF patients were identified from ILD clinics. Idiopathic pulmonary fibrosis in new and known patients had to fulfil the internationally accepted definitions as proposed by the European Respiratory Society (ERS) and American Thoracic society (ATS) [5]:

Major Criteria:

- Exclusion of other known causes of ILD such as certain drug toxicities, environmental exposures and connective tissue disease
- Abnormal pulmonary function studies that include evidence of restriction (reduced VC, often with an increased FEV1/FVC ratio) and impaired gas exchange (increased $P(Aa)O_2$, decreased PaO_2 with rest or exercise or decreased TLCO)

- Bibasilar reticular abnormalities with minimal ground glass opacities on HRCT scans
- Trans-bronchial lung biopsy or BAL showing no features to support an alternative diagnosis

Minor Criteria:

- Age> 50 years
- Bibasilar inspiratory crackles (dry or 'Velcro' type in quality)
- Insidious onset of otherwise unexplained dyspnoea on exertion
- Duration of illness > 3 months

The CF group of patients would include all adult patients (age >16 years). The principal exclusion criteria are:

- Patients in respiratory failure
- Patients with a coexisting respiratory disorder
- Patients with overt congestive cardiac failure
- Patients regarded unfit for any other clinical reason by their respiratory physician

3.2.2 Sample Size

The recruitment targets were 20 IPF and 20 CF patients. This was based on the number of patients attending clinic and the incidence within the region. This is an empirical sample size suggested from previous studies as there is insufficient data available to calculate formal sample size through power calculations. The results will be collated by the research team and simple descriptive statistics produced. A statistician will then be consulted with regard to the most appropriate method of analysis.

3.2.3 Consent and Information

All patients that agreed to be recruited into the study were provided with an information pack. This was either given to them in the clinic if they were recruited from the clinic or sent in the post if they were recruited over the phone. The information pack provided a detailed explanation of the investigations and the consent form, which was returned at the time the patient returned to the hospital for their investigations.

3.3 Oesophageal Investigations

3.3.1 8-Channel Manometry

Patients underwent manometry after a minimum four hour fast from solids and at least two hours free from liquids [135]. Patients were able to take their regular medication on the morning of the test with a sip of water only. The system consisted of a 3.9mm eight lumen single-use catheter, a water perfused manometry system (MMS system) and data displayed on a computer using the MMS programme. The catheter consisted of 4 radial ports arranged at the same level and 4 lateral ports spaced 4cm apart. The 4 radial ports are used to characterise the lower oesophageal sphincter (LOS). Before the start of the investigation each of the eight lumens were flushed and the catheter assembly was connected to the 8 channels of the air pneumo-hydraulic low compliance perfusion pump. The system pumped distilled water through the catheter at a constant rate of 0.6ml/s and the system was calibrated with the 'zero' pressure point being at the level of the a patient's sternal angle. A transducer system was connected to the MMS computer, a Windows compatible computer.

8-Channel manometry standard technique [36]

Patients attended a specific oesophageal physiology laboratory based at the Royal Victoria Infirmary. After discussing the procedure once again with the patient, patients were asked to sit upright on the bed and the catheter, lubricated at the tip was passed horizontally through the nostril into the nasopharynx [135]. The patient was asked to tilt their head forward with their chin touching their chest. As the catheter was advanced the patient was asked to take a few sips of water through a straw and swallow. This technique helps the catheter progress through the cricopharyngeus and into the oesophagus. Whilst the patient was positioned upright the catheter was advanced into the stomach to a distance of 70cm from the nostrils. The patient was then asked to lie in a semi recumbent position as this is the validated position for taking manometry measurements. The presence of all the channels in the stomach is confirmed by a positive deflection in the channels in response to the patient taking a deep breath.

8-Channel manometry LOS position

The catheter was withdrawn at 1cm intervals every thirty seconds [136] until the high pressure zone of the LOS was reached. The lower margin of the LOS was detected first. The catheter was then withdrawn by a further 1cm and a 5ml bolus of water was given to the patient to assess the LOS activity, in particular paying attention to the degree of

relaxation. As the catheter was withdrawn the top of the LOS was represented by a drop in pressure as the catheter exits the high pressure zone. The length and resting pressure were calculated manually using the trace on the MMS programme. The lower oesophageal sphincter end expiratory pressure was defined as the difference between basal tone pressure and the average of the end-expiratory resting pressures found in each port whilst in the high pressure zone.

8-Channel manometry oesophageal peristalsis

With the catheter positioned 5cm above the top of the LOS, ten swallows consisting of 5ml boluses of water were performed. The motility was evaluated for normal peristalsis, simultaneous contractions, aperistalsis or non-specific dysmotility. Mean distal oesophageal peristaltic amplitude was calculated based on the average of all swallows performed at 5cm. mean proximal peristaltic amplitudes were based on the average of all swallows performed at 15cm above the lower oesophageal sphincter. Traces were analysed and categorised using the definitions in the table below. Figure 3- 1 illustrates a section of an 8-channel manometry trace.

Table 3-1: Classification of Oesophageal Peristalsis [36]

Normal Peristalsis	Normal peristalsis >70% of the time
Mild Ineffective Oesophageal Motility	Abnormal peristalsis 30-70% of the time
Severe Ineffective Oesophageal Motility	Normal peristalsis <30% of the time
Aperistalsis	Abnormal peristalsis 100% of the time
Diffuse Oesophageal Spasm	>10% of swallows simultaneous with mean amplitudes over 30mmHg
Nutcracker Oesophagus	Mean amplitude of peristalsis >180mmHg
Hypertonic Lower Oesophageal Sphincter	>45mmHg but relaxing
Hypotonic Lower Oesophageal Sphincter	<10mmHg
Achalasia	Hypertonic LOS, absent or incomplete relaxations >70-80% of the time. Simultaneous contractions or aperistalsis in the oesophageal body

3.3.2 High Resolution Manometry

Patients underwent High Resolution Manometry (HRM) after a minimum four hour fast from solids and at least two hours free from liquids [135]. Patients were able to take their regular medication on the morning of the test with a sip of water only. The system consisted of a manometric catheter connected to a series of pressure transducers which were all connected to a water perfused manometry system (MMS system) and data displayed on a computer using the MMS programme. As with the 8-channel manometry the principles of the system are identical. The pressure in the oesophagus is converted to an electrical signal by the pressure transducers. The computer programme then amplifies and filters the signals so that it can be displayed on the screen in an interpretable manner. In the case of HRM, the measurements are presented as a spatiotemporal Oesophageal Pressure Topography plot as in Figure 3- 2 . This allows more accurate and efficient placement of the catheter [137].

Two main types of manometric catheters can be used for HRM studies, solid state and water-perfused. We used single-use water perfused catheters. The catheter is an extruded silicone catheter containing 20 individual channels spaced 1cm apart in a unidirectional sensor orientation (measuring the pressure at the point of the channel hole). As with the 8-channel manometry, the catheter is perfused with distilled water driven by a pneumatic pressure pump. Each channel opens into the oesophageal lumen at different points and pressures from each of the points is transmitted back to the transducers to be interpreted by the computer. Water perfused catheters are less sensitive to rapidly changing pressures like those found in the upper oesophageal sphincter (UES) and interpretation of pressure changes at the UES have to be treated judicially.

HRM standard procedure

- Equipment preparation – Prior to the arrival of the patient, the perfusion reservoir and the pump were filled with water and the reservoir is pressurised to drive the water through the catheter capillaries. This allows the clinician to check that all the channels are perfusing to ensure an accurate trace. The catheter was perfused with water for several minutes until the pressures in all the channels were stable. Before the study the recording channels were referenced to atmospheric pressure by placing the catheter at the level of the subject's oesophagus, and the system was 'zeroed'.

- Subject Preparation – As with the traditional 8-channel manometry, subjects were fasted for 4 hours prior to the study. Clear instructions were provided to the patient in order to be able to tolerate the procedure including an awareness of some minor discomfort on intubating the nostrils

- Introduction of the catheter - The HRM catheter was introduced through the nostrils in an identical manner to the 8-channel probe. The HRM catheter was lubricated and slowly introduced into one of the subject's nostril whilst the subject was sat upright. A glass of water was available with a straw to aid the insertion of the catheter. The subject was asked to take sips and swallow continuously with their chin placed close to the chest whilst the catheter was inserted in a steady manner through the upper and lower sphincters until it is in the stomach.

- Positioning of the catheter and completion of the study – The catheter was positioned correctly for HRM when both the upper and lower sphincters can be recognised and when at least 2 pressure sensors are in the stomach. The position of the diaphragm can be determined by examining the pressure inversion point (PIP). During inspiration, pressure in the thorax decreases as abdominal pressure increases. The point where the pressure changes with inspiration meet is the PIP and is generally located at the diaphragm [137].

  After the catheter was correctly placed it was secured in this position with tape as a pull-through technique is not required with HRM. The subject was asked to lie down in the semi-recumbent position. Once the patient wass comfortable, the LOS resting pressure was assessed over 30 seconds with the patient asked not to swallow. After this, standard evaluation of oesophageal motility was performed with 10 'wet' swallows using 5ml boluses of water given to the subject via a syringe body. Swallows should be recorded at 20-30 second intervals as this is when the previous peristaltic wave has terminated and the LOS has returned to baseline pressure. After the 10 swallows have been recorded, the catheter was removed.

HRM Analysis

The analysis and interpretation of the 10 swallows HRM test are based on a set of measurements and normal values. These are then classified into groups defined by the Chicago classification criteria [138]. The terms necessary to use the classification a described in the table below:

Table 3-2: HRM measurements

Integrated Relaxation Pressure (IRP - mmHg)	The mean pressure at the O-G junction measured over 4s in the 10 seconds following UOS relaxation. Equates to LOS relation pressure in conventional manometry
Distal Contractile Integral (DCI – mmHg/s/cm)	Amplitude x duration x length of the distal oesophageal contraction. Equates to peristaltic amplitude in conventional manometry
Contractile Deceleration point (CDP)	The inflection point along the swallow where propagation speed slows down and is the point of transition between oesophageal peristalsis and oesophageal emptying.
Contractile Front Velocity (CFV – cm/s)	The gradient of the peristaltic body representing the speed of the swallow.
Distal Latency (DL – s)	Interval between UOS relaxation and the CDP
Peristaltic Breaks (cm)	Gaps in the HRM peristaltic contraction between the UOS and LOS

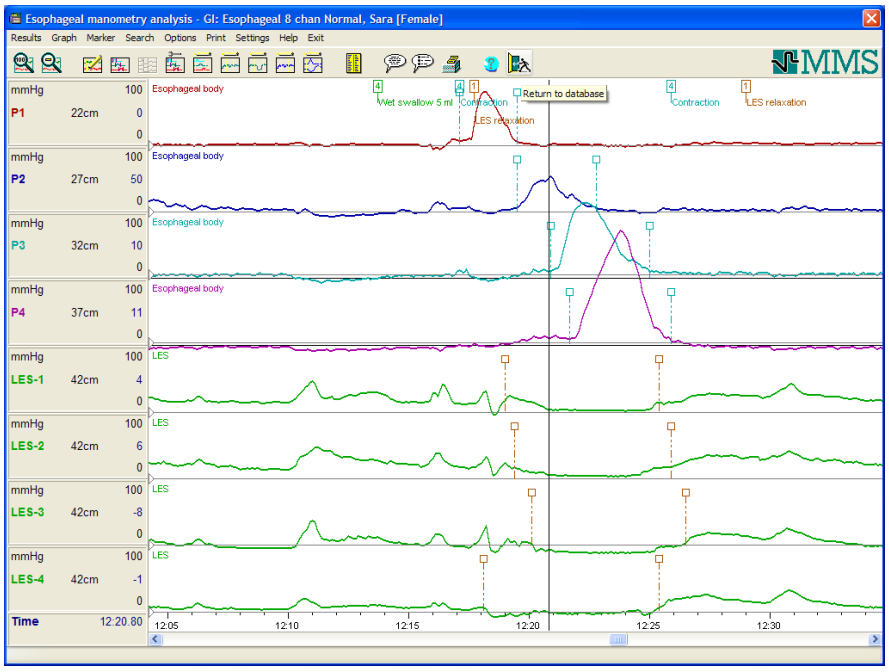
Figure 3- 2 on page 80 illustrates a single peristaltic wave on HRM with the anatomical landmarks and measurement points. After these individual measurements are made they are analysed with the normal values and each swallow is characterised in terms of the integrity of the contraction and the contraction pattern as summarised in the table below:

Table 3-3: Table illustrating the components of the peristaltic contraction that help define the nature of the swallow

Integrity of Contraction	Contraction Pattern
Intact – No peristaltic breaks Weak – Large (>5cm) or small (2-5cm) peristaltic breaks Absent – Minimal integrity of contour plot	Premature (DL < 4.5s) Hypercontractile (DCI > 8000mmHg/cm/s) Rapid Contraction (CFV > 9cm/s) Normal Contraction (none of the above apply)

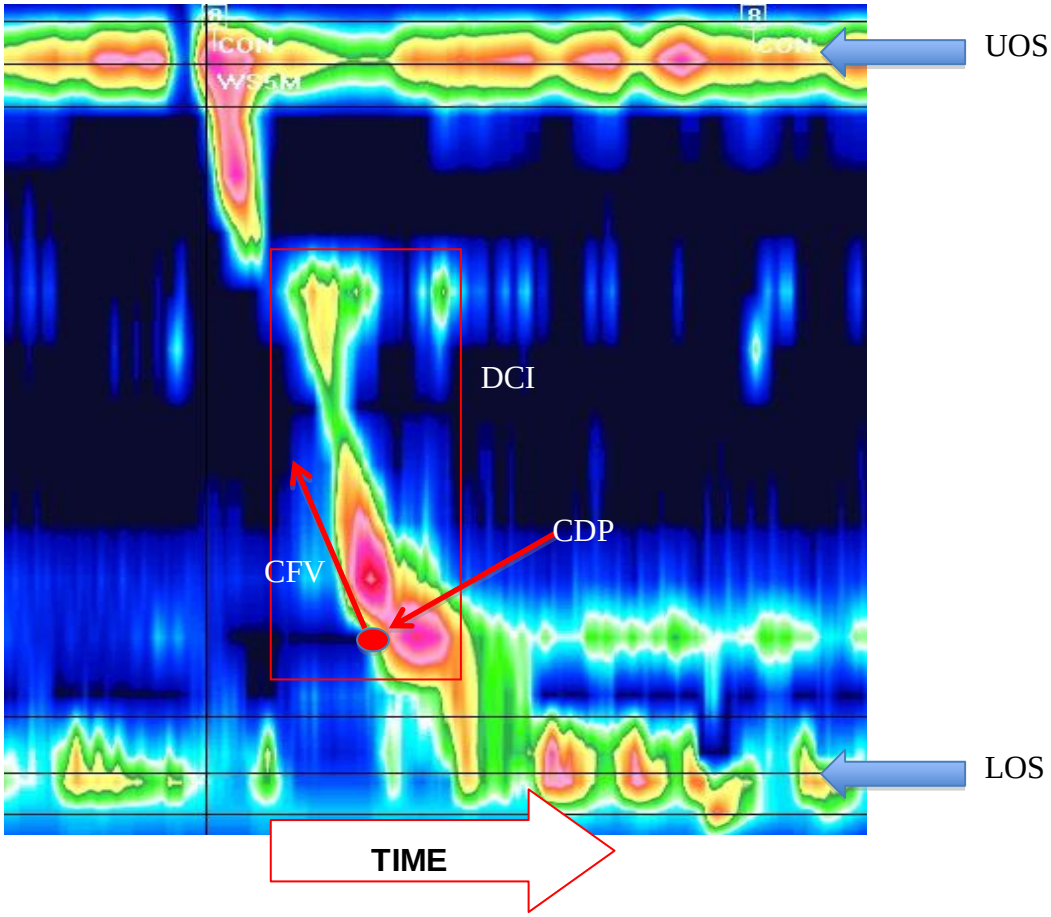
The individual characterisation each swallow is used to compute an overall diagnosis as defined by the Chicago classification using the algorithm in Figure 3- 3 .

Figure 3- 1 : A normal peristaltic wave demonstrated by 8-channel manometry [36].



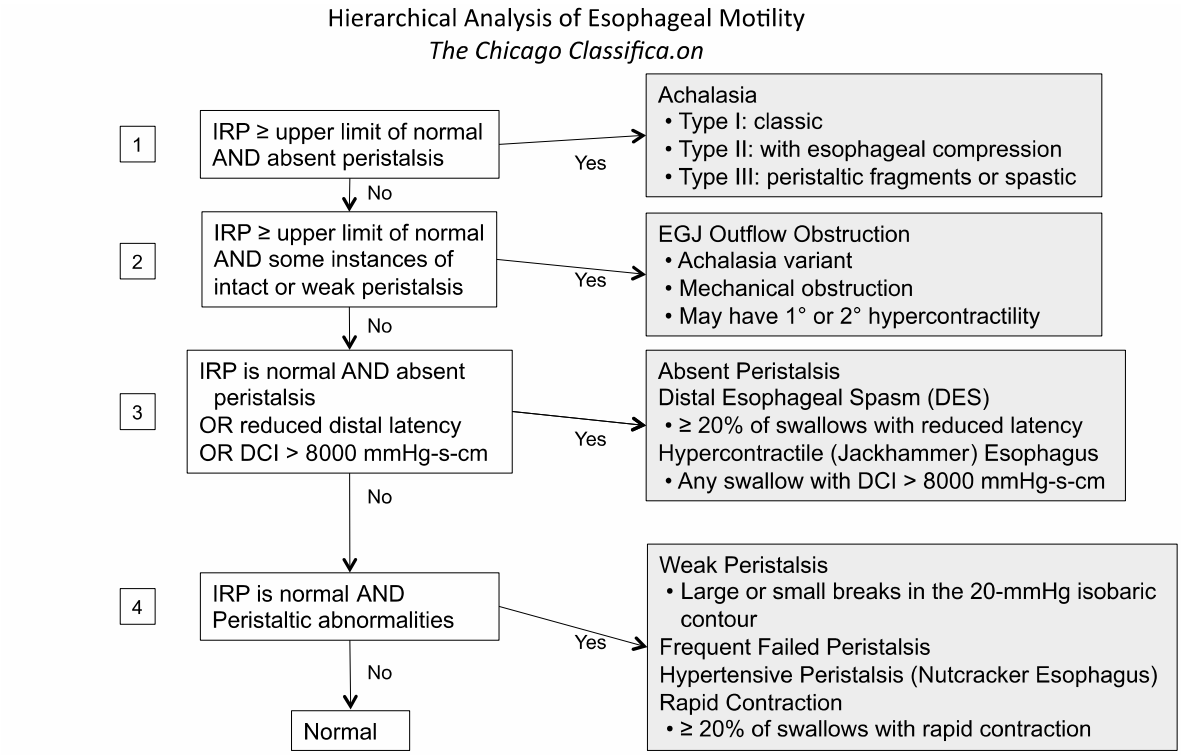
Legend: The propagation of the wave is illustrated from channel P1 to P4 in the oesophageal body and the radial 4 channels mark the position of the LOS. The time (GMT) is on the horizontal axis.

Figure 3- 2 : A High Resolution Manometry trace illustrating a normal peristaltic wave with the landmarks and measurement points identified.



Legend: An HRM trace for a normal peristaltic swallow where UOS is the upper oesophageal sphincter and LOS is the lower oesophageal sphincter. As time progresses on the horizontal axis the swallow migrates from the UOS toward the LOS as a peristaltic wave represented in a topographic form. The contractile deceleration point (CDP) marks the point where the swallow decelerated between the lower oesophageus and LOS prior to emptying into the stomach. The distal contractile integral (DCI) is represented by the red box and is a measure of the amplitude of the wave form (swallow). The speed of the swallow is represented by the gradient of the wave (red arrow) also know as the contractile front velocity (CFV).

Figure 3- 3 : Flow diagram illustrating the analysis algorithm according to Chicago classification[138]



3.3.3 Ambulatory impedance/pH Studies

After performing the oesophageal manometry, the information was used to determine the location of the lower oesophageal sphincter. Combined 24-hour ambulatory. Multichannel Intraluminal Impedance is a technology that measures changes in oesophageal intraluminal resistance and bolus transit. It consists of a catheter with several metal rings (Figure 3- 4). Changes in resistance between these rings are detected. Gas causes an increase in resistance and liquids cause a decrease in resistance. The direction of these changes allows the direction of movement of the bolus to be determined. This device also has a pH probe that allows reflux events to be classified as acidic, weakly acidic or non-acid (Figure 3- 4)

Impedance-pH standard technique [36]

Ambulatory impedance-pH was performed using the MMS Ohmega device and a Pharsiflex (Z61A\ZNIS-8R) catheter. The Ohmega device is simply a portable recording box and the catheter is a 1.9mm diameter single-use catheter consisting of 6 impedance rings (3,5,7,9,15 and 17cm) and a pH probe. The impedance rings at 15 and 17cm were used to identify proximal reflux.

The catheter is initially connected to the Ohmega device and calibrated in a standardised fashion. This begins with a ten-minute pre-soak of the probe in de-ionised water and then the pH probe is calibrated with pH 4 and pH 7 buffer solutions at room temperature. The Impedance-pH catheter was inserted in a similar manner to the manometry catheter and secured so that the pH probe was located 5cm above the upper border of the LOS the location of which was determined from manometry.

During Impedance-pH monitoring, patients were encouraged to maintain their usual eating habit but avoid fresh citrus juices (i.e. very acidic) and chewing gum. The Ohmega device has several buttons allowing the patients to record symptoms, meals and position (upright or supine). They were also given a standardised patient diary to complete. After the 24 hour period, patients returned to the lab and the catheter was removed from the patient. The Ohmega box was then connected to a Windows compatible computer with the MMS software and uploaded. The trace was reviewed manually and the electronic diary was verified with the paper diary and edited appropriately. After the trace was reviewed the MMS software provided an automatic analysis and summary of impedance-pH events and symptoms scores.

The table below shows the main components of analysis provided by the Ohmega device.

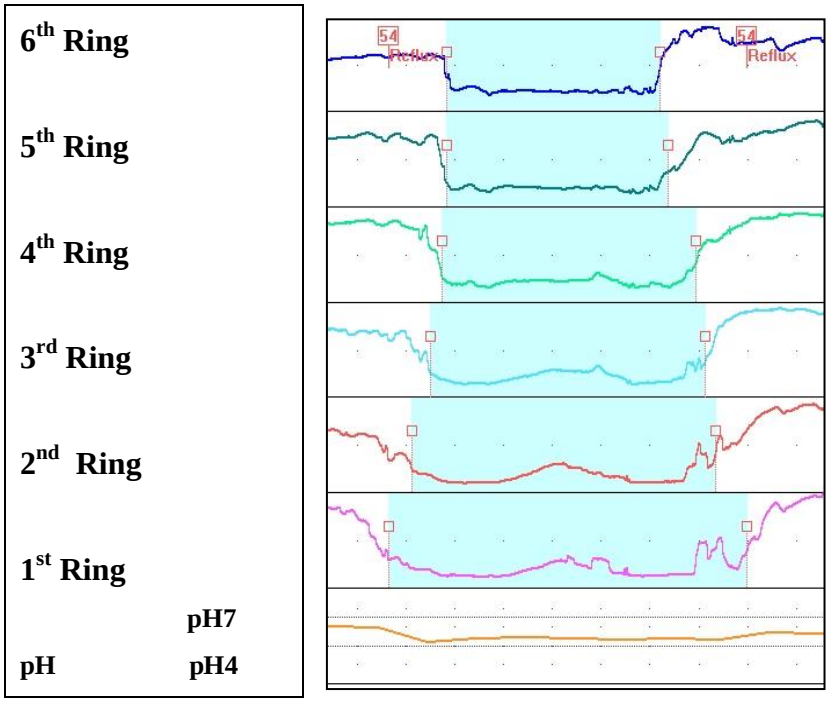
Table 3-4: The components of the 24 hour pH-impedance analysis

pH Analysis	pH results are analysed by comparing them to normal values as defined by Johnson and Demeester [10]. **An abnormal study is defined as a pH < 4 for more than 4.5% of the study duration**.
Impedance Analysis	Impedance traces were manually analysed by the research clinician and compared to normal values as defined by Zerbib [139] **An abnormal study is defined as volume exposure >1.2%**
Symptom Index (SI)	This is calculated using the number of symptomatic episodes associated with a reflux event as a percentage of the total number of symptomatic episodes. **50% is the defined threshold for a positive result** [15].
Symptom sensitivity index (SSI)	This is calculated as the number of reflux events associated with symptoms as a percent of acid reflux events. It accounts for the limitation of the symptom index [15]. **A positive result is an SSI > 10%.**
Symptom Associated Probability (SAP)	A statistical calculation using the data recorded. It uses a Fisher exact test based on 4 distributions (Symptom with reflux/Symptom without reflux/reflux with symptoms/Reflux without symptoms). The test evaluates whether the distribution occurs by chance. **SAP >95% is a positive result**. The test provides a more accurate understanding of the association between reflux and symptoms [15].

The overall analysis of the pH trace used several impedance-pH indices to determine if the patient had pathological reflux. The important distal reflux parameters were oesophageal acid exposure and oesophageal volume exposure. Oesophageal acid exposure is defined as the percentage of time that the pH is less than 4, 5cm above the lower oesophageal sphincter over a 24-hour period (normal <4.5%). Oesophageal volume exposure is defined as the percentage of time that impedance detects refluxate within the oesophagus over a 24-hour period (normal < 1.2%). Distal reflux was present when either the oesophageal acid exposure or oesophageal volume exposure was abnormal. Patients with abnormal oesophageal volume exposure but normal oesophageal acid exposure were likely to have weakly acid reflux [36].

Impedance-pH provides a valuable assessment of proximal reflux i.e. reflux events reaching the impedance ring located 17cm above the LOS. Patients with more than 17 of these events were deemed to have significant proximal reflux.

Figure 3- 4 : A weakly acidic liquid reflux event on impedance-pH.



3.4 Reflux Questionnaire Assessments

Questionnaires have been designed to detect symptoms suggestive of both oesophageal and extra-oesophageal reflux (*appendix 6*). These were used to assess severity of symptoms and responses to treatment. Three questionnaires have been validated for the assessment of patient symptoms and were used in this study:

- The DeMeester Reflux Questionnaire is a validated straightforward tool to assess basic reflux symptoms [57]. It is based on a score of 0-3 for symptoms of reflux, regurgitation and dysphagia.
- A validated questionnaire which focuses on extra-oesophageal reflux symptoms is the reflux symptom index (RSI). This is a 9-item questionnaire which is easily administered and highly reproducible**.** A limitation of this questionnaire is that 5 points can be attributed to heartburn. Thus, the RSI is not limited to extra-oesophageal reflux symptoms but can be elevated in patients with typical reflux symptoms. A RSI score of greater than 13 is abnormal[56].
- The gastrointestinal quality of life index (GIQLI) was developed by *Eypasch et al* in Germany. It is a well established, tested and validated tool which has been shown to be reproducible [140]. The use of GIQLI is recommended for the assessment of anti-reflux surgery by the European Association for Endoscopic Surgery and has been validated for this purpose [141]. A normal score is between 121 and 130.

The questionnaires were performed at the time of recruitment, therefore, if patients are on PPI therapy this was accounted for by a repeat questionnaire assessment at the time of the oesophageal studies before which the patient had stopped their PPI therapy for two weeks.

3.5 Patient Sample Collections

3.5.1 Bronchoscopy

Bronchoscopy was performed on the day the patient returned following their 24-hour pH study, providing continuity and minimal imposition on the patient. The patient was provided with an information leaflet prior to the procedure and requested to fast for 4 hours prior to the test. After receiving informed consent, the patient was taken to the procedure room and intravenous access with a blue venflon was gained.

Adequate sedation was achieved with up to 10mg intravenous midazolam. In addition, local anaesthetic was applied in the form of 4% lignocaine to the nose, pharynx and larynx and just below the vocal cords. Oxygen saturations were monitored with a pulse oximeter and supplemental oxygen was administered via nasal cannulae.

Bronchoscopy was then performed in a supine position and intubation was achieved through one of the nares. A 4.9mm external diameter, 2mm internal diameter fibre-optic bronchoscope was used for the procedure and passed through the nostrils into the larynx and trachea. Three photos were taken of the larynx and vocal folds and were externally reviewed. The bronchoscope was then passed into the lingular bronchus or the bronchus of the right middle lobe.

3.5.2 Bronchoalveolar Lavage

Bronchoalveolar lavage (BAL) was performed in a standardised manner in accordance with BTS guidelines [142]. Three samples of 60ml of sterile saline were injected into the lobe and whilst the standardised lavage was being performed, a series of receptacles connected to the system was used to collect the retrieved lung fluid. The majority of the retrieved BAL was retained for research with a small amount (10ml) reserved for clinical purposes.

3.5.3 Sputum:

CF patients provided a sputum sample on the day of their oesophageal investigations.

3.6 Laboratory Investigations

3.6.1 BAL processing:

The BAL sample was processed immediately after collection using a validated standard operating procedure [143]. This has been produced and extensively used in clinical

practice at the Freeman Hospital's Sir William Leech Centre. The principles of this procedure are to:

- Measure the volume of BAL fluid received and establish the initial cell count
- Prepare 12 cytospins onto glass slides to allow staining and differential cell counts
- Prepare 25 x 600µl aliquots (stored at -20 ºC) to allow pepsin and bile salt assays to be performed
- Storage of cell pellets with up to 6 x 3 million cells (stored at -20 ºC).

The BAL fluid was first filtered through a thin layer of gauze into 2 x 50ml centrifuge tubes and the total volume recorded. The two centrifuge tubes were filled to the same level and then placed into a centrifuge for 6 minutes at 4°C at a speed of 1250rpm. The supernatant was then divided equally into 2 x 50ml centrifuge tubes, being careful not to disturb the cell pellet. The supernatant was placed back in the centrifuge for a further 6mins at 4°C but at a speed of 2500rpm. The resultant supernatant was then further divided into twenty-five 600µl micro centrifuge tubes and the excess divided into 5ml tubes and stored at -20°C for further analysis.

The cell pellets in the two centrifuge tubes were combined and mixed with Dulbecco's PBS to give an opaque suspension. A small aliquot of the suspension was placed on a Neubauer counting chamber and the total cell concentration calculated by counting all the cell in the 4 large squares. Using the information from the cell concentration calculation the suspension was made up to 0.5million cell/ml. Twelve cytospins were then prepared using 100µl of the re-suspended cells at 300rpm for 3 minutes at room temperature. Two cytospins were fixed in acetone for 10 minutes and allowed to air dry.

3.6.2 Sputum processing:

The sputum collected was processed immediately after collection using a validated standard operating procedure [144]. This has been produced and extensively used in clinical practice at the Freeman Hospital's Sir William Leech Centre. The principles of this procedure are to:

- Produce a sputum plug that can be processed
- Produce 25 x 600µl aliquots (stored at -80 ºC) to allow pepsin and bile salt assays to be performed

- Process the cell pellet to determine an initial cell count (viable and non-viable cells)
- Prepare 12 cytospins onto glass slides to allow staining and differential cell counts

Once the sputum had been collected and taken to the lab, time was invested in the initial processing to produce a decent sputum plug. The processing of the sputum was where possible by me but much of the processing was completed by a PhD student, Miss. Gemma Crossfield. The sputum was transferred to a petri dish and using a blunt forceps the thick mucus strands were condensed into a dense plug. The weight of this plug was then measured and the plug suspended in Dulbeccos PBS, using a vortex machine to form a suspension.

The suspension was then centrifuged at 2500rpm for 10 minutes at 4°C and the supernatant is decanted off into a new tube being careful not to displace the sputum pellet. The supernatant is then centrifuged in the same conditions at 2500rpm and the resultant supernatant is divided into 600µl to store at -20°C for future studies.

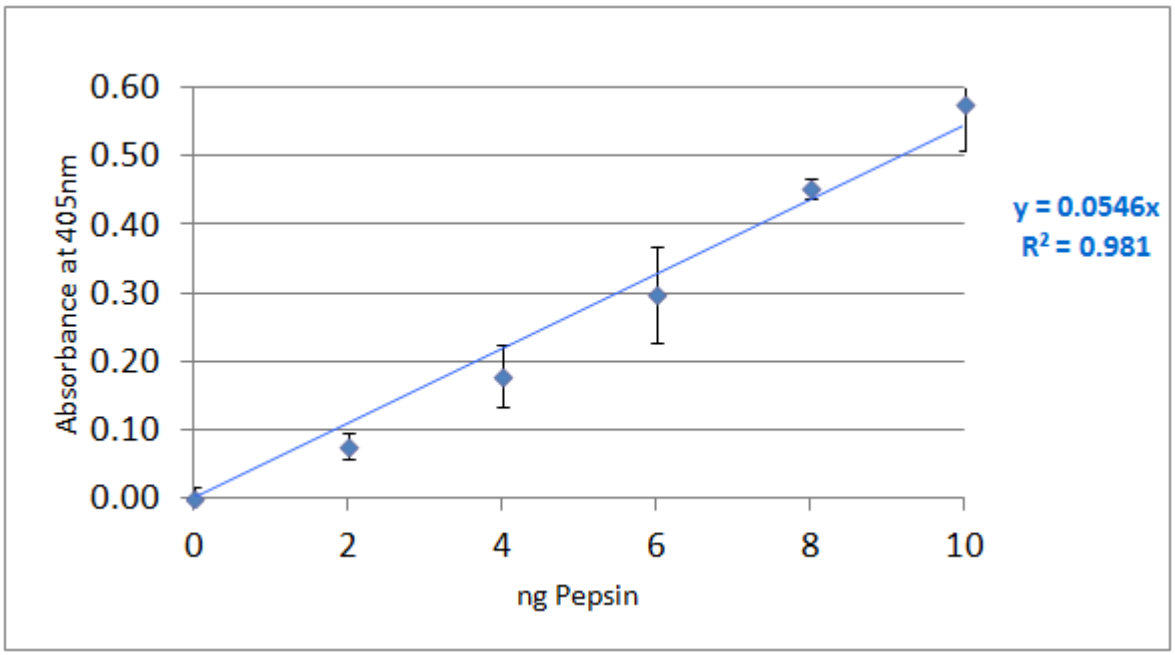
The sputum pellet was suspended in 0.2% sputolysin, which has to be prepared as detailed in the standard operating procedure (*appendix 1*). The sputum pellet was thoroughly mixed with the sputolysin using the vortex machine. A small volume of Dulbeccos PBS was added and a further mix in the vortex machine was performed. The suspension was filtered through a thin nylon gauze and the resultant solution centrifuged at 2000rpm for 10 minutes at 4°C. The supernatant was then discarded and the remaining pellet suspended in a small volume of Dulbeccos PBS to form an opaque suspension and 20µl of this was mixed with the same volume of Trypan Blue dye. A small aliquot of the suspension was placed on a Neubauer counting chamber and the total cell concentration calculated by counting all the cell in the 4 large squares. The cells were recorded as viable (colourless) or non-viable (blue) leucocytes and squamous cells. The total cell count per gram of sputum was calculated. The suspension was centrifuged at 800rpm for 10 minutes at 4°C and the supernatant discarded. A further small volume of Dulbeccos PBS was added to the cell pellet to form a solution in which the cell concentration was 0.5 million cells per ml. Twelve cytospins were then prepared using 100µl of the re-suspended cells at 450rpm for 3 minutes at room temperature. Two cytospins were fixed in acetone for 10 minutes and allowed to air dry.

3.6.3 Pepsin ELISA

The pepsin assay used was developed and extensively calibrated, tested and verified [145]. The principle steps to the pepsin ELISA were:

- 100µl of standards diluted in phosphate buffered saline (PBS) or 20µl of sample, added to 80µl of PBS were added to coat a 96 well micro-plate (Maxisrop, Nunc). The plate was sealed and incubated overnight at room temperature.
- The following day each well was aspirated and washed with 400µl wash buffer repeating the process twice for a total of three washes, followed by two more washes of 1% PBS. The plate was then blocked by adding 300µl of block buffer (1% bovine serum albumin in PBS) to each well and incubated at room temperature for 1 hour. Aspiration and wash were repeated.
- Primary antibody (antipepsin, Biodesign International, USA) was diluted to a working concentration (1 in 2000) in reagent buffer (0.1% BSA, 0.05% Tween 20 in PBS) and 100µl was added to each well. The plate was covered with parafilm and incubated for 2 hours at room temperature. Aspiration and wash were repeated.
- 100µl of the secondary detection antibody (horse radish peroxidase-conjugated anti sheep/goat antibody, Sigma, UK), diluted in reagent dilutant (1 in 10,000), was then added to each well. This was covered and incubated for 2 hours at room temperature. Aspiration and wash were repeated.
- 100µl of substrate solution (2,2'-azino-bis(3-ethylbenzothiazoline-6-) sulfonic acid) was added to each well. This was incubated for 20 minutes at room temperature, avoiding direct light.
- 100µl of stop solution (1% sodium dodecyl sulphate) was added to each well.
- Optical density of each well was determined immediately using a microplate reader set to 405nm [145].
- Negative controls were analysed. These samples were analysed identically apart from omitting the primary antibody. In addition a correction factor is used to correct for the difference in primary antibody affinity to human compared to pig pepsin [145].

Figure 3- 5 : Standard curve produce with pepsin ELISA



3.6.4 Bile Salt analysis

Because bile salts were likely to be essentially undetectable by spectrophotometric based approaches, a more sensitive tandem mass spectrometry method was used at a nationally accredited external laboratory, blind to the study; Sheffield Children's Hospital, UK. Tandem mass spectrometry is a technique that allows the analysis of metabolites and proteins in blood samples. The lower limit of detection limit was 0.01µmol/l but the procedure was further modified to improve the assay sensitivity to 1nmol/l using an extraction based protocol as follows [36]:

450µl of BAL was added to 10ml of distilled water containing 150µl of deuterated taurocholate (internal standard). This solution was loaded onto a C18SPE column (Supelco LC-18) washed with 5ml water and 2ml hexane. The bile salts were eluted with 10ml of methanol and evaporated to dryness. They were then reconstituted in 1ml of 90% acetonitrile. 30µl was injected directly onto tandem mass spectrometry with 50% acetonitrile as running buffer. The bile salts were measured using negative ion mode and multiple reaction monitoring scans, giving sensitivity down to 1nmol/l.

3.7 Cell Staining and Counting

3.7.1 Giemsa 2 (Romanovsky) stain

The principle of this stain is to identify the nucleus of all types of inflammatory cells allowing a differential cell count to be performed.

The method of staining each of the cytospins was through a standard operating procedure (*appendix 2*). The cytospins used for the Romanovsky stain were fixed in acetone. The working stain solution is produced by mixing two stock solutions which were produced as follows:

Stock Solution A: Azure B thiocyanate 1.5g with DMSO 200mls. This mixture was warmed to 37°C until the Azure B has dissolved

Stock Solution B: Eosin Y (VWR BDH 341972Q) 0.5g with methanol 300ml.

Stock solution A was slowly added to stock solution B. This is a concentrated mixture and a 10:1 dilution using PBS/Tween 20 (pH7.4) producing the working dye.

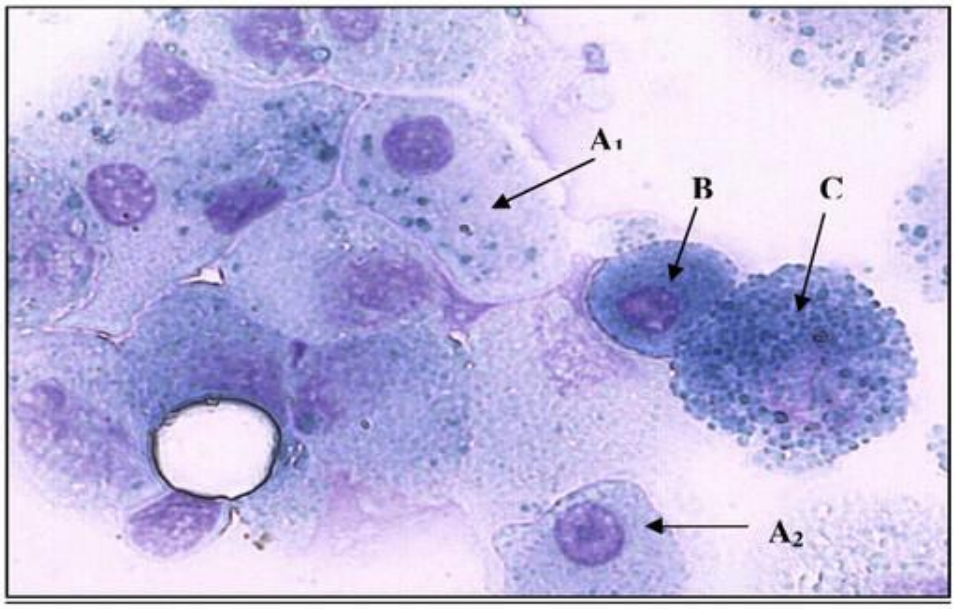
The acetone fixed cytospin was flooded with the dye solution and left for 10 minutes. After this, distilled water was used to wash the slide and it was left to air dry. Once dry, DPX was used to mount the cover slip and the differential count can be performed under a microscope. The table below (Table 3-5) summarises the colours seen for the individual cell components:

Table 3-5: Differential cell count key

Nuclei	Purple
Cytoplasm	Shades of Blue
Cytoplasmic Granules	Shades of Pink
Eosinophilic Granules	Red
Mast Cells	Metachromatic purple red

The diagram on the following page (Figure 3- 6) illustrates the appearance of a Geimsa stained cytospin under high power magnification with the various cell types identified.

Figure 3- 6 : BAL samples stained with Giemsa stain to visualize cell morphology



A_1 and A_2 = Macrophages, B = Monocyte, C = Basophil

3.7.2 Oil Red O

The principle of this stain is to demonstrate intracellular lipid within the macrophages. The staining mechanism of this polyazo dye is a function of the physical property of the dye being more soluble in the lipid than in the solvent. The presence of lipid in alveolar macrophages may be the result of microaspiration secondary to GOR [146].

The method of producing cytospins correctly stained with Oil Red O is through following the standard operating procedure (*appendix 2*). This stain requires the cytospin to be fixed in formalin which takes 10-15minutes at the start of the procedure. The Oil Red O stock solution was produced by dissolving 0.5 grams of Oil Red O in 100mls of 60% isopropanol using very gentle heat.

The working Oil Red O solution was made by diluting the stock solution with distilled water in a 3:2 ratio and filtering the resultant mixture prior to staining. Once the cytospins have been fixed they were washed, first in water and then 60% isopropanol. The cytospin was then flooded with the Oil red O stain and left for 15 minutes. A second wash with water followed by 60% isopropanol was performed before a light

application of Harris Haematoxylin counterstain was added to the slide. After a final rinse with water the cover slip was mounted using a glycerin based aqueous mount. Under the microscope the nuclei appear blue and the lipid appears red.

Counting the macrophages and achieving a lipid-laden alveolar macrophage (LLAM) score is performed using the method as described by Colombo and Hallberg [147]. Using the system described by the authors, a total of 300 macrophages are screened for cytoplasmic lipid granules, and the macrophages are graded to their content of lipid stained: 0 = cytoplasm not opacified, 1 = up to ¼ opacified, 2 = up to ½ opacified, 3= up to ¾ opacified and 4 = totally opacified cytoplasm. Thus LLAM scores could be a maximum of 1200. The percentage of LLAM can then be calculated for 300 macrophaes.

3.7.3 Hemosiderin (Perls Prussian Blue)

The principle of this stain is to specifically stain the released ferric iron from protein bound tissue deposits which in the presence of ferrocyanide ions is precipitated as *potassium ferric ferrocyanide* Prussian Blue. Detection of chemically active iron released from ferritin stores and nitric oxide-derived radicals maybe an indication of oxidative stress in these cells [148]. More specifically elevated levels of haemosiderin laden macrophagesmaybe a sign of occult alveolar haemorrhage secondary to pulmonary veno-occlusive disease, a form of pulmonary hypertension seen in IPF [149].

The method of producing cytospins stained with Perls Prussian Blue was through following the standard operating procedure (*appendix 2*). This stain requires the cytospin to be fixed in acetone which takes 10-15minutes at the start of the procedure. The Perls reagent was produced by mixing 2% hydrochloric acid with 2% potassium hexacyanoferrate (II) trihydrate (potassium Ferrocyanide). A counterstain is also required to provide a neutral control. This was 1% neutral red and is a combination of Industrial methylated spirit and Xylene.

The working solution must be made fresh. Once the cytospins have been fixed and allowed to air dry for 15 minutes they were washed first in distilled water. The cytospin was then flooded with the Perls reagent and left for 15 minutes. A second wash with water followed by the 1% neutral red was performed and left for 30 seconds. A final wash with distilled water is required before the cytospin is mounted in DPX. Under the

microscope the nuclei appear red and the ferric iron appears blue. Red blood cells appear yellow.

A haemosiderin score (HS) was calculated as described by *Reid et al* [148]. In total, 200 macrophages were examined on each slide and each cell was ranked for haemosiderin content using a scale from 0 to 4 as follows: 0 = no colour, 1 = faint blue, 2 = deep blue in a minor portion of the cell, 3 = deep blue in most of the cytoplasm and 4 = deep blue throughout the cell. The total value for all cells was calculated and divided by 2 to obtain a score for an average of 100 cells. In addition, the simple percentage of cells staining positive was also recorded.

3.8 Pulmonary Function Tests

During the assessment of patients in clinic, pulmonary function tests were performed by clinical physiologists in accordance with the standardised European guidelines [138]. During the tests patients were seated with a specialised mouthpiece and nose clip to prevent air escaping during expiration. After a period of a few minutes of adjustment, the patient was asked to take a maximal breath in followed by a hard fast breath out to full expiration. In order for the test to be accurate it was essential that expiration was both forceful and prolonged [36]. The test was repeated for a minimum of three and a maximum of eight times to improve the accuracy.

The simple spirometry provided a graph of volume against time from which the FEV_1 and FVC were calculated. These measurements are defined below in Table 3-6. The FEF_{25-75} was extrapolated from the graph by taking the points at 25% and 75% of the vital capacity and drawing a line between them. The gradient of this line gives the mid expiratory flow FEF_{25-75}. (Figure 3- 7).

The flow-volume curves were measured using a Collins Owl body plethysmography connected to pnemotach device to give a flow signal (Figure 3-8)) which was then integrated with Raptor software to provide volume measurements as defined in the table below:

Table 3-6: Definitions of pulmonary function tests [36]

FVC (litres)	Maximal volume of air exhaled with maximally forced effort from a maximal inspiration, expressed in litres at body temperature and ambient pressure saturated with water vapour (BTPS)
FEV_1 (litres)	Maximal volume of air exhaled in the first second of a forced expiration from a position of full inspiration, expressed in litres at BTPS.
FEV_1/FVC (%)	Ratio of FEV_1 as a percentage of FVC
FEF_{25-75}	Mean forced expiratory flow between 25 and 75% of FVC – known as maximal mid-expiratory flow

Figure 3- 7 : Volume-time graph for a normal subject. The red line shows the FEF $_{25-75}$ [36].

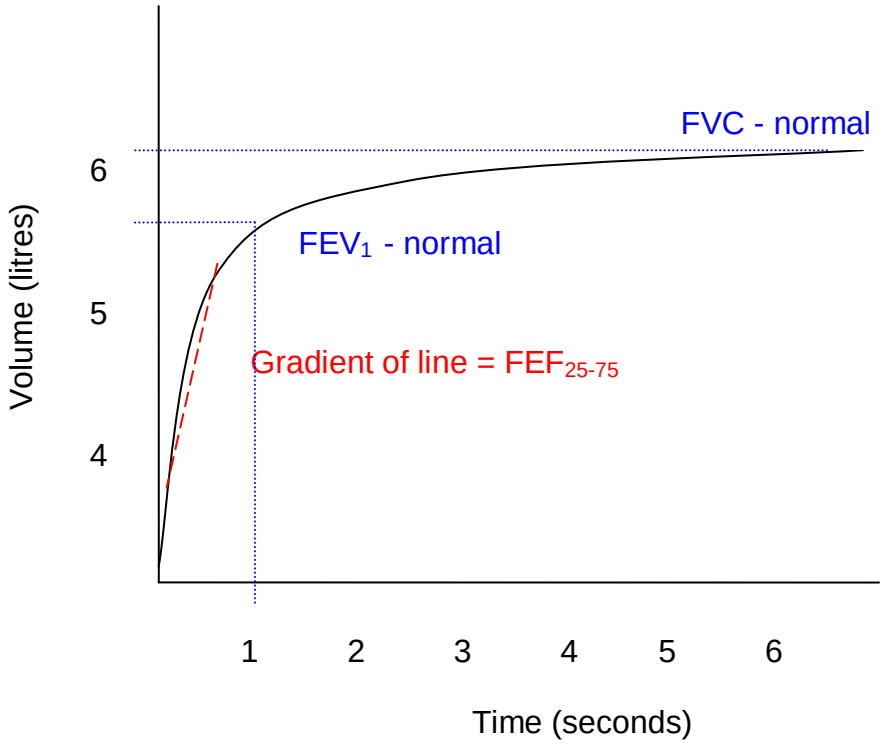
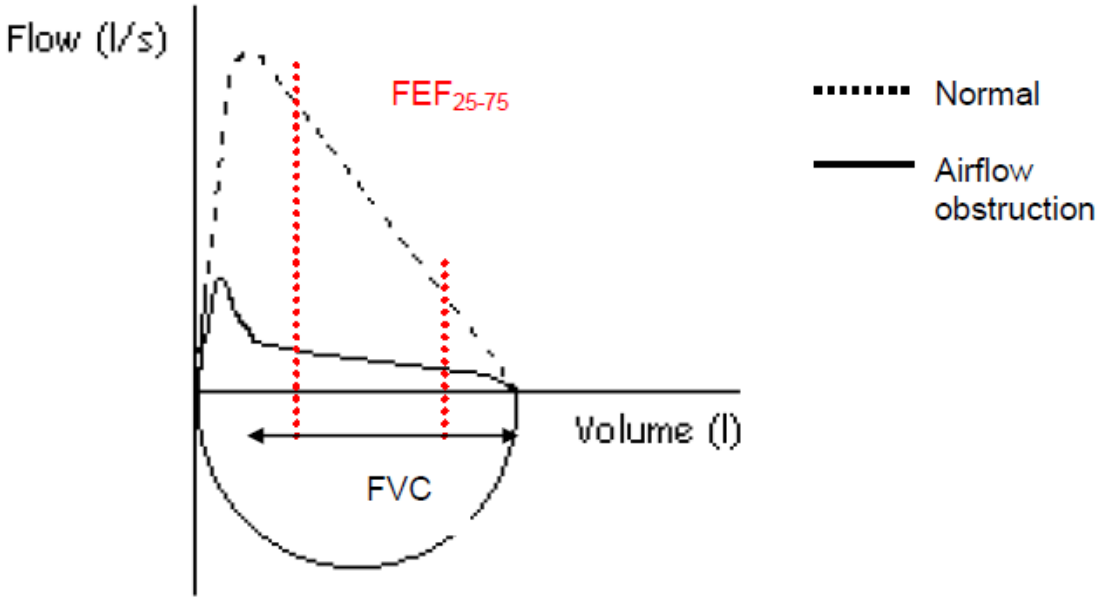


Figure 3- 8 : Flow-volume curve for a normal subject and subject with obstructive air flow disease. Legend: [36].



3.9 Statistical analysis

All the data was recorded onto an excel spreadsheet and the statistical analysis performed on Minitab 16 (State College, Pennsylvania, USA). Due to the very small sample sizes used basic descriptive statistics were extrapolated from the data and for the IPF and CF results Pearson correlation tests was performed to provide a correlation coefficient and p value for the relationship. For the lung transplant patients before and after surgery a non-parametric paired t-test (Wilcoxon) was performed on the data.

4 Idiopathic Pulmonary Fibrosis Results Section

4.1 Introduction

A potential relationship between idiopathic pulmonary fibrosis (IPF) and gastro-oesophageal reflux (GOR) was first demonstrated by Mays et al [73] when they noted that hiatus hernia is more common in IPF patients. Tobin et al [74] demonstrated in 17 patients with biopsy-confirmed IPF, that 94% had reflux confirmed with 24-hour manometry, 75% of these patients reported no reflux associated symptoms. Recently this has been confirmed in a larger cohort of 65 patients by Raghu et al [71]. Their study demonstrated GOR was characterised on 24-hour pH monitoring in 87% of their subjects. Interestingly Raghu et al showed abnormal oesophageal acid exposure in 63% of their patients who remained on a proton pump inhibitor during the pH studies. The most recent guidelines from the American Thoracic Society, 2011 [6] regarding the diagnosis and management of IPF, recognised the complication of GOR in IPF, and encouraged further studies to determine the exact nature of the reflux. The role of microaspiration in IPF is not clearly understood as very few human studies exist looking in particular at this disease.

This section aimed to identify the incidence and nature of reflux in IPF patients and develop an understanding of the role of microaspiration in this patient group.

4.2 Methods

Patients diagnosed with IPF as defined by the internationally accepted criteria attended a specialist ILD clinic at the Royal Victoria Infirmary. Between July 2010 and March 2012 all patients with IPF from this clinic that fulfilled the inclusion criteria as defined in the previous chapter were approached to be recruited to the study.

My protocol was to comprehensively assess for GOR using assessments of symptoms, objective physiological assessments of reflux and putative markers of aspiration . I used a set of validated reflux questionnaires, oesophageal manometry and pH/impedance measurements. In tandem with these assessments a bronchoscopy and lavage was performed to assess markers of aspiration and airway inflammation. Those patients on proton pump inhibitor (PPI) therapy were requested to stop their medication 2 weeks prior to the investigations. In addition, they were asked to complete a set of questionnaires whilst they were taking the PPI. Results were then compared with markers of aspiration in the bronchoalveolar lavage (BAL) samples and differential cell counts from the BAL cytospins. Pulmonary function tests were also available over the time the patient had attended the ILD clinic and these were used in the comparison analysis.

4.3 Results

4.3.1 Recruitment

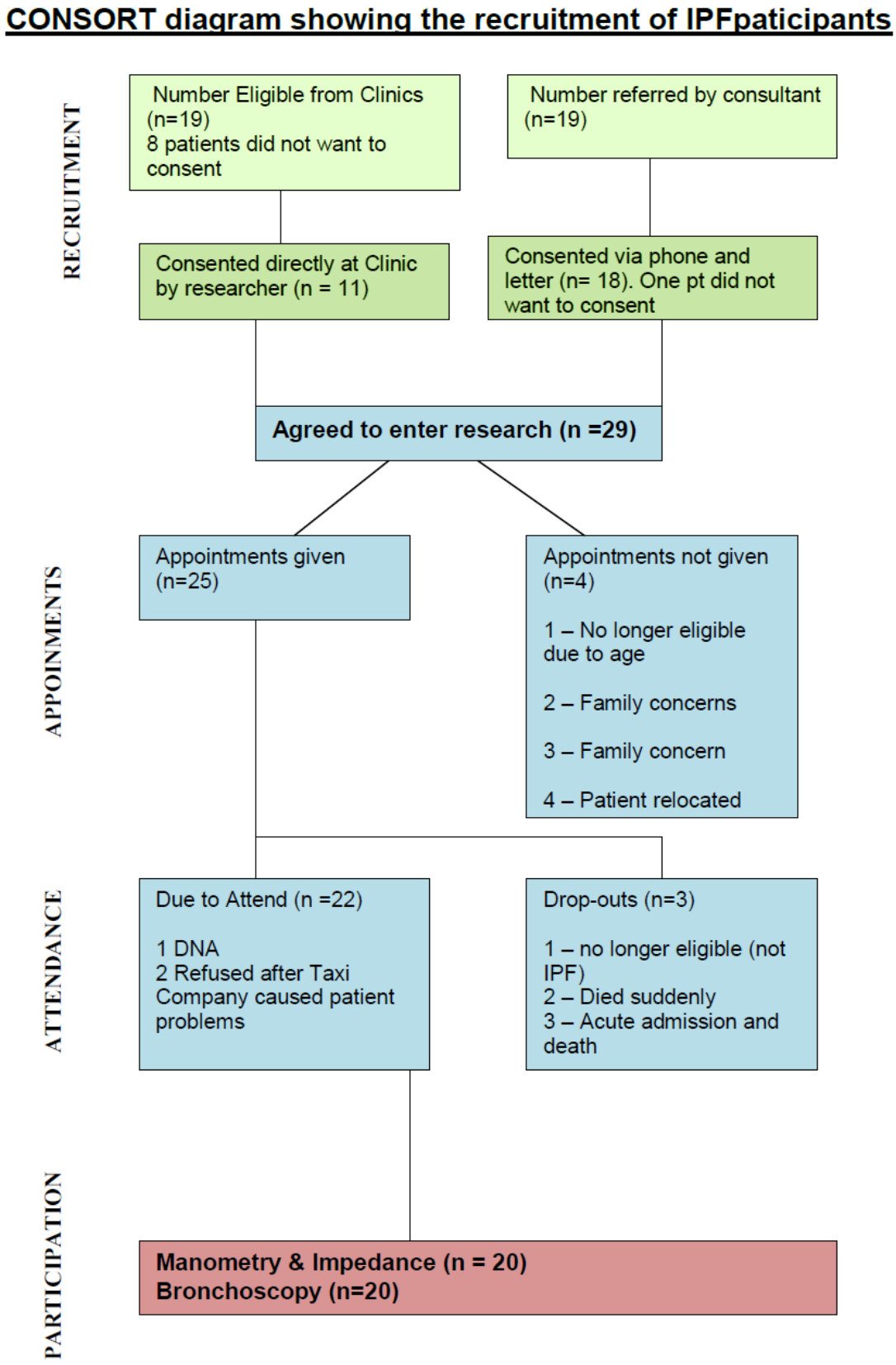
This is summarised in Figure 4- 1 .

The recruitment of IPF patients was initially instigated via the specialist clinic. In July 2010, a specialist IPF clinic was held every 2 weeks with approximately ten patients. Suitable patients for recruitment were selected by the specialist through the study inclusion criteria described in the previous chapter. The principal researcher would approach these patients individually in another clinic room to discuss recruitment into the study. In total 19 patients were approached in this way and 11 consented to the study. After the start of 2011, clinics were reorganised and took place on a weekly basis with several specialists. In this setting it was no longer practical to have the researcher in the clinic setting and the specialist would inform the patient that they would be contacted via telephone. Through this method of referral 19 patients were contacted and only one patient did not consent. In total 29 patients consented to the study.

Of the 29 patients that consented to the study, 4 patients dropped out before an appointment was given for their investigations. Two of the four patients dropped out after family concerns having discussed the study at home. One patient relocated outside the region and was no longer able to participate in the study. The last patient to drop out at this stage was actually a cancellation by the specialist who felt the patient's frailty deemed him unfit for the study investigations

Of the 25 patients given appointments, 3 dropped out and 2 did not attend (DNA). Of the 3 drop outs, two died prior to their appointment date and one patient had their diagnosis changed from IPF to obstructive airway disease and was therefore no longer eligible. Of the 2 DNAs, one was due to a disagreement with the taxi company on the day they were due to attend for their investigations.

Figure 4- 1 : Consort Diagram of IPF patient recruitment



4.3.2 Demographics

Twenty patients were therefore studied (Table 4-1) (14men, 6 women) with a median age of 69 years (range 44-81). Two patients were active smokers at the time of recruitment, six patients stated that they had never smoked and the majority were ex-smokers with a Fangerstorm score of 5-6. This scoring system indicates the level of nicotine dependence with a score over 5 indicating moderate to severe dependence [33]. Only four patients had documented evidence of gastro-oesophageal reflux. Median forced expiratory volume in one second (FEV_1) was 1.94L (Range 1.4-3.55L) and median vital capacity (VC) was 2.53L (Range 1.65-4.35L). Five patients were on steroids and six patients were taking N-acetylcysteine as part of their active IPF treatment. 15/20 patients were taking a proton-pump inhibitor (PPI). All 20 patients completed the investigations.

Table 4-1: Demographics of study patients

	Age	Sex	Smoking status	Fagerstrom Score (SCA)	Known GORD	PPI	Steroids	N-Ace	FEV_1	VC
IPF1	61	male	Current	3	YES	YES	YES	YES	2.45	2.85
IPF2	44	male	Ex-	5	NO	YES	YES	YES	1.95	3.05
IPF3	71	femal	Ex-	6	NO	YES	NO	NO	1.85	2.1
IPF4	81	femal	Ex-	6	NO	YES	NO	NO	1.5	1.65
IPF5	58	male	Ex-	6	YES	YES	NO	NO	3.55	4.35
IPF6	58	male	Never	1	NO	YES	NO	YES	1.82	2.1
IPF7	72	femal	Ex-	6	NO	YES	NO	NO	1.85	2.3
IPF8	47	femal	Ex-	5	NO	YES	YES	NO	1.65	1.9
IPF9	69	male	Never	1	NO	YES	YES	YES	1.4	2.3
IPF10	78	femal	Never	1	YES	YES	NO	YES	1.86	2.23
IPF11	74	male	Ex-	6	NO	YES	NO	NO	2.79	4
IPF12	66	male	Ex-	6	NO	NO	NO	NO	2.99	3.34
IPF13	77	femal	Ex-	6	NO	YES	NO	NO	1.93	2.21
IPF14	72	male	Ex-	6	NO	NO	NO	NO	2.7	3.1
IPF15	73	male	Never	1	YES	YES	NO	NO	1.85	2.16
IPF16	47	male	Never	1	NO	YES	YES	YES	2.31	3.5
IPF17	80	male	Never	1	NO	NO	NO	NO	1.82	2.41
IPF18	65	male	Ex-	6	NO	NO	NO	NO	2.8	3.13
IPF19	65	male	Current	4	NO	YES	NO	NO	2.91	3.38
IPF20	73	male	Ex-	6	NO	NO	NO	NO	2.17	2.64

4.3.3 Oesophageal Manometry

8-channel manometry

11 patients underwent traditional 8 channel manometry as described in the previous chapter. Overall 64% of patients (7/11) had normal oesophageal physiology. No complications were attributed to the procedure.

- Lower oesophageal Sphincter

The median lower oesophageal sphincter length was 4cm (range 3-4cm). Sphincter pressure was within normal limits (6-25mmHg) in the majority of the patients (8/11) with an average sphincter pressure of 21.9mmHg (Range 13-32mmHg). Three patients had a hypertonic LOS and the remaining patients had a normotonic sphincter. Only one patient had complete relaxation of the LOS on swallowing with a median percentage relaxation of 32% (range 0-100%).

- Oesophageal Peristalsis

The median percentage of normal swallows was 90% (range 11-100%). In total 7 patients had normal peristaltic activity (two of these had hypertonic oesophageal peristalsis characterised by high pressure amplitudes), four had non-specific oesophageal dysmotility with 3 of these patients having simultaneous oesophageal contractions in over 20% of the swallows (Figure 4- 2)

Table 4-2: Oesophageal peristaltic amplitudes

	Median (mmHg)	Range (mmHg)	Normal Values (mmHg)
Minimum Oesophageal Amplitude	23	12-51	
Maximum Peristaltic Amplitude	157	104-282	
Average Peristaltic Amplitude	65	40-44	30-180
Distal Oesophageal Amplitude (5cm above the lower oesophageal sphincter)	53	32-109	30-180
Proximal Oesophageal Amplitude (15cm above the lower oesophageal sphincter)	68	22-282	30-180

Median peristaltic amplitudes are shown in Table 4-2. One patient had a hypotonic proximal oesophagus but had a normotonic distal oesophagus. Another patient had a hypertonic proximal oesophagus but they had a normotonic distal oeosphagus. All the other patients had proximal and distal amplitudes within the normal range.

4.3.4 High Resolution Manometry (HRM)

9 patients underwent HRM as described in the previous chapter. Overall 44% of patients (4/9) had normal oesophageal physiology as defined by the Chicago classification. No complications were attributed to the procedure.

- Lower oesophageal Sphincter

The median lower oesophageal sphincter length was 3.9cm (range 2.8-4.3cm). Sphincter pressure was within normal limits (10-45mmHg for HRM) in the majority of the patients (6/9) with an average sphincter pressure of 17.9mmHg (Range 1.7-51mmHg). Two patients had a hypotonic LOS, one patient had a hypertensive LOS and the remaining patients had a normotonic sphincter. In addition, HRM provided details of the intra-abdominal length of LOS and the presence of a hiatus hernia. The median intra-abdominal length of LOS was -1.8cm (a negative value simply implies that the LOS lies above the true pressure inversion point i.e. Suggestive of a hiatus hernia and

is thus NOT intra-abdominal). Six patients (66.7%) had hiatus hernias detected on HRM with a mean hernia length of 2.8cm.

- Oesophageal Peristalsis

The characterisation of the oesophageal peristalsis was determined by a set of measurements taken on HRM as described in table 3.3. The median percentage of normal swallows was 93% (range 7 -100%). In total 4 patients the contraction pattern was normal in 80-100% of swallows. The remaining 5 patients had a mixture of rapid and premature contractions. In four patients there was intact peristalsis in 100% of swallows. The Chicago classification of the oesophageal motility in these 9 patients is shown below (Figure 4- 3).

Table 4-3: HRM key results

	Median	Range	Normal Values
Distal Latency (DL) - s	6.4	5 – 7.8	>4.5
Distal Contractile Integral (DCI) – mmHg.s.cm	488	160 – 2088	<8000
Peristaltic Breaks - cm	0.7	0 – 5.7	<2cm
Integrated Relaxation Pressure (IRP4s) - mmHg	5	-3.8 – 15.1	<15

Figure 4- 2 : Oesophageal Peristalsis

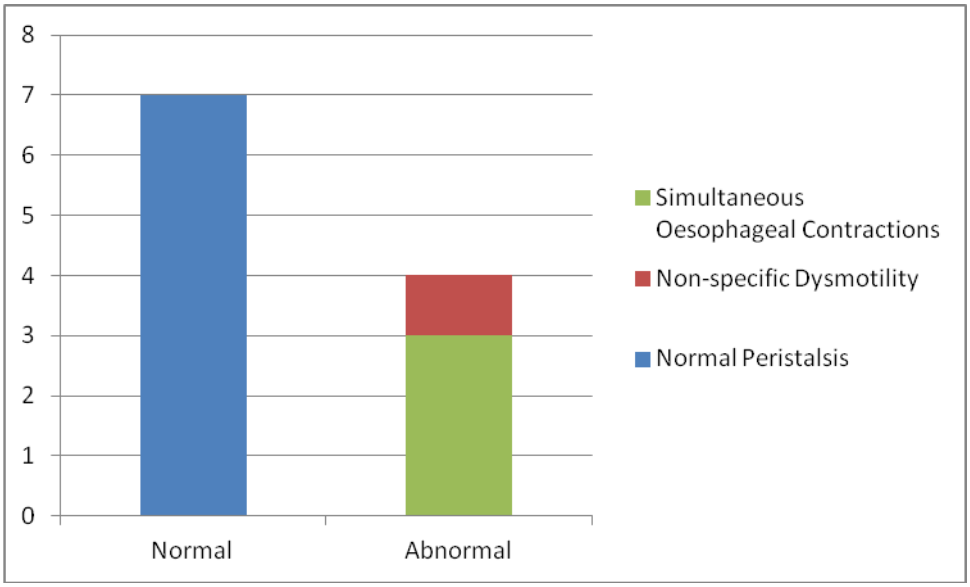
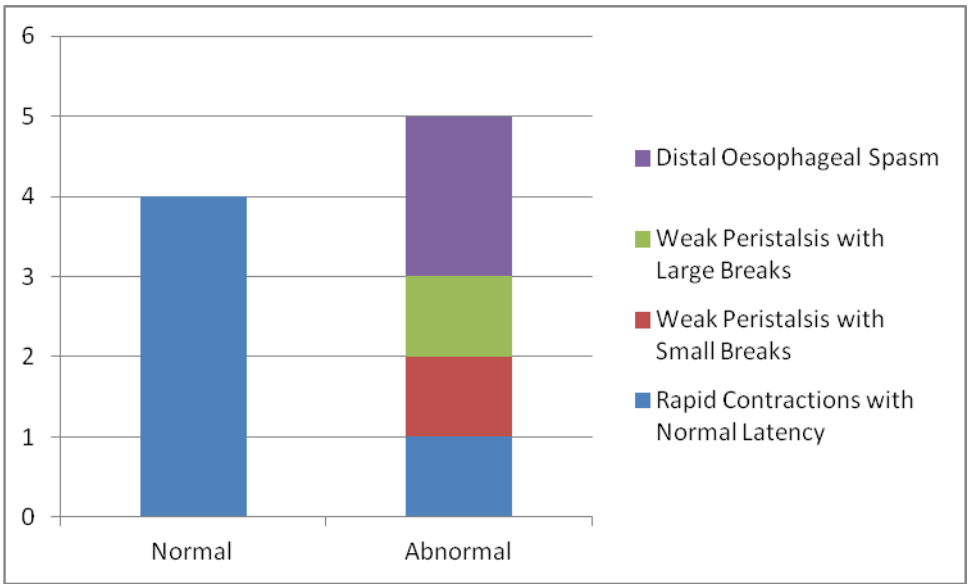


Figure 4- 3 : HRM Oesophageal Peristalsis



In summary just under half the patients (9/20) with IPF demonstarted abnormal oesophageal motility on manometry

4.3.5 Reflux Data

Reflux Questionnaires

Fifteen of the twenty IPF patients were taking PPIs at the time of recruitment. The doses are listed below in Table 4-4. Patients were requested to stop their PPI for 2 weeks prior to the oesophageal physiology investigations. All 15 patients were compliant with this request. Questionnaires were completed by the patient 'ON' and 'OFF' PPI. The median daily dose of lansoprazole was 30mg (Range 15 – 60mg) and omeprazole was 20mg (Range 10-80mg). The total daily dose of PPI were compared to reflux questionnaire scores having adjusted the dosages for lansoprazole to omeprazole equivalents for purpose of comparison; 15mg lansoprazole = 20mg omeprazole, 30mg lansoprazole = 40mg omeprazole and 60mg lansoprazole = 80mg omeprazole [150].

Table 4-4: The variation of PPI dosage in study patients

PPI or H2 Receptor Antagonist Dose	Number of Patients
No Medication	**5**
lansoprazole 30mg od	**6**
lansoprazole 15mg od	**1**
lansoprazole 15mg bd	**1**
lansoprazole 30mg bd	**2**
omeprazole 10mg od	**1**
omeprazole 20mg od	**3**
omeprazole 40mg bd	**1**

The RSI questionnaires were completed by 19 patients prior to their investigations. One patient did not complete this questionnaire and so was excluded from the analysis. All 15 patients who were on PPI therapy completed the RSI questionnaire before the investigation having stopped their medication for 2 weeks before. Eight patients (42%) had a positive RSI score (RSI>13).The median RSI score was 10 (Range 0 to 39). The 15 patients on PPI therapy completed a questionnaire whilst on their treatment. Whilst on their PPI nine patients (60%) had a positive RSI score. The median score was 18 (range 4 to 32). The differences in RSI score 'on' and 'off' PPI did not reach statistical significance (p=0.45).Therefore, a greater proportion of patients had symptomatic reflux

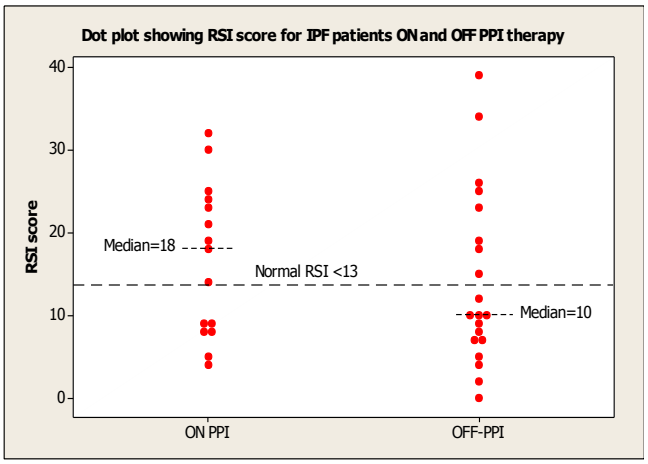
as determined by the RSI despite taking PPI medication. Figure 4- 4 i shows the RSI scores for the IPF patients ON and OFF their PPI. For these patients the median difference of the RSI score on and off PPI was +1 (range -30 to 17). For the 15 patients on PPI no significant relationship was demonstrated between RSI score and the daily dose of PPI (P = 0.645). The scatter plot (Figure 4- 5 i) demonstrates no clear relationship to indicate higher PPI dose reduces RSI score.

The Demeester questionnaires were completed by all 20 patients prior to their investigations. All 15 patients who were on PPI therapy completed the Demeester questionnaire before the investigation having stopped their medication for 2 weeks before. The median Demeester score was 2 (Range 0 to 7). The 15 patients on PPI therapy completed a questionnaire whilst on their treatment. Whilst on their PPI the median score was 2 (range 0 to 5). Therefore, the patients on PPI gained no additional symptom improvement as determined through the Demeester questionnaire. Figure 4- 4 ii shows the Demeester questionnaire scores for the IPF patients ON and OFF their PPI. For these patients the median difference of the Demeester score on and off PPI was -1 (range -4 to 5). Figure 4- 5 ii shows that for these 15 patients on PPI no significant relationship was demonstrated between Demeester score and the daily dose of PPI (P = 0.231).

The GIQLI questionnaires were completed by all 20 patients prior to their investigations. All 15 patients who were on PPI therapy completed the GIQLI questionnaire before the investigation having stopped their medication for 2 weeks before. Fifteen patients (75%) had a score below the normal range (121-130). The median GIQLI score was 95 (Range 49 to 138), indicating health-related quality of life specific to the gastrointestinal system was much lower in the IPF patient group. The 15 patients on PPI therapy completed a questionnaire whilst on their treatment. Whilst on their PPI Thirteen patients (87%) had a GIQLI score below the normal range (121-130). The median score was 108 (range 60 to 135). The differences in GIQLI score ‘on’ and ‘off’ PPI did not reach statistical significance (p=0.41). Therefore, PPI therapy makes very little difference to the quality of life of these individuals. Figure 4- 4 iii shows the GIQLI scores for the IPF patients ON and OFF their PPI. For these patients the median difference of the GIQLI score on and off PPI was 16 (range -41 to 51). Figure 4- 5 iii shows that for the 15 patients on PPI no significant relationship was demonstrated between GIQLI score and the daily dose of PPI (P = 0.595).

Figure 4- 4 : Dot plots showing: i) RSI score (y-axis) for IPF patients ON and OFF PPI therapy (x-axis). The dotted line indicate the upper limit of mormal, abovbe this indicate abmormal RSI scores. ii) Demeester Score (y-axis) for IPF patients ON and OFF PPI therapy (x-axis) iii) GIQLI score (y-axis) for IPF patients ON and OFF PPI therapy (x-axis) The dotted lines indicate the upper and lower linits of the normal GIQLI score, and values below the lower line are abnormal.

i)



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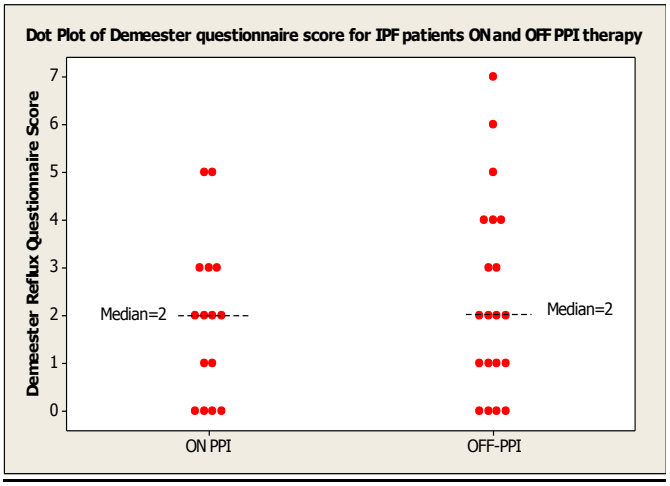

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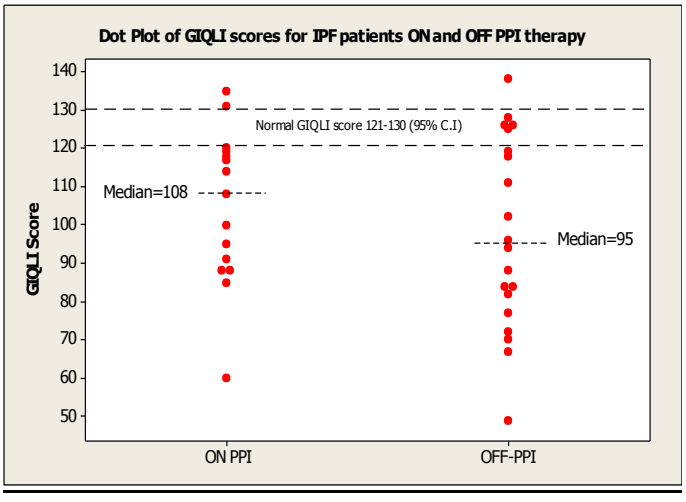
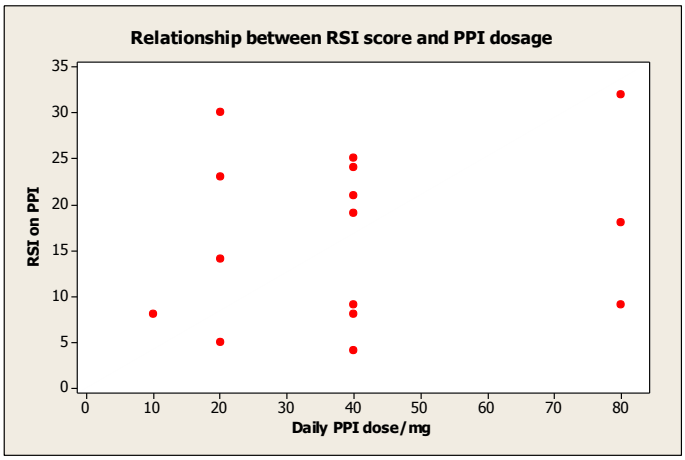


Figure 4- 5 : Scatterplots showing: i) the relationship between the daily dose of PPI (x-axis) and RSI score (y-axis) ii) the relationship between the daily dose of PPI (x-axis) and Demeester score (y-axis) iii) the relationship between the daily dose of PPI (x-axis) and GIQLI score (y-axis)

i)



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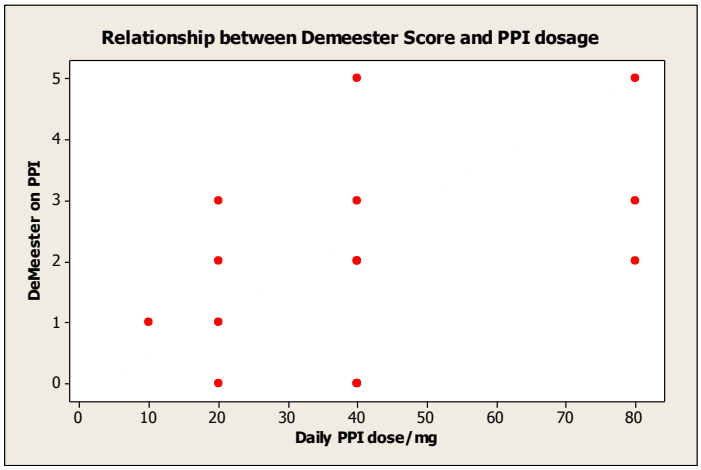
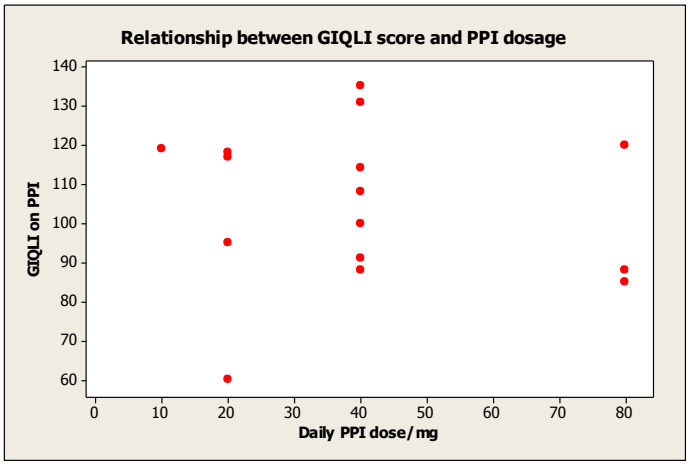


iii)



pH – Impedance

All twenty IPF patients completed the 24 hour recordings. Of the twenty patients, 60% patients had evidence of reflux as determined by an abnormal Demeester score (Figure 4- 6). A summary of the median reflux indices per 24 hours for the pH part of the study are shown in the table below (Table 4-5). Most refluxes were in the upright rather than supine position (62% vs. 38%).

Table 4-5: Median Reflux Indices for pH part of study

	Median	Range	Normal Values	No. of patients with abnormal results
Demeester Score	**20.7**	**0.2-201.6**	**<14.72**	**12/20**
Acid Exposure (%). (% of time pH<4, in 24hrs)	**7**	**0-60**	**<4.2**	**12/20**
Number of Reflux Periods in 24 hours	**58.1**	**0-326.7**	**<50**	**11/20**
Number of long Refluxes /24hours (>5min)	**4.15**	**0-39.4**	**<4**	**10/20**
Longest Reflux	**15.5**	**0-164.3**	**<9.2**	**14/20**

A summary of the median reflux indices as detected by oesophageal impedance is shown in table 3-6. Just over half the patients (60%) had reflux on impedance. Seven patients had weakly acid reflux. Two patients had abnormal amounts of both acid and weakly acid reflux. Six of the twenty patients had abnormal proximal (Figure 4- 7) oesophageal reflux (30%). Of these six, four had abnormal Demeester scores.

The majority of reflux events confirmed from impedance analysis were in the upright rather than in the supine position (medians 33.15 vs. 3.4), but these are within the normal range for a 24 hour period. However, in these 20 patients nine had an abnormal number of supine events compared to only 5 patients with an abnormal number of upright events. Most proximal reflux events were in the upright position 8.6 (0-37.3) vs. 1.1 (0-10.6). The majority of reflux events were mixed (liquid and gas) 26.3 (7.5-89.8) vs. 8.75 (0-42.1) for liquid reflux alone. There is a positive correlation between the proximal reflux score and the number of liquid and mixed reflux events (Figure 4- 8).

The correlation is significant for the number of mixed events and proximal reflux score ($p<0.005$).

Table 4-6: Median Reflux Indices as demonstrated by Oesophageal Impedance

	Median	Range	Normal Values	No. of patients with abnormal results
Oesophageal Volume Exposure (%)	**0.63**	**0.15-1.75**	**0.4 -1.2**	**4/20**
Total Number of Reflux events/24hours	**37.45**	**10.8-119.20**	**25-58**	**6/20**
Number of Acid Refluxes/24 hours	**17**	**0-86.8**	**10-35**	**5/20**
Number Weakly Acid Refluxes/24hours	**11.25**	**0-89.8**	**5-18**	**7/20**
Bolus Clearance Time (s)	**11.5**	**5.5-17.5**	**8-13**	**6/20**
Proximal Reflux Events	**11.15**	**0-44.5**	**4-17**	**6/20**
Liquid Reflux Events	**8.75**	**0-42.1**	**10-32**	**1/20**
Mixed Reflux Events	**26.3**	**7.5-89.8**	**11-26**	**10/20**
Upright Reflux Events	**33.15**	**0-101.1**	**23-52**	**5/20**
Supine Reflux Events	**3.4**	**0-22.7**	**1-6**	**9/20**

Two patients with a positive RSI score (RSI>13) had pathological proximal reflux; Six patients with a positive RSI had no pathological proximal reflux. Four patients with a negative RSI score had abnormal proximal reflux and eight patients had a negative RSI score and a proximal reflux score which fell within the normal range (<17) (Table 4-7).

Table 4-7: The predictive value of the RSI score for proximal reflux

	Proximal Reflux	No proximal reflux	
RSI positive	**2**	**6**	**PPV= 25%**
RSI negative	**4**	**8**	**NPV= 67%**
	Sensitivity= 33%	**Specificity= 43%**	

PPV= Positive Predictive Value, NPV=Negative Predictive Value

No correlation existed between RSI and the Demeester score (P = 0.419) (Figure 4-9 i). In addition, no significant correlation existed between RSI score and proximal reflux measured on oesophageal impedance (P = 0.971). (Figure 4-9 ii).

Manometry to reflux indices

No significant relationship was demonstrated between the length of the LOS and both distal and proximal reflux scores (P= 0.863 and P= 0.712 respectively) (Figure 4-10). The correlations between the LOS resting pressure and Demeester or proximal reflux (Figure 4-11) were not significant (P = 0.801 and P = 0.466 respectively). Of the nine IPF patients who had HRM, distal and proximal reflux did not appear to correlate to intra-abdominal LOS length (P = 0.765 and P = 0.286 respectively).

Relationship between use of PPI therapy and reflux symptoms

Automatic symptom analysis using the MMS software could not be performed due to poor compliance of patients with the symptom button and diary. Symptoms were studied using the questionnaires only. Of the 15 patients who completed the initial symptom questionnaires whilst taking PPI therapy, 60% had an elevated RSI score (>13). No difference was seen in the Demeester questionnaires scores when these patients completed the questionnaire 'on and 'off' their PPI. GIQLI assessment showed 85% had below normal scores whilst on their PPI. The indications from the symptom scores suggest very little improvement whilst taking the PPI.

Figure 4- 6 : Graph Showing Patient Demeester Scores

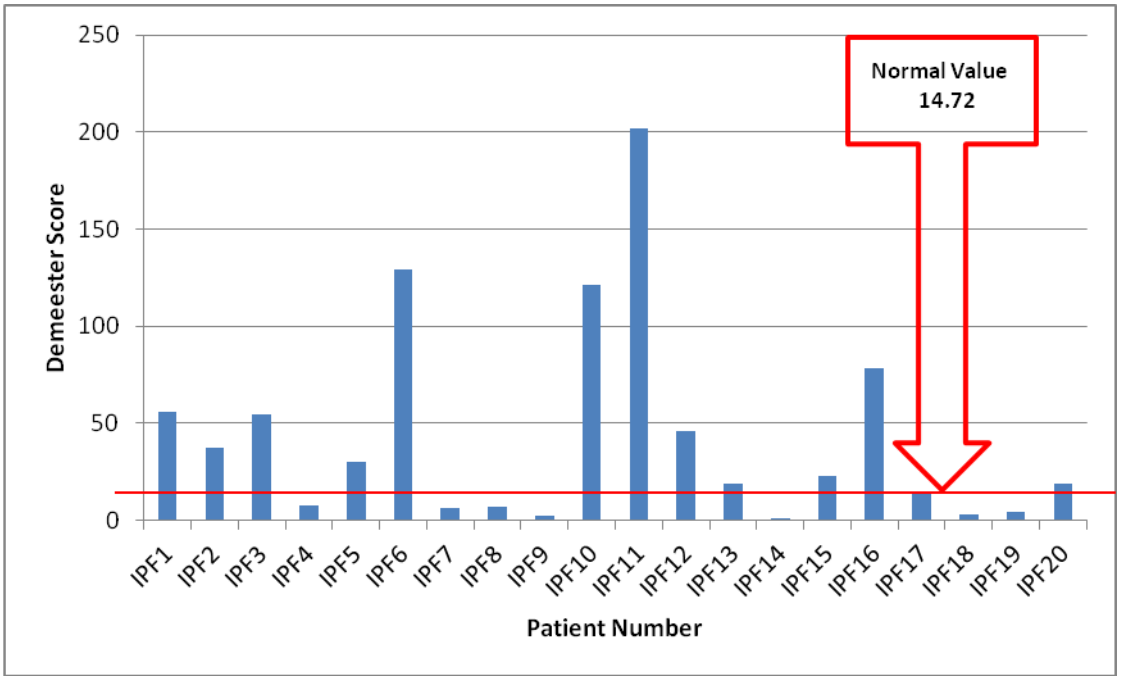


Figure 4- 7 : Graph showing proximal Relfux scores

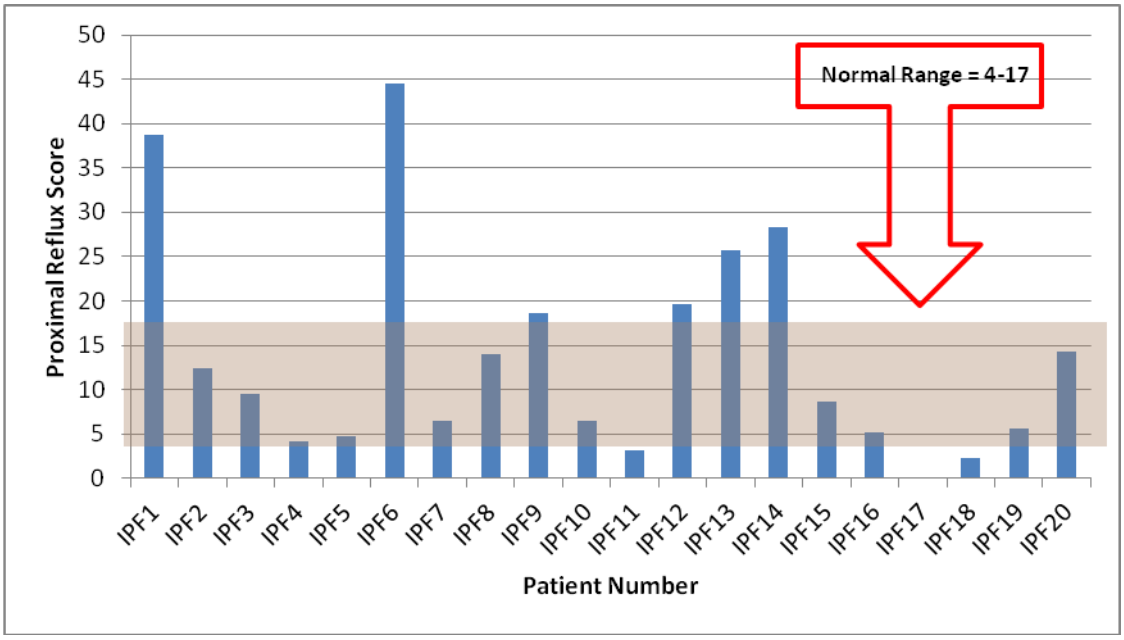
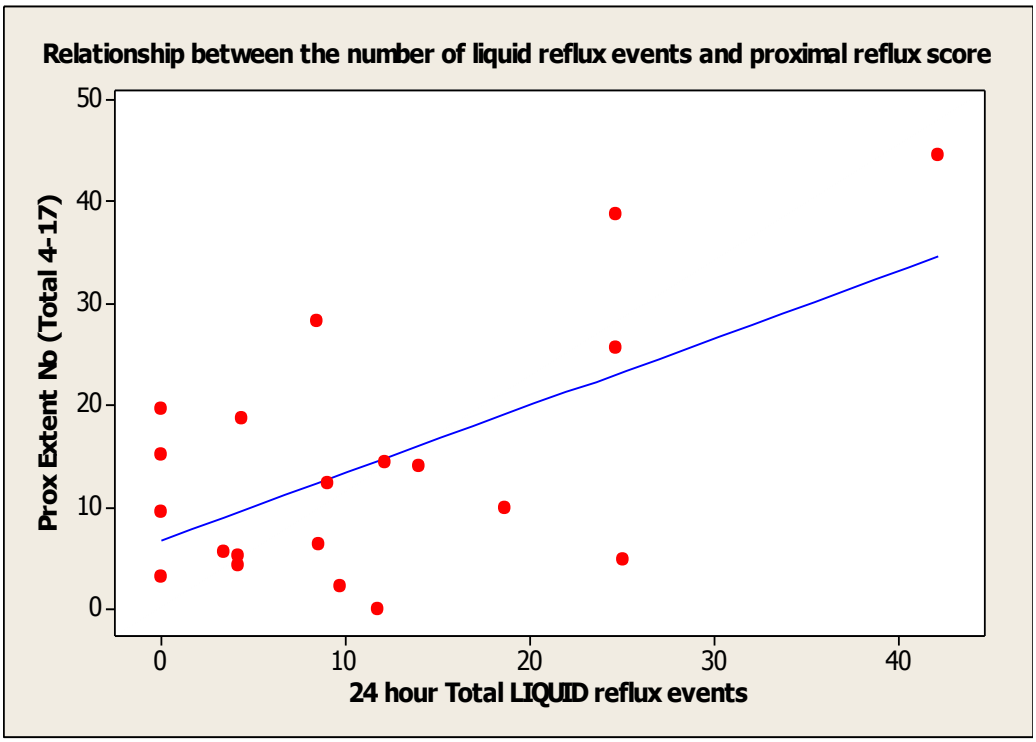


Figure 4- 8 : Scatter plots showing: i) the relationship between liqid reflux events (x-axis) and proximal reflux (y-axis) ii) the relationship between mixed reflux events (x-axis) and Proximal Reflux (y-axis).

i)



ii)

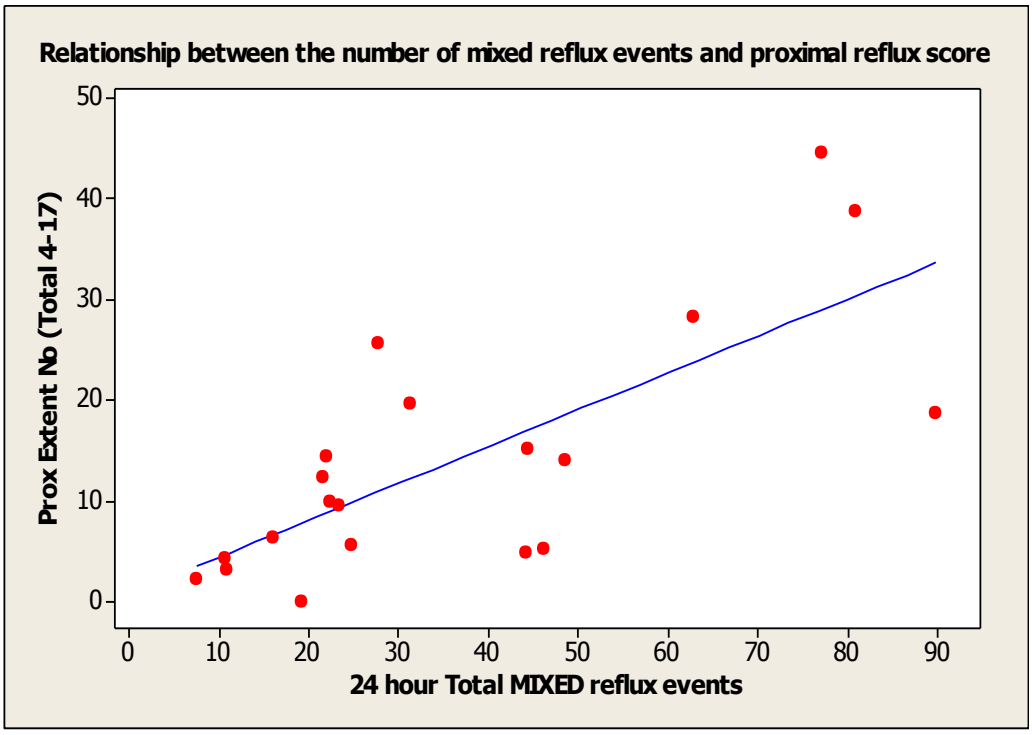
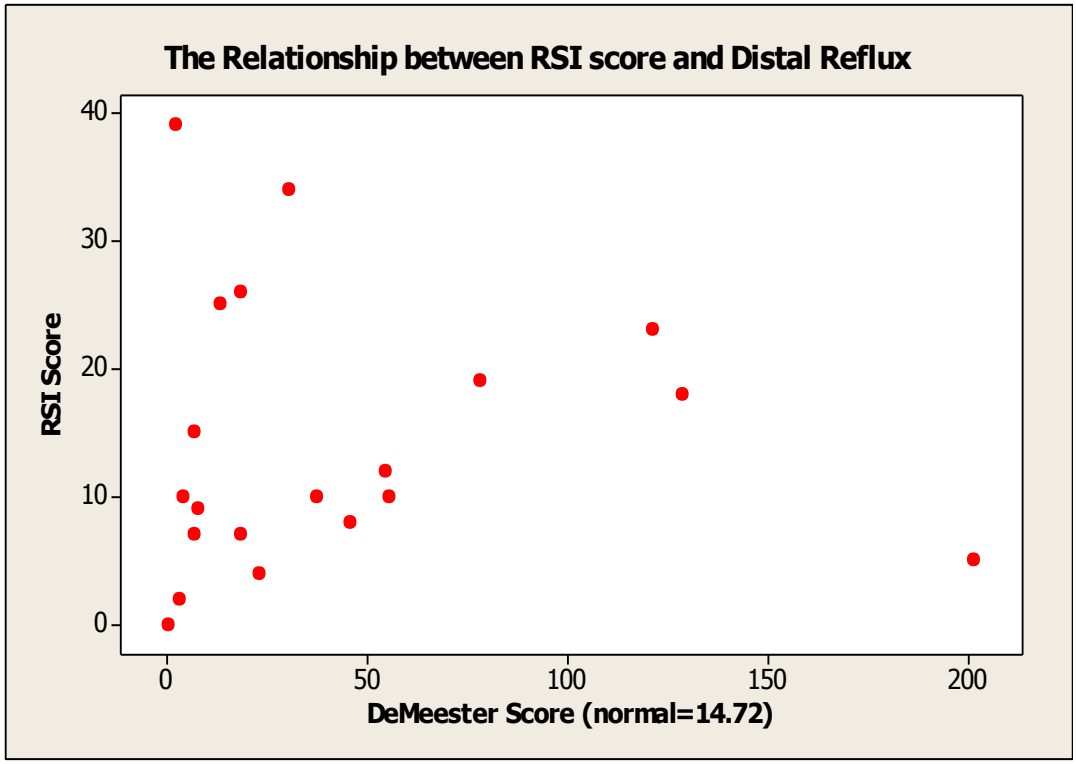


Figure 4- 9 : Scatter plots showing: i) the relationship between the RSI score (x-axis) and distal reflux as defined by Demeester score (y-axis) ii) the relationship between the RSI score (x-axis) and ProximalReflux (y-axis).

i)



ii)

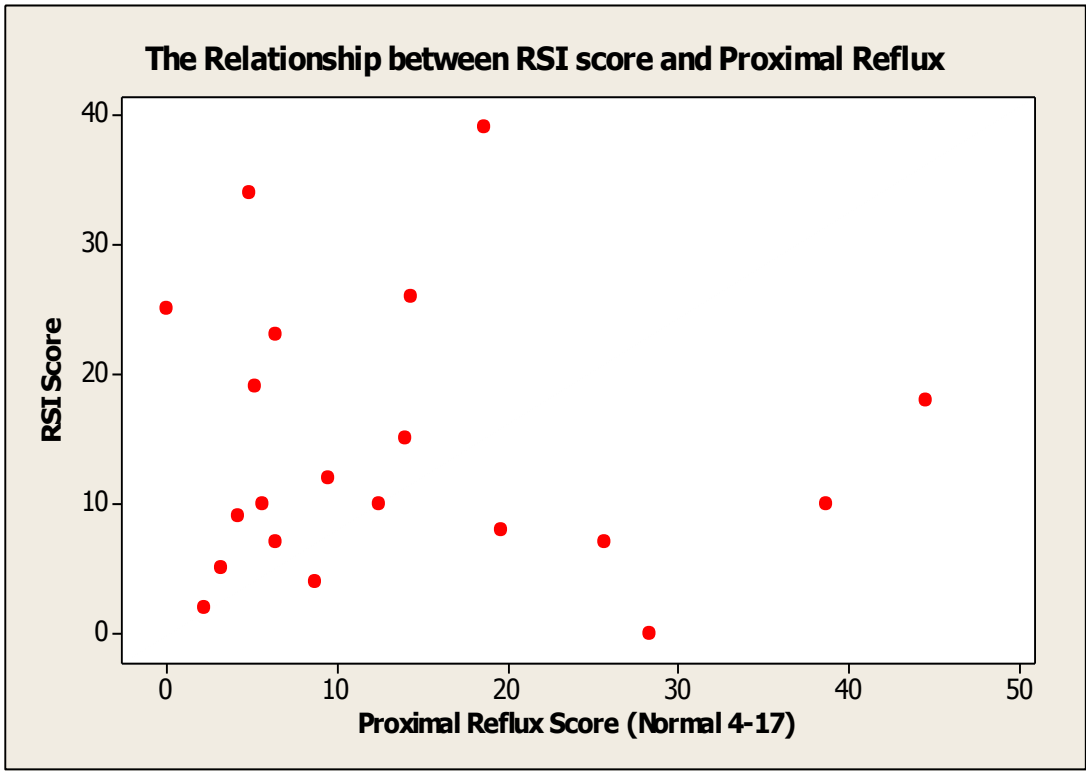
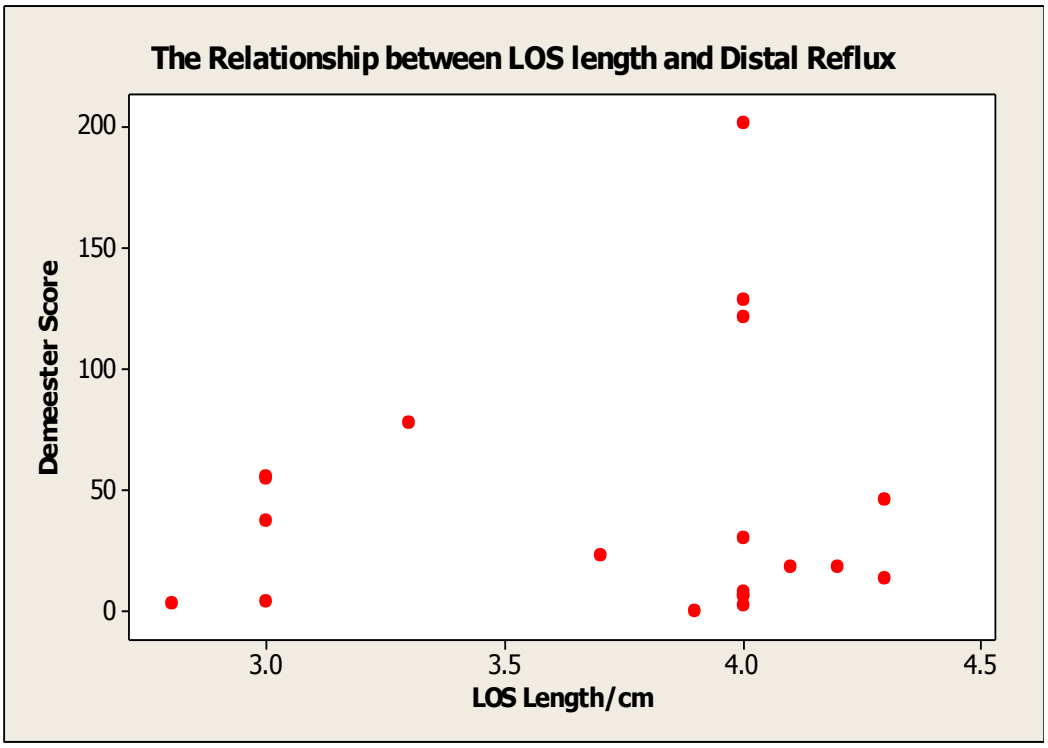


Figure 4-10 : i) The relationship between LOS length (x-axis) and distal reflux as indicated by Demeester score (y-axis); ii) the relationship between LOS length (x-axis) and proximal reflux (y-axis)

i)



ii)

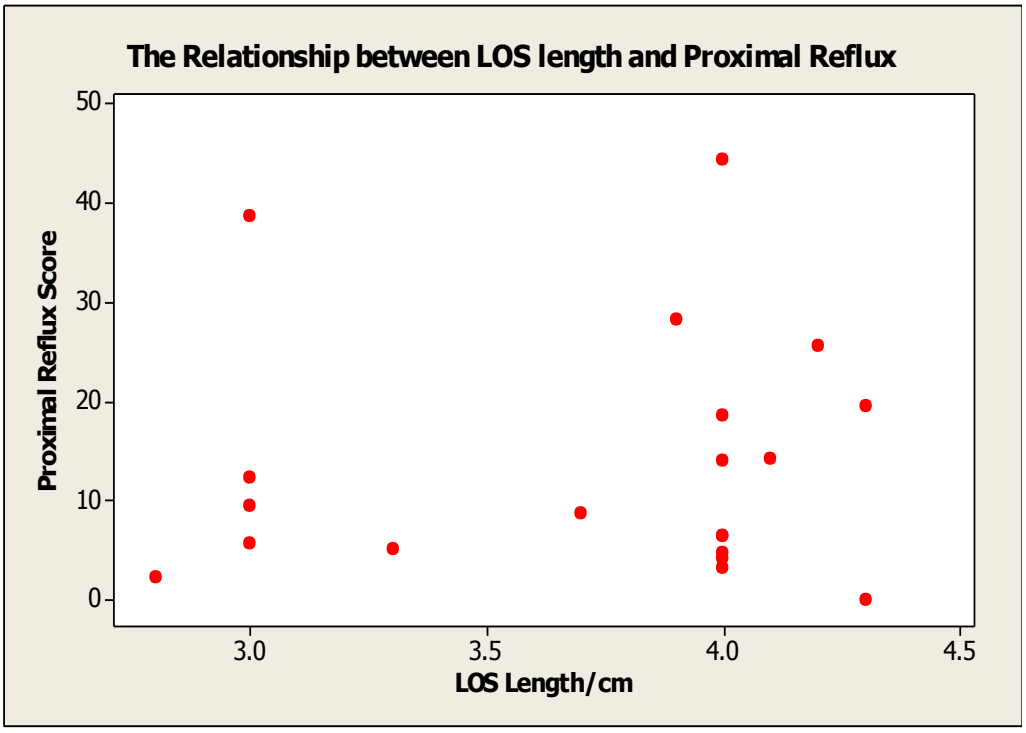
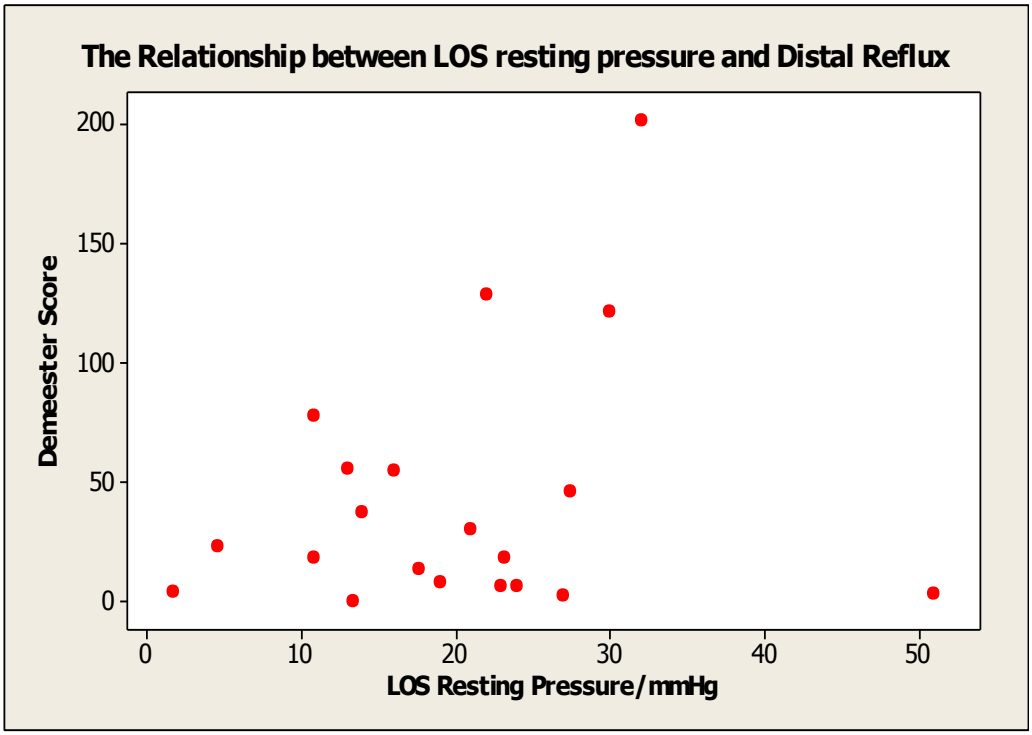
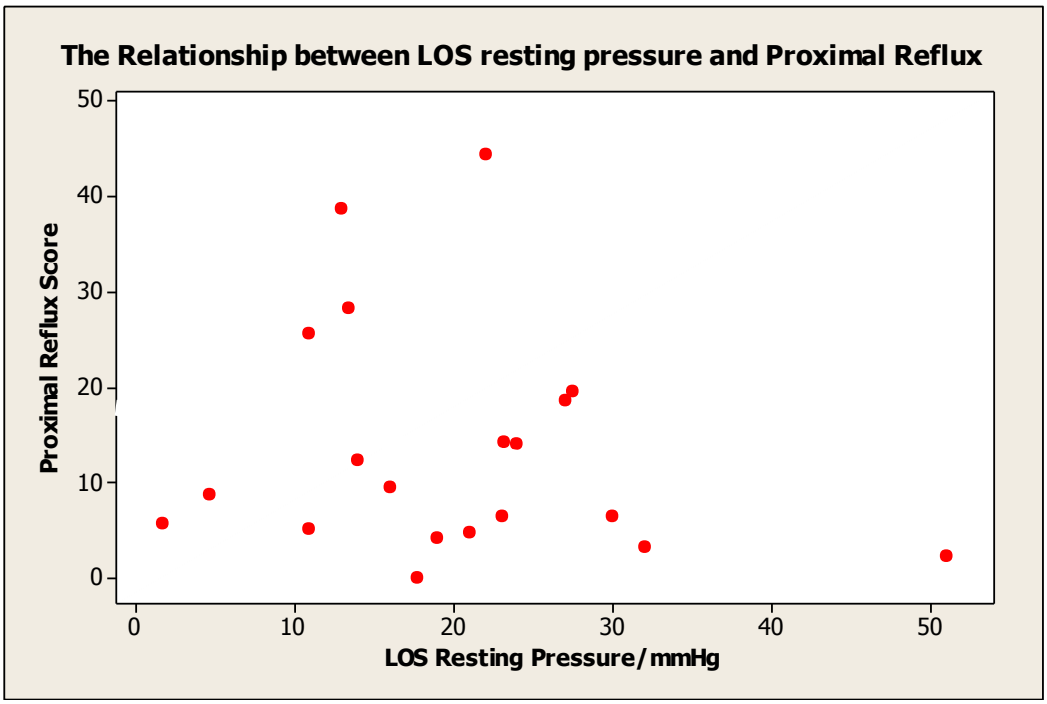


Figure 4-11: i) The relationship between LOS pressure (x-axis) and distal reflux as indicated by Demeester score (y-axis); ii) the relationship between LOS pressure (x-axis) and proximal reflux (y-axis)

i)



ii)



4.3.6 Lavage Processing Data

Cell Counts

All 20 patients with IPF successfully completed a bronchoalveolar lavage (BAL) under sedation as a day case patient with no complications post procedure. All lavages were performed with a standard 3 x 60ml (180ml) 0.9% saline solution. The median volume of BAL return was 90ml (Range 45 -120ml). All lavages were successfully processed and the differential cell counts performed and shown in the table below (Table 4-8).

Table 4-8: The median total cell and differential cell count

	IPF patients	Normal Values [151]
Total BAL cell count (cellsx10^4/ml)	**16.8 (1.8–236)**	**14 (12-16)**
Neutrophils (%)	**7.5 (1-56)**	**2.1 (1.6-2.6)**
Lymphocytes (%)	**3 (1-58)**	**20 (14-26)**
Macrophages (%)	**83 (34-97)**	**73 (66-80)**
Eosinophils (%)	**2.5 (0-12)**	**1.1 (0-2.2)**

There were increased percentages of neutrophils, macrophages and eosinophils but decreased levels of lymphocytes when compared to stable controls [151]. No correlation existed between the percentages of neutrophils, macrophages or eosinophils and proximal reflux score (P= 0.705, P= 0.620 and P=0.449 respectively).

Cell Stains

Table 4-9: Haemosiderin and Oil Red stain median values

	IPF patients	Normal Values [148, 152]
Haemosiderin stained macrophage %	**20 (2-98.5)**	**0 (0-1.5)**
Haemosiderin Score	**31.8 (3 -236.5)**	**0 (0-2)**
Oil Red (lipid laden macrophage) %	**3.3 (0-47)**	**2.63 (0-20)**
Oil Red Positive Macrophage Score	**15.5 (0-310)**	**5.47(0-49)**

All 20 IPF patients had an elevated haemosiderin (HS) score outside the range seen for normal subjects as described by *Reid et al* [148] (Table 4-9). However, there was no

significant correlation to either proximal or distal reflux scores (P= 0.734 and P= 0.295 respectively) or total reflux episodes detected on impedance (P= 0.405) or pH analysis (P= 0.444). (Figure 4-13)

All 20 IPF patients were also scored with regard to lipid laden macrophages as detected by Oil Red staining. The median percentage of Oil Red positive macrophages and median score were both above the values observed in a control population [152] (Table 4-9). However, only 5/20 patients had a lipid laden macrophage score outside the normal range. There was no significant correlation to either proximal or distal reflux scores (P= 0.592 and P= 0.942 respectively) or total reflux episodes detected on impedance (P= 0.781) or pH analysis (P= 0.678) (Figure 4-14).

In summary, all 20 IPF patients successfully completed bronchoscopy and lavage. All BAL samples were processed to produce cytospins and differential cell counts and specific stains as described above were performed on all patient samples. Table 4-9 summarises the individual patient results and Figure 4-15 illustrates the Haemosiderin (HS) and Oil red (OR) percentages found in the IPF patient group when compared to normal controls (median values). Actual slide photos are shown in Figure 4-16.

Table 4-10: Summary of cell processing, differential counts and stain scores for IPF 1-20.

Patient No	BAL Vol/ml vo/ml/mlused/ml	Cell Count(x10^4/ml)	%M	%L	%N	%E	HS score	OR-score
IPF1	35	32.5	48	2	38	12	19.5	52
IPF2	90	22	86	4	8	2	236.5	23
IPF3	80	16	84	3	9	4	4.5	6
IPF4	75	2.5	41	18	31	10	19.5	8
IPF5	90	23.3	89	3	3	5	9.5	139
IPF6	80	13.6	88	9	2	1	8	0
IPF7	90	46.75	82	10	6	2	67	13
IPF8	77.5	17.5	91	2	7	0	115.5	57
IPF9	95	236	57	28	15	0	89	12
IPF10	47	22	43	31	24	2	11.5	34
IPF11	55	14.32	36	58	3	3	109.5	30
IPF12	85	15.3	89	1	3	7	9	0
IPF13	105	8.6	74	9.5	16	0.5	207.5	73
IPF14	40	27.25	54	5	36	5	6.5	0
IPF15	75	5.55	86	3	5	6	44	7
IPF16	45	5.86	78	1	9.5	11.5	3	18
IPF17	86	19.3	94	2	3	1	46.5	11
IPF18	52	1.82	34	1	56	9	71.5	5
IPF19	75	30.13	97	2	1	0	4.5	310
IPF20	82	6.71	96	1	3	0	74.5	24

KEY: %M=macrophages, %L=lymphocytes, %N=neutrophils, %E=eosinophil

Figure 4-１２: Dot Plot to illustrate the haemosiderin score and Oil Red score in IPF patients (n=20) with the upper limit of the normal values indicated.

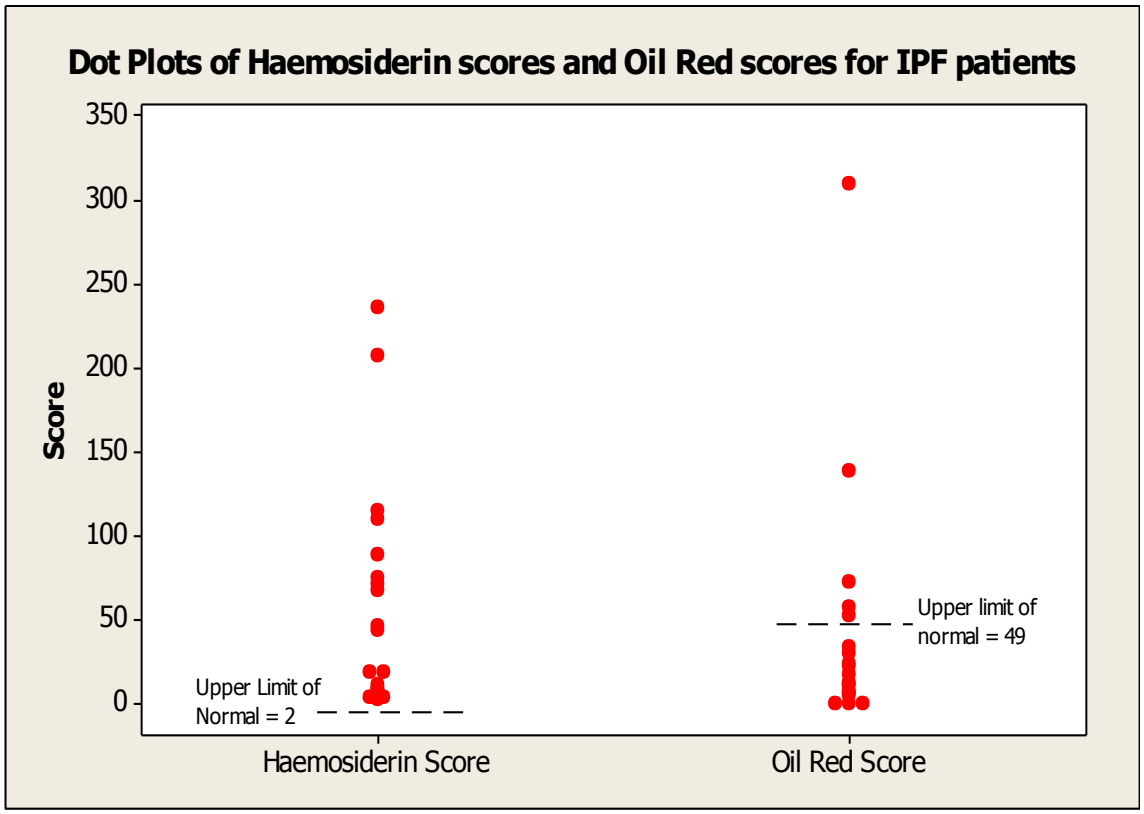
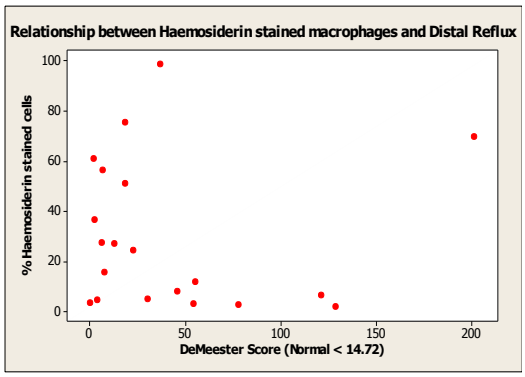
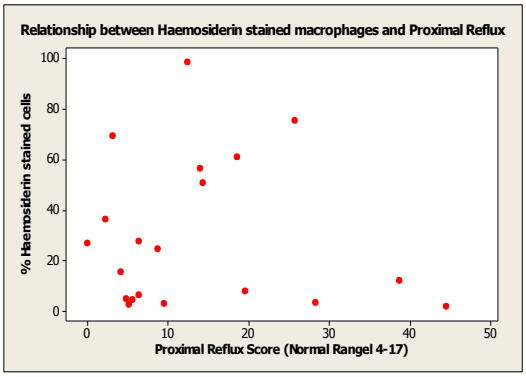


Figure 4- 1 3 : Scatter plots of reflux parameters versus percentage of Haemosiderin stained macrophages (in % 200 macrophages; n =20) i) distal reflux as defined by Demeester score versus % Haemosiderin stained macrophages; ii) proximal reflux score versus % Haemosiderin stained macrophages; iii) Total reflux periods on impedance versus % Haemosiderin stained macrophages; iv) Total reflux periods on pH analysis versus % Haemosiderin stained macropahges.



i)



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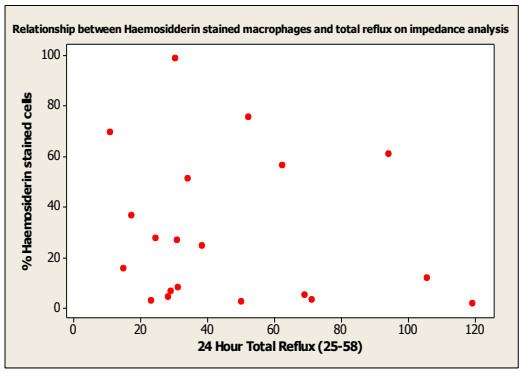
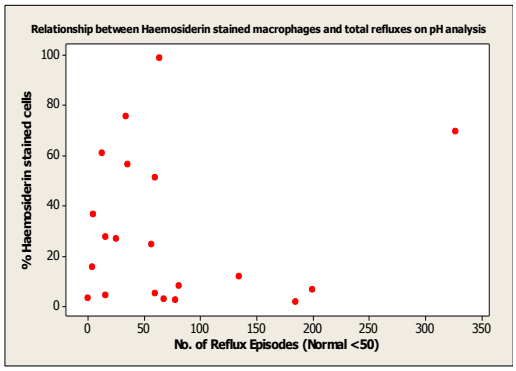
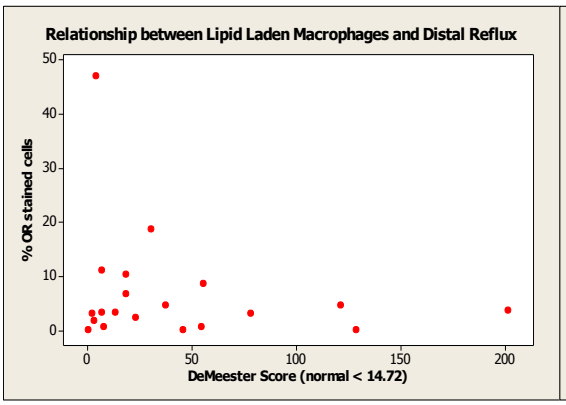


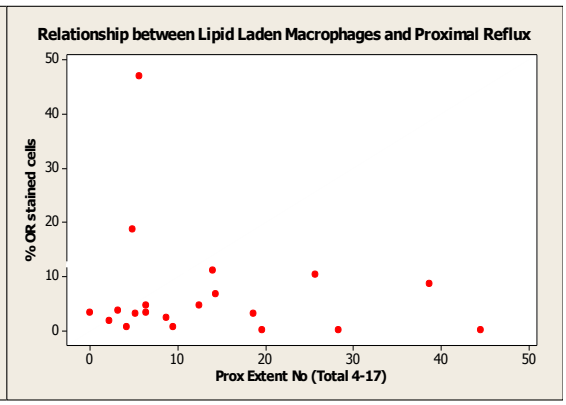
iii)



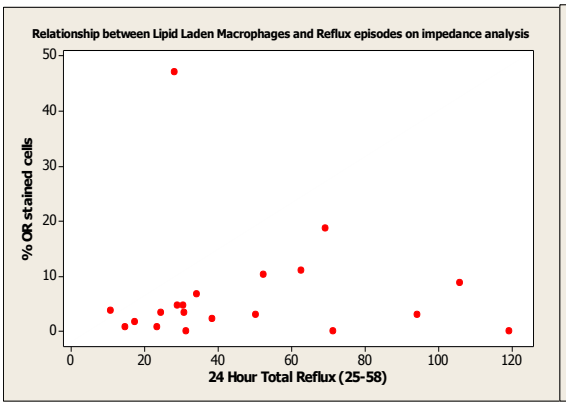
iv)

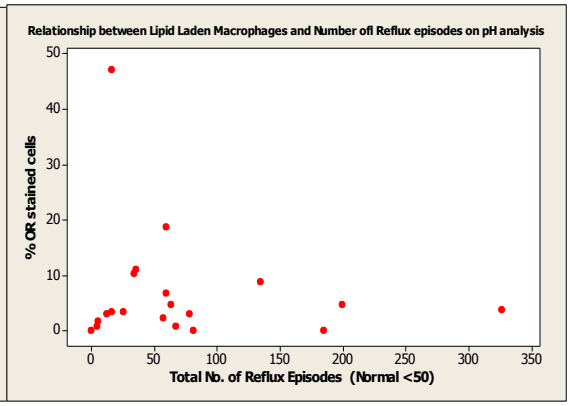
Figure 4- 1 4 : Scatter plots of reflux parameters versus percentage of Oil Red stained macrophages (in % of 300 macrophages; n =20) i) distal reflux as defined by Demeester score versus % Oil red stained macrophages; ii) proximal reflux score versus % Oil red stained macrophages; iii) Total reflux periods on impedance versus % Oil red stained macrophages; iv) Total reflux periods on pH analysis versus % Oil red stained macrophages.





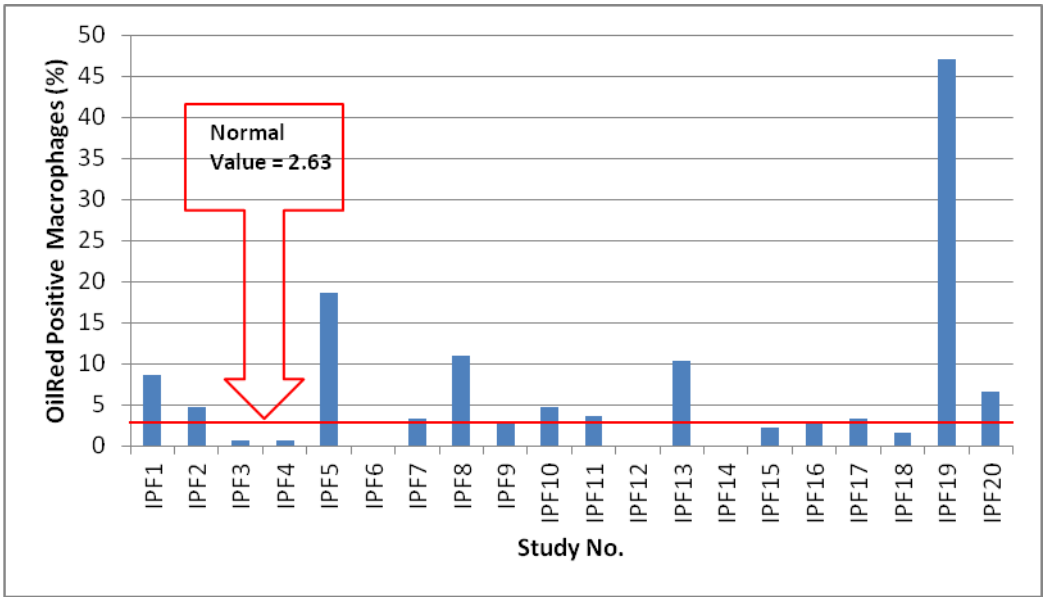
i) ii)



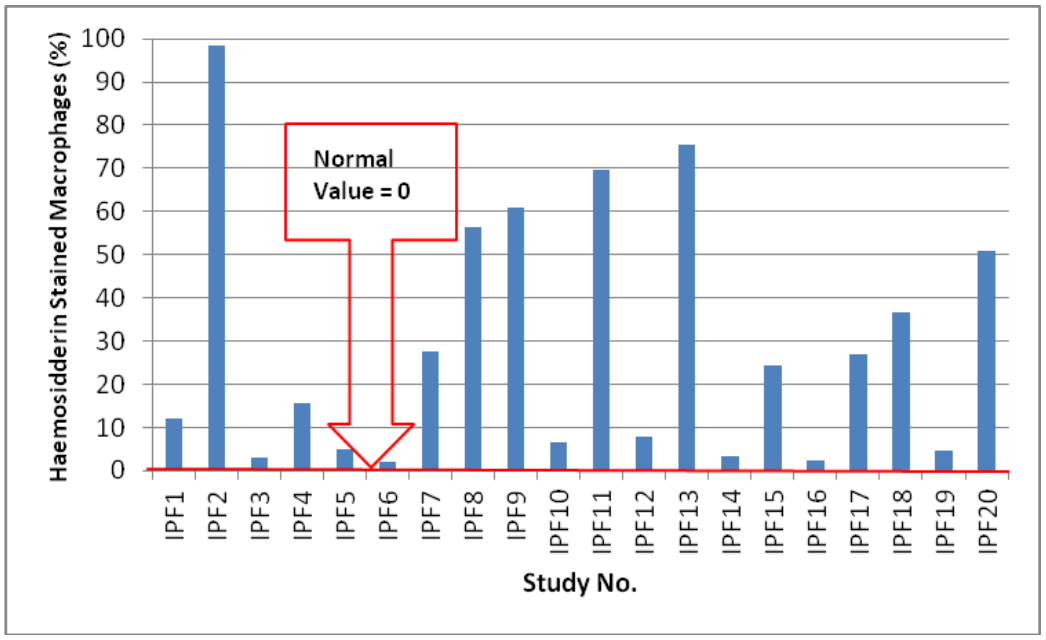


iii) iv)

Figure 4-15 : i) Graph showing percentage of Oil Red positive macrophages (y-axis) for each IPF patient compared to the percentage seen in a control population [152]; ii) Graph showing percentage of Haemosidderin positive macrophages (y-axis) for each IPF patient compared to the percentage seen in a control population [148]

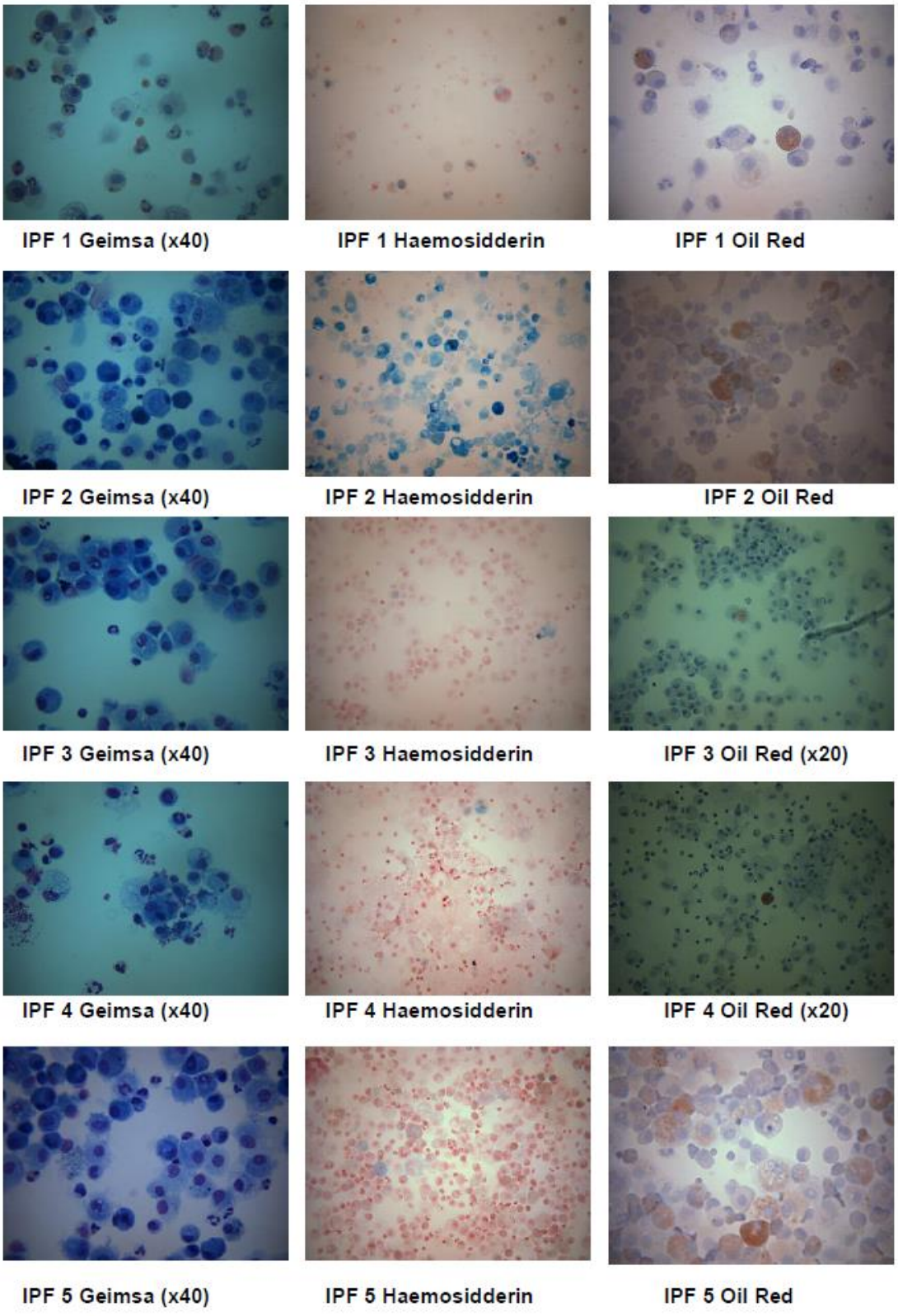


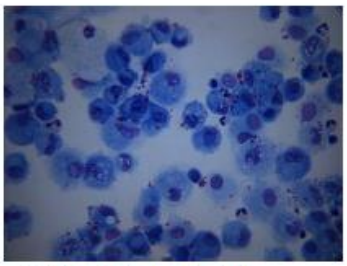
i)



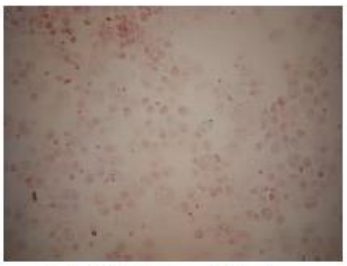
ii)

Figure 4-１６: Slide pictures showing Geimsa, Haemosidderin and Oil Red Staining for IPF 1-20

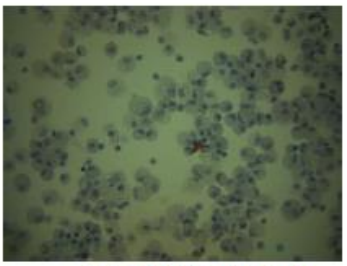




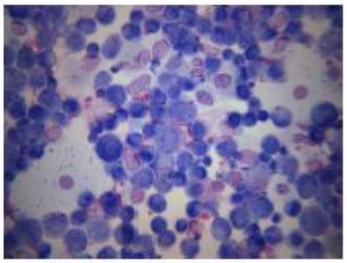
IPF 6 Geimsa (x40)



IPF 6 Haemosidderin



IPF 6 Oil Red (x20)



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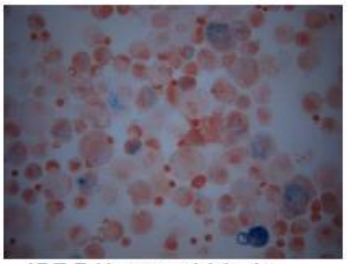
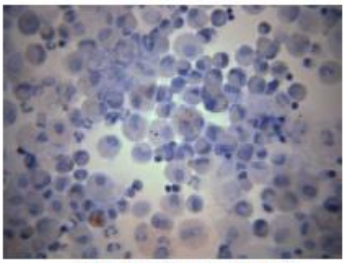
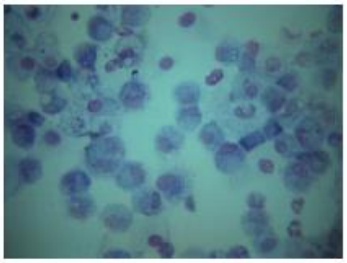


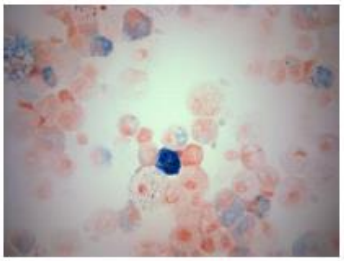
IPF 7 Haemosidderin



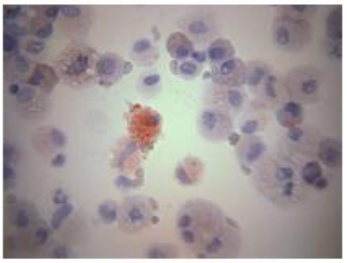
IPF 7 Oil Red (x20)



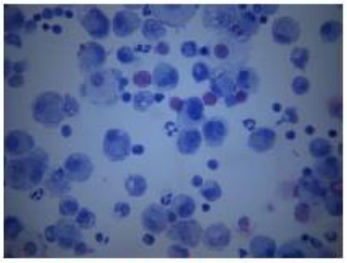
IPF 8 Geimsa (x40)



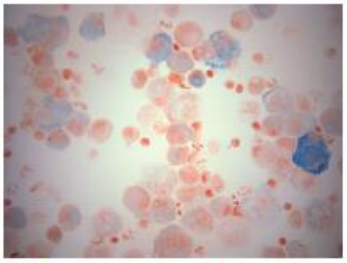
IPF 8 Haemosidderin



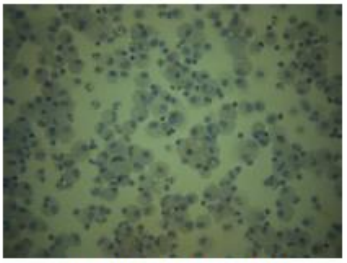
IPF 8 Oil Red



IPF 9 Geimsa (x40)



IPF 9 Haemosidderin



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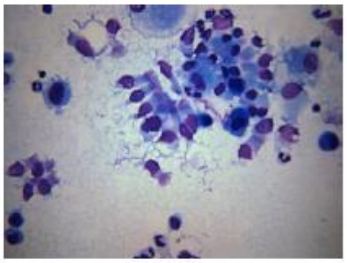
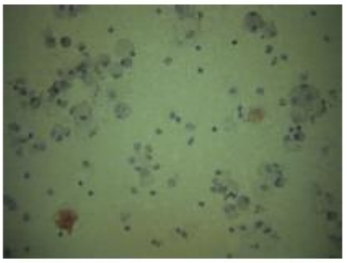


IPF 10 Geimsa (x40)

IPF 10 Haemosidderin



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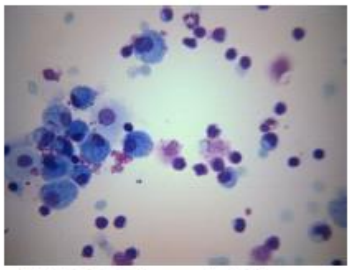

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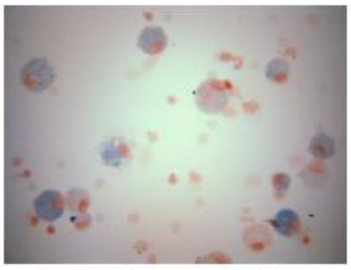

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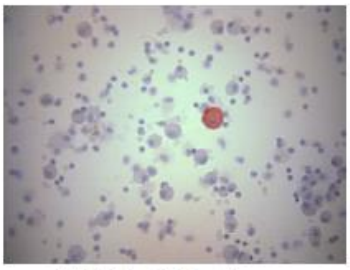

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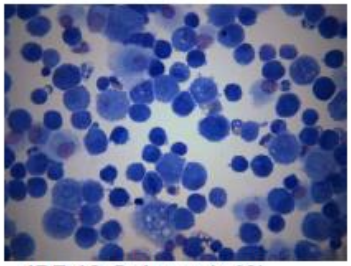

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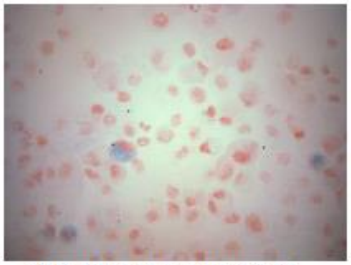

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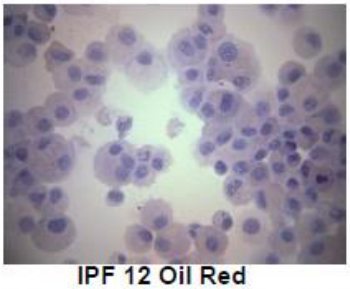

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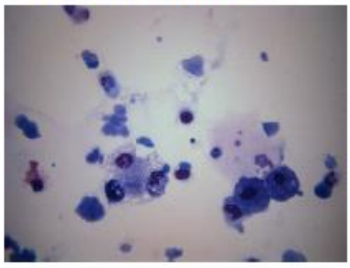

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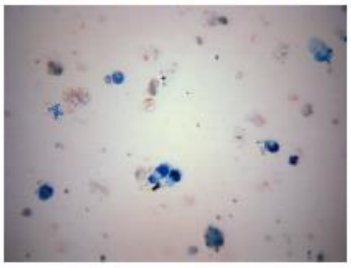

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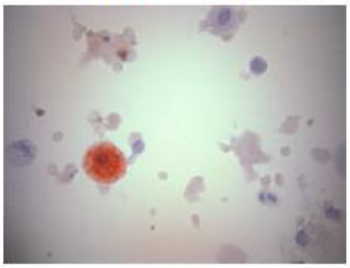

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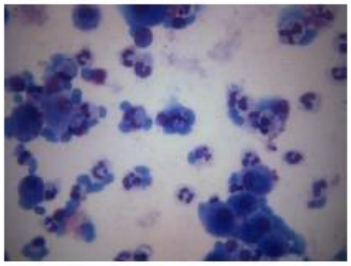

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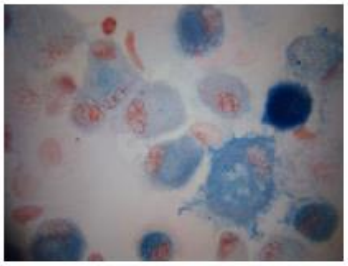

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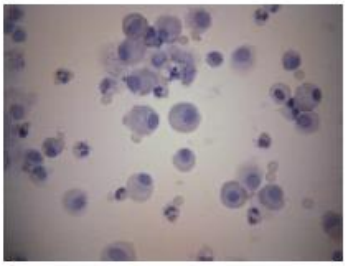

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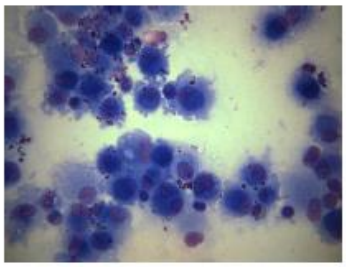

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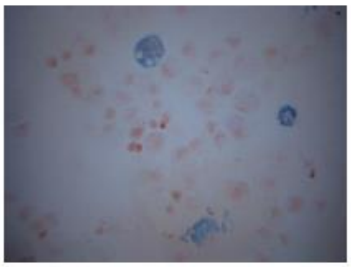

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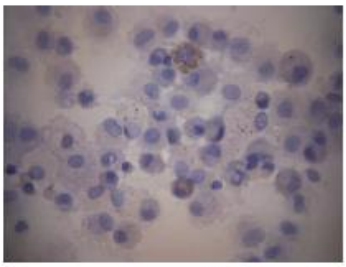

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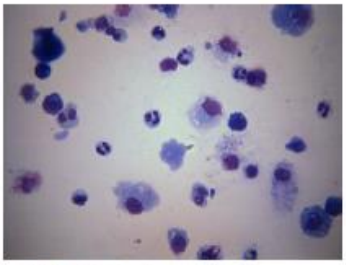

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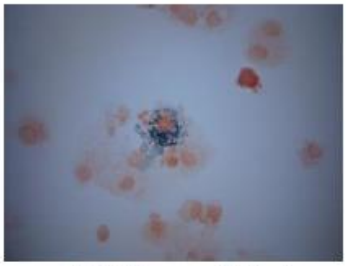

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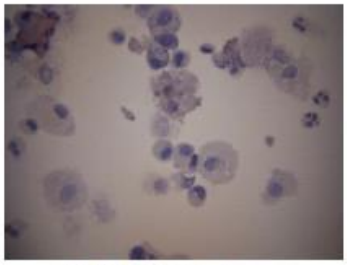

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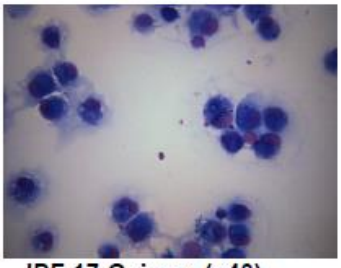

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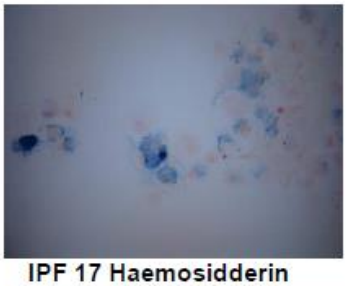

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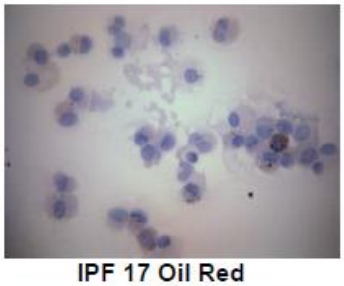

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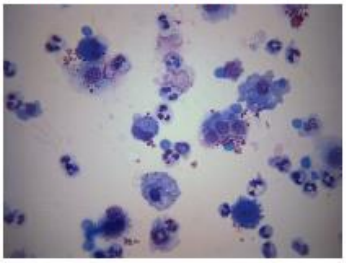

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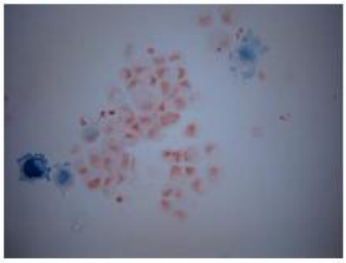

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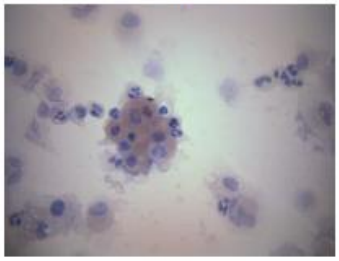

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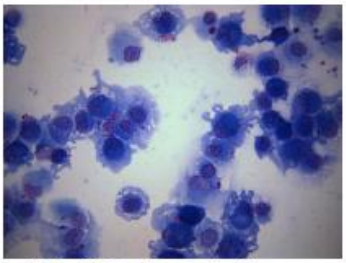

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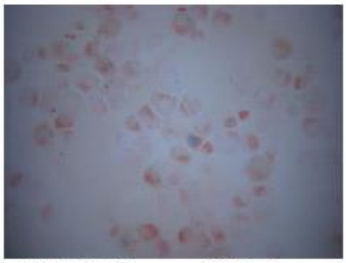

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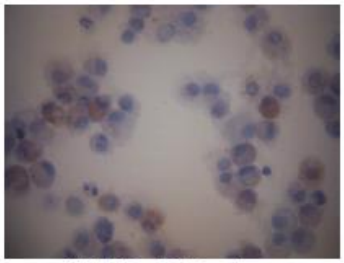

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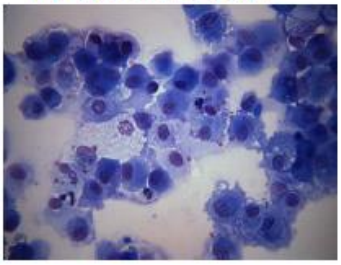

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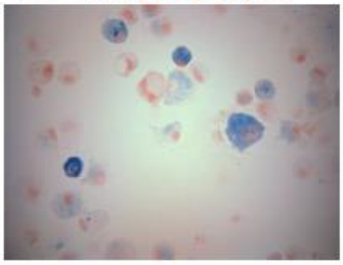

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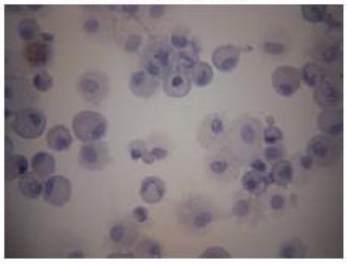


IPF 20 Oil Red

4.3.7 Markers of aspiration

Bile Salts

BAL samples from all 20 IPF were analysed using a combination of tandem mass spectrometry allow the sensitivity of detecting bile salts to be increased to a minimum level of 0.01µmol/L [98]. Our samples were also processed using a specialised extraction technique and the lower limit of detection was 0.001µmol/L. Concentrations of the individual bile salts (glycodeoxycholate, glycocholate, taurodeoxycholate and tauracholate) were added together to give the total bile salt concentration. The concentration of free lithocholate was also available using the extraction technique described in the previous chapter. The table on the following page (Table 4-11) shows the concentration of bile salts identified in the BAL of IPF subjects 1-20 and four normal controls.

All 20 patient samples showed 'detectable' bile salts and 17/20 showed detectable free lithocholate. The highest bile salt concentration was 0.7449µmol/L and the highest free lithocholate concentration was 0.05µmol/L. The median value for bile salts in the 20 IPF patients was 0.0087µmol/L which was similar to the median level detected in the four normal controls (0.0065µmol/L). One patient had much higher levels (0.745µmol/L) than the other patients. The median free lithocholate concentration in the twenty IPF patients was 0.012µmol/L which was lower than the levels detected in the normal controls (0.025µmol/L). It is clear from the table that although levels were detectable, the amounts identified in most patients were in the region of the lower limit of detection (0.001µmol/L) and is essentially a negligible concentration.

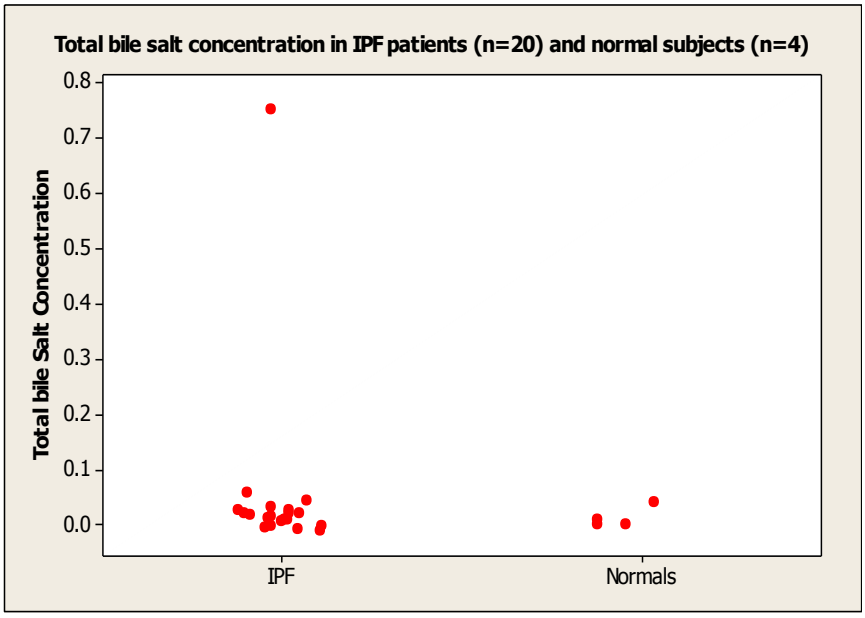
Table 4-11: Bile salt concentration in the BAL for IPF 1-20 and four normal controls

	G-DHC µmol/l	G-THC µmol/l	T-DHC µmol/l	T-THC µmol/l	Total Conc. µmol/l	Free Lithocholate µmol/l
IPF1	0.003	0.003	0.003	0.003	0.012	ND
IPF2	0.006	0.002	0.005	0.003	0.016	ND
IPF3	0.003	0.001	0.050	0.004	0.058	0.02
IPF4	0.010	0.003	0.008	0.004	0.025	0.05
IPF5	ND	ND	0.004	0.002	0.006	ND
IPF6	0.460	0.145	0.111	0.029	0.745	0.016
IPF7	0.002	0.001	0.002	0.000	0.005	0.020
IPF8	0.004	0.001	0.011	0.001	0.017	0.012
IPF9	0.000	0.000	0.003	0.001	0.005	0.008
IPF10	0.000	0.001	0.006	0.001	0.007	0.033
IPF11	0.003	0.001	0.000	0.001	0.004	0.012
IPF12	0.004	0.002	0.002	0.002	0.010	0.011
IPF13	0.002	0.000	0.001	0.001	0.004	0.008
IPF14	0.006	0.001	0.001	0.001	0.010	0.014
IPF15	0.004	0.001	0.002	0.001	0.008	0.017
IPF16	0.002	0.001	0.002	0.001	0.006	0.006
IPF17	0.015	0.004	0.003	0.000	0.022	0.012
IPF18	0.035	0.012	0.007	0.003	0.057	0.013
IPF19	0.002	0.001	0.000	0.000	0.004	0.009
IPF20	0.004	0.001	0.001	0.001	0.006	0.012
Normal 1	0.013	0.020	0.003	0.005	0.041	0.01
Normal 2	0.001	0.001	ND	0.001	0.003	ND
Normal 3	0.004	0.002	0.002	0.002	0.010	0.06
Normal 4	0.001	ND	0.002	ND	0.003	0.04

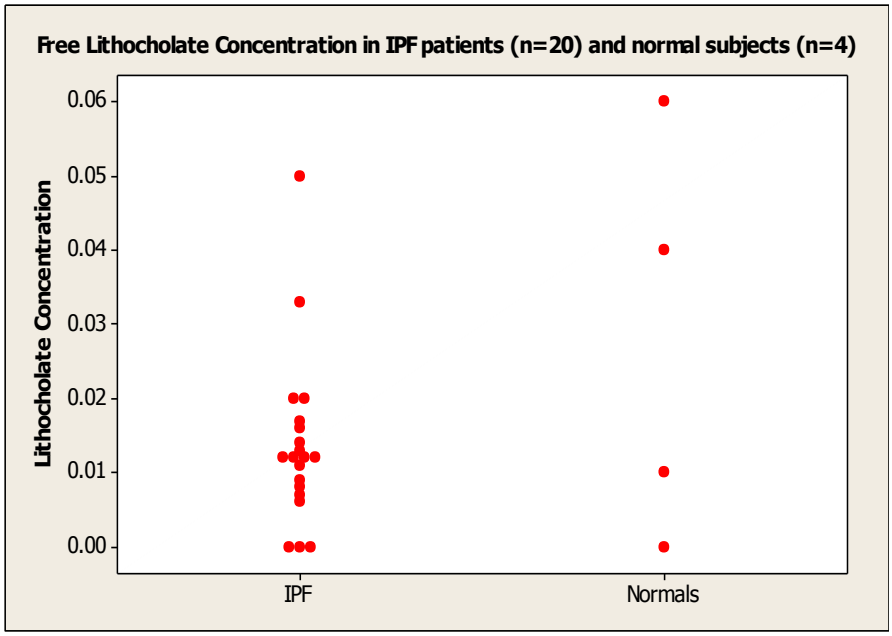
KEY: G-DHC = glycodeoxycholate, G-THC = glycocholate, T-DHC = taurodeoxycholate, T-THC = taurocholate. ND = not detected

Figure 4- 1 7 : Dot plots showing i) Bile salt concentration (y-axis) in the BAL of IPF patients compared to normal subjects (x-axis) ii) Free lithocholate concentration (y-axis) in the BAL of IPF patients compared to normal subjects (x-axis)

i)



ii)



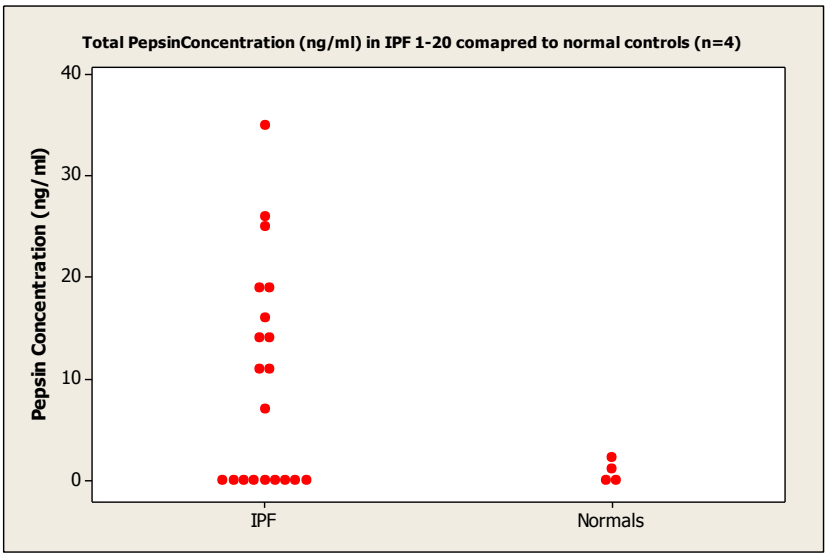
Pepsin

BAL samples from all 20 IPF were analysed using an ELISA technique to detect pepsin. Table 4-12 shows these pepsin values compared against reflux study results and lung function decline. 11/20 patient samples showed detectable pepsin. The highest pepsin concentration was 35ng/ml. The median pepsin concentration in the 20 IPF patients was 9.0ng/ml which was higher than the median level detected in normal controls (1.1ng/ml)[106].

Table 4-12: Pepsin concentrations in BAL samples for IPF1-20

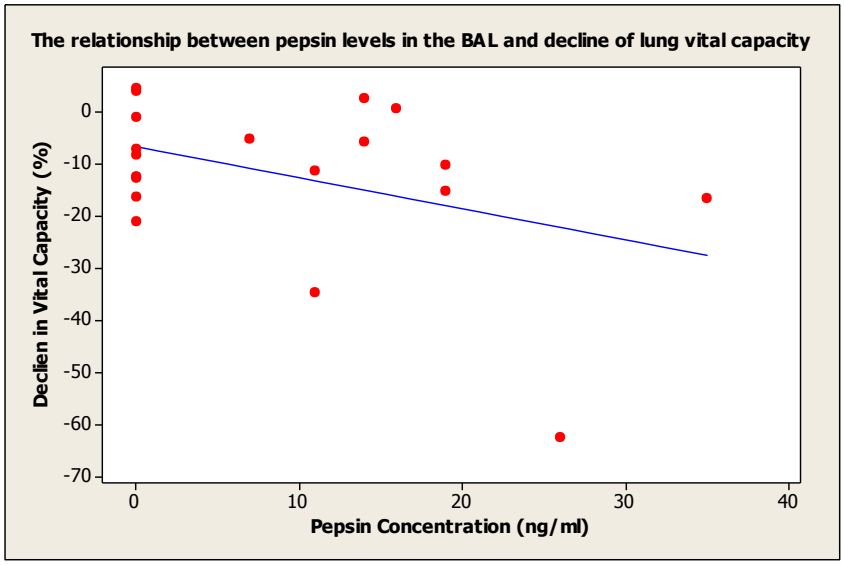
	Pepsis Concentration ng/ml	Demeester Score (<14.72)	Total Reflux Time (<4.2%)	Proximal reflux Score (4-17)	% Decline FEV_1	% Decline of vital Capacity in 12 months
IPF1	0	55.63	16.00	38.7	-6.7	4.5
IPF2	25	37.15	11.00	12.4	N/A	N/A
IPF3	7	54.47	18.00	9.5	-7.2	-5.3
IPF4	0	7.79	2.00	4.2	1.7	3.9
IPF5	16	30.29	8.00	4.8	-4.1	0.4
IPF6	14	128.92	37.00	44.5	-0.5	-5.8
IPF7	19	6.56	2.00	6.4	-5.4	-10.4
IPF8	0	6.78	2.00	14	-1.1	-1.3
IPF9	11	2.03	1.00	18.6	5.1	-11.6
IPF10	0	121.29	39.00	15.2	-21.7	-12.7
IPF11	0	201.56	60.00	3.2	-5.1	-8.5
IPF12	35	45.88	17.10	19.6	-15.2	-16.8
IPF13	0	18.5	6.00	25.7	-16.8	-21.2
IPF14	0	0.2	0.00	28.3	-6.0	-12.8
IPF15	26	22.84	7.40	9.9	-29.6	-62.8
IPF16	14	78.22	20.00	5.2	-12.7	2.4
IPF17	11	13.21	3.10	0	-10.3	-34.9
IPF18	19	2.85	0.70	2.2	-8.5	-15.5
IPF19	0	4.03	0.90	5.6	-8.8	-16.6
IPF20	0	18.51	6.60	14.3	3.9	-7.4

Figure 4-１８: Dot plots showing i) pepsin concentration (y-axis) in the BAL of IPF patients compared to normal subjects (x-axis)



Eleven out of the 20 IPF patients had elevated pepsin concentrations in the lavage compared to pepsin concentrations in BAL of normal controls. Of these eleven patients, 7 had high Demeester scores indicating significant reflux and 3 patients had proximal reflux. Ten of the eleven IPF patients with elevated pepsin levels also had lung function data available. Nine patients showed a decline in FEV_1 and eight showed a decline in vital capacity. Pearson's test showed no correlation between pepsin levels and either Demeester and proximal reflux scores. The regression analysis shows a small degree of association between decline in vital capacity and pepsin levels (p=0.085) and this is illustrated in Figure 4-１９

Figure 4-１９: Scatter plot showing pepsin concentrations (x-axis) for IPF 1-20 against percentage decline of lung vital capacity (y-axis).



4.3.8 Lung Function

Serial lung function results were collected for 19 of the 20 IPF patients. IPF 2 was only seen in the specialist clinic on one occasion and therefore serial lung function tests could not be used to illustrate the rate of decline of lung function. The individual FEV_1 and VC (vital capacity) were plotted against the time period in weeks to reveal a regression line with a formula in the format $y=mx+c$. The values of t=0 and t=52 (1year) were re-inputted into the regression formulas and the percentage decline of lung function per year was calculated for each patient (Table 4-13).

The FEV_1/FVC ratio was greater than 80% in 15/20 people at the time of recruitment. The decline in lung function showed predominantly a greater reduction in FVC over time resulting in abnormal restrictive function. (median loss of vital capacity = 10%/yr).

Table 4-13: Summary of lung function decline as measured using $FEV_1$and VC with corresponding reflux scores for IPF 1-20.

Patient No	Yearly % Decline FEV_1	Yearly % Decline of vital Capacity	Demeester Score (norm <14.72)	Proximal Reflux Score (norm 4-17)
IPF1	-6.7	4.5	55.63	38.7
IPF3	-7.2	-5.3	54.47	9.5
IPF4	1.7	3.9	7.79	4.2
IPF5	-4.1	0.4	30.29	4.8
IPF6	-0.5	-5.8	128.92	44.5
IPF7	-5.4	-10.4	6.56	6.4
IPF8	-1.1	-1.3	6.78	14
IPF9	5.1	-11.6	2.03	18.6
IPF10	-21.7	-12.7	121.29	15.2
IPF11	-5.1	-8.5	201.56	3.2
IPF12	-15.2	-16.8	45.88	19.6
IPF13	-16.8	-21.2	18.5	25.7
IPF14	-6.0	-12.8	0.2	28.3
IPF15	-29.6	-62.8	22.84	9.9
IPF16	-12.7	2.4	78.22	5.2
IPF17	-10.3	-34.9	13.21	0
IPF18	-8.5	-15.5	2.85	2.2
IPF19	-8.8	-16.6	4.03	5.6
IPF20	3.9	-7.4	18.51	14.3

*IPF 2 was excluded from analysis as only a single lung function was performed on this patient

Decline of FEV_1

The median percentage decline of FEV_1 per year was 7% with the largest decline of FEV_1 being 30%. There was no significant correlation between the percentage decline of FEV_1 and proximal reflux score (Pearson correlation = 0.114, p=0.642). There was no significant relationship between the percentage decline of FEV_1 and Demeester score (Pearson correlation = -0.209, p =0.391). In addition, there was no significant relation between the reflux symptom index (RSI score) and decline of FEV1 (p=0.158).

Decline of Vital Capacity (VC)

The median percentage decline of VC per year was 10.4% with the largest decline of FEV_1 being 62.8%. There was no significant correlation between the percentage decline of VC and proximal reflux score (Pearson correlation = 0.054, p=0.825). There was no significant relationship between the percentage decline of VC and Demeester score (Pearson correlation = 0.314, p =0.19). In addition, there was no significant relation between the reflux symptom index (RSI score) and decline of VC (p=0.152).

Although TLco (Transfer factor of the lung for carbon monoxide) is a more useful measurement in assessing lung function in IPF patients this was not measured in all patients at their lung function tests and so the data was not available for analysis.

4.3.9 Reflux Finding Scores

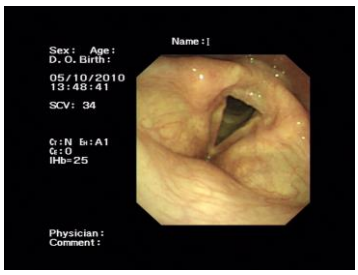
The reflux finding score (RFS) is an 8-item clinical severity scales based on the visual findings during bronchoscopy (Figure 4-２０). The scoring allows another mode of assessing potential laryngopharyngeal reflux (LPR). The scoring was performed by Dr. Julian Mcglashan at Nottingham University based on the validated RFS system produced and validated by *Belafsky et al* in 2001[153] and are documented with RSI score in the table below. The RFS for the IPF group is also illustrated in the dot plot (Figure 4-２１). The Normal RFS score is 7. Seven out of twenty patients had abnormal RFS scores.

Table 4-14: RFS and RSI scores for IPF patients 1-20

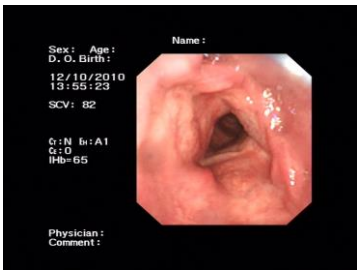
Patient No	Vocal fold oedema	Diffuse laryngeal oedema	Posterior Commissure hypertrophy	Granuloma/ granulation tissue	RFS Total	RSI Score
IPF1	1	0	0	0	3	6
IPF2	2	2	2	0	9	18
IPF3	1	1	1	0	5	10
IPF4	2	1	1	0	9	18
IPF5	3	2	3	0	11	22
IPF6	2	1	1	0	4	8
IPF7	1	1	1	0	5	10
IPF8	2	0	0	0	4	8
IPF9	2	2	3	0	11	22
IPF10	3	2	2	0	11	22
IPF11	1	1	1	0	5	10
IPF12	0	0	0	0	0	0
IPF13	3	2	1	0	9	18
IPF14	2	1	0	0	6	12
IPF15	1	2	1	0	10	20
IPF16	0	0	1	0	1	2
IPF17	2	1	1	0	7	14
IPF18	0	0	1	0	1	2
IPF19	2	1	1	0	7	14
IPF20	1	1	1	0	6	12

There was a positive correlation between the RFS scores calculated by an external source and the RSI scored from the research questionnaires but this was not significant (Pearson correlation = 0.289, p=0.217). There was no significant relationship between RFS scores and proximal reflux scores.

Figure 4-２０: Bronchoscopy images for RFS scoring



IPF 1



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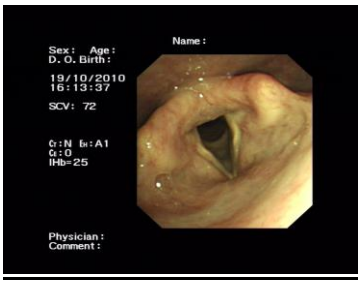
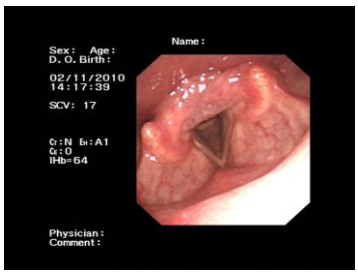


IPF 3



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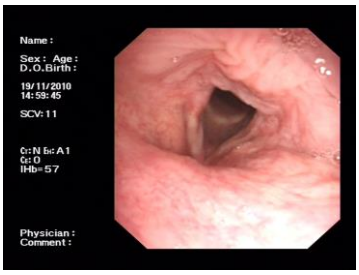

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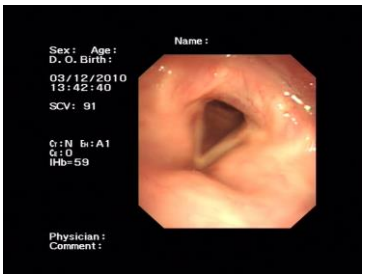

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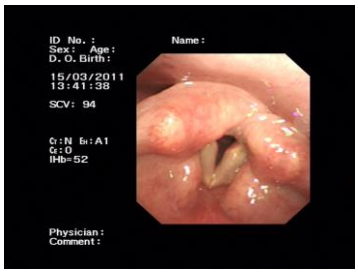
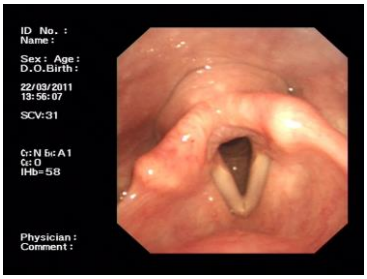


IPF 7



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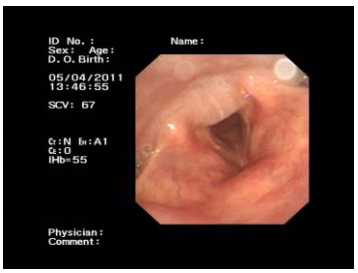

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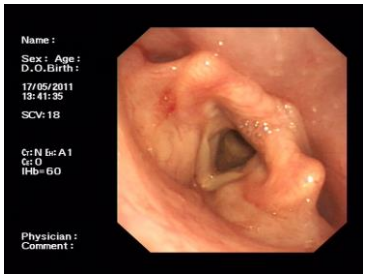

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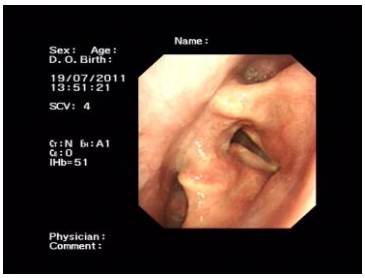

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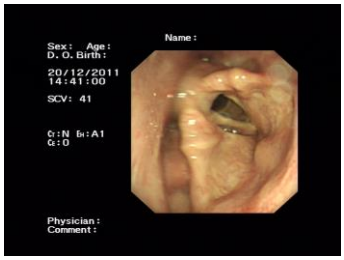

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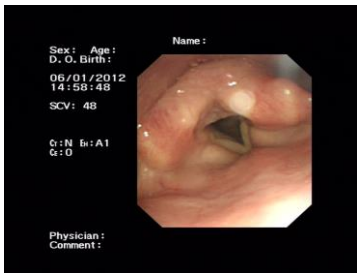

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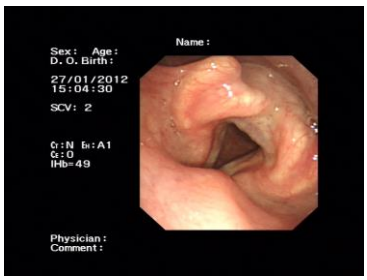

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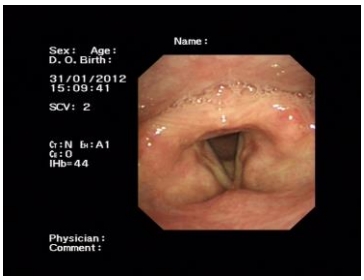
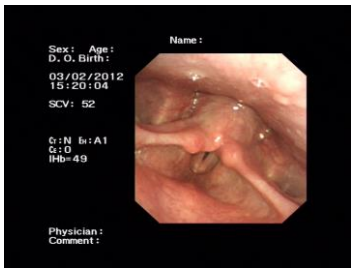


IPF 15



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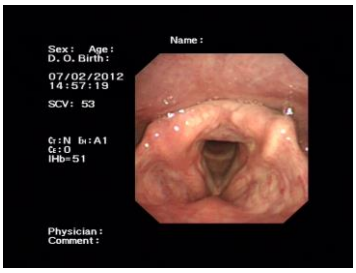

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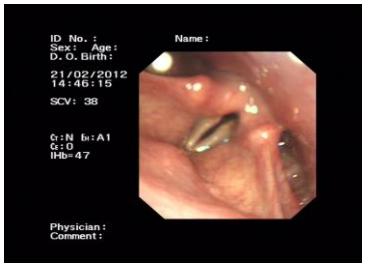

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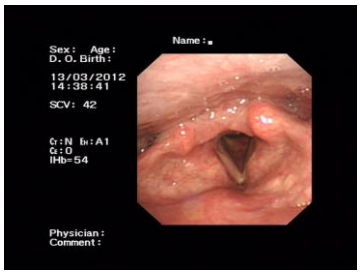

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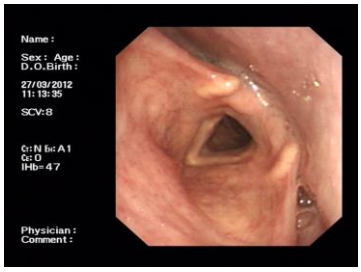


IPF 20

Figure 4-２１: Dot plot of individual Reflux Finding Scores for IPF 1-20 with normal control score indicated

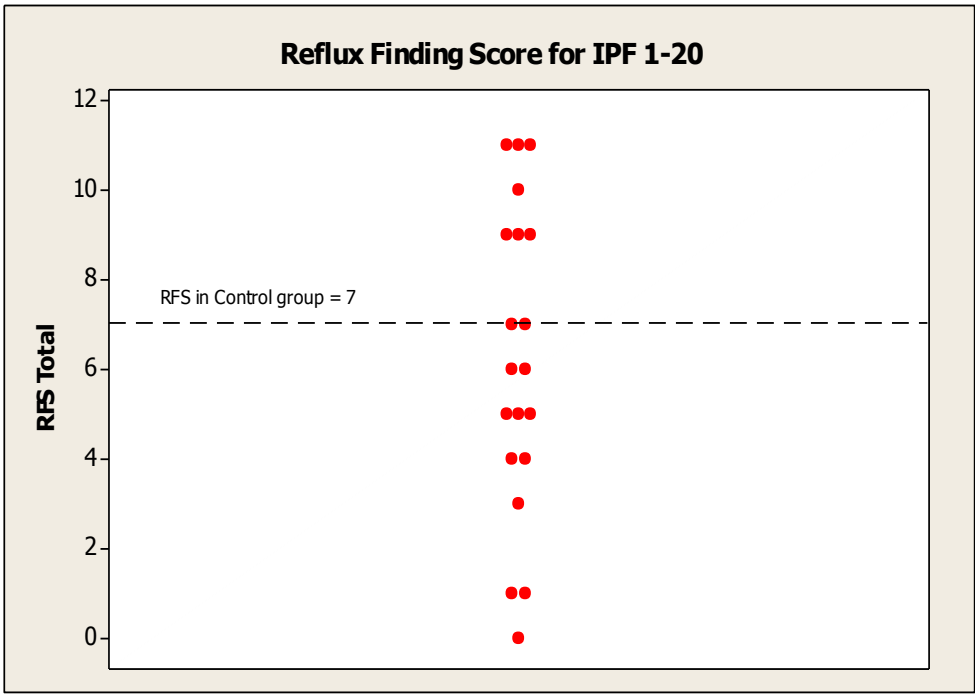
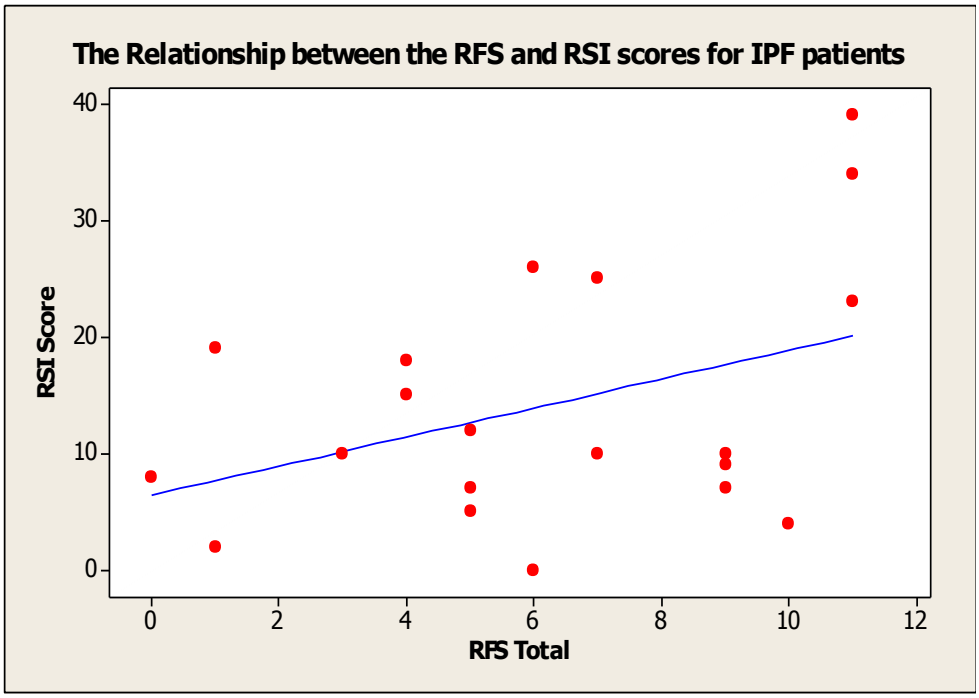


Figure 4-２２: Relationship between RFS scores and RSI scores in IPF patients



4.3.10 Radiological Information

Table 4-15: HRCT information and reflux scores of IPF patients 1 to 20

Patient No	HRCT reviewed	Hiatus hernia details	IPF symmetry	Demeester Score (norm<14.72)	Proximal Reflux Score
IPF1	Yes	Small Sliding	Symmetrical	55.63	38.7
IPF2	Yes	Moderate Sliding	Symmetrical	37.15	12.4
IPF3	Yes	Small sliding	Asymmetrical Left >right	54.47	9.5
IPF4	Yes	Large (most of stomach)	Symmetrical	7.79	4.2
IPF5	Yes	No Hiatus Hernia	Symmetrical	30.29	4.8
IPF6	Yes	No Hiatus Hernia	Symmetrical	128.92	44.5
IPF7	Yes	Moderate Sliding	Symmetrical	6.56	6.4
IPF8	Yes	Small sliding	Symmetrical	6.78	14
IPF9	No	No hiatus hernia on Ba. swallow	Symmetrical (CXR)	2.03	18.6
IPF10	Yes	Small sliding	Symmetrical	121.29	15.2
IPF11	Yes	Small Hiatus Hernia	Asymmetrical Right > left	201.56	3.2
IPF12	Yes	No Hiatus Hernia	Symmetrical	45.88	19.6
IPF13	Yes	Small sliding	Symmetrical	18.5	25.7
IPF14	Yes	Small sliding	Symmetrical	0.2	28.3
IPF15	Yes	Small Hiatus Hernia	Symmetrical	22.84	9.9
IPF16	No	No hiatus hernia on Ba. swallow	Symmetrical (CXR)	78.22	5.2
IPF17	Yes	Small Hiatus Hernia	Symmetrical	13.21	0
IPF18	Yes	Small sliding	Symmetrical	2.85	2.2
IPF19	Yes	Small sliding	Symmetrical	4.03	5.6
IPF20	Yes	No Hiatus Hernia	Symmetrical	18.51	14.3

An independent consultant radiologist (Dr. Hilary Spence, RVI) who was not involved in the initial CT diagnosis of IPF was asked to review the high resolution CT scan (HRCT) of the IPF patient group and comment on the presence of any hiatus hernia and the symmetry of the disease. Eighteen of the twenty patients had CT scans which were accessible on the local PACS system for review. However, the 2 patients who did not have a CT scan had a chest x-ray and barium swallow test in order for the radiologist to make comment.

From HRCT evidence, 14/18 patients had evidence of a hiatus hernia. Of these, eight patients had objective evidence of reflux from pH-impedance. Two patients had evidence of asymmetrical IPF on HRCT. Both these patients had hiatus hernias and objective evidence of reflux. All four patients with no hiatus hernia visible on HRCT had objective evidence of reflux.

The two patients who did not have a CT scan available for review (IPF 9 and IPF 16) both had symmetrical disease on their chest x-ray. A barium swallow had been performed on these patients during their attendance at the IPF centre and in both patients no hiatus hernia was present. Both patients did have objective evidence of reflux on pH-impedance.

4.4 Summary of IPF Results

4.4.1 Clinical Results

Between July 2010 and March 2012, twenty patients formally diagnosed with Idiopathic Pulmonary Fibrosis through a multi-disciplinary meeting were studied. This included fourteen males and six females who had a median age of 69 years. Baseline median lung function for the group was a FEV_1 of 1.96 litres and a Vital Capacity (VC) of 2.53 litres. Fifteen patients were on proton pump inhibitor (PPI) therapy prior to their recruitment into the study; only four had documented evidence of gastro-oesophageal reflux (GOR) in their notes.

All twenty patient successfully completed oesophageal manometry and Impedance-pH studies. Eleven patients demonstrated normal oesophageal peristalsis on either traditional 8-channel manometry or High Resolution Manometry (HRM). The most common abnormality detected on 8-channel manometry was simultaneous swallows but on HRM distal oesophageal spasm was seen most commonly in those patients with abnormal peristalsis. Of the twenty patients, twelve had objective evidence of reflux on impedance-pH. Seven patients had weakly acid reflux and six patients had evidence of abnormal proximal reflux. Most reflux events were mixed (liquid and gas). The incidence of reflux was not related to lower oesophageal sphincter (LOS) length or resting pressure.

Patient symptoms and the effect on quality of life were studies using validated questionnaires. Fifteen patients were already on PPIs before they entered the study and the questionnaires were completed ‘on’ and ‘off’ PPI. Reflux symptom index (RSI) scores, assessing symptoms of extra-oesophageal reflux were higher for patients taking PPI with over 60% having a positive RSI score (RSI > 13). Demeester questionnaire scores for patients ‘on’ and ‘off’ PPI were identical. Quality of life scores were assessed with the Gastro-Intestinal Quality of Life Index (GIQLI). The median score was slightly higher for those patients on PPI therapy (108 vs. 95); however, over 85% of patients on PPI had GIQLI scores below the normal range.

Lung function tests were performed on all 20 IPF patients but serial analysis of FEV_1 and VC were performed on nineteen patients. Using the raw lung function data, the percentage decline of FEV_1 and VC over one year was calculated. 16/19 patients had a decline of FEV_1 with the largest decline being 29.6%. Ten of these patients had an abnormal Demeester score and five patients had abnormal proximal reflux scores.

Fifteen patients demonstrated a reduction in VC. 8 patients with a reduction in vital capacity had elevated Demeester scores and five demonstrated abnormal levels of proximal reflux. The percentage decline of FEV_1 and VC did not correlate directly with abnormal Demeester Scores or elevated levels of proximal reflux. Lung function was abnormal in 5/20 patients (FEV_1/FVC ratio <80% predicted) and the disease progression was a rapid loss of FVC over time of over 10% per year.

The reflux finding scores (RFS) were calculated for all the IPF patients using photos of the patient's larynx taken at bronchoscopy. This score may indicate the presence of laryngopharngeal reflux. The formal scoring was performed by an external specialist from another centre. Seven patients demonstrated abnormal RFS (>7) and all of these patients had elevated reflux symptom index (RSI) scores, a marker of extra-oesophageal reflux. Although a positive correlation was demonstrated between RFS and RSI scores the relationship did not reach statistical significance. There was no correlation between RFS scores and proximal reflux on impedance-pH.

High resolution CT was used in the diagnostic assessment of the IPF patients. In 18/20 of our patients the CT images were available for an independent radiologist to review the presence or absence of a hiatus hernia. Fourteen of these patients had a hiatus hernia on their CT scans and eight of these had objective evidence of reflux on Impedance-pH testing. Four patients had objective reflux in the absence of hernia. The two patients who did not have an accessible CT image had barium swallows. Neither patient was demonstrated to have a hiatus hernia but both had reflux on their impedance-pH studies.

4.4.2 Laboratory based studies

All 20 IPF patients had bronchoscopy performed with a standardised 3 x 60ml saline lavage. This was processed using a standard operating procedure so that a differential cell count could be performed. In addition, pepsin and bile salt assays were performed on the BAL supernatant. The principal cell type identified in the BAL was macrophages and these were stained with Prussian Blue (Haemosiderin) and Oil Red O (lipid-laden) stains. The percentages of cells that stained positive for these stains were higher than the percentages seen in normal controls. There was no correlation between the Haemosiderin or Oil Red scores and reflux levels for the IPF group. All 20 IPF patients showed detectable bile salt and the median levels were higher than the levels seen in normal controls. Free lithocholate was detected in 17/20 patients but the median levels were lower than those seen in normal controls. Eleven out of twenty IPF patients had

pepsin levels in the lavage samples higher than the normal controls, in some patients the levels were over ten times that of normal controls.

5 Cystic Fibrosis Results Section

5.1 Introduction

GOR has been reported as early as the 1970s in patients with CF and currently the prevalence is estimated to be between 35-81% [81]. Over the last 30 years advances in the care of patients with CF have resulted in a growing adult population with CF. There is a higher incidence of GOR in children with CF than in the general population [82], about 1 in 5 newly diagnosed CF infants have pathological reflux, [22] but there are very few comparable studies in the adult CF population. Most of the studies performed to date in this population use 24 hour pH monitoring which only allows the detection of acidic GOR The abnormal CFTR regulation in cystic fibrosis may influence the nature of the reflux including whether it is acidic or weakly acidic reflux. There have been limited studies performed using pH impedance in CF patients [81] with interesting results. *Blondeau et al* performed pH impedance studies on 23 CF patients and demonstrated that up to 80% had acid GOR with subgroup having increased weakly acid reflux.

This section aimed to identify the incidence and nature of reflux in CF patients and develop an understanding of the role of microaspiration in this patient group.

5.2 Methods

Adult patients with diagnosed CF are reviewed at specialist clinics at the Royal Victoria Infirmary. Several respiratory consultants who are specialists in cystic fibrosis work with a team of nurses and physiotherapists creating a multidisciplinary clinic setting. Between June 2011 and April 2012 all patients with typical (Δ508) CF that fulfilled the inclusion criteria as described in the previous chapter were approached to be recruited to the study.

My protocol was to assess for GORD using a set of validated reflux questionnaires, oesophageal manometry and pH/impedance measurements. At the same time as these assessments were made a sputum sample was requested. Those patients on proton pump inhibitor (PPI) therapy were requested to stop their medication 2 weeks prior to the investigations. In addition, they were asked to complete a set of questionnaires whilst they were taking the PPI. Results were then compared with markers of aspiration in the sputum sample, microbiology, and differential cell counts from the sputum processing. Pulmonary function tests were also available over the time the patient had attended the CF clinic and these were used in the analyses.

5.3 Results

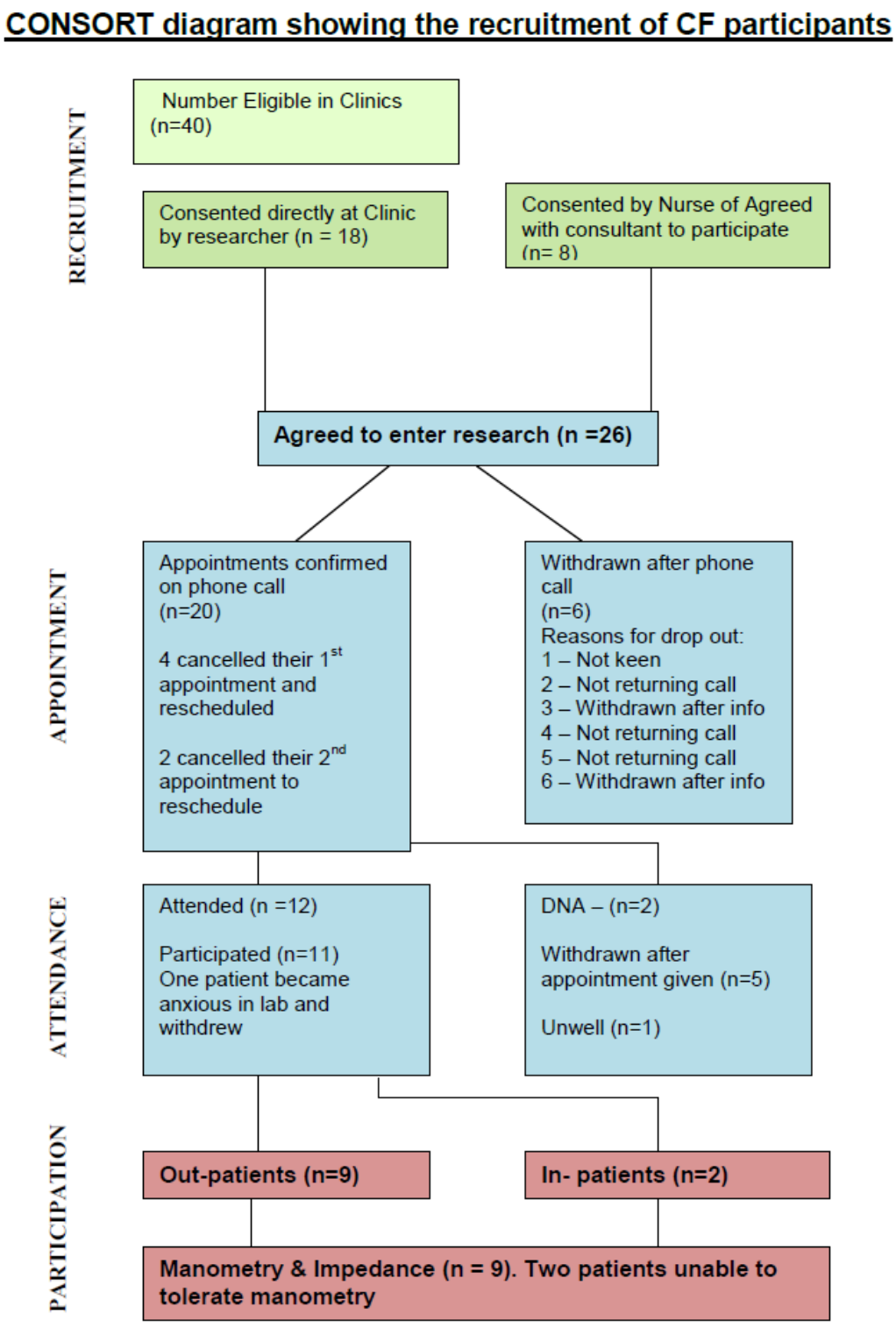
5.3.1 Recruitment

The recruitment of CF patients was initially instigated via the specialist clinic. In June 2011, specialist CF clinics were being held regularly, normally a morning or afternoon clinic dependent on clinician. The CF lead's clinic, held twice a week was chosen as the clinic where the research patients would be recruited from. This allowed confirmation that the inclusion criteria including genotype were strictly adhered to. Suitable patients for recruitment were selected by the specialist using the study inclusion criteria described in the previous chapter. The principal researcher would approach these patients individually in another clinic room to discuss recruitment into the study. In total 40 patients were approached this way and 18 consented to the study. Further recruitment was done by the CF specialist nurse in the absence of the principal researcher. Through this method of referral 8 patients consented to the study. In total 26 patients consented to the study (Figure 5- 1).

Of the 26 patients that consented to the study, 6 patients dropped out before an appointment was given for their investigations. Three of the six patients dropped out after reading the information leaflet. Three other patients were not contactable on the telephone numbers they had provided at the time of consent.

Of the 20 patients given appointments, 8 dropped out which included 2 not attending (DNA), one patient becoming unwell and five patients changed their minds after the appointment was given. Of the 12 patients that did attend, 11 patients actually participated as one patient became extremely anxious on the day of the test and no longer wished to participate.

Figure 5- 1 : Consort Diagram of CF patient recruitment



5.3.2 Demographics

Eleven typical CF patients (Δ508 genotype) were therefore studied (Table 5-1) (6 men, 5 women) with a median age of 29 years (range 44-81) and a median BMI of 22Kg/m^2. Only four patients had documented evidence of gastro-oesophageal reflux either through clinical letters or a recent endoscopy. Median forced expiratory volume in one second (FEV_1) was 1.86L (Range 0.86-3.08L) and median vital capacity (VC) was 2.15L (Range 1.38-5.17L). All the patients had pancreatic insufficiency and 10/11 patients were on azithromycin therapy. All the patients were colonised with *Pseudomonas aeruginosa*. Five patients had CF induced diabetes mellitus and were taking insulin. All patients were taking acid-suppression therapy at the time of recruitment. Nine of the eleven patients completed both oesophageal manometry and pH-impedance; two patients were unable to tolerate the manometry.

Table 5-1: Demographics of study patients

	Age	Sex	BMI Kg/m^2	Genotype	Pancreatic Insufficiency	Known GORD	PPI	On-insulin	On azithromycin	Baseline FEV_1	Baseline VC
CF1	24	Female	18.7	(Δ508/-)	YES	NO	YES	YES	YES	1.6	2.15
CF2	22	Male	18.8	(Δ508/Δ508)	YES	NO	YES	NO	YES	3.08	5.17
CF3	29	Female	20.6	(Δ508/N1303K)	YES	YES	YES	YES	YES	1.2	2.1
CF4	31	Male	23.4	(Δ508/Δ508)	YES	YES	YES	YES	YES	1.86	2.07
CF5	40	Male	26.65	(Δ508/Δ508)	YES	YES	YES	YES	YES	2.26	4.82
CF6	21	Male	19	(Δ508/NMD)	YES	NO	YES	NO	YES	3	5
CF7	25	Female	22	(Δ508/2184delA)	YES	NO	H_2A	NO	YES	1.31	1.88
CF8	35	Male	22.16	(Δ508)	YES	NO	YES	NO	NO	2.25	3.2
CF9	19	Female	29.7	(Δ508/9551D)	YES	NO	YES	YES	YES	2.46	2.85
CF10	59	Female	20.4	(Δ508/D1152H)	YES	YES	YES	NO	YES	0.86	1.38
CF11	32	Male	22.9	(Δ508)	YES	NO	YES	NO	YES	1.08	2.15

5.3.3 High Resolution Manometry (HRM)

9 patients underwent HRM as described in the previous chapter. Overall 66% of patients (6/9) had abnormal oesophageal physiology as defined by the Chicago classification (Table 5-2). No complications were attributed to the procedure.

- Lower oesophageal Sphincter

The median lower oesophageal sphincter length was 3.8cm (range 3.0-4.4cm). Sphincter pressure was within normal limits (10-45mmHg for HRM) in five patients with an average sphincter pressure of 14.54mmHg (Range 4.6-30.6mmHg). Four patients had a hypotonic LOS. In addition, HRM provided details of the intra-abdominal length of LOS and the presence of a hiatus hernia. The median intra-abdominal length of LOS was 1.3cm (Range -2.7- 4.1cm). Five patients (55.6%) had hiatus hernias detected on HRM with a mean hernia length of 2.12cm.

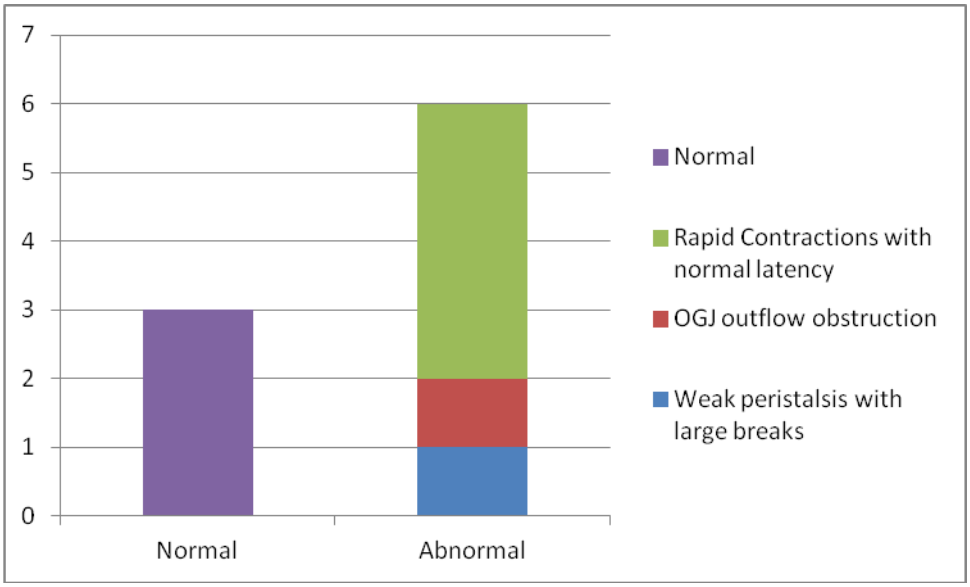
- Oesophageal Peristalsis

The characterisation of the oesophageal peristalsis was determined by a set of measurements taken on HRM as described in table 3.3. The median percentage of normal swallows was 87% (range 33 -100%). In 5 patients the contraction pattern was normal in 80-100% of swallows. The remaining 4 patients had a mixture of rapid and premature contractions. In four patients there was intact peristalsis in over 90% of swallows. The Chicago classification of the oesophageal motility in these 9 patients is shown below (Figure 5- 2).

Table 5-2: HRM key results

	Median	Range	Normal Values
Distal Latency (DL) - s	7.1	5.8-9.0	>4.5
Distal Contractile Integral (DCI) – mmHg.s.cm	522	79-2314	<8000
Peristaltic Breaks - cm	1.0	0.1-5.7	<2cm
Integrated Relaxation Pressure (IRP4s) - mmHg	4.9	1.5-22.3	<15

Figure 5- 2 : HRM oesophageal peristalsis



5.3.4 Reflux Data

Reflux Questionnaires

Ten of the eleven CF patients were taking PPI at the time of recruitment. One patient took Ranitidine, a histamine H_2-receptor antagonist. The doses are listed below in Table 5-3. Patients were requested to stop their acid suppression medication for 2 weeks prior to the oesophageal physiology investigations. Questionnaires were completed by the patient 'ON' and 'OFF' their medication. The median daily dose of lansoprazole was 60mg (Range 15 – 60mg) and omeprazole was 40mg (Range 40-80mg). The total daily dose of PPI were compared to reflux questionnaire scores having adjusted the dosages for lansoprazole to omeprazole equivalents for purpose of comparison; 15mg lansoprazole = 20mg omeprazole, 30mg lansoprazole = 40mg omeprazole and 60mg lansoprazole = 80mg omeprazole [150].

Table 5-3: The variation of PPI dosage in study patients

PPI or H2 Receptor Antagonist Dose	Number of Patients
lansoprazole 15mg once daily	**1**
lansoprazole 30mg once daily	**1**
lansoprazole 30mg twice daily	**3**
omeprazole 20mg twice daily	**3**
omeprazole 40mg once daily	**2**
Ranitidine 300mg once daily	**1**

The RSI questionnaires were completed by all 11 patients prior to their investigations and then repeated on the day of the oesophageal physiology having stopped their gastric acid suppression medication for 2 weeks. 8 patients (72%) had a positive RSI score (RSI>13).The median RSI score was 19 (Range 8 to 36). Whilst on their medication 6 patients (55%) had a positive RSI score. The median score was 17 (range 5 to 32). The differences in RSI score 'on' and 'off' PPI did not reach statistical significance (p=0.34). Use of acid suppression did result in a reduction of the reflux symptom score, although over half the patients still had above normal symptom scores on medication. Figure 5- 3 i shows the RSI scores for the eleven CF patients ON and OFF their acid

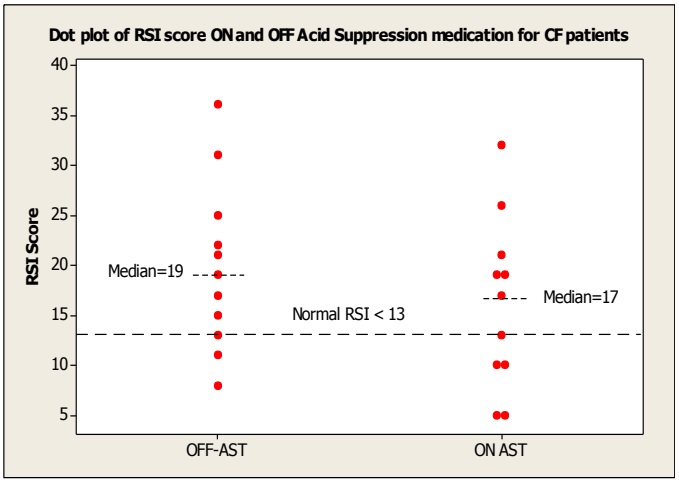
suppression therapy. For these patients the median difference of the RSI scores on and off medication was -4 (range -15 to 8). Figure 5- 4 i shows that for the 10 patients on PPI acid suppression no significant relationship was demonstrated between RSI score and the daily dose of PPI (P = 0.287).

The Demeester questionnaires were completed by all 11 patients prior to their investigations and then repeated on the day of the oesophageal physiology having stopped their gastric acid suppression medication for 2 weeks. The median Demeester questionnaire score was 3 (Range 1 to 7). Whilst on their medication the median score was 2 (range 1 to 8). The use of acid suppression medication makes minimal difference to the Demeester questionnaire score in these patients. Figure 5- 3 ii shows the Demeester questionnaire scores for the CF patients ON and OFF their medication. For these patients the median difference of the Demeester scores on and off medication was 0 (range -3 to 3). Figure 5- 4 ii shows that for the 10 patients on PPI acid suppression no significant relationship was demonstrated between Demeester questionnaire score and the daily dose of PPI (P = 0.231).

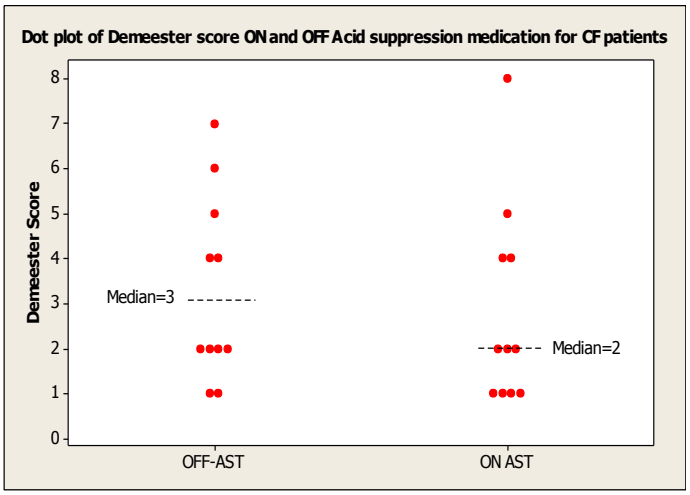
The GIQLI questionnaires were completed by all 11 patients prior to their investigations and then repeated on the day of the oesophageal physiology having stopped their gastric acid suppression medication for 2 weeks. Ten of the eleven patients (91%) had a score below the normal range (121-130). The median GIQLI score was 93 (Range 31 to 122). Whilst on their medication (82%) had a GIQLI score below the normal range (121-130). The median score was 102 (range 47 to 132). The differences in GIQLI score 'on' and 'off' PPI did not reach statistical significance (p=0.39). Figure 5- 3 iii shows the GIQLI scores for the CF patients ON and OFF their medication. For these patients the median difference of the GIQLI score on and off medication was 10 (range -10 to 24). Figure 5- 4 iii shows that for the 10 patients on PPI acid suppression no significant relationship was demonstrated between GIQLI score and the daily dose of PPI (P = 0.595).

Figure 5- 3 : Dot plots showing: i) RSI score (y-axis) for CF patients ON and OFF acid suppression therapy (AST) (x-axis) ii) Demeester Score (y-axis) for CF patients ON and OFF acid suppression therapy (AST) (x-axis) iii) GIQLI score (y-axis) for CF patients ON and OFF acid suppression therapy (AST)(x-axis).

i)



ii)



iii)

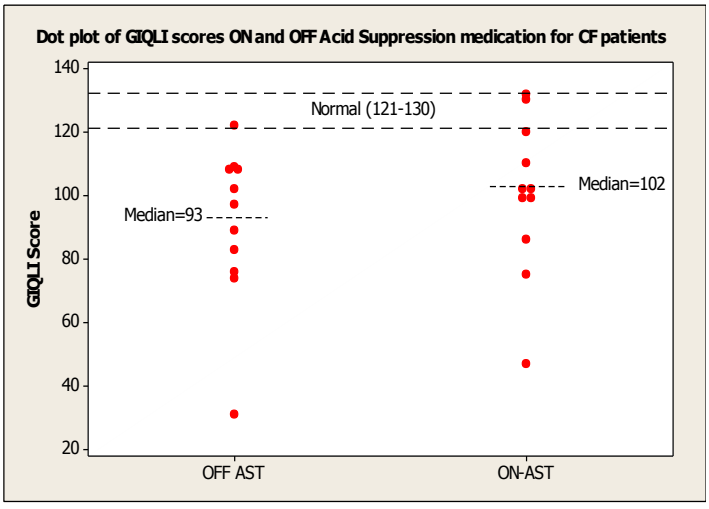
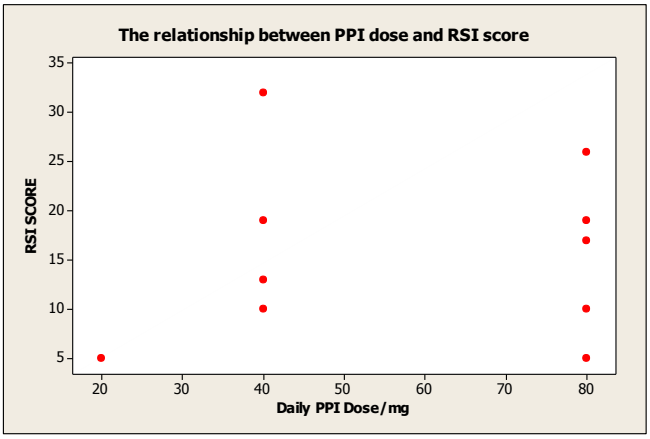
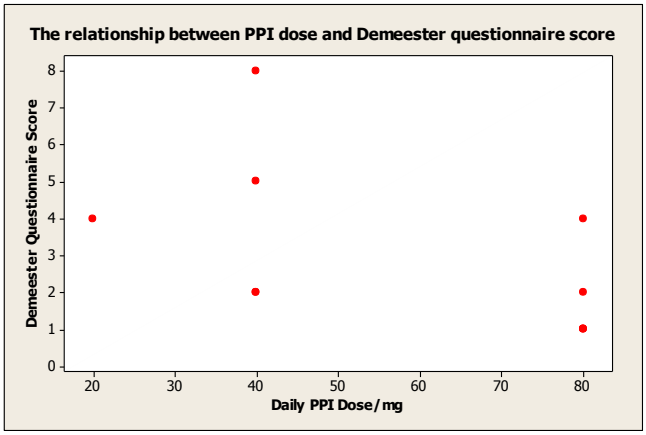


Figure 5- 4 : Scatter plots showing: i) the relationship between the daily dose of PPI (x-axis) and RSI score (y-axis) ii) the relationship between the daily dose of PPI (x-axis) and Demeester score (y-axis) iii) the relationship between the daily dose of PPI (x-axis) and GIQLI score (y-axis)

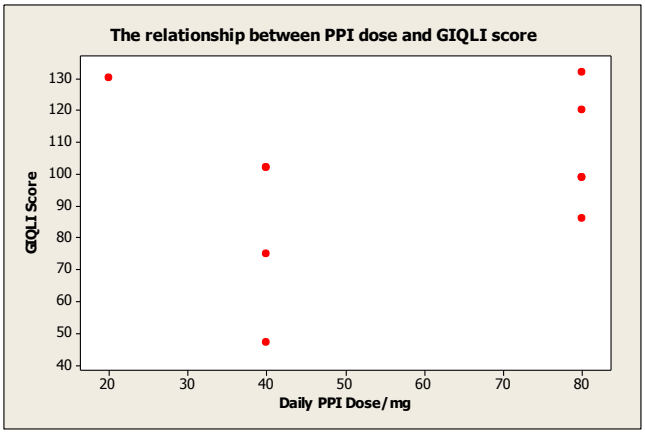
i)



ii)



iii)



pH – Impedance

All eleven CF patients completed the 24 hour recordings. 9 of 11 patients (82%) patients had reflux determined by an abnormal Demeester score (Figure 5- 5). A summary of the median reflux indices per 24 hours for the pH part of the study are shown in the table below (Table 5-4). Most refluxes were in the upright rather than supine position (median no of reflux periods 37 vs. 7).

Table 5-4: Median Reflux Indices for pH part of study

	Median	Range	Normal Values	No. of patients with abnormal results
Demeester Score	**16.81**	**0.2-45.55**	**<14.72**	**9/11**
Acid Exposure (%). (% of time pH<4, in 24hrs)	**6**	**0-16.5**	**<4.2**	**8/11**
Number of Reflux Periods in 24 hours	**64.8**	**0-109.6**	**<50**	**9/11**
Number of long Refluxes /24hours (>5min)	**2**	**0-6.4**	**<4**	**3/11**
Longest Reflux	**6.9**	**0-55.7**	**<9.2**	**5/11**

A summary of the median reflux indices as detected by oesophageal impedance is shown in Table 5-5. Five patients had weakly acid reflux. Two patients had abnormal amounts of both acid and weakly acid reflux. Five of the eleven patients had abnormal proximal (Figure 5- 6) oesophageal reflux (45%). Of these 5, all had evidence of distal reflux.

The majority of reflux events confirmed from impedance analysis were in the upright rather than in the supine position (medians 50.1 vs. 8.1), but the median number of supine events for this group of patients is outside the normal range for a 24 hour period. In addition, in these 11 patients seven had an abnormal number of supine events compared to only 5 patients with an abnormal number of upright events. Most proximal reflux events were in the upright position 12.4 (0-32.2) vs. 2.2 (0-4.8). The majority of reflux events were mixed (liquid and gas) 44.4 (12.1-78.8) vs. 17.9 (0-47.3) for liquid reflux alone. There is a borderline positive correlation between the proximal reflux score and the number of liquid (r=0.391) and mixed reflux (r=0.573) events (

Figure 5- 7). The correlation is almost significant for the number of mixed events and the proximal reflux score (p=0.066).

Table 5-5: Median Reflux Indices as demonstrated by Oesophageal Impedance

	Median	Range	Normal Values	No. of patients with abnormal results
Oesophageal Volume Exposure (%)	**0.76**	**0.02-7.64**	**0.4 -1.2**	**2/11**
Total Number of Reflux events/24hours	**54.5**	**30.9-96.8**	**25-58**	**5/11**
Number of Acid Refluxes/24 hours	**41.2**	**0-71.7**	**10-35**	**7/11**
Number Weakly Acid Refluxes/24hours	**17.1**	**0-44.1**	**5-18**	**5/11**
Bolus Clearance Time (secs)	**10.0**	**7-17**	**8-13**	**0/11**
Proximal Reflux Events	**15.8**	**0-32.2**	**4-17**	**5/11**
Liquid Reflux Events	**17.9**	**0-47.3**	**10-32**	**1/11**
Mixed Reflux Events	**44.4**	**12.1-78.8**	**11-26**	**9/11**
Upright Reflux Events	**50.1**	**19.3-96.8**	**23-52**	**5/11**
Supine Reflux Events	**8.1**	**0-23.4**	**1-6**	**7/11**

Three patients with a positive RSI score (RSI>13) had pathological proximal reflux; Five patients with a positive RSI had no pathological proximal reflux. 2 patient with a negative RSI score had abnormal proximal reflux and 1patient had a negative RSI score and a proximal reflux score which fell within the normal range (<17) (Table 5-6).

Table 5-6: The predictive value of the RSI score

	Proximal Reflux	No proximal reflux	
RSI positive	**3**	**5**	**PPV= 37.5%**
RSI negative	**2**	**1**	**NPV= 33%**
	Sensitivity= 60%	**Specificity= 16.7%**	

PPV= Positive Predictive Value, NPV=Negative Predictive Value

No significant correlation existed between RSI and the Demeester score (P = 0.133) (Figure 5- 8 i). In addition, no significant correlation existed between RSI score and proximal reflux measured on oesophageal impedance (P = 0.433). (Figure 5- 8 ii).

Automatic symptom analysis using the MMS software could not be performed due to poor compliance of patients with the symptom button and diary. Symptoms were studied using the questionnaires only.

Comparison of manometry with reflux indices

For the nine patients who were able to tolerate HRM, the findings of their manometry were compared to their reflux assessments. A sphincter length over 3.5cm appears to result in higher levels of both distal and proximal reflux but this relationship was not significant (P= 0.342 and P= 0.431 respectively) (

Figure 5- 9). A larger intra-abdominal sphincter length appears to result in a lower level of reflux (A negative value simply implies that the LOS lies above the true pressure inversion point i.e. Suggestive of a hiatus hernia and is thus NOT intra-abdominal). A longer intra-abdominal sphincter length was related to a lower Demeester score (p=0.004), but intra-abdominal sphincter length did not appear to determine proximal reflux extent (Figure 5- 1 0). Five patients had measurable hiatus hernias on HRM. There was no significant relationship between the LOS resting pressure and distal or proximal reflux (Figure 5- 1 1) (P = 0.932 and P = 0.308 respectively).

Figure 5- 5 : Dot plot Showing CF patient Demeester Scores (n=11)

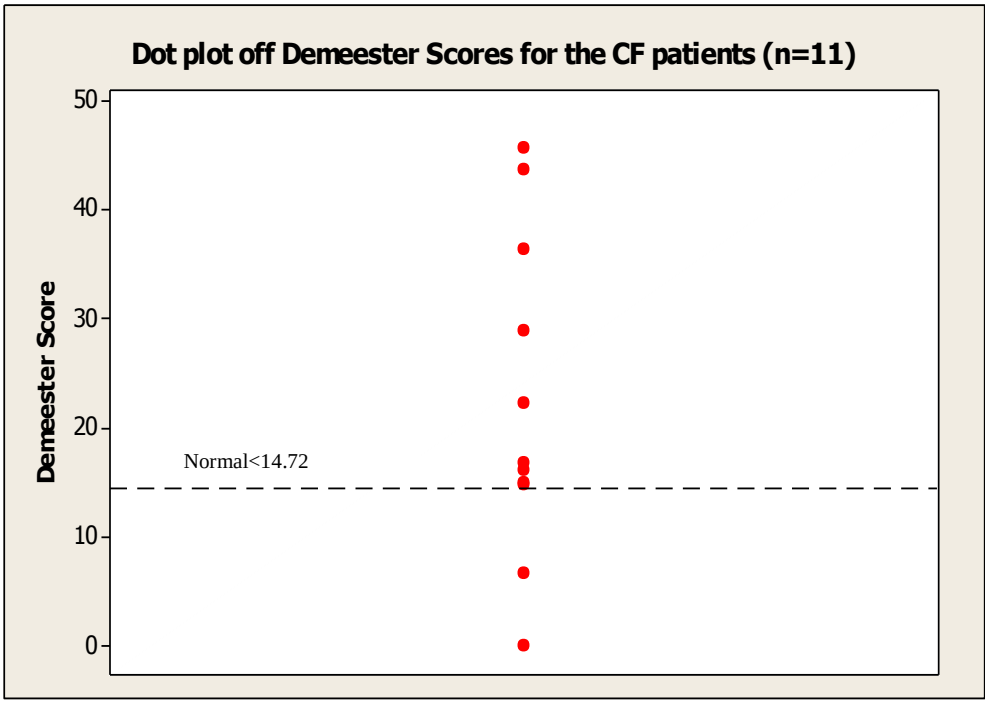


Figure 5- 6 : Dot plot showing showing CF patient proximal Relfux scores (n=11)

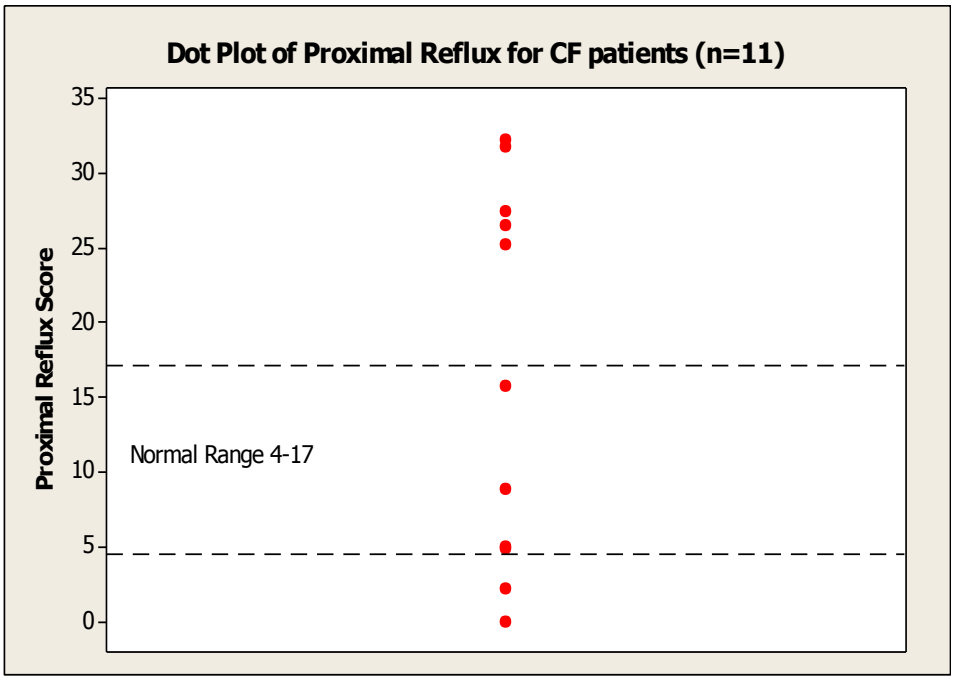
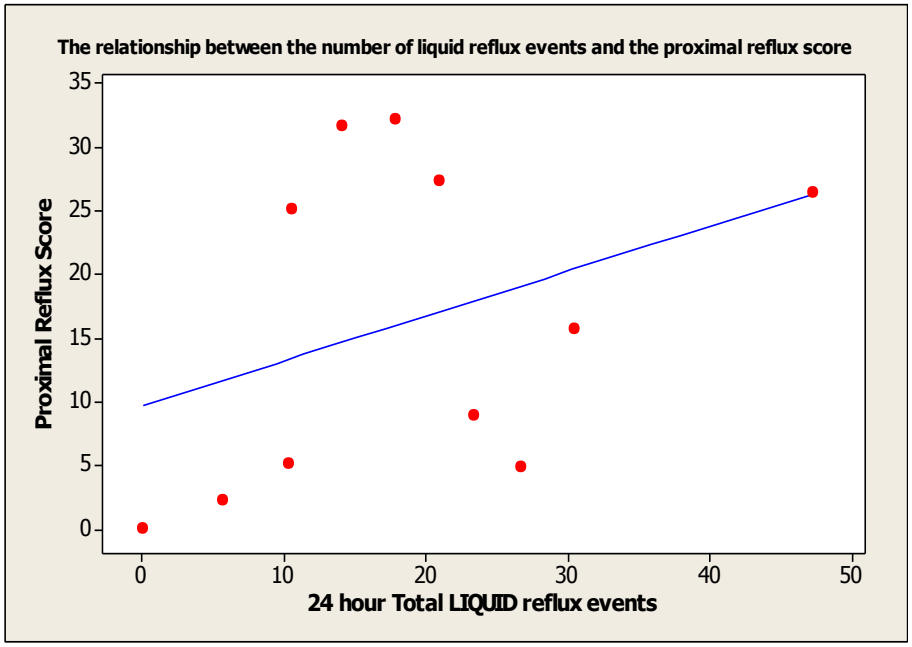


Figure 5- 7 :Scatter plots showing: i) the relationship between liqid reflux events (x-axis) and proximal reflux (y-axis) ii) the relationship between mixed reflux events (x-axis) and Proximal Reflux (y-axis).

i)



ii)

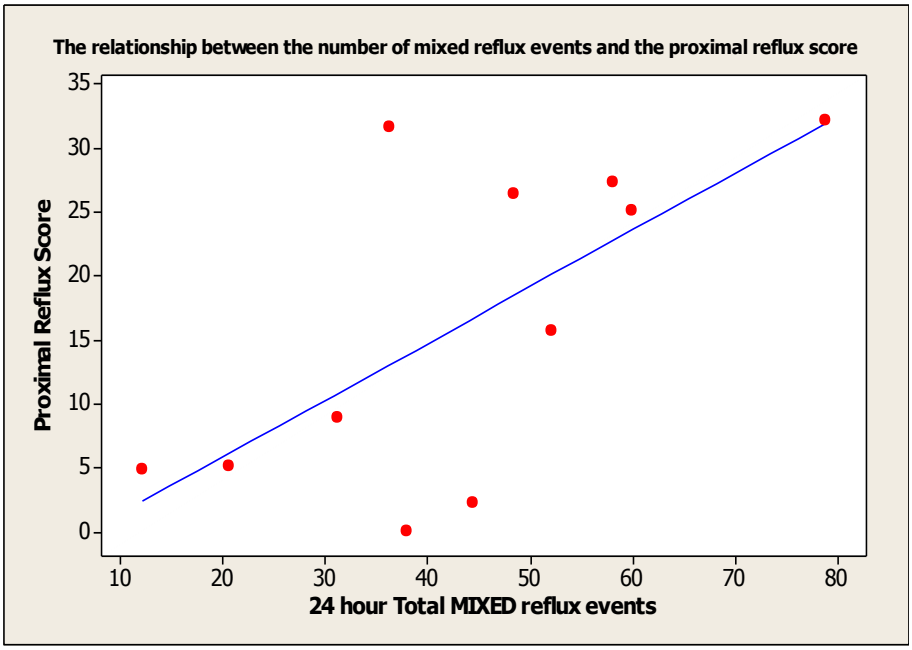
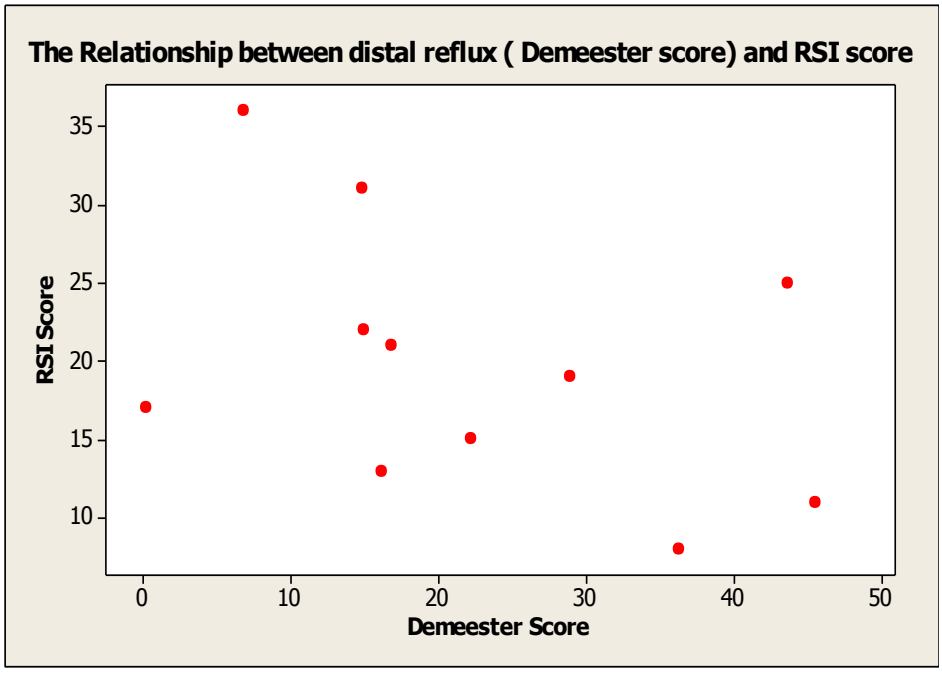


Figure 5- 8 : Scatter plots showing: i) the relationship between the RSI score (x-axis) and distal reflux as defined by Demeester score (y-axis) ii) the relationship between the RSI score (x-axis) and Proximal Reflux (y-axis).

i)



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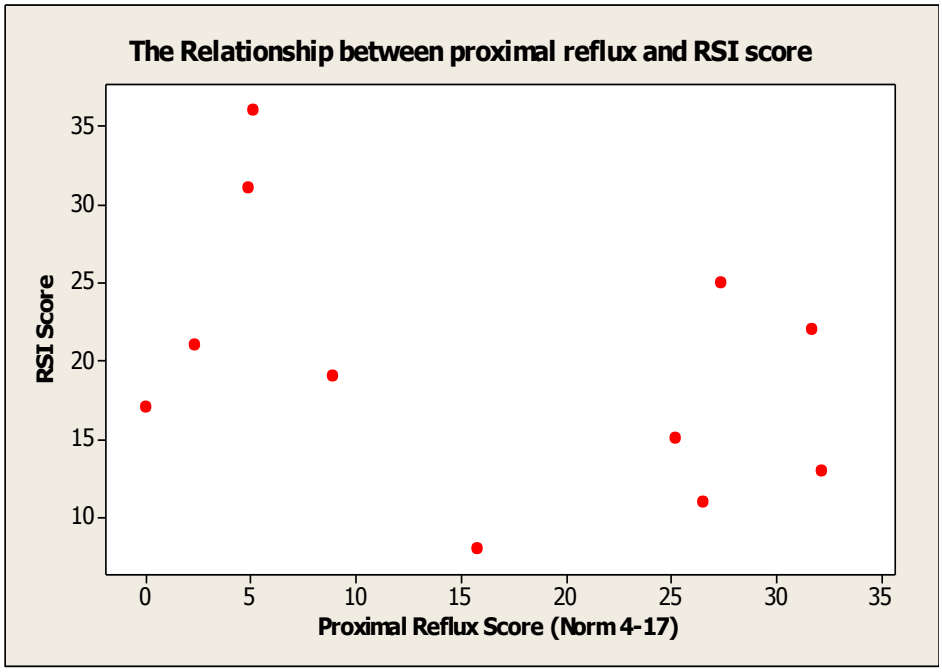
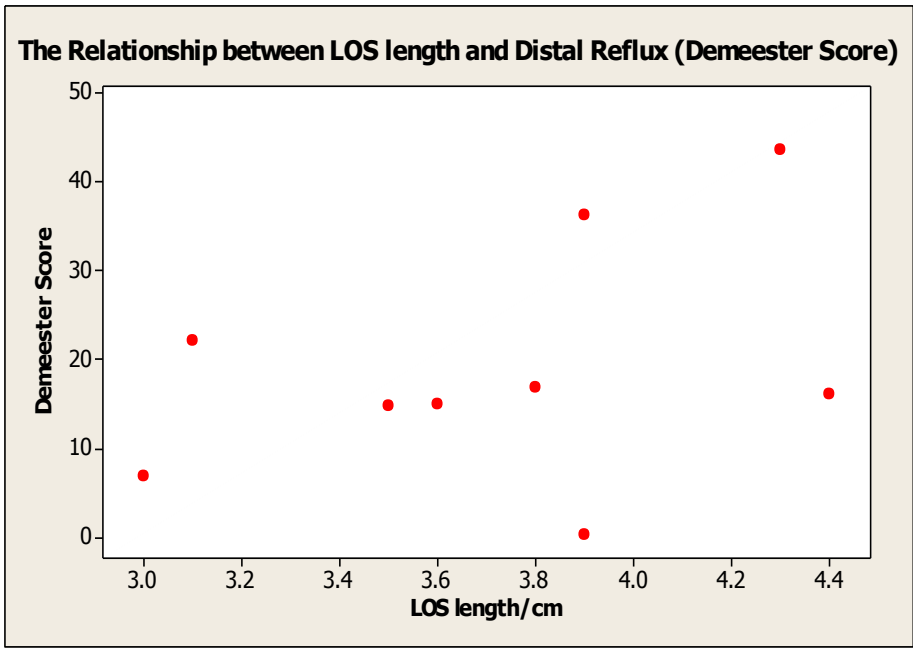


Figure 5- 9 : i) The relationship between LOS length (x-axis) and distal reflux as indicated by Demeester score (y-axis); ii) the relationship between LOS length (x-axis) and proximal reflux (y-axis)

i)



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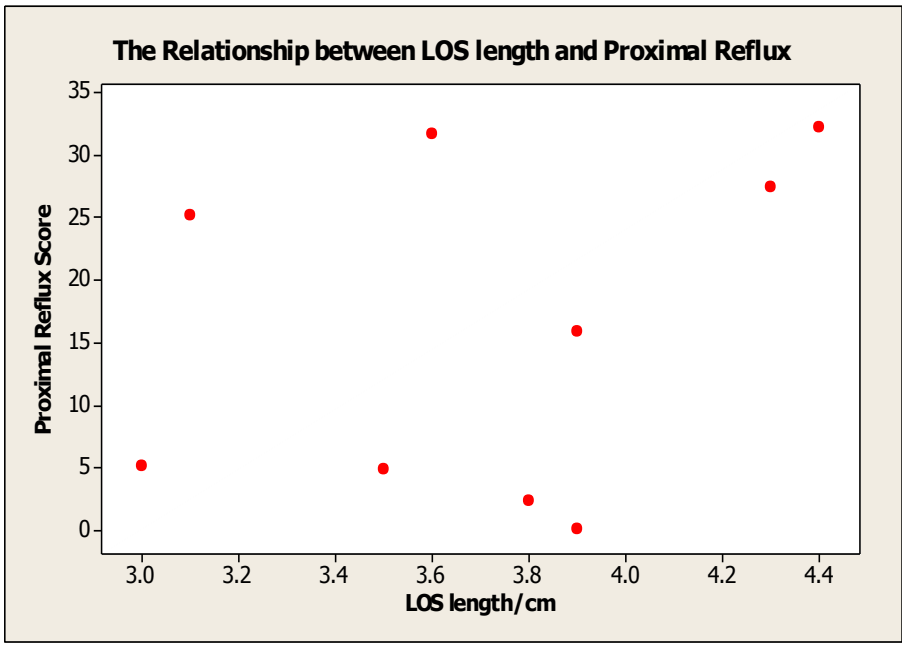
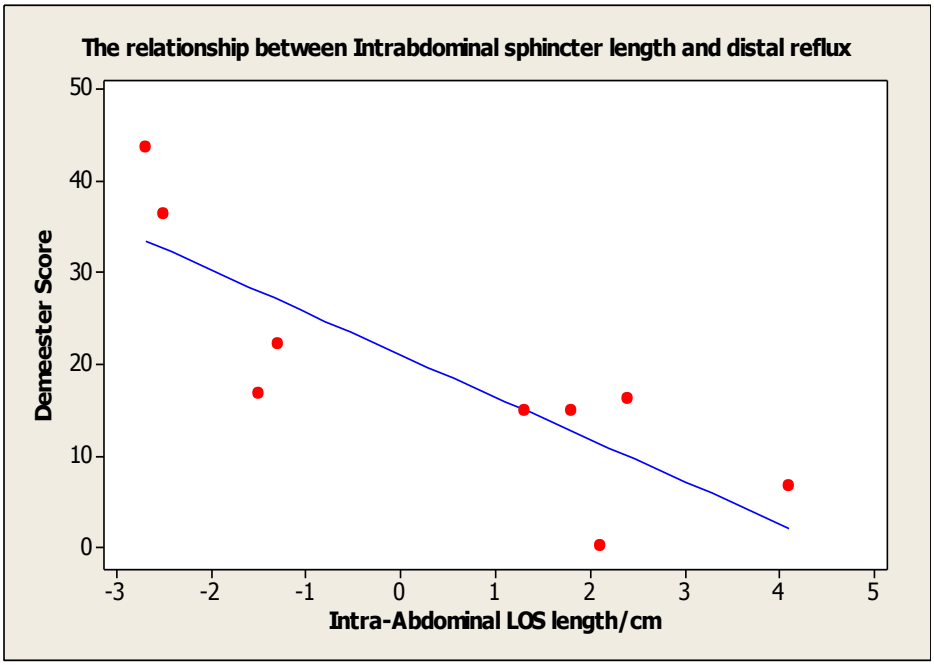


Figure 5-10 : i) The relationship between intra-abdominal LOS length (x-axis) and distal reflux as indicated by Demeester score (y-axis); ii) the relationship between intra-abdominal LOS length (x-axis) and proximal reflux (y-axis)

i)



ii)

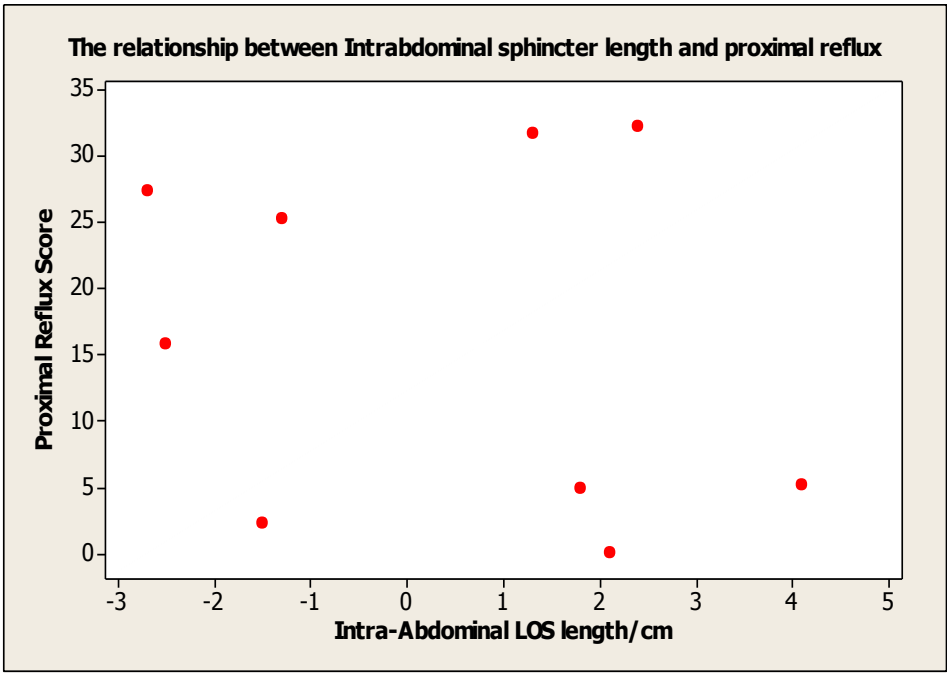
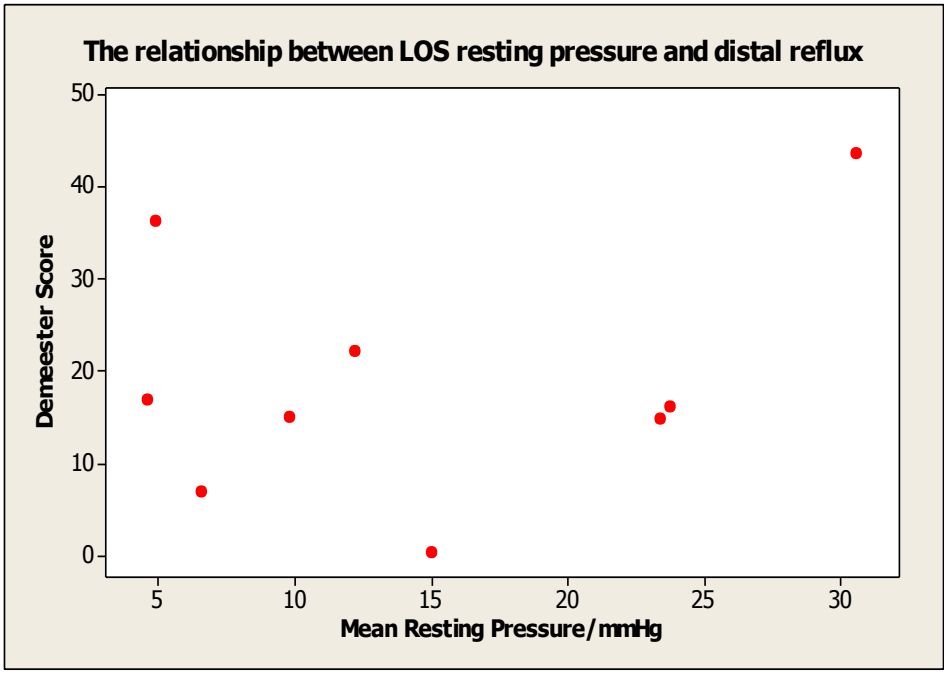
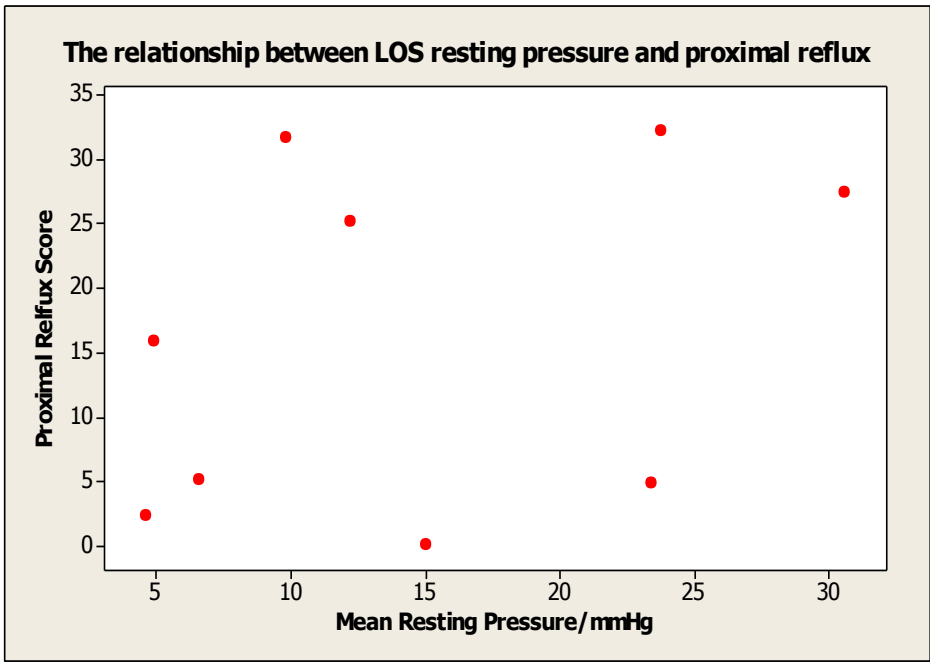


Figure 5- 1 1 :i) The relationship between LOS pressure (x-axis) and distal reflux as indicated by Demeester score (y-axis); ii) the relationship between LOS pressure (x-axis) and proximal reflux (y-axis)

i)



ii)



5.3.5 Sputum Processing Data

Cell Counts

All eleven patients with CF were able to produce a sample of sputum prior to their oesophageal investigations and this was processed so that a differential cell count could be performed. Due to the quality of the sputum only ten samples were suitable for performing a differential cell count.

Table 5-7:The median total cell and differential cell counts in sputum

	CF patients	Normal Values [154]
Total Sputum cell count (cellsx10^6/g)	**23.1**	
Neutrophils (%)	**100**	**33.6**
Lymphocytes (%)	**0.6**	**1.25**
Macrophages (%)	**0**	**57.8**
Eosinophils (%)	**0**	**0.3**

The majority of cells in the sputum of CF patients were neutrophils. No additional staining was performed due to the low percentages of macrophages (See Table 5-7 and Table 5-8).

Table 5-8: Summary of differential cell counts for CF 1-20.

Patient No	Sputum Vol/ml	Cell Count(x10^6/g)	%Macrophages	%Lymphocytes	%Neutrophils	%Eosinophils
CF1	0.76	23.1	0	0	100	0
CF2	0.69	123.36	0	0	100	0
CF3	0.68	16.6	0	1	99	0
CF4	0.49	32.52	0	0	100	0
CF5	0.86	16.37	0	0	100	0
CF6	0.43	2.03	0	0.6	99.4	0
CF7	0.64	23.4	0	0	100	0
CF8	1.22	38.79	0	0.6	99.4	0
CF9	0.48	0.67	N/A	N/A	N/A	N/A
CF10	1.39	31.59	0	0	100	0
CF11	1.6	2.29	0	0	99.6	0

5.3.6 Markers of aspiration

Bile Salts

Two sets of sputum samples were collected and processed for bile salts analysis. The sample taken on the days of the investigation, and a further sample taken 24hours later after the oesophageal investigation were complete. Sputum samples from all 11 CF patients were analysed using a combination of tandem mass spectrometry followed by a specialised extraction technique to allow the sensitivity of detecting bile salts to be increased to a minimum level of 0.001µmol/L. Concentrations of the individual bile salts (glycodeoxycholate, glycocholate, taurodeoxycholate and tauracholate) were added together to give the total bile salt concentration. The concentration of free lithocholate was also available using the extraction technique described in the previous chapter. The table below (Table 5-9) shows the concentration of bile salts identified in the sputum samples of CF.

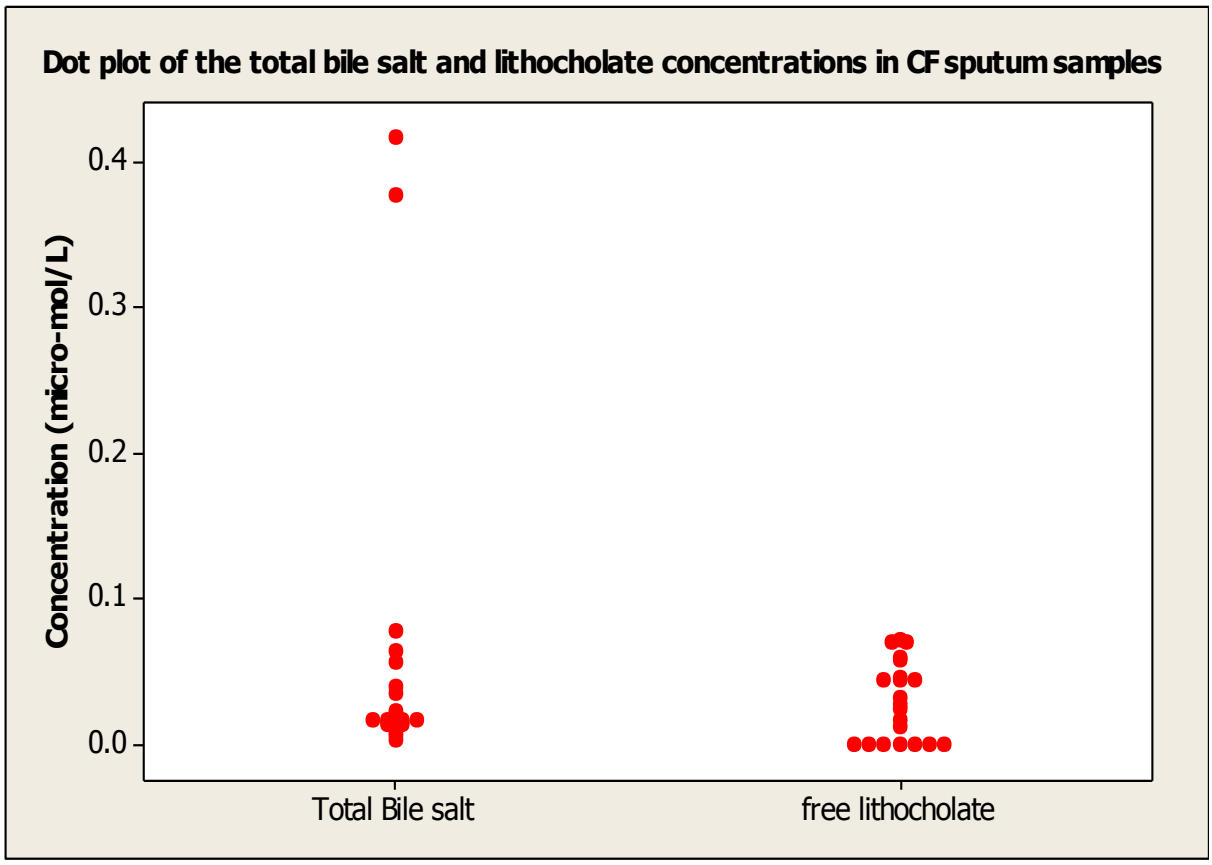
Of the 22 patient samples taken, twenty-one were suitable for analysis. All 21 samples showed detectable bile salts and 14/20 showed detectable free lithocholate (Figure 5-12). The highest bile salt concentration was 0.416µmol/L and the highest free lithocholate concentration was 0.072µmol/L. The median value for bile salts in the 21 CF sputum samples analysed was 0.016µmol/L. The median free lithocholate concentration in the 21 CF sputum samples analysed was 0.027µmol/L.

Table 5-9: Bile salt concentration in the sputum samples of CF patients (n=11)

	G-DHC µmol/l	G-THC µmol/l	T-DHC µmol/l	T-THC µmol/l	Total Conc. µmol/l	Free Lithocholate µmol/l
CF1	0.114	0.117	0.007	0.139	0.377	0.000
CF1-2	0.041	0.286	0.011	0.079	0.416	0.072
CF2	0.007	0.019	0.006	0.007	0.039	0.000
CF2-2	0.001	0.000	0.004	0.015	0.020	0.032
CF3	0.000	0.001	0.007	0.015	0.022	0.000
CF3-2	0.000	0.001	0.002	0.003	0.005	0.000
CF4	0.000	0.009	0.000	0.013	0.021	0.000
CF4-2	0.005	0.005	0.001	0.004	0.015	0.044
CF5	0.001	0.000	0.003	0.004	0.007	0.000
CF5-2	0.000	0.001	0.000	0.002	0.003	0.016
CF6	Insufficient Sample	Insufficient Sample	Insufficient Sample	Insufficient Sample	Insufficient Sample	Insufficient Sample
CF6-2	0.007	0.002	0.002	0.005	0.016	0.059
CF7	0.007	0.002	0.004	0.003	0.015	0.000
CF7-2	0.046	0.021	0.005	0.006	0.078	0.070
CF8	0.024	0.030	0.004	0.006	0.064	0.046
CF8-2	0.008	0.003	0.000	0.006	0.016	0.011
CF9	0.001	0.000	0.010	0.005	0.016	0.027
CF9-2	0.008	0.003	0.002	0.003	0.016	0.024
CF10	0.005	0.001	0.003	0.004	0.013	0.070
CF10-2	0.032	0.003	0.013	0.008	0.056	0.044
CF11	0.005	0.000	0.002	0.006	0.013	0.044
CF11-2	0.022	0.007	0.002	0.004	0.035	0.057

KEY: G-DHC = glycodeoxycholate, G-THC = glycocholate, T-DHC = taurodeoxycholate, T-THC = taurocholate. ND = not detected

Figure 5-１２: Dot plots showing Bile salt concentration and free lithocholate concentration (y-axis) in the sputum samples of CF patients.



Pepsin

Sputum samples from all 11 CF were analysed using an ELISA technique to detect pepsin. Table 5-10 shows theses pepsin values compared against reflux study results and lung function decline. 7/11 patient samples showed detectable pepsin . The highest pepsin concentration was 324ng/ml. The median pepsin concentration in the 11 CF patients was 88ng/ml which was higher than the median level detected in normal controls (7.7ng/ml) [137].

Of the seven patients with elevated pepsin levels in the sputum 6 had high Demeester scores indicating significant reflux and 3 patients had proximal reflux. Five patients showed a decline in FEV_1. Pearson's test showed no correlation between pepsin levels and either Demeester and proximal reflux scores.

Table 5-10: Pepsin Concentrations in the sputum samples of CF patients (n=11)

Patient No	Pepsin Conc (ng/ml)	Demeester Score (<14.72)	Total Reflux Time (<4.2%)	Proximal reflux Score (4-17)	%Decline FEV_1
CF1	0	14.84	4.10	4.9	-16.5
CF2	152	43.65	16.50	27.4	-14.2
CF3	88	14.95	4.70	31.7	-3.6
CF4	0	45.55	13.50	26.5	-11.6
CF5	0	22.24	6.00	25.2	-3.5
CF6	324	28.95	8.4	8.9	-18.2
CF7	196	16.18	7.5	32.2	-30.9
CF8	112	6.81	1.6	5.1	-14.9
CF9	0	0.2	0	0	-4.0
CF10	80	16.81	5.7	2.3	1.2
CF11	111	36.3	7.9	15.8	5.0

5.3.7 Lung Function

Serial lung function results (3 per patient) were collected for all eleven CF patients. The individual FEV_1 values were plotted against the time period in weeks to reveal a regression line with a formula in the format $y=mx+c$. Where y= FEV_1 m= gradient of the plot created from serial lung function= and c= the constant .The values of t=0 and t=52 (1year) were re-inputted into the formulas and the percentage decline of lung function per year was calculated for each patient from the FEV_1 values at t=0 and 1 year (Table 5-11).

Table 5-11: Summary of lung function decline as measured using FEV_1 with corresponding reflux scores for CF 1-11.

Patient No	% Decline FEV_1	Demeester Score (norm <14.72)	Proximal Reflux Score (norm 4-17)
CF1	-16.5	14.84	4.9
CF2	-14.2	43.65	27.4
CF3	-3.6	14.95	31.7
CF4	-11.6	45.55	26.5
CF5	-3.5	22.24	25.2
CF6	-18.2	28.95	8.9
CF7	-30.9	16.18	32.2
CF8	-14.9	6.81	5.1
CF9	-4.0	0.2	0
CF10	1.2	16.81	2.3
CF11	5.0	36.3	15.8

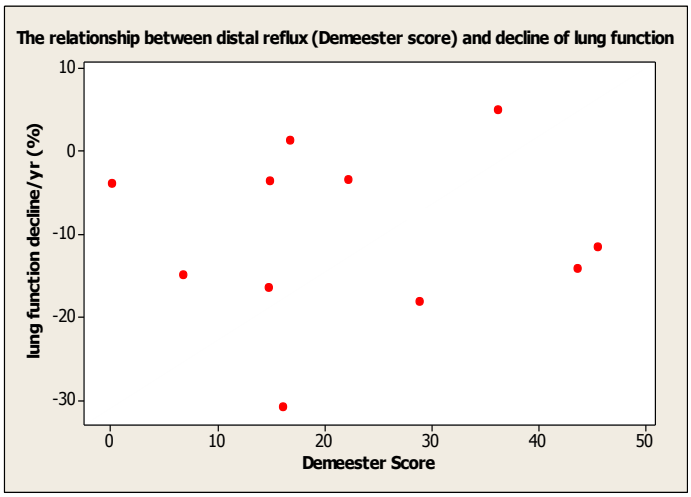
Decline of FEV_1

The median percentage decline of FEV_1 per year was 11.6% with the largest decline of FEV_1 being 30.9%. Two patients were shown to have an increase in FEV_1/year with the largest percentage gain being 5.0%.

There was no relationship between the percentage decline of FEV_1 and Demeester score (Pearson correlation = 0.173, p =0.612).The degree of proximal reflux was not related with decline of lung function (Pearson correlation = -0.191, p=0.574). RSI score did not correlate with a larger percentage decline of lung function (Pearson correlation = -0.309, p=0.355) (Figure 5-13).

Figure 5- 1 3 : Scatter plots showing: i) the relationship between the decline in lung function (y-axis) and distal reflux as defined by Demeester score (x-axis) ii) the relationship between the decline in lung function (y-axis) and Proximal Reflux (x-axis) iii) the relationship between the decline in lung function (y-axis) and RSI score (x-axis).

i)



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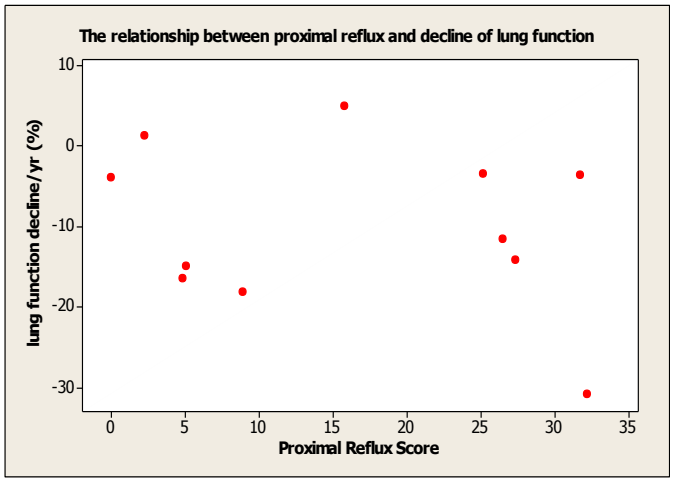
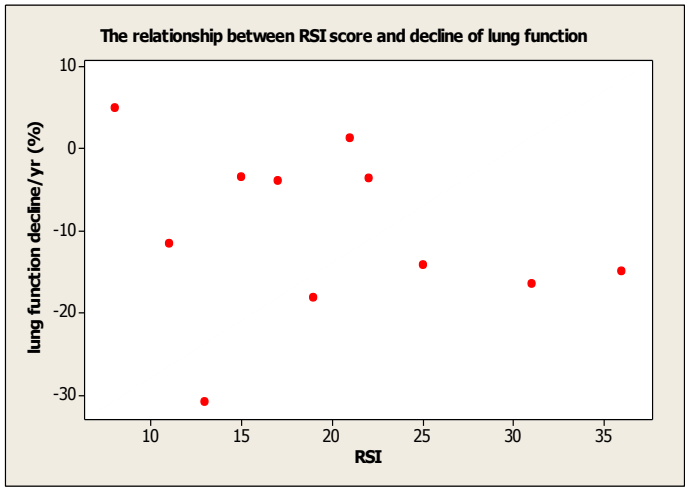


iii)



5.4 Summary of CF Results

5.4.1 Clinical Results

It was hard to get data in this population of 40 patients deemed to be suitable and the 26 patients actually approached only 11 were successfully studied. Between June 2011 and March 2012, eleven patients with Cystic Fibrosis were studied. This included six males and five females who had a median age of 29 years. Baseline median lung function for the group was a FEV_1 of 1.86 litres and a Vital Capacity (VC) of 2.15 litres.

All 11 patients were on acid suppression therapy prior to their recruitment into the study; only four had documented evidence of gastro-oesophageal reflux (GOR) in their notes. All the patients studied were classified as having typical CF with a Δ508 mutation and all had pancreatic insufficiency. Five of the eleven patients had insulin dependent diabetes.

Nine patients successfully completed oesophageal manometry and all eleven completed Impedance-pH studies. Three patients demonstrated normal oesophageal peristalsis High Resolution Manometry (HRM). The most common abnormality detected on HRM was rapid contractions. Of the eleven patients, nine had objective evidence of reflux on impedance-pH. Five patients had weakly acid reflux and five patients had evidence of abnormal proximal reflux. Most reflux events were mixed (liquid and gas). The incidence of reflux was not related to lower oesophageal sphincter (LOS) resting pressure but an increased intra-abdominal LOS length was associated with a lower Demeester score.

Patient symptoms and the effect on quality of life were studied using validated questionnaires. All eleven patients were already on acid suppression before they entered the study and the questionnaires were completed ‘on’ and ‘off’ treatment for ten of the patients. Reflux symptom index (RSI) scores, assessing symptoms of extra-oesophageal reflux were abnormal (RSI > 13) in eight patients off therapy and abnormal in six patients whilst on their medication. Demeester questionnaire scores for patients ‘on’ and ‘off’ were 3 and 2 respectively. Quality of life scores were assessed with the Gastro-Intestinal Quality of Life Index (GIQLI). The median score was below the normal range (121-130) for patients ‘on’ and ‘off’ their treatment; whilst on acid suppression treatment 82% had a score below the normal range as opposed to 90% whilst off their treatment. Although all patients were on medical treatment to suppress gastric acid

production, nearly all had objective evidence of reflux and the symptom questionnaires indicate regular high dose acid suppression medication has very little effect.

Lung function tests were performed on all 11 CF patients. Using the raw lung function data, the percentage decline of FEV_1 over one year was calculated. 9/11 patients had a decline of FEV_1 with the largest decline being 30.9%. Seven of these patients had an abnormal Demeester score and five patients had abnormal proximal reflux scores. The percentage decline of FEV_1 did not correlate directly with abnormal Demeester Scores. Proximal reflux and RSI scores were not associated with a greater percentage decline of FEV_1 in the low numbers of patients studied.

5.4.2 Laboratory based studies

All 11 CF patients had sputum samples taken and processed using a standard operating procedure so that a differential cell count could be performed. In addition, pepsin and bile salt assays were performed on the resultant supernatant. The principal cell type identified in the sputum was the neutrophil and the median percentages of these were much higher than found in normal controls (100% vs. 33.6%).

Bile salt and pepsin analysis were performed on 22 samples taken in the 24 hour period the patients attended for their oesophageal tests. Of the 22 patient samples taken, twenty-one were suitable for analysis. All 21 samples showed detectable bile salts and 14/20 showed detectable free lithocholate. The highest bile salt concentration was 0.416µmol/L and the highest free lithocholate concentration was 0.072µmol/L. Elevated pepsin concentrations were detected in seven of the eleven CF patients with a median pepsin concentration of 88ng/ml, over 10 times higher than the concentrations of pepsin found in the sputum of healthy controls [137].

6 Anti-reflux surgery in lung transplant patients

6.1 Introduction

The previous sections of this results chapter focused on the assessment of reflux in the patients with idiopathic pulmonary fibrosis and cystic fibrosis. The latest data from the International society of heart and lung transplantation (ISHLT) suggests IPF patients represent 30.2% of all lung transplants performed in the adult population and CF patients represent 14.4% of all lung transplants performed between 2011 and 2012 [155]. Newcastle data indicates that more CFs than this are transplanted, up to 30% [156]. Thus a considerable percentage of patients with IPF and CF make up the lung transplant population. This section comprises of work initiated by *Robertson at al* [36] and then completed by myself. The combined work and results in this section of the thesis have been presented in a peer-reviewed publication [97].

Up to 75% of lung transplant patients have demonstrable gastro-oesophageal reflux disease (GORD) [128-132]. In routine practice, anti-reflux surgery has been shown to improve symptoms and quality of life. In lung transplant recipients it is hypothesised that early anti-reflux surgery may also lead to protection of lung function and increased survival, through preventing microaspiration. Most of the current evidence of the effects of fundoplication in lung transplant patients originates from Duke University [132]. More recently in our unit work by *Robertson at al* [36] in a small number of patients demonstrated that anti-reflux surgery improves both reflux and extra-oesophageal reflux symptoms.

However, there is a lack of basic information in this patient group including safety and assessments of quality of life. Such information is particularly important in this patient group who have already endured many years and months of chronic ill-health as well as the post-operative stresses after their transplantation. Physiological post-operative complications of anti-reflux surgery include temporary dysphagia, nausea[134], discomfort from gas bloat and increased flatulence[129] and are common post-fundoplication, This puts these patients at risk of physiological dysfunction and reduced quality of life after surgery.

Early data from our unit had demonstrated that fundoplication in a small group of lung transplant patients had resulted in improved lung function [36] .This part of my thesis focuses on work which complements the study and initial findings of *Robertson et al*

[36] by assessing the safety of fundoplication in lung transplant recipients and its effects on quality of life and lung function.

6.2 Methods

All lung transplant recipients referred to the Northern Oesophago-Gastric Unit for reflux assessment and consideration for anti-reflux surgery between 1st June 2008 and 31st December 2010 were studied. Between June 2008 and December 2009 recruitment was performed by Mr. A.G.N. Robertson and continued by myself until December 2010. Surgery was considered for patients with symptomatic reflux alone, or for reflux associated with deteriorating lung function.

Reflux status was assessed on proton pump inhibitor therapy, by oesophageal manometry, pH-impedance and endoscopy. Patients underwent a thorough pre-operative assessment to ensure fitness for surgery. Reflux status was defined by the presence of symptoms combined with objective evidence of GORD on pH-impedance and/or endoscopy. Pulmonary function tests and bronchoscopy were routinely performed in the preoperative work-up.

Patients were followed up clinically with emphasis on lung function, satisfaction with treatment and quality of life. The validated questionnaires described in the previous chapter were used; the DeMeester Reflux Questionnaire, the Reflux Symptom Index (RSI) questionnaire and the Gastro-Intestinal quality of life index (GIQLI). These were completed pre-operatively, 6 weeks and 6 months post-operatively. Pre and post-fundoplication BMI were recorded. Patient satisfaction was assessed by directly questioning of patients.

Lung function was assessed in accordance with European standardised spirometry guidelines [157]. Bronchiolitis obliterans syndrome scores were calculated using FEV_1 in accordance with International Society for Heart and Lung Transplantation guidelines [127, 158].

Surgical technique

Laparoscopic Nissen fundoplication was performed using the same surgeon with the same operating technique as follows. A 4-port access technique was used with the epigastric incision allowing for the Nathanson retractor to retract the liver. The oesophageal hiatus was dissected to mobilise the oesophagus with care taken to preserve the posterior vagus nerve. A surgical window was created behind the oesophago-gastric junction to allow a loose 360^o wrap to be tailored. The wrap was secured with 3 sutures and the posterior crura were repaired to tighten the hiatus. One

further suture was used to anchor the wrap to the oesophagus and right crus. Local anaesthesia was inserted into the peritoneal cavity and infiltrated in the wounds at the end of the procedure [36].

6.3 Results

6.3.1 Demographics

Between the 1st June 2008 and the 31st December 2010, 109 lung transplants took place in total. 16 patients were referred to the Northern oesophagogastric unit for reflux assessments and consideration for anti-reflux surgery. Of the sixteen patients studied, ten were female and six were male with a median age of 38 years. The majority of patients had a background of cystic fibrosis as their condition requiring lung transplant (n=10). Patients with a background of fibrotic lung disease made up one-quarter of the lung transplant group studied (n=4). Thirteen patients had a single sequential lung transplant, one patient had left single lung transplant and two patients had right single lung transplants.

Table 6-1: Demographics of the lung transplant group

Demographics	
Age	Median 38years (range 24-63)
Sex	
-Male	6
-Female	10
Underlying Pathology	
-Cystic Fibrosis	10
-Pulmonary fibrosis	4
-COPD/Asthma	2
#Transplant	
-SSLT	13
-LSLT	1
-RSLT	2

6.3.2 Oesophageal Manometry

All 16 patients underwent traditional 8 channel manometry as described in the previous chapter. Overall 81.3% of patients (13/16) had normal oesophageal physiology on manometry. No complications were attributed to the procedure.

- **Lower oesophageal Sphincter**

The median lower oesophageal sphincter length was 2.75cm (range 1.5-4.3cm). Sphincter pressure was within normal limits (6-25mmHg) in the majority of the patients (11/16) with an average sphincter pressure of 24.7mmHg (Range 9.3-55.36mmHg). Five patients had a hypertonic LOS and the remaining patients had a normotonic sphincter. The median of the mean distal amplitude of the swallows was 60.95mmHg (Range 18-165.9mmHg).

6.3.3 pH-Impedance

All sixteen lung transplant patients completed the 24 hour recordings whilst on PPI therapy. 15 of 16 patients (94%) patients had pathological distal reflux as determined by an abnormal Demeester score (Figure 6-1). Over half the patients had evidence of proximal reflux (Figure 6-2). A summary of the median reflux indices as determined by impedance monitoring is shown in the table below (Table 6-2).

Table 6-2: Median Reflux Indices as determined by pH-impedance analysis

	Median	Range	Normal Values	No. of patients with abnormal results
Demeester Score	**52.8**	**7.47-115.22**	**<14.72**	**15/16**
Acid Exposure (%). (% of time pH<4, in 24hrs)	**15.45**	**1.6-33.1**	**<4.2**	**14/16**
Total Reflux Events on impedance	**62**	**10-125**	**25-58**	**9/16**
Proximal Refluxes	**24**	**2-71**	**4-17**	**9/16**
Oesophageal Volume Exposure	**1.09**	**0.16-3.84**	**0.4-1.2**	**6/16**

6.3.4 Lung function

The rate of decline in FEV_1 was calculated in standardised method [159] using serial FEV_1 readings from the patient's lung function tests before fundoplication up to the final FEV_1 readings available after fundoplication. First of all the FEV_1 values were plotted from the baseline FEV_1 level to the time fundoplication was performed and the gradient between points was calculated in millilitres per month. The same was done for the FEV_1 measurements after fundoplication.

6.3.5 Other Assessments

All patients had a diagnostic gastroscopy (OGD). 15/16 patients had a hiatus hernia on OGD (2-6cm). 8/16 had oesophagitis (grade A n=4), (grade B n=3), (grade C n=1). Grade C is the most severe erosive oesophagitis. One patient had a small tongue of Barrett's oesophagus confirmed on histological assessment. Three patients had oesophageal candidiasis which was treated pre-operatively.

Figure 6- 1 : Dot plot Showing lung transplant patient Demeester Scores (n=16)

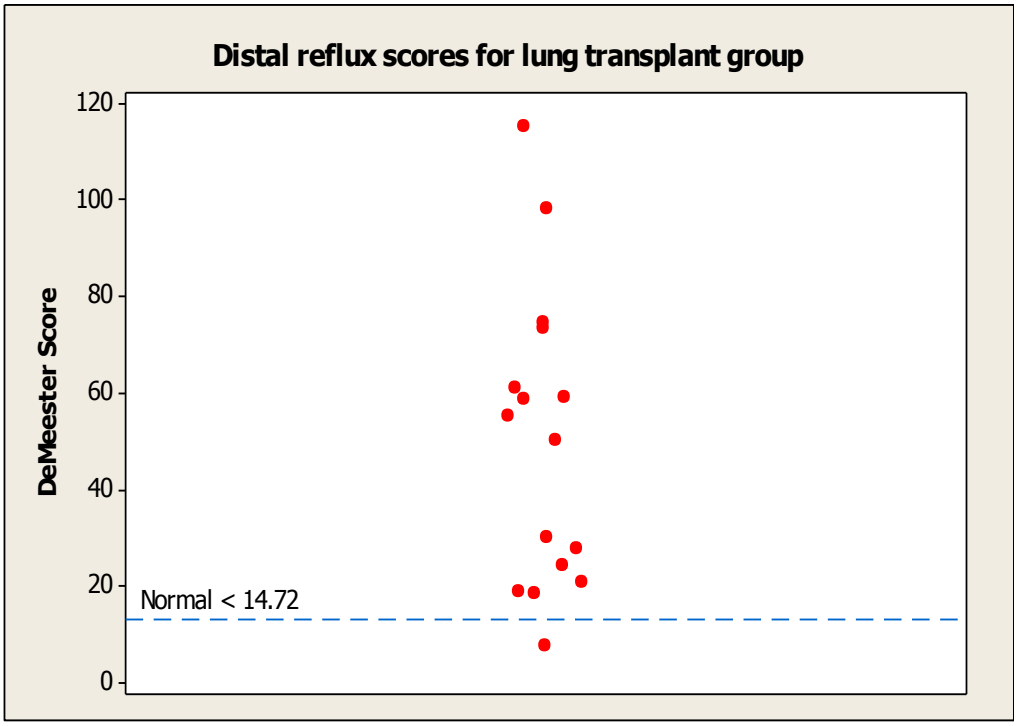
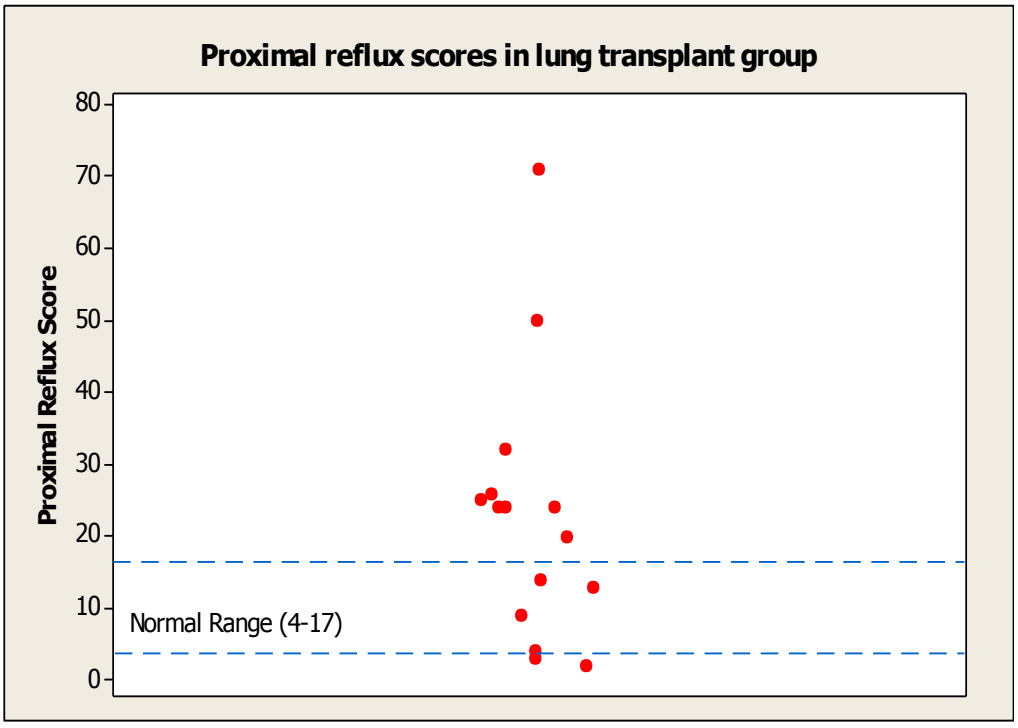


Figure 6- 2 : Dot plot Showing lung transplant patient proximal reflux scores (n=16)



6.3.6 Reflux Questionnaires

There was a statistically significant improvement in symptoms and quality of life scores over the first six months post-fundoplication. Questionnaires were completed by 15/16 patients. One patient, despite reporting high levels of satisfaction with their result, did not wish to spend time completing these questionnaires.

Table 6-3: Median quality of life questionnaire scores before and after anti-reflux surgery

	Pre-operative	**Six weeks**	**Six months**
GIQLI	106 (65-132)	118 (63-133)	128 (75-142)
DeMeester	4 (1-6)	1 (0-4)	1 (0-2)
RSI	15 (8-23)	3.5 (2-18)	2 (0-18)

Reflux Symptom Index questionnaire

Pre-fundoplication RSI was positive on 8/15 patients and this decreased to 3/15 being positive for RSI by six weeks and 2/15 being positive at six months. The median RSI underwent a statistically significant improvement from 14 (range 1-23) pre-operatively to 4 (range 0-25) at six weeks post-fundoplication (p=0.01) and 2 (range 0-20) at six months (p=0.0005) (Figure 6- 3 i).

DeMeester reflux questionnaire score

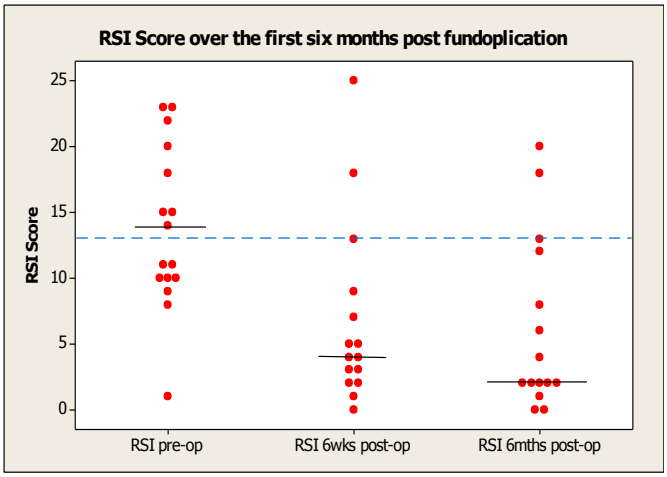
There was a statistically significant improvement in median DeMeester reflux questionnaire score from 4 (range 1-6) pre-operatively to 1 (range 0-5) at six weeks (p=0.007) and 1 (range 0-3) at six months (p=0.001) (Figure 6- 3 ii).

GIQLI

There was an improvement in median GIQLI score from 106 (range 54-132) pre-operatively to 116 (range 61-133) at six weeks (p=0.06). This was a statistically significant improvement by six months 127 (range 75-142) (p=0.004) (Figure 6- 3 iii). There was a statistically significant improvement from six weeks to six months (p=0.03).

Figure 6- 3 :Individual Dot plots showing: i) the change in RSI score (y-axis) 6 months after fundoplication ii) the change in Demeester questionnaire score (y-axis) 6 months after fundoplication iii) the change in GIQLI score (y-axis) 6 months after fundoplication

i)



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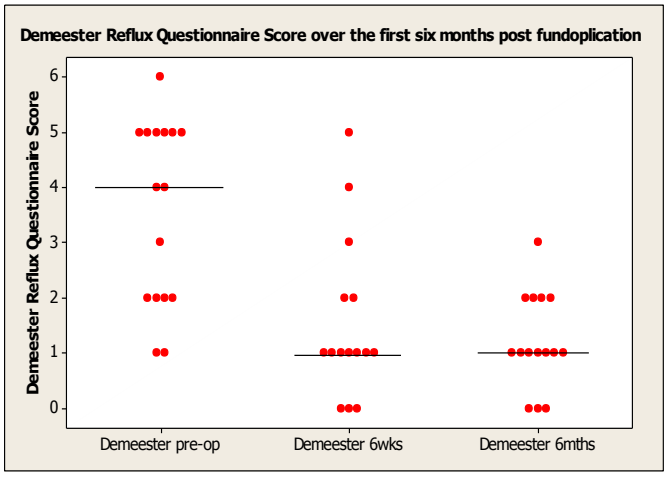

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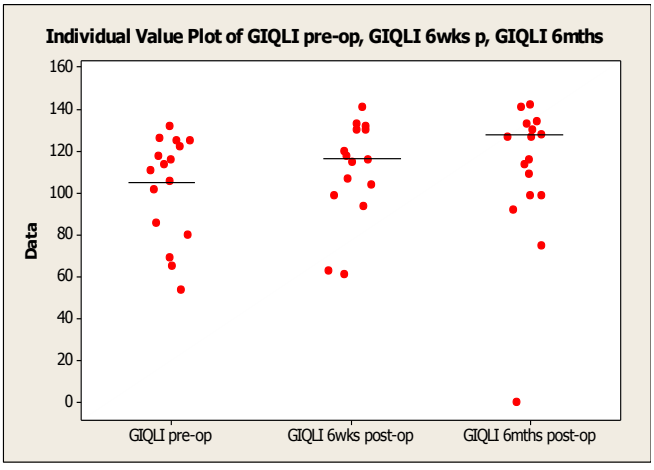


6.3.7 Body mass index

Median BMI significantly decreased from 23.4 (range 18.5-33.2) pre-fundoplication to 21.6 (range 17.6-32.9) at six months post-fundoplication ($p<0.001$) (Figure 6-4).

6.3.8 Lung function

Patients were followed up for a median of 502 days post-fundoplication (range 177-923days). Median FEV_1 was similar pre-fundoplication 2.05L (range 0.74-5.12L) and post-fundoplication 2.13L (range 0.73-5.21L) ($p=0.09$). Eight patients were operated on for deteriorating lung function. Of these eight, one patient had a reversal of BOS, two had a stabilisation of lung function and five had a decrease in the rate of deterioration. There was a statistically significant decrease in the rate of decline of FEV_1 per day post fundoplication from a median change of -132.3ml/month (range -4.5 to -242.4ml/month) pre-fundoplication to a median change post fundoplication of +6.9ml/month (range -22.5 to +117ml/month) post-fundoplication ($p=0.008$) (Figure 6-5).

6.3.9 Operation parameters and patient satisfaction

Fundoplication was performed at a median of 405 days post transplant (range 178-3235 days). Median intra-operative time was 90minutes (range 60-125minutes). All patients had blood loss of less than 100ml. 5/16 patients were admitted electively to our High Dependency Unit for observation for 24 hours but none of the patients required an ITU stay. Median hospital stay was 2 days (range 2-4 days). Longer stays were due to post-operative pain, peri-operative dysphagia (n=1), a return to theatre or difficulty arranging transport home.

Morbidity and mortality

There were no deaths or serious post-operative complications. Two patients developed post-operative dysphagia. One of these patients returned to theatre the following day and underwent a laparoscopy and minor revision of the wrap and subsequently made an uneventful recovery. In the other patient, barium swallow revealed no significant hold-up and symptoms subsequently resolved spontaneously.

Overall satisfaction with fundoplication

Overall 15/16 patients reported being satisfied at 6 months follow-up.

Figure 6- 4 : Individual dot plot showing the change in BMI (y-axis) after fundoplication

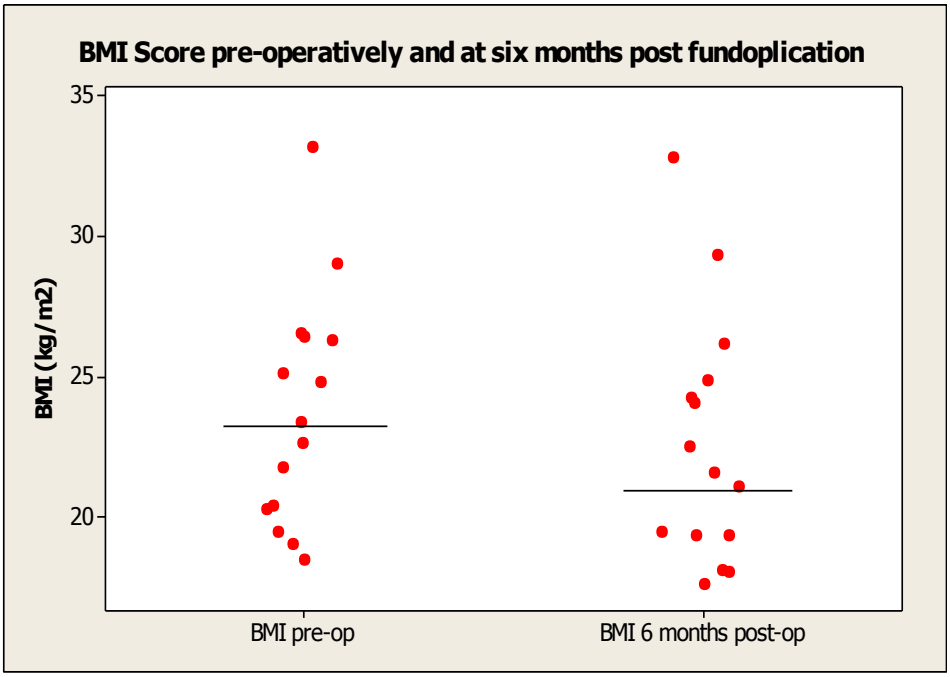
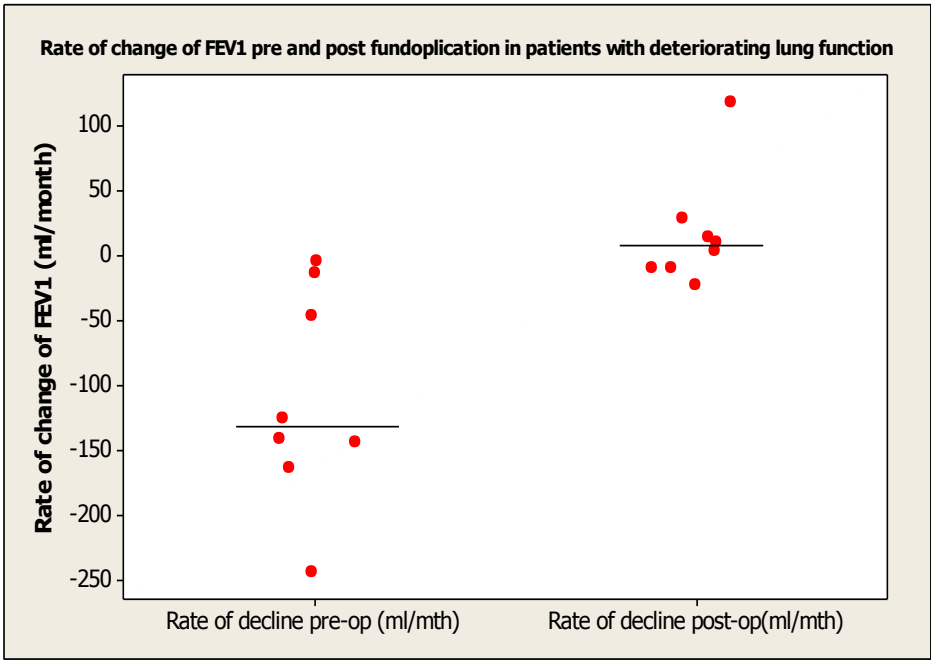


Figure 6- 5 : Individual dot plot showing the rate of change of FEV_1 (y-axis) after fundoplication



6.4 Summary of lung transplant patient Results

6.4.1 Reflux Findings

Between the 1st June 2008 and the 31st December 2010, 16 lung transplant patients were assessed at the Northern oesophagogastric. Of the sixteen patients studied, ten were female and six were male with a median age of 38 years. 15/16 patients had evidence of reflux with an abnormal Demeester score and 9/16 had significant proximal reflux. Most patients (13/16) had normal oesophageal physiology when assessed with 8-channel manometry. The significant incidence of reflux was confirmed on endoscopy with half the patients having visible signs of inflammation.

6.4.2 Operative Outcomes

All patients successfully underwent laparoscopic fundoplication with no permanent morbidity or mortality. 15/16 patients expressed their satisfaction with the operation when directly questioned. Reflux questionnaire assessments showed significant improvement of RSI, Demeester questionnaire and GIQLI scores after the operation. This improvement was seen as early as 6 weeks post fundoplication. In addition, the rate of decline of FEV_1 was significantly reduced after fundoplication.

7 Discussion

7.1 Idiopathic Pulmonary Fibrosis patients

7.1.1 Recruitment to study

Between July 2010 and March 2012 38 patients diagnosed with idiopathic Pulmonary Fibrosis (IPF) were approached to enter the study. Twenty nine patients initially consented to the study but in total nine patients dropped out. In total, 20 patients were actually investigated. Despite this relatively high dropout rate, recruitment was considered to have been very successful. This provided experience of recruiting this specialised group of patients to a comprehensive "aerodigestive" investigation. To our knowledge this was the first such systematic study. I feel that the results of this study are timely, coinciding with increasing international calls for research into IPF in general and the potential role of aspiration in particular [90].

The median age of our IPF group was 69 years, however, three patients who dropped out were in their eighth and ninth decades of life and although they had initially consented to the study in the discussion with the clinician, their relatives had influenced their drop out after the review of the information leaflet. Many relatives believed the investigations may have been too exhausting for their elderly family members. Although these patients were clinically suitable, the views of the relatives were taken very seriously and the patients left the study. This clearly indicates the nature of IPF as a disease principally affecting patients in the later decades of life has an influence on their ability to participate in the research.

Idiopathic pulmonary fibrosis may be complicated by serious acute exacerbation resulting in acute hospital admission. One patient who had consented was admitted one week before their research appointment and subsequently died. Only two patients failed to attend their appointment after confirmation. This may be accounted by the fact that the disease is idiopathic and those suffering from it are keen to identify a cause. The majority of the patient group were just into their retirement and prior to the diagnosis had been quite well. The low 'did not attend (DNA)' rate indicates the desire for patients to return to their original level of fitness and thus their willingness to be recruited into the research.

7.1.2 Reflux in IPF – Clinical Findings

Twenty patients attended and completed the oesophageal physiology tests, which included oesophageal manometry and pH impedance. Only four patients had previously documented evidence of gastro-oesophageal reflux disease (GORD), but fifteen were on proton pump inhibitor (PPI) treatment at the start of the study. This was considerably more than the number of patients on steroid treatment which is often an indication to have PPI therapy for gastric lining protection (n=5). This implies many patients were on empirical PPI therapy without clear evidence of reflux.

Our results showed 12 patients had evidence of reflux and in six there was evidence of proximal reflux. As early as the 1970s, reflux had been demonstrated in patients with IPF [73] with more recent studies demonstrating over 80% of patients having evidence of reflux [71]. Our study demonstrated slightly lower percentage of IPF patients with reflux (60%) but using pH impedance identified both acid and weakly acid reflux in patients with IPF. This is consistent with the findings of *Salvarino et al*, [160] who showed that 83% of IPF patients had abnormal distal acid exposure compared to 43% of non-IPF subjects but more importantly showed both acidic and weakly acidic reflux episodes were higher in IPF . This illustrates the value of impedance-pH monitoring. In their study they observed a high frequency of both acid and non-acid reflux in the IPF group compared to non-IPF patients.

In our study, 9 patients had some degree of oesophageal dysmotility identified either by traditional 8-channel manometry (n= 4) or high resolution manometry (HRM) (n=5). In almost all the patient (n=16) lower oesophageal sphincter pressure (LOS) resting pressure was within the normal range. However, HRM detected a hiatus hernia in 67% of IPF patients. These findings are consistent with other recent studies [160] comparing manometric studies in IPF patients and healthy volunteers. In their study 55% of IPF patients had hiatus hernia detected on manometry compared to only 14% in healthy volunteers.

Our study also used the HRCT images to identify hiatus hernias in the IPF patients. The presence of hiatus hernia is well known to be associated with increased reflux by affecting the integrity of the LOS [24]. For this reason we used a radiologist specialising in gastrointestinal imaging to review the HRCT images. In 78% of our IPF group, hiatus hernias were identified on their CT images. Over half of these patients had objective evidence of reflux on Impedance-pH. *Noth et al* [161] identified 39% of IPF patients in their study had hiatus hernias on CT scans. Although modern CT has been recognised as

a method of diagnosing hiatus hernias barium swallows have been the standard method of determining the presence of hiatus hernias with clear identification of the LOS and its relationship to the diaphragmatic crus. As a result the high percentage of hiatus hernias diagnosed in our study must be interpreted with caution. In addition, the interpretation of a hiatus hernia on CT scans between radiologists varies considerably; the use of single radiologist reviewing our scans limits the interpretation of these results. A recommendation for further study out with my thesis would be the comparison of HRCT and barium swallows for assessing hiatus hernia in this patient group.

Lee et al [19] retrospectively studied 204 patients with IPF of which 45% had a history of reflux and 34% reported symptoms of reflux. Of these 47% were taking anti-reflux medications. Using regression modelling they concluded that use of gastro-oesophageal reflux medication was associated with longer survival. In our study, only 4/20 patients had an established diagnosis of gastro-oesophageal reflux disease but 15/20 were already on PPI medication. Although oesophageal physiology was performed off-PPI medication, RSI scores for those patients when taking PPI were shown to be abnormal in 60% indicating extra-oesophageal reflux symptoms persisted despite use of PPI medication. In addition, Demeester questionnaire scores assessing classical reflux symptoms remained unchanged in PPI users once off their medication. To my knowledge this is the first study to prospectively evaluate reflux symptoms on and off PPI in this patient group. Although some studies suggest a survival benefit in long term PPI use in IPF patients [19], it is clear from work on lung transplant patients that PPIs may not reduce volume reflux and surgical treatment maybe more valuable [95].

There was no significant change in symptom scores with increasing dose of PPI. However, when PPI use is compared to the rate of decline of the vital capacity (VC), there is a positive correlation between the daily dose of PPI and the rate of decline of VC which reached statistical significance ($p=0.003$). The findings of my study suggest that a proportion of patients may not benefit from taking a PPI as the reflux they have is weakly acid or non-acid reflux. However, in our study some patients had acid reflux and PPI use may help these patients and control symptoms which in turn reduces the deterioration of lung function, particularly the vital capacity and as *Lee et al* [19] suggest contribute to long term survival. Finally quality of life scores for PPI users with IPF were only slightly higher than those not taking any anti-reflux medication, but more importantly 85% of PPI users had GIQLI scores below the normal range, questioning the overall efficacy of medically managed reflux disease in IPF patients.

In our study we also used a validated scoring system to identify laryngopharngeal reflux. *Belafsky et al* [56] validated the reflux finding score (RFS) by demonstrating excellent inter and intra observer reproducibility when assessing the effectiveness of PPI therapy in 40 patients clinically proven reflux. The score is an 8-item clinical severity scale based on findings from fibre optic inspection of the larynx. Scores range from 0 to 26, with an abnormal score being above 7.

Nine out of the twenty IPF patients had an RFS score of 7 or above. There was no relationship between objective reflux scores diagnosed on impedance and RFS scores. Unlike the above authors' study, anti-reflux medication does not appear to have any relationship to the scores. However, all patients with an abnormal RSI score (>13) had a RFS score of 7 or above. Although the correlations between the two scores did not reach significance, this could simply be a reflection of the small sample sizes in this study; the findings of elevated RSI scores corresponding to possible changes at the level of the laryngopharynx may be evidence of refluxate irritating the upper airways raising the suspicion of microaspiration in these patients.

7.1.3 Reflux in IPF – Cellular Findings

Differential Cell Counts

The clinical application of differential cell counts in BAL is widely accepted and recommended in the clinical guidelines [6] as a diagnostic tool for IPF in specialist centres. More recently the American Thoracic Society (ATS) produced guidelines for the inclusion of BAL in clinical practice [162]. The use of BAL differential cell counts alone cannot be used to make a diagnosis of IPF but knowledge of the cellular composition together with radiological and clinical information can help in the diagnosis and differentiating between the ILD subtypes. *Meyer et al* [162] suggest that a diagnosis of IPF can be associated with a BAL neutrophil count of >3%. From our patients (Table 4-8), the median neutrophil count is 7.5%, supporting the diagnosis of IPF as described in the guidelines. More specifically, the guidelines suggest when compared to differential counts in normal individuals, IPF is characterised by elevated alveolar macrophages, elevated neutrophils and possibly elevated eosinophils with a lack of prominent lymphocytosis or eosinophilia. The results of our BAL cell counts when compared to the normals [151] appears to support this description of an IPF diagnosis. However, when individual IPF patient BAL cell counts are reviewed, four individuals had a lymphocytosis (>15% lymphocyte) count. This may suggest a

different diagnosis including sarcoidosis. One individual demonstrated a lymphocytosis > 50% in combination with neutrophilia > 3% which maybe more consistent with acute hypersensitivity pneumonitis [162]. As the authors suggest, BAL cell counts alone cannot easily differentiate between the various subtypes of ILD and clinical and radiological correlation must be used. The majority of the cells within the lavage sample were macrophages, consistent with the findings in normal individuals. IPF patients did have a slightly higher percentage of neutrophils. Elevated neutrophil counts are most often associated with acute inflammation, but none of the IPF patients were on treatment for chest infections at the time of bronchoscopy. In our study the median age was 69 years and consisted of 12 individuals who were ex-smokers and two who were smoking at the time of the study. Elderly patients and asymptomatic smokers can have a higher percentage of neutrophils in the BAL [163] and may be another factor that accounts for the neutrophil distribution in the study group.

In the guidelines *Meyer at al* [162] describes the recommended BAL procedure. They suggest that the volume of normal saline instilled should be between 100 and 300ml, divided into three to five aliquots. Optimal sampling should retrieve over 30%. We used 3 x 60ml normal saline lavages and the median retrieval was 50% in our study. As recommended in the guidelines prompt processing of BAL provides optimal results and all of the study samples were processed within 30 minutes of the BAL. Although the methodology used in my study for the collection and processing of the BAL strongly adheres to the recommendations of the guidelines, we collected the lavage from the standard sites, the right middle lobe or lingual. *Meyer et al* [162] suggest using the HRCT to find a target site for the BAL as this is more likely to yield a diagnostic specimen and hence suggest BAL should be completed within 6 weeks of the HRCT. The use of the traditional site of BAL in my study may account for some of the cellular variations seen between individual patient samples.

Oil Red O – Lipid Laden Macrophages

Several studies have suggested the use of Oil Red O staining in BAL to identify exogenous lipid as a possible surrogate marker for GORD [146]. In their study of 34 lung transplant patients, *Hopkins et al* performed 24-hour pH studies to diagnose GORD and used Oil red O staining of the macrophages to calculate the lipid index given as the lipid laden macrophage score. They used a lipid index of >150 as being significant for reflux and showed 83.3% sensitivity and 76.4% specificity when compared to 24-pH study results. *Hayes et al* [164] also demonstrate a relationship between clinically

occult reflux disease and lipid laden macrophage score. In their retrospective review of 17 patients with cystic fibrosis they showed that surgical management of reflux resulted in reduction of the lipid index, supporting the evidence of *Hopkins et al* [146] that lipid laden macrophage score is a useful adjunct in assessing reflux disease.

In our study, only 5 patients had a lipid laden macrophage score outside the upper limit seen in normal controls; only one individual having a score over 150. Two patients with elevated scores did not have reflux on pH-impedance. There was no correlation between lipid-laden macrophage scores and proximal or distal reflux. *Kitz et al* [165], in their retrospective analysis of 448 children support this finding and showed no correlation between lipid laden macrophage scores and pH monitoring. Contrary to the finding of *Hayes et al* [164], *Rosen at al* [166] assessed 50 children in which fundoplication had been performed in thirteen. They hypothesised that with treatment reflux should decrease and the lipid laden macrophage score should also decrease. However, after fundoplication, those patients without a symptomatic improvement had an increase in the lipid laden macrophage score, suggesting that lipid laden macrophages may be a marker of lung inflammation rather than specifically reflux related disease. It is clear that the lipid laden macrophage score cannot be used as a gold standard to assess reflux related aspiration. Not only do studies suggest variable findings, but lipid deposits in macrophages can be of endogenous origin as suggested in studies on patients with pneumonia [167] and may not be an accurate discriminator of aspiration in patients with reflux disease.

Prussian Blue – Haemosiderin Laden Macrophages

Oxidative stress and the effect this has on lung tissue has been investigated by *Reid et al* [148] in lung transplant patients. They suggested that the generation of free radicals and reactive oxygen species (ROS) by activated neutrophils contributes to the inflammatory process which may ultimately result in bronchiolitis obliterans syndrome (BOS). It is believed that the generation of free radicals and ROS originates from the release of 'free iron' from ferritin under inflammatory conditions. Alveolar macrophages (AM) attempt to protect against this iron-catalysed oxidative stress by scavenging the iron and sequestering it has an inert form called hemosiderin [148]. Therefore, the detection of hemosiderin laden macrophages can be used as a marker of oxidative stress and possible inflammation. In their study they showed the BAL cells from the lung transplant subjects and BOS subjects had a significantly higher hemosiderin score compared to normal subjects. In our study we found that IPF patients had a very high percentage of

hemosiderin stained macrophages and a hemosiderin score 15 times higher than the upper limit seen in normal individuals. However, the findings of elevated hemosiderin scores in IPF did not correlate to either distal or proximal reflux. This suggests as with lung transplant patients, IPF patients are subject to an inflammatory insult leading to the disruption of iron homeostasis and oxidative stress. *Kim et al* [168] suggested that increased alveolar septal capilleries and hemosiderin deposition may be useful predictor of pulmonary hypertension in IPF patients. They evaluated a cohort of 154 IPF cases, of which hemosiderin scores were calculated in 149 cases. They demonstrated that increased iron deposition was associated with elevated right ventricular systolic pressure, an early indicator of pulmonary hypertension. The mechanism of this is secondary to the remodelling of post-capillary pulmonary vessels in non-fibrotic areas of explanted lungs from IPF patients. They showed that hemosiderin scores provided a better predictor to the degree of pulmonary hypertension in IPF than either HRCT or lung function assessment. Important to note, the hemosiderin scoring system used by the authors is a variation of the standard scoring system described by *Kahn et al* and used by *Reid et al* [148] as well as in our study. Therefore, it limits the ability to directly compare with other studies and questions whether hemosiderin scores can accurately predict the degree of pulmonary hypertension. *Puxeddu et al* [149] studied 47 IPF patients against 14healthy controls. They demonstrated higher levels of haemosiderin laden macrophages in the IPF patients with no significant differences between smokers and non-smokers. Previous theories had indicated high levels of iron-laden macrophages were assocaiated with tobacco smoke as a reaction to oxidative stress. *Puxeddu et al* [149]suggest high numbers of haemosiderin laden macrophages in the IPF group is indicative of occult alveolar haemorrhage secondary to pulmonary veno-occlusive disease. Elevated haemosiderin scores in IPF form an important tool in the diagnosis and management of the disease suggesting further discussion on the use N-acetylcysteine (NAC), a tripeptide that scavenges oxygen free-radicals. The most recent ATS guidelines [6] only give a weak recommendation for NAC monotherapy in IPF but some studies have demonstrated both radiological and symptomatic improvements in IPF using aerosolised NAC which acts directly on the alveoli as an anti-oxidant.

It is clear that the staining of cells to identify lipid laden macrophages and hemosiderin deposits can provide useful information in IPF patients as well as patients with other lung diseases. Many of the studies were based on a paediatric population, where the mechanisms of reflux as well as the extent of lung pathologies varies considerably to

the adult population, particularly the elderly patients as seen with IPF. Both types of stain may be more useful in the assessment of the lung disease rather than specifically being used to identify reflux disease.

Bile Salts and Pepsin

There are a limited number of studies attempting to identify the relationship between reflux and aspiration and many of these have focused on lung transplant patients. Elevated bile salts have been identified in patients post lung transplant [93], with up to 43% having elevated bile salt levels 3 months after surgery. In addition to bile, pepsin has also been identified as a biomarker of gastric aspiration with elevated levels being identified in lung transplant recipients compared to normal controls [106]. Very few studies have clearly identified whether these markers of aspiration account for the pathophysiological changes seen in IPF. *Lee et al* [112] have compared pepsin levels in a case control study using 24 cases with acute exacerbation of IPF and 30 controls with stable IPF. They showed that the median level of BAL pepsin in the acute exacerbation group was higher than in the stable controls, (46.8ng/ml vs. 35.4ng/ml). Although the difference did not reach statistical significance the authors do conclude that elevated BAL pepsin is predictive of acute exacerbation of IPF, basing this on a subgroup of 8 patients with very high pepsin levels (>70ng/ml). Secondly the authors showed in 7 patients with an acute exacerbation, previous pepsin levels from lavages taken when these patients were diagnosed with IPF were no different in 6 out of the seven patients questioning the validity of the conclusion the authors have drawn. More importantly this study does not have any objective reflux assessment and this is a key component in ascertaining the possibility of gastric aspiration. In my study BAL was not performed in patients with acute exacerbations. All of the study patients were clinically stable at the time of investigation. Many of the early studies assessing reflux and IPF used pH studies to assess reflux but very few also analysed BAL for biomarkers of aspiration.

My study combined impedance-pH assessment of reflux with assessment of bile salts and pepsin in lavage samples. I demonstrated that more than half of the IPF patients had elevated pepsin levels in the lavage compared to normal controls; impedance-pH confirmed that eight patients of the eleven had reflux (5 distal reflux only, 2 both distal and proximal reflux, 1 proximal only). There was no correlation between reflux scores and pepsin when analysed for the whole group but a suggestion within the subgroup of eleven there appears to be a relationship between pepsin levels and reflux. Correlation

statistics on such small numbers of patients must be interpreted with caution and further work is indicated.

Several studies more recently have also confirmed the presence of pepsin in the BAL of IPF patients. *Savarino et al* [160] comment that in a study of 40 consecutive IPF patients with IPF had a higher amount of pepsin than non-IPF patients ($p>0.03$). However, it is important to say that only 21 IPF patients had bronchoscopy and lavage not the forty described in the abstract. In addition, the authors have not used a standardised lavage; they used '*at least 100ml of sterile saline*' which certainly alters the accuracy that these results can be interpreted with. I used a standardised lavage on all 20 patients which was 3x60ml sterile saline. The detection of pepsin is more accurately performed using an ELISA and this certainly supersedes the accuracy of commercially available kits e.g. Peptest^{TM} (lower limit of detection 16ng/ml), as used by *Savarino et al*. Interestingly, *Fahim et al* [75] only identified pepsin in 2/17 patients with IPF. Their study used an exhaled breath condensate and then a Peptest^{TM}. Not only could the Peptest^{TM} affect the lower limit of detection but an exhaled breath condenser to detect pepsin relies on the sample being taken in conjunction with a reflux event which with single sample testing increases the chance of missing most events and questions the reliability of the authors result particularly with regard to pepsin measurement. In my study, using standardised lavages and an ELISA to identify pepsin indicates IPF patients have detectable pepsin levels within the lavage which may be affecting lung function. However, my results should also be interpreted with caution. Although our ELISA test produced accurate standard plot where $R^2= 0.981$, further patients are required with repeated ELISA tests on the samples to support our initial findings.

Using the technique of tandem mass spectometry the lower limit of detection of bile salts in 0.01µmol/L[102]. An extraction technique further increased the lower limit of detection to 0.001µmol/L. 17/20 of my study IPF patients had detectable, very low levels of bile salts.These levels are so close to the lowest level of detection, they can be regarded as negligible amounts. There was no relationship between bile salt concentrations in the BAL and reflux (both proximal and distal scores). In addition, bile salt concentration in BAL had no relationship to the decline of lung function. Very few studies have attempted to isolate bile salts in IPF patients to determine if it is a marker of microaspiration. *Savarino et al* [160] showed that 13/21 patients with IPF had bile salts in the BAL compared to no patients in the non-IPF group. The authors used a

commercial assay called Bioquant for the detection of bile acids, quoting the lowest level of detection as 0.2µmol/L. We evaluated the kit used by *Savarino et al*, while setting up our study design. The manufacturer of this kit actually claims a lower level of detection of 1.0 µmol/L. The results from our group and others indicate that outcomes of lower than 5 µmol/L may not be reliable [102]. This is in contrast to the latest work by *Savarino et al* which state sensitivities of 0.2umol/L [160].

In contrast my study using a sensitive analytical chemistry approach documented bile acid levels at orders of magnitude lower than the study by *Savarino et al*. These levels were not different to levels found in normal BAL [169] . Overall I would conclude that levels of bile acids were not raised in my series of patients and that appropriately sensitive methods are required for BAL analysis of bile aspiration rather than kits designed for reporting circulating levels of bile salts in pathology.

Only two of the IPF patients had bile salt levels above the upper limit of normal. Only eight patients with reflux had bile salt levels above the median value seen in normal. we used a very accurate method of detecting bile salts the levels seen in our subjects and the relationship with refux indicate:

1. Bile reflux (duodenogastric reflux) is not significant in IPF
2. BAL measurement may not be the optimal method for this. My results demonstrated elevated haemosiderin scores which together with elevated protein in IPF BAL may mean that solute measurements are difficult to interpret due to loss of lung barrier function.
3. Elevated pepsin levels may be important in IPF indicating gastro-oesophageal reflux.

In summary my results illustrate that objective pH-impedance measurements can be performed safely and identify patients with both acid and non-acid reflux. Bronchoalveolar lavage was well tolerated in all our patients allowing cellular profile and stains to be performed as well labarotory analysis of markers of aspiration. Staining cells with both Oil Red O and Prussian blue may be a useful adjunct is assessing the inflammatory process taking place in IPF. Accuarate and standardised measurements of pepsin and bile salts are required to confirm the use of these markers in assessing microaspiration as a pathological process in IPF.

7.2 Cystic Fibrosis patients

7.2.1 Recruitment to Study

Between June 2011 and March 2012 40 patients diagnosed with Cystic Fibrosis (CF) were approached to enter the study. Initially 26 patients consented to participate in the research but less than half of this number actually attended (n=11). Of the eleven patients, two were recruited as inpatients from the CF ward. One patient who had consented in clinic became extremely anxious in the clinical setting of the lab and chose to withdraw from the study. Two patients withdrew their consent at the time of phoning to confirm their appointment. The other dropouts consisted mainly of patients failing to attend. The median age of the group was 29 years and all had the ΔF508 mutation.

There are several reasons why the drop-out rate was high compared to the IPF group. This is a much younger population, many of whom were in work or higher education. With regular clinic appointments and attendance for lung function tests, many may not have the time for further attendance to hospital for research purposes. Cystic fibrosis centres are often conducting research and clinical trials and this relies on patients consenting to several studies at a time; thus precluding further participation in research. Having reviewed several patient forums it is apparent that many young patients with CF feel institutionalised, spending a significant proportion of their adolescent lives in hospital. This may have an impact of recruitment to research taking place within the hospital.

Four patients rescheduled after their first appointment was given and then a further two patients rescheduled their second appointment. In all the cases it was secondary to chest infection requiring intravenous antibiotics either within the community or as an inpatient. It was therefore in the best interest of the patient to perform the research tests after their treatment was completed.

7.2.2 Reflux in CF – Clinical Findings

In total eleven patients attended the oesophageal physiology tests, nine patients completed oesophageal manometry and pH impedance, two were unable to tolerate the manometry and had impedance-pH studies only. Only four patients had previously documented evidence of GORD, but all eleven patients were on gastric acid suppression medication (10 on PPI, 1 on Ranitidine) at the start of the study. However, nearly all patients with CF have exocrine pancreatic insufficiency that requires pancreas enzyme replacement therapy. Therefore, the routine use of gastric acid suppression medication

was used to increase fat absorption despite the use of pancreas enzyme replacement [170].

Our results showed 9 patients had evidence of reflux and in five there was evidence of proximal reflux. Five patients had weakly acid reflux and in two there was significant volume reflux. This shows that reflux is far more prevalent that initially thought amongst patients attending a dedicated CF unit. Early studies demonstrated a high incidence of reflux in CF patients. *Faithi et al* [80] studied 30 adult CF patients. Eighteen were considered to have reflux. These patients were not all objectively studied and the information was based on a reflux questionnaire validated by the same unit. In fact only five patients were studied using 24-hour pH monitoring; four had a high Demeester score indicating reflux. This study uses a very small number of patients to objectively assess reflux. *Ledson et al* [100] used a similar number of patients as our study (n=11) and demonstrated 8/11 had reflux. Both these studies relied on pH monitoring only rather than pH-impedance and whilst our patients (10/11) stopped their acid suppression medication for 2 weeks, *Ledson et al* [100] only stopped the medication for 48 hours prior to the test. *Blondeau et al* [81] studied 33 patients with CF using pH-impedance as in our study and also demonstrated that the majority of patients had acid reflux (67%), slightly lower than in our study. They demonstrated both weakly acid reflux and proximal reflux in their study but the number of patients affected was slightly lower than our study (15% vs. 45% and 36% vs. 45% respectively). It is clear from both our study and the literature that reflux is common in CF. The reflux is mainly acid reflux but there is weakly acid reflux in a large proportion of patients and it is likely that refluxate is actually a mixture of acid and weakly acid content. Several studies have demonstrated elevated gastric acid secretion associated with the ΔF508 mutation [171]. Other studies have suggested that the absence of CFTR-mediated bicarbonate secretion in the duodenum together with the CFTR protein on parietal cells causing gastric acid secretion via CFTR-modulated cAMP-dependent pathway in which K^+ is exchanged for H^+, leads to a drop in the pH, possibly contributing the incidence of acid reflux in CF [172].

All 9/11 patients had high resolution manometry (HRM) performed to ascertain the degree of oesophageal function. Only three patients had normal oesophageal motility as described in the Chicago classification for HRM. The majority of patients with abnormal oesophageal motility were categorised as having ‘rapid contractions with normal latency’, very similar in appearance to a simultaneous swallow. Five patients out

of the six with abnormal oesophageal motility had reflux, including 3 patients with proximal reflux. Two patients with normal oesophageal motility had reflux. Over half the patients (5/9) had a hiatus hernia detected on manometry. It is well understood that both significant oesophageal dysmotility and hiatus hernia can predispose to reflux [24]. The two factors affecting the pathophysiology of reflux are the loss of the anti-reflux mechanism and diminished oesophageal clearance. An excess of transitory LOS relaxations (TLOSR) and a hypotonic LOS lead to loss of the anti-reflux mechanism. Development of a hiatus hernia with this exacerbates any reflux symptoms [173]. There are very few studies that describe the incidence of oesophageal dysmotility in CF, but it is a well known phenomenon in patients with chronic respiratory disease predisposing to poor oesophageal clearance and increased reflux symptoms[174]. As well as the functional integrity of the oesophagus contributing to reflux it is useful to remember that most of our CF patients had diabetes which either by causing autonomic neuropathy or smooth muscle dysfunction leads to an increase prevalence of oesophageal dysmotility.

Sabati et al [175] prospectively studied 201 patients with CF using two validated questionnaires; the Mayo GER questionnaire (GERQ) to assess the prevalence and severity of reflux symptoms. The GERQ revealed 53% of patients suffered heartburn and 33% suffered acid regurgitation. Patients on acid suppression medication in fact had more symptoms than those not taking acid suppression tablets. We used three validated questionnaires to assess reflux in CF patients. The RSI score for extra-oesophageal reflux symptoms was abnormal in 72% of our patients and despite acid suppression, 55% still had an abnormal RSI scores on PPIs. Typical reflux symptoms assessed using the Demeester questionnaire did not appear to be significantly affected by acid suppression treatment. The GIQLI assessment is abnormal in our CF patients both with and without treatment with use of medication resulting in only a small increase in the score. There was no significant change in symptom scores with increasing dose of PPI. The daily dose of PPI was not related to the rate of decline of lung function (FEV_1). My results suggest that a proportion of patients may not benefit from PPI use and this may be due to the presence of weakly acid or non-acid reflux. However, in those patients who had acid reflux, PPI use may help control symptoms. Finally quality of life scores for PPI users with CF were only marginally higher than those not taking any anti-reflux medication, but more importantly 82% of PPI users had GIQLI scores below the normal

range, questioning the overall efficacy of medically managed reflux disease in CF patients.

7.2.3 Reflux in CF – Cellular Findings

Patients were asked to provide sputum prior to their oesophageal physiology tests having fasted for 4 hours. The sputum was processed with assistance from Miss. Gemma Crossfield, a PhD student, using the standard operating procedure (*appendix2*), carefully separating the sputum plug from the saliva. Despite using a meticulous methodology to process the sputum in order to provide a good quality cytospin for staining, only 10/11 samples could be analysed and all were heavily concentrated with neutrophils. This made further staining using Oil Red O and Prussian Blue impossible due to lack of macrophages within the sample. *Blondeau et al* [81] collected saliva from CF patients to analyse rather than sputum but saliva does not represent the fluid found in the large airway. As a result we used sputum but clearly the method of collecting sputum can influence the quality of the sample the differential counts. Spontaneous sputum analysis as in our study is a recognised technique for cytological diagnoses but the presence of large quantities of dead cells can affect the accuracy of the count [163]. *Balbi et al* [163] reviewed the literature regarding sputum collection and international guidelines on sputum collection studies suggest induced sputum as providing more representative cell counts, however they also conclude that the induced sputum technique can result in a neutrophilia and thus affect the overall accuracy of the cell differential counts.

It is believed that duodenogastric reflux of bile is common in cystic fibrosis and is associated with cholelithiasis (gallstones), a common complication of CF [176]. *Hallberg et al* [176] investigated 8 adults with CF and compared them to 7 healthy volunteers without reflux disease. They collected gastric aspirates in these subjects and analysed them for bilirubin. Where the bilirubin concentration was high, a bile acid profile was performed using mass spectrometry. They showed that the median bile acid concentration of the gastric aspirates was nine time greater in CF patients than healthy subjects concluding that duodenogastric bile reflux is more common in CF. This is a very small study and more importantly, the healthy subjects did not actually have a bile acid profile performed as they did not have detectable bilirubin in the aspirate. The authors made the assumption that the bile acid concentration would be low or negligible as the bilirubin concentration had not been higher than 1.5μmol/L. The theory of bile reflux being more prevalent in CF patients have formed the basis of several other

studies attempting to detect bile salts in saliva, sputum or BAL samples from CF patients. *Blondeau et al* [81] studied 71 CF patients concluding that reflux and aspiration was common in CF. However, this 71 consisted of 10 lung transplant patients with a background of CF. It is only these ten patients that had both reflux assessment using pH-impedance and BAL analysis of bile salts. Eight patients from this group had reflux and six had detectable bile in the lavage. From this small number it is not possible to accurately conclude that reflux and aspiration occur in CF. In addition, these were lung transplant patients post significant surgery and the findings may not be applicable to the more widespread non-transplanted CF patients. The authors did study a further 61 patients but separated them into two groups, analysing the saliva for bile acids in 38 patients and performing impedance-pH studies on a separate group of 23 patients. They identified 20/23 patients to have reflux and 16/38 to have detectible bile acids in the saliva. As the authors have separated the groups it is difficult the relationship between the reflux and the detection of bile acids in saliva. Although the authors comment that the detection of bile acids in saliva may be a useful surrogate for proximal reflux, saliva is not representative of lung fluid and thus aspiration; the use of sputum or BAL analysis for the markers of aspiration are preferable [104, 177].

In our study two separate samples of sputum were taken from the patients for bile salt analysis. Of the 22 samples taken, 21 were processed and used for analysis; there were detectable bile salts in all 21 samples. Two patients who did not have objective evidence of reflux still had detectable bile salts in their sputum. There was no significant relationship between bile salt concentrations and either Demeester or proximal reflux ($p=0.554$ and 0.337 respectively). The detection of these bile salts in the sputum is supported in the work by *Pauwels et al* [177]. In this prospective study they compared bile salt concentrations in the induced sputum samples of CF patients, healthy volunteers, asthmatics and chronic cough patients. 56% of CF patients compared to 13% of healthy volunteers had elevated bile salt levels in the sputum. 28% of asthmatics also had elevated bile salt levels. In the CF patients they demonstrated that elevated bile salt levels were associated with a higher degree of lung function impairment. Although the authors comment on the median concentration of bile salts being significantly elevated in CF patients compared to the other groups, the dot plot of their results illustrated that the highest concentrations of bile salts were actually in the chronic cough group with many patients within the chronic cough and asthma groups having elevated levels; elevated bile salts in sputum may be common to patients with chronic respiratory

disorders and not only in CF patients. The groups were not matched in terms of patient numbers, therefore limiting the accuracy of comparing the groups. Finally, the authors conclude that the elevated bile salt levels are indicative of aspiration of duodenogastric contents. However, no objective evidence is available in this study to demonstrate duodenogastric reflux and the use of pH-impedance would have greatly added value to their study. In both the studies described above, the measurement of bile salts was performed using a commercially available enzyme assay (Bioquant). These kits are not as accurate or sensitive when compared to mass spectrometry and therefore the results should be interpreted with care. Our study is unique in providing detailed analysis of bile salts in sputum using mass spectrometry and having available the objective evidence of reflux assessment to determine if reflux and aspiration were responsible for deteriorating lung function in these patients. Although no significant statistical correlation was demonstrated between bile salt concentration and reflux scores or lung function and reflux scores, bile salts were present in all the patients with evidence of reflux and this could be very important for future studies. Correlation statistics have to be interpreted judicially when performed on such small patient groups.

Very few studies have used sputum as a medium to detect pepsin. *McNally et al* [104] studied 31 patients with CF and compared the pepsin levels in bronchoalveolar lavage with 15 controls. The patients were all children with a mean age of 10.4 years. The lavage was performed with 1ml/Kg normal saline with an average return of 40%. The mean pepsin level in the BAL was higher in the CF group than the control group. However, pepsin was detected in the control group and 12/31 CF patients had pepsin quantities comparable to the control group. The authors therefore used the 95^{th} percentile for the controls as the cut-off for elevated levels of pepsin (10.4ng/ml); levels above this were considered 'high' and seen in 19/31 CF patients. The authors suggest that these findings of elevated pepsin concentration in over half of their subjects are in keeping with aspiration. It is difficult to accurately confirm aspiration and as this study lacks objective reflux assessment it is difficult to determine the significance of the finding in this study. Although our study uses much smaller numbers we identified pepsin in the sputum of 7/11 CF patients with levels almost three higher than the pepsin concentrations detected in the study above. Six patients had objective evidence of reflux on pH-impedance assessment. Three patients had evidence of proximal reflux. In conclusion, although our study uses small numbers of patients and there is no control group for comparison, it has been demonstrated that gastro-oesophageal reflux is

important in CF. In addition, the elevated concentrations of pepsin in sputum of CF patients who also have identifiable reflux provides much stronger evidence of microaspiration being an important pathological process in these patients.

7.3 Lung Transplant Patients

7.3.1 Reflux in Lung Transplant patients

Between June 2008 and December 2010, 16 patients who had undergone lung transplant were referred for reflux investigations. Nine patients were initially studied by Mr. A.G.N. Robertson as part of a PhD [36] and further recruitment continued by myself focusing on the safety and efficacy of fundoplication in lung transplant patients [97]. All 16 completed 8 channel manometry, thirteen having completely normal investigations. Very little is published with regards to oesophageal motility after lung transplant but there is a high prevalence of foregut motility problems in patients with end-stage lung disease [132, 178]. D'ovidio et al demonstrated that up 80% (60/78) of these patients had oesophageal dysmotility and or a hypotensive lower oesophageal sphincter [179]. *Basseri at al* [180] demonstrated the problems with dysmotility seen in end-stage lung disease patients were as high as in the lung transplant candidates. This study evaluates oesophageal manometry post-lung transplant using HRM and shows 76.7% of patients to have oesophageal dysmotility. Both hypotensive and aperistaltic swallows were six times higher in the 30 lung transplant candidates compared to the 10 control subjects; this is in keeping with pre-transplant findings. Only 3/16 of our patients had abnormal peristalsis on 8-channel manometry and 5/16 had hypertonic lower oesophageal sphincters. The majority of our lung transplant patients had normal manometry and this discrepancy in results may be explained by different equipment and different reference values used particularly when trying to compare HRM and 8-channel manometry findings.

We have used combined pH-impedance to assess these patients as this is the most accurate way currently to assess reflux [39]. All patients were assessed whilst on PPI medication [36]. The use of pH-impedance allowed the assessment of both mildly acidic, non-acid reflux events and proximal reflux events, which may be physiologically and pathologically important, especially if it leads to aspiration in this vulnerable population [108]. Previous studies have shown increased prevalence and severity of GOR post lung transplantation [181, 182] with up to 75% of patients having demonstrable reflux on pH monitoring [130, 181, 183]. In our 16 patients the post-transplant level of GOR was 94% and 56% had proximal reflux on pH-impedance, despite the use of PPI. *Davis et al* [184] also demonstrated half of their subjects suffered from proximal reflux. Following endoscopy half of our patients had evidence of oesophagitis which is of concern considering the regular use of PPI medication and 15/16 had some evidence of a hiatus

hernia which is contrary to *Davis et al* [184] in which no patients were found to have a hiatus hernia.

7.3.2 Fundoplication after Lung Transplant

There is no consensus regarding fundoplication in lung transplant recipients [185]. We chose to operate in patients with symptomatic reflux and those with evidence of reflux and deteriorating lung function [97]. A laparoscopic Nissen fundoplication was favoured in our practice [36]. This study demonstrates that laparoscopic fundoplication in a transplant setting is safe. Of the sixteen patients operated on 15/16 patients reported a high level of satisfaction with the results of surgery at six weeks and at six months. This study also demonstrated that in this specialised patient population laparoscopic anti-reflux surgery is effective in reducing symptoms of GORD and improves quality of life. Our study also supports the possibility that fundoplication may impact positively on the loss of lung function seen in BOS, as 8/16 operations were performed for deterioration of lung function and all responded positively after surgery.

With regard to safety our study had comparable results to the Duke's group [186] with no significant mortality or morbidity experienced. In addition, our operative times and blood loss figures were comparable to the Duke group[186]. Increased length of stay in the transplant population and a higher readmission rate, due to transplant co-morbidity are reported in some studies[186] . Our patients' long post-operative stay may be partially explained by the fact that some transplant patients had to travel greater distances than a local population and for practical purposes spent longer in hospital. Overall our results suggest that laparoscopic fundoplication is safe in selected lung transplant recipients.

In terms of patient outcomes, three questionnaires were used as described in the previous section; the European Association has recommended the GIQLI questionnaire for the assessment of quality of life after fundoplication[141]. The DeMeester Reflux Questionnaire is validated to assess reflux symptoms and the RSI has been validated in non-transplant patients as a marker of extra-oesophageal reflux and has been used to assess the effects of fundoplication on extra-oesophageal reflux [187, 188]. The median GIQLI, Demeester and RSI scores all showed considerable improvement over time reaching statistical significance. Median BMI significantly decreased from 23.4 pre-fundoplication to 21.6 at six months post-fundoplication. The Melbourne group's study [189] of fundoplication in lung transplantation described a decrease in mean BMI from

23kg/m^2 six months pre-operatively to 21kg/m^2 six months post-operatively. This may indicate the need for specialist dietary advice and intervention with this patient group. Lung function was assessed in accordance with European Respiratory Society guidelines. Eight patients were operated on for deteriorating lung function. Of these eight, one patient had a reversal of BOS, two had a stabilisation of lung function and five had a decrease in the rate of deterioration. There was a statistically significant decrease in the rate of decline of FEV_1 per day post fundoplication which supports some of the work from the Duke University Transplant Group suggesting that anti-reflux surgery may lead to increased survival and improved lung function post-transplantation[132].

Our study shows that in this small group of lung transplant patients the intervention of laparoscopic fundoplication is safe and can result in an improvement of quality of life. Reflux may be contributing to the decline of lung function and the development of BOS and these results may indicate that anti-reflux surgery could play a role in reducing this. However, our current study has several limitations. The numbers involved were small and the study wasn't randomised so there was no control group to compare and determine the true effect of the surgery. Fundoplication was performed at different times after transplant and no patients were operated on within 90 days of transplant, the suggested optimum time for intervention [65]. Further studies could include a focus on the effects of early fundoplication (within 90 days) on allograft function and long-term survival.

7.4 Conclusions and Future work

I have studied three very specific groups of patients and have demonstrated that in patients with severe lung disease, reflux investigations can be performed safely. Working within a specialist upper gastrointestinal unit with sophisticated equipment for assessing reflux and oesophageal function we have shown that patients can be referred, counselled and investigated safely with close liaison with the CF, IPF and transplant clinics. Most patients are keen to attend for these investigations despite the invasive nature of the tests and certainly with the lung transplant patients the oesophageal physiology investigations have formed the basis of their surgical management.

The use of pH-impedance and high resolution manometry have demonstrated that in both CF and IPF reflux can be identified in the majority of patients and can include acid, non-acid and proximal reflux. This is extremely important as recent evidence suggests that despite PPI use reflux can persist, in particular non-acid type, predisposing to *Pseudomonas* infection and a deterioration of lung function [190]. Our results indicate in all three groups of patients, objective evidence of reflux and symptoms of reflux persist despite PPI use. Further work is required to evaluate the role of medical treatment of reflux in advanced lung disease including identifying the role of dysmotiltiy agents such as dompridone and metochlopromide in controlling reflux symptoms.

These groups of patients may have their deteriorating lung conditions treated with a lung transplant and considering that over 90% of the lung transplant patients we studied had reflux, this relationship could be very significant. It may indicate that patients with end-stage lung disease have reflux and consideration of surgical management in carefully selected patients prior to transplantation. Anti-reflux surgery prior to transplantation may reduce the incidence of BOS in the allograft.

It is clear that the laboratory studies attempting to identify microaspiration either through specialised stains or through bile salt and pepsin assays need much more development and global consensus. Certainly the results from this pilot study indicate that BAL fluid and sputum analysis can yield useful results but there are numerous improvements that can be made; the collection of sputum, the standardisation and accuracy of ELISA in order to develop reference ranges, the use of mass spectrometry for bile salts in other centres to ensure reproducibility of tests and comparisons to be made.

This study has managed to take three separate groups of clinical patients and safely recruit them, perform clinical tests and then incorporate that information into a host of laboratory tests. The patients were studied using the most up to date methods of assessing reflux including the use of HRM to provide better characterisation of oesophageal motility. The BAL was performed as recommended in the current guidelines and the close proximity of the endoscopy unit to the laboratory allowed the prompt processing of BAL ensuing good quality cytospins [162]. This study highlights the use of specialised cell staining, particularly Prussian Blue, both as a diagnostic tool as well as assessing the response to therapy. The positive findings of the research have altered the clinical management of lung transplant patients, improving their quality of life.

There are clearly weaknesses to this study including the lack of a control group to compare with the IPF and CF patients. The poor recruitment in the CF group resulted in only 11 patients attending from a designated national unit. Clearly the CF patients are a vulnerable group with other comorbidities and closer liaison with a patient's specialist nurse may aid recruitment and individual patient's confidence in future reflux studies. It is clear that reflux is a problem in all three groups of patients. Only the transplant group were studied on PPI therapy but the findings from this study question the efficacy of PPI treatment in IPF and CF patients. Clearly objective assessment of reflux performed in these patients whilst they were on their medication would have been helpful in this study and would have answered important clinical questions on the role of PPI therapy in these patients. The processing of BAL was performed very efficiently but the diagnostic quality of the sample may have been improved with targeted BAL using the HRCT. The collection of sputum also needs to be improved to ensure that the quality of the sample is consistent between patients and to allow for more accurate cytological analysis.

At our centre future work is focusing on the establishment of an aerodigestive unit in which the respiratory physicians, transplant team and upper gastrointestinal surgeons work closely in a multidisciplinary setting to enhance the clinical management of patients. In addition, a close liaison should be maintained with the university laboratories, trying to develop the techniques used to analyse the samples for a variety of markers and inflammatory proteins. Future work will inevitably include larger studies in IPF, CF and transplant patients with the aim to conduct randomised controlled trials of both medical and surgical treatment of reflux. We hope to develop clinical trials

that may elucidate the exact role reflux has in the pathophysiology of chronic lung disease and the importance of identifying markers of microaspiration early in the disease process. We believe early surgery may have a crucial role in the management of these patients. However, patients with advanced lung disease and those post lung-transplants have to be carefully considered before surgical intervention is offered as many of these patients are frail, elderly and suffer multiple co-morbidities making surgical intervention high risk in many of these patients. Therefore a multidisciplinary team approach to managing these patients must be encouraged to allow careful and safe decision making.

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Appendix

Appendix 1

Standard Operating Procedures for BAL and Sputum processing

Appendix 2

Standard Operating Procedures for Geimsa, Oil Red O and Perls (Prussian Blue) cell staining

Appendix 3

Ethical Approval and NHS R&D approval

Appendix 4

Patient information sheet and consent forms

Appendix 5

Successful Grant Application

Appendix 6

Reflux Questionnaires

Appendix 7

Publication related to thesis

Appendix 1

Standard operating protocols for BAL and Sputum processing

Sir William Leech Centre and Freeman Hospital Standard Operating Procedures

- BAL processing SOP index S 01. Version 3
- Sputum processing SOP index S 19. Version 2

Sir William Leech Centre
Freeman Hospital
Standard Operating Procedures

BAL Processing
SOP Index **S 01.** version 3

	Name	Signature	Date
Author	G Johnson		12.1.11
Approved by	C Ward		26.1.11
Effective from	12.1.11		

Summary and Reason for change

Training Requirements:
Read all relevant SOPs.
The technique should be demonstrated by experienced personnel.

Linked SOPs
C01 Routine cleaning and sterilization of class 2 cabinet
C03 Routine cleaning and disinfecting of Shandon Cytospin 3
C04 Routine cleaning and disinfecting of MSE 3000i centrifuge
C 05 Disposal of clinical waste
C06 Disposal of Sharps
C07 In the event of spillage within the MSE 3000i centrifuge
S17 Use of the MSE 3000i centrifuge

Review Date

Jan 2016

SOP S 01.version 3

BAL Processing

Principle

To measure the volume of BAL fluid received, to count the total number of cells and prepare cytospins.

To store the processed supernatant in

(1). 25 x 600µl aliquots at -80°C

(2). 4 X 5ml aliquots at -80°C

To store the cells

(1). Up to 6 x 3 million cells at –80°C

For transfer to university

(1) Brushings in RPMI

(2) 20 ml BAL

Personnel

BMS, Research Personnel

Specimens

BAL fluid, Brushings

Equipment

Class 2 safety cabinet

Gloves Nitrile White coat Plastic apron

Ribbon gauze 30 x 7 cms Pastettes

Centrifuge tubes 4 x 50 mls, 5ml tubes 4 x 5ml

Neubauer Counting Chamber with cover glass.

Microcentrifuge tubes x 31

Glass slides x 6 Filter cards x 6 Cytofunnels x 6

CH2 Microscope with x 40 objective

Centrifuge 3000I

MSE Microcentaur

Cytospin 3

Reagents

Dulbeccos Phosphate buffered saline

Virkon

Trigene

RPMI

Quality Control

The BAL fluid can be stored at 4°C for up to a maximum of 1 hour before processing.

Risk Assessment

This procedure has been examined under COSSH guidelines. There is a potential BIOLOGICAL HAZARD. Disposal and decontamination procedures should be followed.

Safety

Before starting the procedure refer to the relevant COSSH assessments relating to handling of BAL

When handling BAL fluid always wear protective clothing, white coat, gloves and disposable plastic apron.

Treat all BAL Fluid as biological hazards.

The procedure should be carried out in a class 2 safety cabinet.

Dulbeccos Phosphate Buffered Saline. Irritating to eyes, respiratory system and skin. Target organs Central Nervous System and kidneys.

Procedure

1. Generate a BAL work sheet and allocate a TW / IF number.

2 **Filter 20 mls BAL fluid**

(If the volume of BAL is > 40 mls). through a layer of gauze into a universal container. Dispose of the gauze into the clinical waste. Label with TW number. For transfer to university.

3. **Filter remaining BAL.**

Measure and record the volume on the BAL work sheet.

4 **Centrifuge the BAL**

at 1250 rpm (183g) for 6 mins at 4° C. (prog 3) MSE Mistral 3000i.

5. **Decant the supernatant**

into 2 x 50 ml centrifuge tubes, taking care not to disturb the cell pellet.

6. **Centrifuge the supernatant**

at 2500 rpm (734g) for 6 mins at 4°C. (prog 4)

7. **Divide the supernatant**

into 600 µl X 25 in micro centrifuge tubes and 4 x 5ml centrifuge tubes, label with TW number.

8. **Add 1 – 50 mls Dulbeccos PBS**

to the cell pellet to give an opaque suspension. Mix gently.

9. **Find the total cell concentration**

using an Improved Neubauer counting chamber. Count the cells in 4 large squares. Adjust the volume to give a final cell concentration of 0.5 million cells per ml.

$$\frac{\text{Total number of cells} \times 10^6}{0.5} = \text{x ml}$$

10. **Prepare cytospins x 6**

using 100ul of re suspended cells at 300 rpm (9g) for 3 mins. (prog 2 Shandon Cytospin 3) Place used cytofunnels in 1% Virkon for sterilisation. Place used filter cards into the clinical waste.

11. **Fix cytospins x 1**

in acetone at room temperature for 10 mins then air dry. The remaining cytospins are air dried overnight, wrapped in cling film and stored at –70°C.

12. **Prepare Cell Pellets (max 6)**

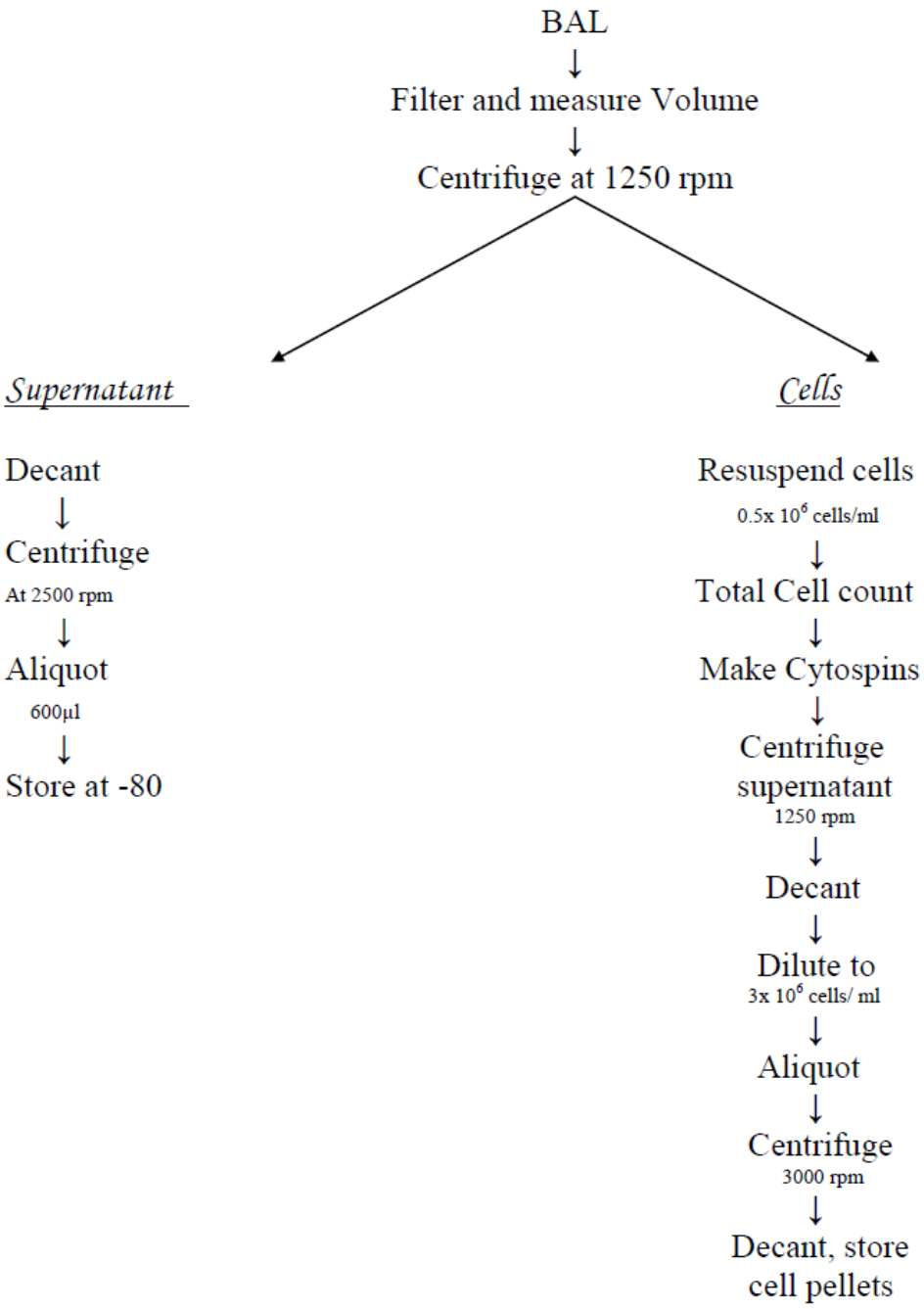
the cell suspension is recentrifuged using prog 3. Discard the supernatant and resuspend the cells in Dulbeccos PBS to give a concentration of 2 – 3 million cells per ml. 6 x 1 ml aliquots are centrifuged at 3000 rpm (352g) for 4 mins MSE Micro Centaur. Discard the supernatant add 1 ml RLT buffer to each pellet and store at –20°C until transfer to –80°C freezer. Cell pellet tubes are labeled with the IF number.

13. Discard all pipettes, tubes etc to clinical waste. Sterilise work surfaces with 1:50 Trigene

Results

25 x 600µl aliquots of acellular BAL fluid stored at –80°C.
4 x 5ml aliquots of acellular BAL fluid
1 x cytospins acetone fixed and stained with Geimsa.
5 x cytospins air dried, wrapped and stored at –20°C
6 cell pellets stored at –80°C

For transfer to University
1 x 20 ml BAL
Brushings


BAL
Filter and measure Volume
Centrifuge at 1250 rpm
Supernatant
Decant
Centrifuge
At 2500 rpm
Aliquot
600µl
Store at -80
Cells
Resuspend cells
0.5x 10^6 cells/ml
Total Cell count
Make Cytospins
Centrifuge supernatant
1250 rpm
Decant
Dilute to
3x 10^6 cells/ ml
Aliquot
Centrifuge
3000 rpm
Decant, store cell pellets

Sir William Leech Centre
Freeman Hospital
Standard Operating Procedures

Sputum Processing
SOP Index **S 19.** version 2

	Name	**Signature**	**Date**
Author	G Johnson		11.4.11
Approved by	C Ward		11.4.11
Effective from	11.4.11		

Summary and Reason for change

Change of protocol

Training Requirements:
Read all relevant SOPs.
The technique should be demonstrated by experienced personnel.

Linked SOPs
C01 Routine cleaning and sterilization of class 2 cabinet
C03 Routine cleaning and disinfecting of Shandon Cytospin 3
C04 Routine cleaning and disinfecting of MSE 3000i centrifuge
C 05 Disposal of clinical waste
C06 Disposal of Sharps
C07 In the event of spillage within the MSE 3000i centrifuge
S17 Use of the MSE 3000i centrifuge

Review Date

April 2016

Location Room 225

Principle
This SOP provides a general description of processing sputum. Separate out the plug of sputum, weigh the selected sputum and disperse the mucous plug, then centrifuge and remove supernatant. Digest, dilute and then filter the suspension through nylon gauze. After centrifugation divide the supernatant into aliquots and calculate the relative numbers of viable and nonviable cells, also the percentage of squamous cells present in the sample.
Store the processed supernatant in 250µl aliquots
Prepare 4 cytospin preps.

Personnel
BMS and research personnel

Specimen
sputum.

Equipment
Class 2 safety cabinet
Gloves nitrile
White coat
Plastic apron
Petri dishes
Forceps
Bench rocker with ice pack
Nylon gauze filter
Plastic filter funnel
Centrifuge tubes 3 x 15 mls
Pastettes
Neubauer Counting Chamber with cover glass.
Micropipette
Tips
Microcentrifuge tubes
Glass slides x 4
Filter cards x 4
Cytofunnels x 4
CH2 Microscope with x 40 objective
Centrifuge 3000I
Cytospin 3

Reagents
Dulbeccos Phosphate buffered saline
Virkon
Trigene
Trypan Blue 0.4% Sigma T8154
Dithiothreitol (DTT) Sputolysin Reagent from Calbiochem cat 560000
Dilute 10ml Sputolysin reagent with 40 ml distilled water.
70% alcohol

Quality Control
The sputum can be stored at 4°C for up to a maximum of 1 hour before processing. If the sputum sample is very dense the volume of DTT added can be increased to a factor of x 8. Record this variation on the sputum work sheet.
Limit sample size to 1 gram if possible.
The viability count should be carried out within 5 minutes of mixing the sample with Trypan Blue.

Risk Assessment
This procedure has been examined under COSSH guidelines. There is a potential BIOLOGICAL HAZARD. Disposal and decontamination procedures should be followed.

Safety
Before starting the procedure refer to the relevant COSSH assessments relating to handling of sputum.
When handling sputum always wear protective clothing, white coat, gloves and disposable plastic apron.
Treat all sputum as a biological hazard.
The procedure should be carried out in a class 2 safety cabinet.

Procedure
1. Generate a sputum work sheet and allocate a sample number.

2. In the Class 2 cabinet separate the dense sputum plugs from any saliva. Transfer the sample to a petri dish, using blunt forceps and a circular motion condense the thick mucous strands into a dense plug. Repeat this process several times if necessary.

3. Transfer the sputum plug to a pre weighed 15 ml polypropylene centrifuge tube and reweigh.

4. Calculate the weight of the mucous plug and add 8 x volumes of Dulbeccos PBS i.e. 1gm sputum / 8 ml D-PBS.

5. Disperse the sputum plug by repeated gentle aspiration with a pastette. Vortex for 15 seconds, attach the tube to an ice pack and place on a bench rocker for 15 minutes.

6. Centrifuge at 2500 rpm for 10 minutes at 4°C (brake off)

7. Carefully remove the supernatant (4 x volume) into a fresh centrifuge tube.

8. Centrifuge at 2500 rpm for 10 minutes at 4°C (brake off)

9. Divide the supernatant into 250µl aliquots and label Pre DTT.

10. Freshly prepare 0.2% sputolysin by diluting 10ml ampoule of sputolysin in 40 ml distilled water. (The diluted Sputolysin may be kept for 1 week at 4°C.)

11. Add 4 volumes (4 x weight of the selected sputum) of 0.2% sputolysin to the sputum pellet.

12. Disperse sputum pellet by repeated aspiration into a plastic pipette (avoid foaming) vortex for 15 seconds, rock on ice for 15 minutes.

13. Vortex for a further 15 seconds and add a volume Dulbeccos PBS equal to the volume of DTT added. i.e. if 1ml of DTT was added to the sample add 1 ml Dulbeccos PBS.

14. Pre wet a 48u nylon gauze filter with Dulbeccos PBS and shake off excess fluid. Using nylon gauze and a plastic funnel filter the sample into a new 15ml centrifuge tube.

15. Centrifuge at 2000 rpm for 10 mins at 4° C with no brakes. (prog 1) MSE Mistral 3000i

16. Carefully decant the supernatant and store 250µl aliquot x 10 and label post DTT, discard any remaining supernatant .

17. Re suspend the cell pellet in Dulbeccos PBS to produce an opaque suspension, mix gently. Record the volume of D PBS for use in total cell calculation.

18. Take a 20µl aliquot of the cell suspension and gently mix with 20µl of Trypan Blue. Use an Improved Neubauer Haemocytometer to count the cells in the 4 large outer squares.
Cells are recorded as
Viable Leucocytes/ macrophages (colourless)
Nonviable " " (blue)
Squamous cells

19. Calculate the percentage of Squamous cells

$$\frac{\text{No of Squamous x 100}}{\text{Total cell count}}$$

20. Calculate the percentage of viable cells

$$\frac{\text{No of Viable cells x 100}}{\text{No of Viable + nonviable cells}}$$

21. Calculate the total number of non squamous cells in the sample

a) $\frac{\text{Viable + nonviable cells}}{4} \times 2 \times \text{Vol D-PBS} = z$

b) $\frac{Z}{100} = \text{total cells} \times 10^6$

22. Calculate the total cells per gram of sputum

$$\frac{\text{Total number of cells x } (10^6)}{\text{Weight of condensed sputum}}$$

23. The remaining cells are washed with D-PBS. Fill the centrifuge tube with D-PBS, gently mix then centrifuge at 800 rpm at 4°C for 10 mins . Carefully decant the supernatant and discard. Adjust the cell concentration to 0.5 X 10^6 cells per ml.

$$\frac{\text{Total number of cells x } 10^6}{0.5} = \text{X ml}$$

Where x is the volume of D PBS added to the cell pellet. Prepare 4 cytospins using 80 µl of the cell suspension, spin at 450 rpm for 6 minutes. Air dry then fix 1 cytospin in acetone for 10 mins.

Results

250µl acellular supernatant stored at –80°C.
1 cytospins acetone fixed and stained with Geimsa or Carbol Chromotrope.
3 cytospins air dried, wrapped and stored at –20°C

24. When the procedure is completed dispose of all biological material, contaminated disposable equipment in the clinical waste bag, place all pipette tips etc in the sharps bin.

25. Sterilise work surfaces with 70% alcohol.

Sputum Processing

Patients Name	Date	Time	Number	Requested By

Tube weight (gms)	**(a)**	
Tube + selected sample weight (gms)	**(b)**	
Selected sputum weight (gms)	**(W)**	
Volume of D-PBS added (mls)	**(8 x W)**	
Volume of DTT added (mls)	**(4 x W)**	
Volume of PBS added (ml)	**(4 x W)**	

Processor's initials

Number of sputum supernatants prepared Store at –70C	

Volume of PBS added to cell pellet **(X)**	

Counts: Improved Neubauer Haemocytometer

Resuspend cell pellet in PBS to give an opaque suspension Record the volume of PBS added, then dilute 20µl cells + 20µl Trypan Blue

Haemocytometer Counts

Cell Counts		Differential		Cell Count		
Squamous	Non Squamous **A**	Number of haemocytometer fields counted **B**	% Squamous	Viable cells for non-squamous cells	Non viable cells for non squamous cells	% viability

Calculation of Number of Cells in Selected Sputum Samples

Weight of Selected Sputum **W**	Volume PBS **X**	Dilution used **Z**	Mean number of cells in one square **Y**	Total cells x 10^6 in samples T	Total Cell Count per gram
			A/B	**X x Y x Z / 100**	**T / W**

Differential Cell Count	
Neutrophils	
Eosinophils	
Macrophages	
Lymphocytes	

Squamous Cell Contamination	
Bronchial epithelial	

Appendix 2

Standard operating protocols for BAL and Sputum processing

Sir William Leech Centre and Freeman Hospital Standard Operating Procedures

- Geimsa 2 processing SOP index S 08. Version 3
- Oil Red O processing SOP index T 15. Version 1
- Perls Prussian Blue processing SOP index T 16. Version 1

Sir William Leech Centre
Freeman Hospital
Standard Operating Procedures

Geimsa 2
SOP Index **T 08.** version 3

	Name	**Signature**	**Date**
Author	G Johnson		4.4.11
Approved by	C Ward		4.4.11
Effective from	4.4.11		

Summary and Reason for change

Change of buffer and staining protocol

Training Requirements:
Read all relevant SOPs.

Review Date
April 2016

Geimsa 2

Principle
To stain acetone fixed cytospin preparations with Romanovsky stain, prior to performing a differential cell count.

Personnel
BMS

Specimen
Acetone fixed cytospins.

Equipment
Gloves nitrile
White coat
Plastic apron
Staining tray

Reagents
Geimsa 2 (Romanovsky) Stain
Stock solution A

Azure B thiocyanate 1.5 gms
DMSO 200mls
In a fume cupboard gently warm the mixture to 37°C until the azure B has dissolved.

Stock solution B

Eosin Y (VWR BDH 341972Q) 0.5 grm
Methanol 300mls.

Working Concentrate.
Add stock solution A slowly to stock solution B. Store at room temperature in a dark glass bottle.

Working dilute solution

Stock dye mixture	50µl – 100µl
PBS/ tween 20 pH 7.4	1 ml

DPX

Quality Control
Check results microscopically. If the staining is weak repeat stain for 10 mins.

Risk Assessment
This process involves little risk if carried out wearing suitable protective clothing. No suitable alternative techniques are available.

Safety
Acetone : highly flammable. Irritating to eyes. Repeated exposure may cause skin dryness or cracking. Vapour may cause drowsiness and dizziness.

Azure B : (Aldrich 41,900-1)Harmful by inhalation. Irritating to eyes, skin and respiratory system.

DMSO : (Sigma D 8418) Do not breathe the vapour.

Methanol :Highly flammable. Toxic by inhalation, in contact with skin and if swallowed. Danger of irreversible effects.

DPX: May cause harm to the unborn child. Possible risk of impaired fertility. Flammable. Irritating to skin. Harmful by inhalation and in contact with skin.

Procedure
1. After fixation air dry cytospin.

2. Stain with diluted working solution 10 mins

3. Rinse briefly in distilled water.

4. Air dry and mount in DPX

Results

Nuclei	purple
Cytoplasm	shades of blue
Cytoplasmic granules	shades of pink
Eosinophilic granules	red
Mast cells	metachromatic purple red

Sir William Leech Centre
Freeman Hospital
Standard Operating Procedures

Oil Red O
SOP Index T 15. version 1

	Name	Signature	Date
Author	G Johnson		10.11.10
Approved by	C Ward		26.1.11
Effective from	26.1.11		

Summary and Reason for change

Training Requirements:
Read all relevant SOPs.

Review Date
Nov 2015

Oil Red O

Principle
To stain formalin fixed cytospin preparations with Oil Red O stain
The staining mechanism of this polyazo dye is a function of the physical property of the dye being more soluble in the lipid than in the solvent.

Personnel
BMS , research personnel

Specimen
Formalin fixed cytospins.

Equipment
Gloves nitrile
White coat
Coplin Jars x 2

Reagents
Neutral Buffered Formalin
60% Isopropanol

Oil Red O Stock solution
Oil Red O 0.5 grms
Isopropanol 100mls
Dissolve the dye oin the isopropanol, using very gentle heat

Working Solution
Stock oil Red O 30mls
Distilled water 20 mls
Allow to stand for 10 minutes then filter into a Coplin jar and cover immediately.
The stain does not keep, make up fresh from stock each time.

Glycerin Jelly Mountant
Gelatin 10 gms
Distilled water 60 mls
Glycerol 70 mls
Phenol 0.25 gms
Dissolve the gelatin in the distilled water, add the glycerol and phenol.
Store at 4°C

Quality Control

Working solution must be made up fresh from stock.
Always include a positive control.

Risk Assessment

This process involves little risk if carried out wearing suitable protective clothing. No suitable alternative techniques are available.

Safety

10% Buffered formalin
Toxic by ingestion, inhalation. Prolonged exposure causes conjunctivitis, laryngitis, bronchitis, pneumonia. Burns to eyes and skin. Ulceration (cracking around finger nails). Carcinogenic, Teratogenic, mutagenic. Reacts violently with HCl to produce carcinogenic compounds.

Isopropanol

Inhalation of vapors irritates the respiratory tract. Exposure to high concentrations has a narcotic effect, producing symptoms of dizziness, drowsiness, headache, staggering, unconsciousness and possibly death.

Oil Red O

Hazardous in case of ingestion. Slightly hazardous in case of skin contact (irritant), of eye contact (irritant).

Procedure

1. In the fume hood Fix cytospin in formalin 10 -15 minutes

2. Rinse in running tap water

3. Rinse briefly with 60% isopropanol

4. Stain in freshly prepared working solution 15 mins.

5. Rinse briefly 60% isopropanol

6. Rinse in water

7. Lightly counterstain with Harris Haematoxylin

8. Wash until blue.

9. Mount in aqueous mountant

Results

Nuclei	blue
Lipid	red

Sir William Leech Centre
Freeman Hospital
Standard Operating Procedures

Perls Prussian Blue
SOP Index **T 16. V1**

	Name	Signature	Date
Author	G Johnson		10.11.10
Approved by	C Ward		26.1.11
Effective from	26.1.11		

Summary and Reason for change

Training Requirements:
Read all relevant SOPs.

Review Date
Nov 2015

Perls Prussian Blue

Principle
Dilute mineral acid hydrolysis releases ferric iron from protein bound tissue deposits, which, in the presence of ferrocyanide ions is precipitated as potassium ferric ferrocyanide Prussian blue.
$FeCl_3 + K_4Fe(CN)_6 = KFeFe(CN)_6 + 3KCl$

Personnel
BMS, Research personnel

Specimen
Acetone fixed cytospins.

Equipment
Gloves nitrile
White coat
Test tube
Staining rack

Reagents
Acetone

Perls Reagent:
a. 2% Hydrochloric Acid
b.2% Potassium hexacyanoferrate (II) trihydrate (Pot Ferrocyanide)
Working Solution
Mix 10mls of solution a and b.

1% neutral red
IMS
Xylene
DPX

Quality Control
Working solution must be made up fresh.
Always include a positive control.
Differentiate Neutral red during dehydration

Risk Assessment
This process involves little risk if carried out wearing suitable protective clothing. No suitable alternative techniques are available.

Safety
Acetone : highly flammable. Irritating to eyes. Repeated exposure may cause skin dryness or cracking. Vapour may cause drowsiness and dizziness.

Potassium Ferrocyanide (Potassium hexacyanoferrate (II) trihydrate)
Irritant (but highly toxic after hydrolysis).

Hydrochloric Acid:
Extremely corrosive. Inhalation of vapour can cause serious injury. Ingestion may be fatal. Liquid can cause severe damage to skin and eyes. TLV 5 ppm.

Neutral red 1%
Harmful by ingestion. Eye irritant. May cause dermatitis. Reacts strongly with oxidisers

IMS (Industrial Methylated Spirit): Highly flammable. Harmful.

Xylene: Flammable. Harmful by inhalation. Irritating to skin.

DPX: May cause harm to the unborn child. Possible risk of impaired fertility. Flammable. Irritating to skin. Harmful by inhalation and in contact with skin.

Procedure

1. Fix cytospin in acetone 10 -15 minutes

2. Air dry then rinse in distilled water

3. Flood slide with freshly prepared Perls reagent 15 minutes

4. Wash well in distilled water

5. Counterstain in filtered 1% Neutral Red 30 seconds

7. Wash well

8. Dehydrate and mount in D.P.X

Results

Nuclei	red
Ferric iron	Prussian Blue
RBC	yellow

Appendix 3

Ethical Approval and NHS R&D approval

- Approval letter from County Durham and Tess Valley 2 Research Ethics committee
- IRAS application
- NHS R&D Trust approval letter
- R&D application form

National Research Ethics Service

County Durham & Tees Valley 2 Research Ethics Committee
The Tatchell Centre
University Hospital of North Tees
Piperknowle Road
Stockton-on-Tees
TS19 8PE

Telephone: 01642 624164
Facsimile: 01642 624164

22 February 2010

Professor S.Michael Griffin
Professor of Gastrointestinal Surgery
Northern Oesophagogastric Unit
Royal Victoria Infirmary
Queen Victoria Road
NE1 4LP

Dear Professor Griffin

Study Title:	**The use of impedance pH measurements to determine the effect of gastro-oesophageal reflux in patients with cystic fibrosis and idiopathic pulmonary fibrosis**
REC reference number:	**10/H0908/8**
Protocol number:	**1**

Thank you for your letter of 18 February 2010, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Vice Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

For NHS research sites only, management permission for research ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk. *Where the only involvement of the NHS organisation is as a Participant Identification Centre, management permission for research is not required but the R&D office should be notified of the study. Guidance should be sought from the R&D office where necessary.*

Sponsors are not required to notify the Committee of approvals from host organisations.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Covering Letter	1	17 December 2009
REC application	IRAS 2.3	17 December 2009
Protocol	1	17 December 2009
Investigator CV	1	17 December 2009
Questionnaire: DeMeester Reflux Questionnaire	Validated	
Questionnaire: Reflux symptom index questionnaire response form	Non Validated	
Questionnaire: Gastrointestinal Quality of Life Index	Validated	
Participant Information Sheet: Patients with Cystic Fibrosis	2	15 February 2010
Participant Information Sheet: Patients with Idiopathic Pulmonary Fibrosis	2	15 February 2010
Participant Consent Form: Patients with Cystic Fibrosis	2	15 February 2010
Participant Consent Form: Patients with Idiopathic Pulmonary Fibrosis	2	15 February 2010
GP/Consultant Information Sheets	1	15 February 2010
Response to Request for Further Information	1	18 February 2010

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Service website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments

- Adding new sites and investigators
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nres.npsa.nhs.uk.

10/H0908/8	**Please quote this number on all correspondence**

Yours sincerely

pp

Mrs Sue Brooks
Vice Chair

Email: leigh.pollard@nhs.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: Mr Amaran Krishnan, Northern Oesophagogatric Cancer Unit, Royal Victoria Infirmary, Queen Victoria Road, Newcastle upon Tyne, NE1 4LP
Research & Development Office, 4th Floor, Leazes Wing, Royal Victoria Infirmary, Queen Victoria Road, Newcastle upon Tyne, NE1 4LP

Welcome to the Integrated Research Application System

IRAS Project Filter

The integrated dataset required for your project will be created from the answers you give to the following questions. The system will generate only those questions and sections which (a) apply to your study type and (b) are required by the bodies reviewing your study. Please ensure you answer all the questions before proceeding with your applications.

Please enter a short title for this project (maximum 70 characters)
Reflux disease in idiopathic pulmonary fibrosis and cystic fibrosis

1. Is your project research?

(•) Yes ( ) No

2. Select one category from the list below:

( ) Clinical trial of an investigational medicinal product
( ) Clinical investigation or other study of a medical device
( ) Combined trial of an investigational medicinal product and an investigational medical device
(•) Other clinical trial or clinical investigation
( ) Study administering questionnaires/interviews for quantitative analysis, or using mixed quantitative/qualitative methodology
( ) Study involving qualitative methods only
( ) Study limited to working with human tissue samples, other human biological samples and/or data *(specific project only)*
( ) Research tissue bank
( ) Research database

If your work does not fit any of these categories, select the option below:

( ) Other study

2a. Please answer the following question(s):

a) Does the study involve the use of any ionising radiation?	( ) Yes	(•) No
b) Will you be taking new human tissue samples (or other human biological samples)?	(•) Yes	( ) No
c) Will you be using existing human tissue samples (or other human biological samples)?	( ) Yes	(•) No

3. In which countries of the UK will the research sites be located? *(Tick all that apply)*

[x] England
[ ] Scotland
[ ] Wales
[ ] Northern Ireland

3a. In which country of the UK will the lead NHS R&D office be located:

(•) England
( ) Scotland

○ Wales

○ Northern Ireland

○ This study does not involve the NHS

4. Which review bodies are you applying to?

☑ NHS/HSC Research and Development offices
☐ Social Care Research Ethics Committee
☑ Research Ethics Committee
☐ National Information Governance Board for Health and Social Care (NIGB)
☐ Ministry of Justice (MoJ)

5. Will any research sites in this study be NHS organisations?

◉ Yes ○ No

5a. Do you want your application to be processed through the NIHR Coordinated System for gaining NHS Permission?

◉ Yes ○ No

If yes, you must complete and submit the NIHR CSP Application Form immediately after completing this project filter, before proceeding with completing and submitting other applications.

6. Do you plan to include any participants who are children?

○ Yes ◉ No

7. Do you plan to include any participants who are adults unable to consent for themselves through physical or mental incapacity? *The guidance notes explain how an adult is defined for this purpose.*

○ Yes ◉ No

8. Do you plan to include any participants who are prisoners or young offenders in the custody of HM Prison Service in England or Wales?

○ Yes ◉ No

9. Is the study, or any part of the study, being undertaken as an educational project?

◉ Yes ○ No

9a. Is the project being undertaken in part fulfilment of a PhD or other doctorate?

◉ Yes ○ No

10. Is this project financially supported by the United States Department for Health and Human Services?

○ Yes ◉ No

11. Will identifiable patient data be accessed outside the clinical care team without prior consent at any stage of the project (including identification of potential participants)?

○ Yes ◉ No

Integrated Research Application System
Application Form for Other clinical trial or investigation

National Patient Safety Agency
National Research Ethics Service

Application to NHS/HSC Research Ethics Committee

The Chief Investigator should complete this form. Guidance on the questions is available wherever you see this symbol displayed. We recommend reading the guidance first. The complete guidance and a glossary are available by selecting Help.

Short title and version number: (maximum 70 characters - this will be inserted as header on all forms)
Reflux disease in idiopathic pulmonary fibrosis and cystic fibrosis

Please complete these details after you have booked the REC application for review.

REC Name:
County Durham & Tees Valley 1 REC

REC Reference Number:
10/H0905/2

Submission date:
17/12/2009

PART A: Core study information

1. ADMINISTRATIVE DETAILS

A1. Full title of the research:

The use of impedance pH measurements to determine the effect of gastro-oesophageal reflux in patients with cystic fibrosis and idiopathic pulmonary fibrosis

A2-1. Give details of the educational course or degree for which this research is being undertaken:

Name and level of course/ degree:
Doctorate of Medicine

Name of educational establishment:
School of Medical Science, University of Newcastle upon Tyne

Name and contact details of academic supervisor:

	Title	Forename/Initials	Surname
	Professor	Jeff	Pearson
Address	Institute for Cell and Molecular		
	Biosciences, Medical School		
	University of Newcastle upon Tyne		
Post Code	NE2 4HH		

E-mail	J.P.Pearson@ncl.ac.uk
Telephone	01912228699
Fax	01912228742

Name and contact details of student:

	Title	Forename/Initials	Surname
	Mr	Amaran	Krishnan

Address	Northern Oesophagogastric Cancer Unit, Royal Victoria Infirmary Newcastle upon Tyne
Post Code	NE1 4LP
E-mail	amaran.krishnan@ncl.ac.uk
Telephone	07932788176
Fax	

A copy of a current CV for the student (maximum 2 pages of A4) must be submitted with the application.

A2-2. Who will act as Chief Investigator for this study?

- ○ Student
- ○ Academic supervisor
- ◉ Other

A3. Chief Investigator:

	Title	Forename/Initials	Surname
	Professor	S.Michael	Griffin

Post	Professor of Gastrointestinal Surgery
Qualifications	MD, FRCS
Employer	Newcastle Hospitals NHS Foundation Trust
Work Address	Northern Oesophagogastric Unit Royal Victoria Infirmary Queen Victoria Road
Post Code	NE1 4LP
Work E-mail	Michael.Griffin@nuth.nhs.uk
* Personal E-mail	
Work Telephone	01912820234
* Personal Telephone/Mobile	
Fax	01912820237

** This information is optional. It will not be placed in the public domain or disclosed to any other third party without prior consent.*

A copy of a current CV (maximum 2 pages of A4) for the Chief Investigator must be submitted with the application.

A4. Who is the contact on behalf of the sponsor for all correspondence relating to applications for this project?
This contact will receive copies of all correspondence from REC and R&D reviewers that is sent to the CI.

Title	Forename/Initials	Surname

	Mr Amaran Krishnan
Address	Oesophageal Laborotory Northern Oesophagogastric Unit Royal Victoria Infirmary, Newcastle
Post Code	NE1 4LP
E-mail	amaran.krishnan@ncl.ac.uk
Telephone	07932788176
Fax	

A5-1. Research reference numbers. *Please give any relevant references for your study:*

Applicant's/organisation's own reference number, e.g. R & D (if available):

Sponsor's/protocol number:

Protocol Version:

Protocol Date:

Funder's reference number:

International Standard Randomised Controlled Trial Number (ISRCTN):

ClinicalTrials.gov Identifier (NCT number):

European Clinical Trials Database (EudraCT) number:

Project website:

Ref.Number Description	Reference Number

A5-2. Is this application linked to a previous study or another current application?

○ Yes ◉ No

Please give brief details and reference numbers.

2. OVERVIEW OF THE RESEARCH

To provide all the information required by review bodies and research information systems, we ask a number of specific questions. This section invites you to give an overview using language comprehensible to lay reviewers and members of the public. Please read the guidance notes for advice on this section.

A6-1. Summary of the study. *Please provide a brief summary of the research (maximum 300 words) using language easily understood by lay reviewers and members of the public. This summary will be published on the website of the National Research Ethics Service following the ethical review.*

The aim of this study is to determine the nature of reflux disease in patients with idiopathic pulmonary fibrosis (IPF) and cystic fibrosis (CF) and understand its contribution to progressive lung damage in these patients. Reflux is when stomach contents travel up the gullet and then enter the airways causing significant lung damage. This reflux may or may not cause symptoms. However, the long term consequences of stomach content within lung tissue can result in severe deterioration of lung function, affecting patients' quality of life. Patients with CF and IPF have been known to suffer from reflux. Unfortunately, there is very little understanding about how bad this reflux can be in these patients. As life expectancy in people with CF increases it becomes much more important to develop our understanding so that we can improve the management of reflux in CF patients.
We aim to use state of the art devices called impedance pH catheters to measure reflux to a very accurate degree. This device detects both acid and non-acid reflux, and will be combined with oesophageal manometry studies which provide details of how the gullet is functioning. These investigations will be compared to patients' lung function tests to determine the relationship between the two.
Both groups of patients will have samples analysed in the lab for bile salts and pepsin; two chemicals, originating from the stomach and found in refluxed material. The IPF group of patients will have provided samples at bronchoalveolar lavage (BAL). This procedure will be performed by a respiratory physician and a small amount of this sample will be

required for research purposes. The CF group of patients will have daily physiotherapy where they would be encouraged to clear their airways. A small amount of sputum will be requested and analysed in the same way.

A6-2. Summary of main issues. *Please summarise the main ethical and design issues arising from the study and say how you have addressed them.*

We aim to work with patients with advanced lung disease (IPF and CF) in order to accumulate the knowledge and information that may explain how reflux disease contributes to deteriorating lung function in these patients. In order to do accomplish this we will have to overcome the following ethical and design issues:

1)Patients must understand the background of their disease and the possible role of acid reflux in its progression. This will be achieved by direct communication from the investigator, their clinician and a take home information sheet.

2)Patients will have to make two additional visits to the hospital for the purpose of oesophageal reflux tests

3)Patients will have to comply with devices used for reflux monitoring for 24 hours

4)Patients with IPF will require a flexible bronchoscopy in order to provide a lavage sample

5)Patients will have to agree to having their bodily fluids tested in a laboratory

6)Patients will be made aware of the results of their reflux tests and where appropriate be referred to a specialist to treat any significant disease.

A10. What is the principal research question/objective? *Please put this in language comprehensible to a lay person.*

The principal research objective is:
We aim to identify the extent of gastro-oesophageal reflux in patients with IPF and CF and determine whether there is a clear relationship between reflux, lung (micro) aspiration and deterioration of lung function. We shall use a combination of common medical investigations together with laboratory analysis of samples provided by these patients.

A11. What are the secondary research questions/objectives if applicable? *Please put this in language comprehensible to a lay person.*

A12. What is the scientific justification for the research? *Please put this in language comprehensible to a lay person.*

BACKGROUND
Numerous small observational studies have shown that gastro-oesophageal reflux is prevalent among patients with advanced lung disease. The main concern is that reflux is a major risk factor for recurrent micro-aspiration which can contribute to deterioration in lung function. There are very few studies which have reliably assessed reflux with impedance tools or performed an assessment of the molecular biomarkers of aspiration. (Sweet, 2009)

SCIENTIFIC JUSTIFICATION
Interstitial lung disease comprises of a group of both acute and chronic disorders characterised by diffuse pulmonary inflammation as well as signs of restrictive lung function. Within this rather broad umbrella term, the more specific diagnosis of idiopathic pulmonary fibrosis (IPF) lies. Recently [Raghu, 2006] demonstrated through 24-h pH monitoring that abnormal GOR was present in up to 87% of patients with IPF. It is thought that chronic aspiration of stomach contents may contribute to chronic lung dysfunction (progressive loss of lung function). This is a fairly recent concept and was first described in 1990. Gastric aspiration has accounted for deterioration in lung function in adult patients with cystic fibrosis (CF) [Blondeau, 2009]. Few studies have been completed to elucidate the exact cause of reflux in CF patients, but an increased abdominal-thoracic pressure gradient during physiotherapy and periods of coughing may be a major contributing factor. [Ledson, 1998].
Impedance is a small device that can be placed in the gullet to measure reflux whether it is acid or not. It is an exciting new technology which is more accurate than current acid detection studies (Wise, 2007). These non acid reflux events may contribute to the development of chronic lung dysfunction. The use of this technology enables us to study reflux in a "real life situation", unlike previous studies where anti-acid therapy has been discontinued artificially, for acid monitoring studies (Davis, 2003). The older technique will miss episodes of non-acid reflux. A recent study [Savarino, 2009] demonstrated using oesophageal impedance on subjects with systemic sclerosis associated ILD, that increased non-acid reflux episodes are linked to the progression of pulmonary disease.
To accurately quantify the levels of pepsin and bile salts being aspirated into the lungs, samples of fluid from

bronchoalveolar lavage (IPF group) and Sputum (CF group) must be taken and analysed. A causal relationship between reflux mediated microaspiration and chronic lung injury has been suggested in transplant patients in which the concentration of bile salts and pepsin in bronchoalveolar lavage predisposed to Bronchiolitis obliterans syndrome (BOS). [Blondeau, 2008]. The molecular evidence we can gather from our study will reinforce the information gathered from our impedance and manometry studies.

IMPORTANCE
This is a very important topic. A recent review article published by Sweet in March 2009, and evidence gathered from a study by Blondeau in March 2008 identify that further information is required to determine the role of gastro-oesophageal reflux in patients with advanced lung disease and cystic fibrosis. Gastro-oesophageal reflux is a disease that, once identified in these patients can potentially be treated; this therefore identifies the importance of this research and its potential contribution to improving the lung function in these patients. In addition, a large number of IPF patients are anecdotally placed on PPI treatment, with no evidence of the type of reflux, its extent and potential harm that may be caused.

A13. Please give a full summary of your design and methodology. *It should be clear exactly what will happen to the research participant, how many times and in what order. Please complete this section in language comprehensible to the lay person. Do not simply reproduce or refer to the protocol. Further guidance is available in the guidance notes.*

Purpose & Theory

Both Symptomatic and asymptomatic reflux is a common feature in patients with advanced lung disease. We hypothesise that, in patients with idiopathic pulmonary fibrosis (IPF) and Cystic fibrosis (CF), this reflux together with the subsequent (micro) aspiration of stomach contents into the lung can lead to long term deterioration of lung function. Detection of reflux using established techniques combined with laboratory measurements of biomarkers in refluxate will identify both the extent and severity of gastrooesophageal reflux (GOR) in these patients. The subsequent treatment of GOR in these identified patients could preserve long-term lung function and improve their quality of life.

Aims

-To measure pH/impedance in patients with IPF and CF to objectively assess reflux disease.

-To measure patient symptoms of reflux disease, using a specific questionnaire.

-To compare objective assessment of reflux disease (impedance) with patient experience of symptoms (questionnaire)

-To compare objective and clinical assessments of reflux and symptoms with markers of aspiration in the fluid removed from the lungs (pepsin, bile salts)

- To correlate the above investigations of reflux with baseline lung function

- To identify patients suitable for specialist referral and subsequent management of reflux disease; and assess the effect of the intervention with regular lung function assessment

Design and methodology

CF Patients will be recruited directly from designated specialist clinics. There are currently two specialists at the Royal Victoria infirmary, and patients will be approached directly by myself, the researcher, and provided with a patient information leaflet. Patients with IPF will be recruited with the aid of an interstitial lung disease(ILD)specialist who will be closely involved in the study. Currently at the Royal Victoria infirmary, ILD clinics are organised twice a month and recruitment of IPF patients will be by the researcher directly from these clinics. Patients will be again provided with a patient information sheet.
Both groups of patients will have regular lung functions assessment, and if they choose to participate in the study they will be requested to attend a routine lung function assessment at the start of the study to assess their baseline function. In addition all patients will be provided with validated questionnaires to assess their reflux subjectively.

Over an 18 month period both groups of patients (CF and IPF) will have reflux and acid levels measured in the gullet using a small probe passed through the nose. This will be performed once only unless there was any significant problem with the first attempt. Patients will be required to attend the oesophageal lab. The first test is well established, pH manometry, and involves insertion of a plastic tube into the patients nose, to sit in the gullet for approximately 20 minutes whilst recordings on oesophageal function takes place. After this the initial plastic tube will be replaced with a finer pH impedance catheter. It is a thin walled tube (2mm in diameter) which will be placed through the nostril into the gullet to look for reflux for a distance of approximately 45cm. The tube consists of a series of small rings which detect

changes of resistance between these rings. Liquids have low resistance gases have a high resistance. This device is able to detect changes in resistance at various points along the tube. This enables this device to distinguish between swallows and reflux events, determine the composition of the reflux event (gas/liquid) and the level of reflux.

Impedance devices have been in use for over 10 years and the devices used in the study have been used in the UK for 3 years in both clinical and research settings. Impedance devices are used routinely throughout the UK and worldwide. UK centres include Glasgow Royal Infirmary, University College London Hospitals, Nottingham, Manchester (paediatrics) and Plymouth. We also use this device clinically at the Northern Oesophago-Gastric Cancer Unit in the Royal Victoria Infirmary for ongoing research studying reflux in lung transplant patients. The impedance device used is CE marked in line with European standards and is manufactured to comply with the European Medical Devices Directive (93/42/EEC) and therefore does not require MHRA approval. There is a completed Pre Purchase Questionnaire (PPQ) from Ardmore Healthcare Ltd that confirms this compliance.

The device itself has been operationally checked by the electronic department on receipt and has been placed on the Newcastle Upon Tyne Hospitals Trust asset register. (Trust Safety Number: Safety Information for Impedance- 155951) There will be no dietary restrictions during this study and patients will be encouraged to try to have a "normal" a diet as possible to allow a "real life" assessment of their reflux. The pH impedance catheter will be placed in the patient for 24 hours and record the data over this period of time. The patients will be asked to return the following day for the catheter to be removed and the data will be downloaded from the device. This data will then be combined with the manometry to provide an objective assessment of reflux.

The degree of reflux detected (how often, how severe, and whether it is acid or not) will be compared with molecular measures of reflux. The detection of pepsin (a protein made in the stomach) and bile salts (from the liver and small intestine) in the lung fluid and the presence of cells of inflammation in the lung fluid sample will be used to assess the relevance of the detected reflux episodes. These samples will be collected differently for the CF and IPF groups as follows:

i)CF patients will be encouraged to express sputum through their routine morning physiotherapy. A small aliquot will be requested for analysis

ii) IPF patients who consent to the study will have a flexible bronchoscopy and 3 x 60ml lavages will be performed. The samples will be sent for routine investigations to assist in the further management of the patient's lung disease, and a small aliquot will be retained for our lab based investigations

Outcomes

The information gathered from the studies above will then be analysed. The study will provide us with a subjective, objective and laboratory based assessment of reflux disease in patients with IPF and CF
Those patients with significant reflux that could warrant treatment would be referred to an upper GI specialist for the most appropriate management.

A14-1. In which aspects of the research process have you actively involved, or will you involve, patients, service users, and/or their carers, or members of the public?

- ☐ Design of the research
- ☐ Management of the research
- ☑ Undertaking the research
- ☐ Analysis of results
- ☑ Dissemination of findings
- ☐ None of the above

Give details of involvement, or if none please justify the absence of involvement.

4. RISKS AND ETHICAL ISSUES

RESEARCH PARTICIPANTS

A17-1. Please list the principal inclusion criteria (list the most important, max 5000 characters).

All IPF patients will be identified from ILD clinics. IPF in new and known patients will fulfill the internationally accepted definitions as proposed by the European and American Societies

Major Criteria:
- Exclusion of other known causes of ILD such as certain drug toxicities, environmental exposures and connective tissue disease

- Abnormal pulmonary function studies that include evidence of restriction (reduced VC, often with an increased FEV1/FVC ratio) and impaired gas exchange (increased P(A-a)O2, decreased PaO2 with rest or exercise or decreased TLCO)

- Bibasilar reticular abnormalities with minimal ground glass opacities on HRCT scans
- Transbronchial lung biopsy or BAL showing no features to support an alternative diagnosis

Minor Criteria:
- Age > 50 years

- Bibasilar inspiratory crackles (dry or 'Velcro'-type in quality)

- Insidious onset of otherwise unexplained dyspnoea on exertion

- Duration of illness > 3 months

The CF group of patients would include all adult patients (age >16 years)

A17-2. Please list the principal exclusion criteria (list the most important, max 5000 characters).

- Patients in respiratory failure

- Patients with a co-existing respiratory disorder

- Patients with overt congestive cardiac failure

- Patients regarded unfit for any other clinical reason by their respiratory physician

RESEARCH PROCEDURES, RISKS AND BENEFITS

A18. Give details of all non-clinical intervention(s) or procedure(s) that will be received by participants as part of the research protocol. *These include seeking consent, interviews, non-clinical observations and use of questionnaires.*

Please complete the columns for each intervention/procedure as follows:

1. Total number of interventions/procedures to be received by each participant as part of the research protocol.
2. If this intervention/procedure would be routinely given to participants as part of their care outside the research, how many of the total would be routine?
3. Average time taken per intervention/procedure (minutes, hours or days)
4. Details of who will conduct the intervention/procedure, and where it will take place.

Intervention or procedure	1	2	3	4
Patient Information Sheet and discussion	1	0	20m	Researcher with the patient's clinician
Consent	1	0	10m	Researcher with the patient's clinician
Oesophageal Reflux Questionnaires	1	0	20m	Provided to patient by researcher

A19. Give details of any clinical intervention(s) or procedure(s) to be received by participants as part of the research protocol. *These include uses of medicinal products or devices, other medical treatments or assessments, mental health interventions, imaging investigations and taking samples of human biological material. Include procedures which might be received as routine clinical care outside of the research.*

Please complete the columns for each intervention/procedure as follows:

1. Total number of interventions/procedures to be received by each participant as part of the research protocol.
2. If this intervention/procedure would be routinely given to participants as part of their care outside the research, how many of the total would be routine?
3. Average time taken per intervention/procedure (minutes, hours or days).
4. Details of who will conduct the intervention/procedure, and where it will take place.

Intervention or procedure	1	2	3	4
Pulmonary Function Tests	1	1	10m	Respiratory Clinic Nurses
Oesophageal Manometry	1	0	20m	Researcher and Specilaist Nurse
Oesophageal Impedance pH Study	1	0	24h	Researcher and Specilaist Nurse
Flexible Bronchoscopy and Bronchoalveolar lavage (IPF patients only)	1	0	4h	Respiratory Consulatant (ILD specialist)

A20. Will you withhold an intervention or procedure, which would normally be considered a part of routine care?

○ Yes ◉ No

A21. How long do you expect each participant to be in the study in total?

From the time of recruitment we would endeavour that each patient may remain in the study for up to one month. This would allow time to complete all the investigations and collect the required samples.

A22. What are the potential risks and burdens for research participants and how will you minimise them?

For all studies, describe any potential adverse effects, pain, discomfort, distress, intrusion, inconvenience or changes to lifestyle. Only describe risks or burdens that could occur as a result of participation in the research. Say what steps would be taken to minimise risks and burdens as far as possible.

Manometry and impedance are low risk procedures. Many patients undergo manometry and pH studies (an old-fashioned measurement similar to impedance) without experiencing any complications. The main risk is of discomfort to the nose, throat or gullet. This is minimised with some light lubrication and an effective technique which has been well practiced by the researcher.

BAL will be carried out on the IPF group of patients and on no patients with severely compromised respiratory function. As such the recent published evidence states the following with regards to potential risks:

- No complications in up to 95%
- Cough
- Transient fever (2.5%)
- Transient chills and myalgias
- Transient infiltrates in most (resolves in 24 hours)
- Bronchospasm (<1%)
- Transient fall of lung function
- Transient decrease in baseline PaO2
- Death (1 in 10000)

All procedures will be carried out following the current guidelines and performed only by the lead researcher (Amaran Krishnan) and consultant respiratory physician (Dr. Ian Forrest).

A23. Will interviews/ questionnaires or group discussions include topics that might be sensitive, embarrassing or upsetting, or is it possible that criminal or other disclosures requiring action could occur during the study?

○ Yes ◉ No

A24. What is the potential for benefit to research participants?

The accurate diagnosis of acid and non-acid reflux in patients with CF and IPF may indicate the need for a specialist referral. The assessment by the upper GI specialist may lead to treatment being offered for the improvement of reflux which may improve the long term lung function and thus the patient's quality of life.

A25. What arrangements are being made for continued provision of the intervention for participants, if appropriate, once the research has finished? *May apply to any clinical intervention, including a drug, medical device, mental health intervention, complementary therapy, physiotherapy, dietary manipulation, lifestyle change, etc.*

Patients with abnormal reflux investigations will have upper GI follow up through the research department with follow up for their lung function tests and possible treatment for their reflux disease.

A26. What are the potential risks for the researchers themselves? *(if any)*

No significant risks

RECRUITMENT AND INFORMED CONSENT

In this section we ask you to describe the recruitment procedures for the study. Please give separate details for different study groups where appropriate.

A27-1. How will potential participants, records or samples be identified? Who will carry this out and what resources will be used?*For example, identification may involve a disease register, computerised search of GP records, or review of medical records. Indicate whether this will be done by the direct healthcare team or by researchers acting under arrangements with the responsible care organisation(s).*

IPF Patients - Patients will be recruited directly from new and follow-up clinics lead by the ILD specialist. Patients will also be contacted through the ILD register for the region and then brought to a clinic for a face to face discussion.

CF patients - Patients will be recruited directly from new and follow-up clinics lead by the CF specialists.

Recruitment will be a two stage process. After the initial discussion patients will receive an information leaflet detailing all the investigations they may need to participate in. They will then be booked into another clinic to be consented and the first investigation, lung function tests will be performed at this point.

A27-2. Will the identification of potential participants involve reviewing or screening the identifiable personal information of patients, service users or any other person?

○ Yes ◉ No

Please give details below:

A28. Will any participants be recruited by publicity through posters, leaflets, adverts or websites?

○ Yes ◉ No

A29. How and by whom will potential participants first be approached?

Potential participants will be approached by me, Amaran Krishnan, principal researcher. They will be both verbally informed and given an information sheet. I shall contact them by their chosen method of communication to confirm their availability for the study

A30-1. Will you obtain informed consent from or on behalf of research participants?

◉ Yes ○ No

If you will be obtaining consent from adult participants, please give details of who will take consent and how it will be done, with details of any steps to provide information (a written information sheet, videos, or interactive material). Arrangements for adults unable to consent for themselves should be described separately in Part B Section 6, and for children in Part B Section 7.

If you plan to seek informed consent from vulnerable groups, say how you will ensure that consent is voluntary and fully informed.

1)Consent will be taken by myself, the researcher, in the presence of the patient's clinician
2)The consent will be written consent and signed by the patient with their physician present and a copy for them and one for the research portfolio will be provided.

If you are not obtaining consent, please explain why not.

Please enclose a copy of the information sheet(s) and consent form(s).

A30-2. Will you record informed consent (or advice from consultees) in writing?

(•) Yes ( ) No

A31. How long will you allow potential participants to decide whether or not to take part?

Approximately 72 hours, but I shall ask participants when it would be appropriate as some may want to speak to their family/friend etc

A32. Will you recruit any participants who are involved in current research or have recently been involved in any research prior to recruitment?

(•) Yes
( ) No
( ) Not Known

If Yes, please give details and justify their inclusion. If Not Known, what steps will you take to find out?

If it is appropriate to do so; does not infer any problems to this study and if the participant is not at any additional risks/inconvenience

A33-1. What arrangements have been made for persons who might not adequately understand verbal explanations or written information given in English, or who have special communication needs? *(e.g. translation, use of interpreters)*

This will be reviewed on an individual basis. Arrangements through the NHS may be required; or patient's family members may need to be present so that there is complete understanding by the patient of the research.

A34. What arrangements will you make to ensure participants receive any information that becomes available during the course of the research that may be relevant to their continued participation?

All results will be communicated directly to the patient at the follow-up respiratory clinic. At this point, if deemed to have significant reflux, the patient, if they agree will be referred to an upper GI specialist.

A35. What steps would you take if a participant, who has given informed consent, loses capacity to consent during the study? *Tick one option only.*

( ) The participant and all identifiable data or tissue collected would be withdrawn from the study. Data or tissue which is not identifiable to the research team may be retained.
(•) The participant would be withdrawn from the study. Identifiable data or tissue already collected with consent would be retained and used in the study. No further data or tissue would be collected or any other research procedures carried out on or in relation to the participant.
( ) The participant would continue to be included in the study.

○ Not applicable – informed consent will not be sought from any participants in this research.

Further details:

If you plan to retain and make further use of identifiable data/tissue following loss of capacity, you should inform participants about this when seeking their consent initially.

CONFIDENTIALITY

In this section, personal data means any data relating to a participant who could potentially be identified. It includes pseudonymised data capable of being linked to a participant through a unique code number.

Storage and use of personal data during the study

A36. Will you be undertaking any of the following activities at any stage (including in the identification of potential participants)? *(Tick as appropriate)*

- ☐ Access to medical records by those outside the direct healthcare team
- ☐ Electronic transfer by magnetic or optical media, email or computer networks
- ☐ Sharing of personal data with other organisations
- ☐ Export of personal data outside the EEA
- ☑ Use of personal addresses, postcodes, faxes, emails or telephone numbers
- ☐ Publication of direct quotations from respondents
- ☐ Publication of data that might allow identification of individuals
- ☐ Use of audio/visual recording devices
- ☑ Storage of personal data on any of the following:
  - ☐ Manual files including X-rays
  - ☑ NHS computers
  - ☐ Home or other personal computers
  - ☐ University computers
  - ☐ Private company computers
  - ☐ Laptop computers

Further details:

A38. How will you ensure the confidentiality of personal data? *Please provide a general statement of the policy and procedures for ensuring confidentiality, e.g. anonymisation or pseudonymisation of data.*

Caldicott Principles for the management of patient information. All data will be encrypted and password secure.

A40. Who will have access to participants' personal data during the study? *Where access is by individuals outside the direct care team, please justify and say whether consent will be sought.*

The Clinical team, the research team, personnel from regulatory authorities or from the sponsor, i.e. the Trust, with their consent.

Storage and use of data after the end of the study

A43. How long will personal data be stored or accessed after the study has ended?

- ○ Less than 3 months
- ○ 3 – 6 months
- ◉ 6 – 12 months
- ○ 12 months – 3 years
- ○ Over 3 years

INCENTIVES AND PAYMENTS

A46. Will research participants receive any payments, reimbursement of expenses or any other benefits or incentives for taking part in this research?

○ Yes ◉ No

A47. Will individual researchers receive any personal payment over and above normal salary, or any other benefits or incentives, for taking part in this research?

○ Yes ◉ No

A48. Does the Chief Investigator or any other investigator/collaborator have any direct personal involvement (e.g. financial, share holding, personal relationship etc.) in the organisations sponsoring or funding the research that may give rise to a possible conflict of interest?

○ Yes ◉ No

NOTIFICATION OF OTHER PROFESSIONALS

A49-1. Will you inform the participants' General Practitioners (and/or any other health or care professional responsible for their care) that they are taking part in the study?

○ Yes ◉ No

If Yes, please enclose a copy of the information sheet/letter for the GP/health professional with a version number and date.

PUBLICATION AND DISSEMINATION

A50. Will the research be registered on a public database?

◉ Yes ○ No

Please give details, or justify if not registering the research.
The research will be registered with the NIHR

A51. How do you intend to report and disseminate the results of the study? *Tick as appropriate:*

- ☑ Peer reviewed scientific journals
- ☐ Internal report
- ☑ Conference presentation
- ☐ Publication on website
- ☑ Other publication

☐ Submission to regulatory authorities

☐ Access to raw data and right to publish freely by all investigators in study or by Independent Steering Committee on behalf of all investigators

☐ No plans to report or disseminate the results

☑ Other (please specify)

If an oppurtunity arises, feedback will also be given to the CF and IPF patients' groups e.e British Lung Foundation patient meeting.

A53. Will you inform participants of the results?

◉ Yes ○ No

Please give details of how you will inform participants or justify if not doing so.
Return to clinic and discuss findings

5. Scientific and Statistical Review

A54. How has the scientific quality of the research been assessed? *Tick as appropriate:*

☐ Independent external review

☐ Review within a company

☐ Review within a multi−centre research group

☑ Review within the Chief Investigator's institution or host organisation

☑ Review within the research team

☐ Review by educational supervisor

☐ Other

Justify and describe the review process and outcome. If the review has been undertaken but not seen by the researcher, give details of the body which has undertaken the review:
The research protocol has been designed using evidence published in journals. Professors Jeffrey Pearson and S.M. Griffin have produced critique with regard to the gastro-oeophageal component of the protocol. Dr Chris Ward, Senior Lecturer and Dr. Ian Forrest, ILD specilaist have helped in the review of the respiratoy component.

For all studies except non-doctoral student research, please enclose a copy of any available scientific critique reports, together with any related correspondence.

For non-doctoral student research, please enclose a copy of the assessment from your educational supervisor/ institution.

A56. How have the statistical aspects of the research been reviewed? *Tick as appropriate:*

☐ Review by independent statistician commissioned by funder or sponsor

☐ Other review by independent statistician

☐ Review by company statistician

☐ Review by a statistician within the Chief Investigator's institution

☐ Review by a statistician within the research team or multi−centre group

☐ Review by educational supervisor

☐ Other review by individual with relevant statistical expertise

☑ No review necessary as only frequencies and associations will be assessed – details of statistical input not required

In all cases please give details below of the individual responsible for reviewing the statistical aspects. If advice has been provided in confidence, give details of the department and institution concerned.

Title Forename/Initials Surname

Department
Institution
Work Address

Post Code
Telephone
Fax
Mobile
E-mail

Please enclose a copy of any available comments or reports from a statistician.

A57. What is the primary outcome measure for the study?

The primary outcome of the study is to identify the association between gastro-oesophageal reflux and lung function in IPF and CF patients.

A58. What are the secondary outcome measures? *(if any)*

A59. What is the sample size for the research? *How many participants/samples/data records do you plan to study in total? If there is more than one group, please give further details below.*

Total UK sample size:
Total international sample size (including UK):
Total in European Economic Area:

Further details:
30 IPF and 30 CF patients. This is based on the number of patients attending clinic and the incidence within the region

A60. How was the sample size decided upon? *If a formal sample size calculation was used, indicate how this was done, giving sufficient information to justify and reproduce the calculation.*

Emperical sample size - suggested from previous studies but no data available to calculate formal sample size

A61. Will participants be allocated to groups at random?

○ Yes ◉ No

A62. Please describe the methods of analysis (statistical or other appropriate methods, e.g. for qualitative research) by which the data will be evaluated to meet the study objectives.

The results will be collated by the research team and simple descriptive statistics produced. A statistician will then be consulted with regard to the most appropriate method of analysis.

6. MANAGEMENT OF THE RESEARCH

A63. Other key investigators/collaborators. *Please include all grant co−applicants, protocol co−authors and other key members of the Chief Investigator's team, including non-doctoral student researchers.*

	Title	Forename/Initials	Surname
	Professor	Jeffrey	Pearson
Post	Professor of Molecular Physiology		
Qualifications	pHD		
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	Title	Forename/Initials	Surname
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	Title	Forename/Initials	Surname
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	Title	Forename/Initials	Surname
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Post	Honorary Lecturer in Surgery		
Qualifications	MBBS, FRCS, pHD		
Employer	Newcastle upon Tyne NHS Foundation Trusts		
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Fax	
Mobile	
Work Email	jon.shenfine@nuth.nhs.uk

Title	Forename/Initials	Surname
Dr	Stephen	Bourke

Post	Consultant in Respiratory Medicine
Qualifications	MBBS, MRCP
Employer	Newcastle upon Tyne NHS Foundation Trusts
Work Address	Department of Repsiratory Medicine
	Royal Victoria Infirmary
	Newcastle upon Tyne
Post Code	NE1 4LP
Telephone	01912336161
Fax	
Mobile	
Work Email	stephen.bourke@nuth.nhs.uk

A64. Details of research sponsor(s)

A64-1. Sponsor

Lead Sponsor

Status:
- (●) NHS or HSC care organisation
- ( ) Academic
- ( ) Pharmaceutical industry
- ( ) Medical device industry
- ( ) Local Authority
- ( ) Other social care provider (including voluntary sector or private organisation)
- ( ) Other

If Other, please specify:

Commercial status: Non-Commercial

Contact person

Name of organisation	Newcastle upon Tyne NHS foundation Trust
Given name	Amanda
Family name	Tortice
Address	Joint research office, Royal Victoria Infirmary
Town/city	Newcastle upon Tyne
Post code	NE1 4LP
Country	UNITED KINGDOM
Telephone	0191 282 5959
Fax	0191 282 4524

E-mail amanda.tortice@nuth.nhs.uk

Is the sponsor based outside the UK?
○ Yes ◉ No

Where the lead sponsor is not established within the UK, a legal representative in the UK may need to be appointed. Please consult the guidance notes.

A67. Has this or a similar application been previously rejected by a Research Ethics Committee in the UK or another country?

○ Yes ◉ No

Please provide a copy of the unfavourable opinion letter(s). You should explain in your answer to question A6-2 how the reasons for the unfavourable opinion have been addressed in this application.

A68. Give details of the lead NHS R&D contact for this research:

	Title	Forename/Initials	Surname
	Ms	Amanda	Tortice
Organisation	Newcastle Upon Tyne Hospitals NHS Foundation Trust		
Address	Joint Research Office		
	4th Floor Leazes Wing		
	Royal Victoria Infirmary		
Post Code	NE1 4LP		
Work Email	amanda.tortice@nuth.nhs.uk		
Telephone	0191 282 5959		
Fax	0191 282 4524		
Mobile			

Details can be obtained from the NHS R&D Forum website: http://www.rdforum.nhs.uk

A69-1. How long do you expect the study to last in the UK?

Planned start date: 01/02/2010
Planned end date: 01/02/2012
Total duration:
Years: 2 Months: Days:

A71-1. Is this study?

◉ Single centre
○ Multicentre

A71-2. Where will the research take place? *(Tick as appropriate)*

☑ England
☐ Scotland

- ☐ Wales
- ☐ Northern Ireland
- ☐ Other countries in European Economic Area

Total UK sites in study

Does this trial involve countries outside the EU?
○ Yes ○ No

A72. What host organisations (NHS or other) in the UK will be responsible for the research sites? *Please indicate the type of organisation by ticking the box and give approximate numbers of planned research sites:*

☑ NHS organisations in England	1
☐ NHS organisations in Wales	
☐ NHS organisations in Scotland	
☐ HSC organisations in Northern Ireland	
☐ GP practices in England	
☐ GP practices in Wales	
☐ GP practices in Scotland	
☐ GP practices in Northern Ireland	
☐ Social care organisations	
☐ Phase 1 trial units	
☐ Prison establishments	
☐ Probation areas	
☐ Independent hospitals	
☑ Educational establishments	1
☐ Independent research units	
☐ Other (give details)	
Total UK sites in study:	2

A75-1. Will a data monitoring committee (DMC) be convened?

○ Yes ◉ No

If Yes, please forward details of the membership of the DMC, its standard operating procedures and summary reports of interim analyses to the Research Ethics Committee which gives a favourable opinion of the study (or to GTAC if applicable).

A75-2. What are the criteria for electively stopping the trial or other research prematurely?

A76. Insurance/ indemnity to meet potential legal liabilities

***Note:* in this question to NHS indemnity schemes include equivalent schemes provided by Health and Social Care (HSC) in Northern Ireland**

A76-1. What arrangements will be made for insurance and/or indemnity to meet the potential legal liability of the sponsor(s) for harm to participants arising from the management of the research? *Please tick box(es) as applicable.*

Note: Where a NHS organisation has agreed to act as sponsor or co-sponsor, indemnity is provided through NHS schemes. Indicate if this applies (there is no need to provide documentary evidence). For all other sponsors, please describe the arrangements and provide evidence.

☑ NHS indemnity scheme will apply (NHS sponsors only)
☐ Other insurance or indemnity arrangements will apply (give details below)

Please enclose a copy of relevant documents.

A76-2. What arrangements will be made for insurance and/ or indemnity to meet the potential legal liability of the sponsor(s) or employer(s) for harm to participants arising from the design of the research? *Please tick box(es) as applicable.*

Note: Where researchers with substantive NHS employment contracts have designed the research, indemnity is provided through NHS schemes. Indicate if this applies (there is no need to provide documentary evidence). For other protocol authors (e.g. company employees, university members), please describe the arrangements and provide evidence.

☑ NHS indemnity scheme will apply (protocol authors with NHS contracts only)
☐ Other insurance or indemnity arrangements will apply (give details below)

Please enclose a copy of relevant documents.

A76-3. What arrangements will be made for insurance and/ or indemnity to meet the potential legal liability of investigators/collaborators arising from harm to participants in the conduct of the research?

Note: Where the participants are NHS patients, indemnity is provided through the NHS schemes or through professional indemnity. Indicate if this applies to the whole study (there is no need to provide documentary evidence). Where non-NHS sites are to be included in the research, including private practices, please describe the arrangements which will be made at these sites and provide evidence.

☑ NHS indemnity scheme or professional indemnity will apply (participants recruited at NHS sites only)
☐ Research includes non-NHS sites (give details of insurance/ indemnity arrangements for these sites below)

Please enclose a copy of relevant documents.

A77. Has the sponsor(s) made arrangements for payment of compensation in the event of harm to the research participants where no legal liability arises?

○ Yes ◉ No

Please enclose a copy of relevant documents.

Part B: Section 5 – Use of newly obtained human tissue(or other human biological materials) for research purposes

1. What types of human tissue or other biological material will be included in the study?

Sputum sample from CF patients and BAL samples following bronchoscopy in IPF patients

2. Who will collect the samples?

CF patients - They will be provided with a receptical to collect sputum after their morning physiotherapy

IPF patients - BAL samples will be collected by the ILD respiratory specialist

3. Who will the samples be removed from?

☑ Living donors
☐ The deceased

4. Will informed consent be obtained from living donors for use of the samples? *Please tick as appropriate*

In this research?
◉ Yes ○ No

In future research?
○ Yes ○ No ◉ Not applicable

6. Will any tissues or cells be used for human application or to carry out testing for human application in this research?

○ Yes ◉ No

8. Will the samples be stored: *[Tick as appropriate]*

In fully anonymised form? *(link to donor broken)*
○ Yes ◉ No

In linked anonymised form? *(linked to stored tissue but donor not identifiable to researchers)*
◉ Yes ○ No

If Yes, say who will have access to the code and personal information about the donor.
Lead Researcher and Consultant respiratory Lead (Dr. Ian Forrest)

In a form in which the donor could be identifiable to researchers?
○ Yes ◉ No

9. What types of test or analysis will be carried out on the samples?

Pepsin and bile salt assays on BAL(IPF) and sputum (CF) samples

10. Will the research involve the analysis or use of human DNA in the samples?

○ Yes ◉ No

11. Is it possible that the research could produce findings of clinical significance for donors or their relatives?

◉ Yes ○ No

12. If so, will arrangements be made to notify the individuals concerned?

◉ Yes ○ No ○ Not applicable

If No, please justify. If Yes, say what arrangements will be made and give details of the support or counselling service.

13. Give details of where the samples will be stored, who will have access and the custodial arrangements.

Locked freezers in lab M1070 at the Institutes of Cell and Molecular Biology and Cellular Medicine at the University of Newcastle

Professor J.P. Pearson and Dr. Chris Ward of the institute will have control of the samples.

14. What will happen to the samples at the end of the research? *Please tick all that apply and give further details.*

☑ Transfer to research tissue bank

(If the bank is in England, Wales or Northern Ireland the institution will require a licence from the Human Tissue Authority to store relevant material for possible further research.)

☐ Storage by research team pending ethical approval for use in another project

(Unless the researcher's institution holds a storage licence from the Human Tissue Authority, or the tissue is stored in Scotland, or it is not relevant material, a further application for ethical review should be submitted before the end of this project.)

☐ Storage by research team as part of a new research tissue bank

(The institution will require a licence from the Human Tissue Authority if the bank will be storing relevant material in England, Wales or Northern Ireland. A separate application for ethical review of the tissue bank may also be submitted.)

☐ Storage by research team of biological material which is not "relevant material" for the purposes of the Human Tissue Act

☐ Disposal in accordance with the Human Tissue Authority's Code of Practice

☐ Other

☐ Not yet known

Please give further details of the proposed arrangements:

PART C: Overview of research sites

Please enter details of the host organisations (Local Authority, NHS or other) in the UK that will be responsible for the research sites. *For NHS sites, the host organisation is the Trust or Health Board. Where the research site is a primary care site, e.g. GP practice, please insert the host organisation (PCT or Health Board) in the Institution row and insert the research site (e.g. GP practice) in the Department row.*

Research site		Investigator/ Collaborator/ Contact	
Institution name	Newcastle Hospitals NHS foundation Trusts	Title	Professor
Department name	Northern Oesophago-gastric Unit	First name/ Initials	S.M.
Street address	Royal Victoria Infirmary	Surname	Griffin
Town/city	Newcastle upon Tyne		
Post Code	NE1 4LP		
Institution name	University of Newcastle upon Tyne	Title	Professor
Department name	Institutes of Cellular and Molecular Bioscience	First name/ Initials	J.P.
Street address	Framlington place	Surname	Pearson
Town/city	Newcastle upon tyne		
Post Code	NE1 4HH		

PART D: Declarations

D1. Declaration by Chief Investigator

1. The information in this form is accurate to the best of my knowledge and belief and I take full responsibility for it.

2. I undertake to abide by the ethical principles underlying the Declaration of Helsinki and good practice guidelines on the proper conduct of research.

3. If the research is approved I undertake to adhere to the study protocol, the terms of the full application as approved and any conditions set out by review bodies in giving approval.

4. I undertake to notify review bodies of substantial amendments to the protocol or the terms of the approved application, and to seek a favourable opinion from the main REC before implementing the amendment.

5. I undertake to submit annual progress reports setting out the progress of the research, as required by review bodies.

6. I am aware of my responsibility to be up to date and comply with the requirements of the law and relevant guidelines relating to security and confidentiality of patient or other personal data, including the need to register when necessary with the appropriate Data Protection Officer. I understand that I am not permitted to disclose identifiable data to third parties unless the disclosure has the consent of the data subject or, in the case of patient data in England and Wales, the disclosure is covered by the terms of an approval under Section 251 of the NHS Act 2006.

7. I understand that research records/data may be subject to inspection by review bodies for audit purposes if required.

8. I understand that any personal data in this application will be held by review bodies and their operational managers and that this will be managed according to the principles established in the Data Protection Act 1998.

9. I understand that the information contained in this application, any supporting documentation and all correspondence with review bodies or their operational managers relating to the application:

    - Will be held by the main REC or the GTAC (as applicable) until at least 3 years after the end of the study; and by NHS R&D offices (where the research requires NHS management permission) in accordance with the NHS Code of Practice on Records Management.
    - May be disclosed to the operational managers of review bodies, or the appointing authority for the main REC, in order to check that the application has been processed correctly or to investigate any complaint.
    - May be seen by auditors appointed to undertake accreditation of RECs.
    - Will be subject to the provisions of the Freedom of Information Acts and may be disclosed in response to requests made under the Acts except where statutory exemptions apply.

10. I understand that information relating to this research, including the contact details on this application, may be held on national research information systems, and that this will be managed according to the principles established in the Data Protection Act 1998.

11. I understand that the main REC or its operational managers may share information in this application or supporting documentation with the Medicines and Healthcare products Regulatory Agency (MHRA) where it is relevant to the Agency's statutory responsibilities.

12. I understand that the summary of this study will be published on the website of the National Research Ethics Service (NRES), together with the contact point for enquiries named below. Publication will take place no earlier than 3 months after issue of the ethics committee's final opinion or the withdrawal of the application.

Contact point for publication*(Not applicable for R&D Forms)*

NRES would like to include a contact point with the published summary of the study for those wishing to seek further information. We would be grateful if you would indicate one of the contact points below.

- [x] Chief Investigator
- [ ] Sponsor
- [ ] Study co-ordinator
- [ ] Student
- [ ] Other – please give details
- [ ] None

Access to application for training purposes *(Not applicable for R&D Forms)*

Optional – please tick as appropriate:

- [x] I would be content for members of other RECs to have access to the information in the application in confidence for training purposes. All personal identifiers and references to sponsors, funders and research units would be removed.

Signature: .....

Print Name: Professor S.M. Griffin

Date: 17/12/2009 *(dd/mm/yyyy)*

D2. Declaration by the sponsor's representative

If there is more than one sponsor, this declaration should be signed on behalf of the co−sponsors by a representative of the lead sponsor named at A64-1.

I confirm that:

1. This research proposal has been discussed with the Chief Investigator and agreement in principle to sponsor the research is in place.
2. An appropriate process of scientific critique has demonstrated that this research proposal is worthwhile and of high scientific quality.
3. Any necessary indemnity or insurance arrangements, as described in question A76, will be in place before this research starts. Insurance or indemnity policies will be renewed for the duration of the study where necessary.
4. Arrangements will be in place before the study starts for the research team to access resources and support to deliver the research as proposed.
5. Arrangements to allocate responsibilities for the management, monitoring and reporting of the research will be in place before the research starts.
6. The duties of sponsors set out in the Research Governance Framework for Health and Social Care will be undertaken in relation to this research.
7. I understand that the summary of this study will be published on the website of the National Research Ethics Service (NRES), together with the contact point for enquiries named in this application. Publication will take place no earlier than 3 months after issue of the ethics committee's final opinion or the withdrawal of the application.

Signature: .....

Print Name: Miss Amanda Tortice

Post: Head of Research and Development

Organisation: Newcastle upon Tyne NHS Foundation Trust

Date: 17/12/2009 *(dd/mm/yyyy)*

D3. Declaration for student projects by academic supervisor

1. I have read and approved both the research proposal and this application. I am satisfied that the scientific content of the research is satisfactory for an educational qualification at this level.

2. I undertake to fulfil the responsibilities of the Chief Investigator and the supervisor for this study as set out in the Research Governance Framework for Health and Social Care.

3. I take responsibility for ensuring that this study is conducted in accordance with the ethical principles underlying the Declaration of Helsinki and good practice guidelines on the proper conduct of research, in conjunction with clinical supervisors as appropriate.

4. I take responsibility for ensuring that the applicant is up to date and complies with the requirements of the law and relevant guidelines relating to security and confidentiality of patient and other personal data, in conjunction with clinical supervisors as appropriate.

Signature: .....

Print Name: Professor J.P. Pearson

Post: Professor of Molecular Physiology

Organisation: Institutes of Cellular and molecular Biosciences

Date: 17/12/2009 *(dd/mm/yyyy)*

The Newcastle upon Tyne Hospitals NHS
NHS Foundation Trust

LRF/JW

The Freeman Hospital
High Heaton
Newcastle upon Tyne
NE7 7DN

Tel: 0191 233 6161
Fax: 0191 213 1968
www.newcastle-hospitals.nhs.uk

18th May 2010

Professor S Griffin
Professor of Gastrointestinal Surgery
Northern Oesophagogastric Unit
Royal Victoria Infirmary

Dear Professor Griffin

Trust R&D Project:	**5183**
Title of Project:	**The use of impedance pH measurements to determine the effect of gastro-oesophageal reflux in patients with cystic fibrosis and idiopathic pulmonary fibrosis**
Principal Investigator	**Professor Michael Griffin**
Number of patients:	**60**
Funder (proposed):	**Own Account**
Sponsor (proposed):	**Newcastle upon Tyne Hospitals NHS Foundation Trust**
REC number:	**10/H0908/8**

Having carried out the necessary risk and site assessment for the above research project, Newcastle upon Tyne Hospitals NHS Foundation Trust grants NHS R&D approval for this research to take place at this Trust dependent upon:

(i) you, as Principal Investigator, agreeing to comply with the Department of Health's Research Governance Framework for Health and Social Care, and understanding their responsibilities and duties (a copy of responsibilities prepared by the Trust R&D Office is enclosed)

(ii) you, as Principal Investigator, ensuring compliance of the project with all other legislation and guidelines including Caldicott Guardian approvals and compliance with the Data Protection Act 1998, Health and Safety at Work Act 1974, any requirements of the MHRA (*eg* CTA, EudraCT registration), and any other relevant UK/European guidelines or legislation (*eg* reporting of suspected adverse incidents).

(iii) where applicable, you, as Principal Investigator, should also adhere to the GMC supplementary guidance *Good practice in research* and *Consent to research* which sets out the good practice principles that doctors are expected to understand and follow if they are involved in research – see http://www.gmc-uk.org/guidance/ethical_guidance/5991.asp

Sponsorship

The Newcastle upon Tyne Hospitals NHS Foundation Trust will act as Sponsor for this project, under the Department of Health's guidelines for research in health and social care.

In addition, the Trust has a Research Governance Implementation Plan, agreed with the Department of Health, in order to fully comply with Research Governance and fulfil the responsibility of a Sponsor.

As the Trust is acting as Sponsor for the research and where some of the research is taking place outside of Newcastle upon Tyne, then all costs must be met for research governance audit visits to those sites. It is the responsibility of the PI to provide confirmation to the Trust of who will pay these costs. Audit is required under the Research Governance Framework for Health and Social Care. (Please note that the Trust randomly audits 10% of approved research projects annually.)

Any changes to the study protocol, other study documents (eg, Patient Information Sheets and Consent forms), or any other amendments to the study must be submitted to the Ethics Committee and MHRA (if relevant) for review – see http://www.nres.npsa.nhs.uk/applications/after-ethical-review/amendments/ for guidance). The R&D office must also review these notices of amendments in parallel with ethical and regulatory review so that implications of the amendment can be assessed. Therefore, you must send a copy of all amendment documents to the R&D office at the same time you are submitting these to the Ethics Committee/MHRA. If changes or amendments to the study have implications for costs or use of resources, you must also submit details of these changes to the R&D office.

It is also the Principal Investigator's responsibility to ensure that all staff involved in the research have Honorary Research Contracts or the necessary letters of access. These need to be issued prior to commencing the research.

In addition, unless otherwise agreed with the Trust, the research will be covered for negligence under the CNST (Clinical Negligence Scheme for Trusts), however cover for no-fault harm is the responsibility of the Principal Investigator to arrange if required.

Please also note that for any NHS employee who generates Intellectual Property *in the normal course of their duties*, it is recognised that the Intellectual Property Rights remain with the employer and not the employee.

Yours sincerely

Sir Leonard R Fenwick CBE
Chief Executive

Enc: Principal Investigator Responsibilities Document

CC: Mr G Regan, Finance Department, Room 203, Cheviot Court, Freeman Hospital
Dr N Thompson, Clinical Director, Freeman Hospital
Mr A Krishnan, Sponsor Representative and coordinator, Royal Victoria Infirmary

The Newcastle upon Tyne Hospitals NHS
NHS Foundation Trust

RESEARCH & DEVELOPMENT PROJECT REGISTRATION FORM (v2.0)

1. REGISTRATION INFORMATION	
PROJECT REGISTRATION NUMBER: (This will be allocated by the R&D Office upon receipt of application, unless you have pre-registered your project and been given a reference number)	5183
University Grants & Contracts number (if applicable, and if known)	

2. STUDY DETAILS	
Full title:	The use of impedance pH measurements to determine the effect of gastro-oesophageal reflux in patients with cystic fibrosis and idiopathic pulmonary fibrosis
Short title or acronym of study:	Oesophageal Reflux in Idiopathic Pulmonary Fibrosis and Cystic Fibrosis
Proposed start date:	02/02/2010
Proposed end date:	01/02/2012

3. PRINCIPAL INVESTIGATOR	
Name (including title):	Mr Amaran Krishnan
Post Held:	Clinical Research Fellow, Northern Oesophago-Gastric Cancer Unit
Who is your employment contract held with (i.e. through whom you are a salaried employed member of staff):	Newcastle Hospitals FoundationTrusts
Contact address:	Northern Oesophagogastric Unit Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP
Contact email:	amaran.krishnan@ncl.ac.uk
Has the PI received GCP training within the last 12 months	**YES** ☐ **NO** ☒
Telephone:	07932788176
If the employer is NOT Newcastle upon Tyne Hospitals NHS Foundation Trust	
Does the PI hold an Honorary Contract with this Trust?	**YES** ☐ **NO** ☒

4. RESPONSIBLE CLINICIAN	
If the PI is not a consultant or senior clinician in the Newcastle upon Tyne Hospitals NHS Foundation Trust:	
Name (including title):	Professor. S.M. Griffin
Post Held:	Professor of Gastrointestinal Surgery
Contact address:	Northern Oesophago-Gastric Cancer Unit, Royal Victoria Infirmary, Queen Victoria Road Newcastle
Contact email:	Michael.Griffin@nuth.nhs.uk
Telephone:	01912820234

5. FUNDER INFORMATION		
Funder name:	Northern Oesophago-Gastric Cancer Unit	
Funder:	**Commercial** ☐ **Non-Commercial** ☒	
Funding amount (whether awarded or requested)	£	**Awarded** ☐ **Requested** ☒

6. SPONSOR INFORMATION	
You are advised to consult the accompanying guidance when completing this section	
Proposed Sponsor	Newcastle upon Tyne Hospitals NHS Foundation Trust
Proposed Sponsor address	

7. CLINICAL TRIALS OF INVESTIGATIONAL MEDICINAL PRODUCTS	
You are advised to consult the guidance accompanying this form as the definition of a 'medicinal product' embraces all kinds of products including pharmaceutical and biological medicines, vaccines, herbal remedies and homeopathic produces and also includes products which have already received marketing authorisation.	
Is this a clinical trial of an Investigational Medicinal Product:	**YES** ☐ **NO** ☒
If yes:	
Please detail arrangements for trial monitoring:	n/a

8. END OF TRIAL TREATMENT INFORMATION	
Please supply the following information if your study is an investigational medicinal produce (IMP) or device trial.	
What treatment will patients receive at the end of the trial (e.g. exit strategy)?	n/a
How will this be provided?	n/a

9. TISSUE SAMPLES	
Are tissue samples being taken:	**YES** ☒ **NO** ☐
If Yes:	

Is any tissue being transferred between institutions (including between Newcastle Hospitals Trust and Newcastle University)	**YES** ☒ **NO** ☐
Is any of the tissue being taken from deceased patients?	**YES** ☐ **NO** ☒
Are the tissue samples being taken for the purposes of: **this study alone (and not retained thereafter)** **This study and then added to an existing tissue bank?**	 ☐ ☒
Which Bank:	University of Newcastle upon Tyne
What consent is being used: **generic** **study specific**	☐ ☒

10. DATA PROTECTION / CALDICOTT ISSUES (please see notes)	
Has Caldicott Guardian approval been given for use or transfer of patient held on/transferred from NHS Trust computers or servers?:	**YES** ☒ **NO** ☐ **N/A** ☐
If Yes:	
Who is the database custodian?	Professor S.M. Griffin

11. FINANCIAL INFORMATION You should complete the information in full below, appending any supporting information to show your calculations. A template guide is available alongside this form and you are advised to consult the guidance accompanying this form for further explanation or clarification.	
Who will administer the research funds (usually Newcastle Hospitals Trust or Newcastle University):	Newcastle Upon Tyne Hospitals NHS Trust
Total Direct Research Staff Costs:	£46,178
Total Direct Research Non-Staff Costs:	£22,000
Total NHS Service Support Staff Costs:	£0
Total NHS Service Support Non-Staff Costs:	£0
Total Excess Treatment Costs:	£
Which Directorate will be covering Excess Treatment Costs:	General Surgery
30% overheads on research costs (commercial only)	£
Non NHS-costs (e.g. University)	£30,000
TOTAL:	£98,178

SECTIONS 12 AND 13 ONLY NEED TO BE COMPLETED IF YOU ARE NOT SUBMITTING A NRES FORM (formerly COREC form) to R&D alongside this registration form.	
12. CHIEF INVESTIGATOR	
As PI above (otherwise complete below):	☐

Name:	
Title:	
Employing Organisation	
Contact address:	
Contact email:	
Telephone:	

13. PATIENT/SAMPLE INFORMATION	
If approval is given, it will be for the stated number of samples/patients. Recruitment to a project/trial is required to stop when the maximum number of participants (patients and control participants if relevant) has been reached.	
Number of tissue samples	
Number of patients	

14. SUBMISSION CHECKLIST				
Necessary documentation:				
			Office use only:	
Original plus four copies of this R&D Application Form	Hard copies only	☒	R&D Received date:	Finance ☐ Radiology ☐ Pharmacy ☐ Lab Services ☐
Copy of NRES form (formerly COREC)	Hard copy only	☒	R&D Received date:	Finance ☐ Radiology ☐ Pharmacy ☐ Lab Services ☐
Five copies of the project protocol (as submitted with NRES form or later version)	Hard copies only	☒	R&D Received date:	Finance ☐ Radiology ☐ Pharmacy ☐ Lab Services ☐
EudraCT Number (if this is a ctIMP – see section 7)				
If commercially-sponsored:				
Copy of the proposed contract:	Hard copy OR electronic[1]	☐ ☐	R&D Received date:	Finance ☐ Radiology ☐ Pharmacy ☐ Lab Services ☐
Indemnity Agreement(s):	Hard copy OR electronic[1]	☐ ☐	R&D Received date:	Finance ☐ Radiology ☐ Pharmacy ☐ Lab Services ☐
Can be forwarded once available (where relevant, evidence required before full approval will be granted):				
Ethics committee opinion	Not required OR hard copy OR electronic[1] to follow	☐ ☐ ☒	R&D Received date:	Finance ☐ Radiology ☐ Pharmacy ☐ Lab Services ☐
Sponsor agreement letter (not required if Newcastle Hospitals Trust or Newcastle University is Sponsor)	Not required OR hard copy OR electronic[1] to follow	☒ ☐ ☐	R&D Received date:	Finance ☐ Radiology ☐ Pharmacy ☐ Lab Services ☐
MHRA Clinical Trials Authorisation (CTA)	Not required OR hard copy OR electronic[1] to follow	☒ ☐ ☐	R&D Received date:	Finance ☐ Radiology ☐ Pharmacy ☐ Lab Services ☐

[1]If submitting document electronically please address to Trust.R&D@nuth.nhs.uk and state the Trust project reference number (if known) AND the PI/full project title.

15. PRINCIPAL INVESTIGATOR SIGNATURE	
I confirm that this information is, to be best of my knowledge, correct. I also confirm that I am aware of the Research Governance Framework for Health and Social Care (available at http://www.dh.gov.uk/PolicyAndGuidance/ResearchAndDevelopment/ResearchAndDevelopmentAZ/ResearchGovernance/ResearchGovernanceArticle/fs/en?CONTENT_ID=4002112&chk=PJlaGg) and the responsibilities I have as Principal Investigator and that by signing I agree to accept these responsibilities for this research project. I am aware that any deliberate misleading statements will lead to the immediate removal of Trust Approval.	
Signature:	Date:

Appendix 4

Patient information sheet and consent forms

- IPF patient information leaflet and consent form (version 2)
- CF patient information leaflet and consent form (version 2)

Study Information for patients with idiopathic pulmonary fibrosis

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for reading this.

What is the purpose of the study?

It is thought that patients with idiopathic pulmonary fibrosis experience reflux. This is when the stomach contents travel up the gullet and enter the airways causing lung damage. This reflux may or may not cause symptoms; however the long term consequences of stomach contents within the lung is the deterioration of lung function, which can have a considerable effect on quality of life. We aim to use state of the art devices called impedance pH catheters to measure the reflux to a very accurate degree. In addition, we would like to use samples from the lung to test for stomach chemicals. Using both these tests we aim to understand the nature and effect of reflux, which might help future treatment.

Why have I been chosen?

You have volunteered for the study which will involve 20 volunteers with idiopathic pulmonary fibrosis who have been assessed to be fit for all the procedures being performed in the study.

Do I have to take part?

Taking part is entirely voluntary. It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and will be asked to sign a consent form. If you do decide to take part you are still free to withdraw at any time and without giving a reason.

What will happen to me if I take part?

If you decide to take part in the study, you will be required to take part in three investigations:

1. **Lung Function Tests –** These tests need to be performed at the start of the study so that we are aware of your lung functions. This will be performed at the time of recruitment and is simply the routine spirometry that you normally have at the start of the clinic.
2. **Oesophageal manometry and impedance pH –** These investigations will be done over the course of 24 hours. Both tests involve passing a narrow plastic tube through the nose which will sit in the gullet. Prior to the tests you will be required to complete a questionnaire so we can assess the nature of any reflux.

   The oesophageal manometry tube allows the passage of four channels of water into the gullet and over 20 minutes this will measure the pressure waves so that we can assess your swallowing. This tube will then be removed and the information used to place the impedance pH tube.

   The impedance pH tube is a much finer tube, and will also be passed into the gullet via your nostril. It is made up of small monitors along a fine tube that can detect the changes in electrical resistance present in liquids and gases. Thus it can detect the presence of gas and liquid in your gullet and whether you are swallowing this gas/liquid or whether it is travelling in the wrong direction. In summary this tube will allow us to accurately measure the reflux from your stomach. The impedance catheter will be placed in your gullet for 24 hours, after which it will be quickly removed and the information transferred to a computer.

3. **Bronchoscopy and bronchoalveolar lavage (BAL) –** This is performed as a day case at the RVI Hospital. Bronchoscopy involves the examination and sampling of the air passages using a narrow fibre-optic camera. The procedure involves application of anaesthetic to the nose and throat to numb these areas. We use a combination of anaesthetic (lignocaine) spray, liquid and gel to do this. A cannula will be placed in a vein in your arm and you will be offered intravenous sedation with a drug called midazolam. It is the aim of this sedation to relax you though not to achieve general anaesthesia and you will not be unconscious. Your heart rate and oxygen levels will be monitored throughout.

   The bronchoscope which is approximately the thickness of a pencil is passed through the nose or mouth. Further local anaesthetic is given through the bronchoscope to the vocal cords to numb these before the bronchoscope is passed into the windpipe. Further local anaesthetic is given to the main air passages (bronchi) to reduce the risk of coughing.

A sample will be taken through the bronchoscope at this stage. 180mls of sterile saline (salty water) is injected through the scope and then sucked back through the scope having washed out the air passages. This is called bronchial lavage. Overall this procedure will take approximately 15-20 minutes to perform and is carried out one lung at a time. It is usually well tolerated and without significant side effects (see below).

What do I have to do?

- **Oesophageal Manometry and Impedance**

  An appointment will be made after you have consented to the study. You will be required to attend the oesophageal physiology laboratory. Before attending you will be required to be off your stomach medications for 2 weeks and 4 hours prior to the appointment have nothing to eat or drink. You will have the impedance tube secured in place for 24 hours and this will be recording the activity of the stomach through the box you shall carry on a belt. You may eat and drink normally with this tube in place. The following day the tube will be removed and the results transferred to a computer.

- **Bronchoscopy and BAL**

  The procedure is performed as a day case. You will be expected to attend the hospital on the morning of the test and would expect to leave in the afternoon. Your stay would usually be around 4 hours in total. We ask you to be fasted when you arrive, no food or drink for at least 4 hours before the bronchoscopy. Prior to the bronchoscopy you will be seen by a doctor to ensure there are no reasons not to perform the procedure on that day. The doctor will also perform a simple breathing test (spirometry) to check your lung function. After the bronchoscopy you will rest in a bed until fully recovered. We recommend that you do not eat or drink for 2 hours after the procedure. You should not drive, operate machinery or make any important decisions within 24 hours. We recommend you avoid alcohol after the procedure. You should be accompanied after the procedure by a responsible adult for the remainder of the study day/overnight. We will arrange a taxi to bring you to/from the hospital if necessary.

What is being tested?

- **Oesophageal Manometry and Impedance**

  The oesophageal manometry allows the assessment of how well the gullet is working, by observing over 20 minutes the changes in pressure as you

swallow. It also provides the information required to place the impedance tube. The impedance device will measure the amount of stomach fluid travelling up the gullet toward the airways, providing us with an assessment of reflux.

- **Bronchoscopy and BAL**

  The procedure you are undergoing is routinely used in transplant recipients and we have a vast experience of bronchoscopy including the research samples we will be taking. We are proposing to obtain the samples from the air passages to test them in the laboratory for stomach chemicals. These are called bile and pepsin.

What are the side effects of the treatment?

- **Oesophageal Manometry and Impedance**

  The possible side effects of the manometry and impedance catheters are discomfort to the nose, throat or gullet. These are normally related to the manometry test which only lasts 20 minutes. For 2 weeks prior to commencing this test it is important that you withhold any antacid treatment (proton pump inhibitor –PPI). This will provide a more accurate assessment of any reflux.

- **Bronchoscopy and BAL**

  When the intravenous cannula is inserted a small amount of pain/discomfort may be felt (similar to having blood taken from a vein).

  The procedure itself is usually free of pain / discomfort. A side effect of the sedation (should you choose to have it) is amnesia. This means you may not recall having had the procedure. However, the amnesia does not extend past this immediate period and you will not forget any important information.

  Coughing may be expected during the procedure and small amounts of bleeding can occur which you may notice coughed up afterwards. Occasionally, you may develop a low grade temperature afterwards that will resolve, by itself, in a day or two. Some patients report a sore throat for a short time after the procedure.

SIDE EFFECTS OF BRONCHOSCOPY

The procedure is extremely safe but is not without some risk. In addition to the minor local side effects outlined above there are potentially some more serious complications of the procedure. These include low oxygen levels, obstruction of the air passages, pneumonia, collapse of the lung, irregular heart beat and lung congestion.

The risks of a serious complication of the bronchoscopy are less than 1 in 1000 and of death less than 1 in 10000. These figures come from studies of patients with lung diseases.

More detailed information can be provided by Dr. Ian Forrest during clinic

What are the disadvantages of taking part?

Involvement involves time to be spent at the hospital, presumably missing work or holiday. The procedures have some minor local side effects as outlined above. There are some limitations on what we recommend you can do after the bronchoscopy. Whilst the procedures are safe, we would not wish to perform bronchoscopy on a female patient who may be pregnant. Women who are at risk of pregnancy may be asked to have a pregnancy test prior to the procedure.

What are the possible benefits of taking part?

The information gathered from these investigations may provide a direct benefit to you; the identification of significant reflux may warrant a specialist referral which may lead to treatment of the reflux. The information gathered will certainly provide benefit to patients in the future that are diagnosed with IPF, by providing us with an understanding of the association of reflux with IPF. Your participation in the study will have NO influence on your existing treatment.

What if something goes wrong?

In the unlikely event of a complication of this study occurring, you will be treated appropriately by the clinicians at the Hospital as an NHS patient. You have the right to claim against NHS Crown Indemnity for any injury that may arise and the normal NHS complaints mechanisms are open to you.

Will my taking part in this study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential. No identifiable data will be used in the study, and all data will be stored on a secure, encrypted database which is password locked. Any samples or information that leaves the hospital will have any information that

identifies you removed. All samples will remain under the direct control of Professor Jeffrey Pearson and Dr Chris Ward at the University of Newcastle, in a secure facility. Outside the research team, your GP will be aware of your involvement in this study.

What will happen to the results of the study?

The results will be discussed at medical meetings and published in scientific journals as they emerge. You can request copies of any published results involving your information from your IPF consultant. You will of course not be identified personally in any form of publication. At the end of this study, we may require the samples (unidentifiable) to be used for further related research.

Who is organising and funding the research?

The research project has been developed under the leadership of Professor Griffin at the Royal Victoria Infirmary. Funding has been provided through the Northern Oesophago-gastric Unit. There is no commercial involvement and no financial incentive to recruit any patient / volunteer exists.

Who has reviewed the study?

The Local Research Ethics Committee has reviewed the study.

Contact for further information

Study Co-Ordinator:	**Professor Griffin, Royal Victoria Infirmary** **Tel 0191 233 6161 ext 20240**
Principal Investigator:	Mr. Amaran Krishnan Oesophageal Laboratory Royal Victoria Infirmary Tel 0191 233 6161 ext 20240
IPF Consultant Lead:	Dr. Ian Forrest Department of Respiratory Medicine Royal Victoria Infirmary Tel 0191 233 6161 ext 20149
Independent Contact:	Amanda Tortice, Research and Development Office Royal Victoria Infirmary. Tel 0191 282 5959

YOU SHOULD RECEIVE A COPY OF THIS INFORMATION SHEET AND A SIGNED CONSENT FORM TO KEEP FOR YOUR REFERNCE

The Newcastle upon Tyne Hospitals NHS
NHS Foundation Trust

Patient Consent Form

STUDY TITLE: The use of impedance pH measurements to determine the effect of gastro-oesophageal reflux in patients with idiopathic pulmonary fibrosis

Lead Investigator: Amaran Krishnan
Supervisors: Prof. S.M. Griffin, Prof. J. Pearson, Mr. Jon Shenfine, Dr. Chris Ward, Dr. Ian Forrest

1. I confirm that I have read and understand the information sheet
   I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that relevant sections of any of my medical notes and data collected during the study may be looked at by responsible individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
4. I understand that all data will be handled with the strictest of confidentiality. ☐
5. I understand that samples taken from me will be stored and maybe used for future related studies. ☐
6. I agree to my GP being informed of my participation in the study. ☐
7. I agree to take part in the above study ☐

Name of Patient	Signature	Date
Name of Person taking consent	Signature	Date

When completed, 1 for patient; 1 for researcher site file; 1(original) to be kept in medical notes.

Study Information for patients with Cystic Fibrosis

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for reading this.

What is the purpose of the study?

It is thought that patients with cystic fibrosis (CF) experience reflux. This is when the stomach contents travel up the gullet and enter the airways causing lung damage. This reflux may or may not cause symptoms; however the long term consequences of stomach contents within the lung is the deterioration of lung function, which can have a considerable effect on quality of life. We aim to use state of the art devices called impedance pH catheters to measure the reflux to a very accurate degree. In addition, we would like to use sputum coughed up from the lung to test for stomach chemicals. Using both these tests we aim to understand the nature and effect of reflux, which might help us, determine the best treatment.

Why have I been chosen?

You have volunteered for the study which will involve 20 volunteers with cystic fibrosis who have been assessed to be fit for all the procedures being performed in the study.

Do I have to take part?

Taking part is entirely voluntary. It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and will be asked to sign a consent form. If you do decide to take part you are still free to withdraw at any time and without giving a reason.

What will happen to me if I take part?

If you decide to take part in the study, you will be required to take part in three investigations:

1. **Lung Function Tests –** These tests need to be performed at the start of the study so that we are aware of your lung functions. This will be performed at the time of recruitment and is simply the routine spirometry that you normally have at the start of the clinic.
2. **Oesophageal manometry and impedance pH –** These investigations will be done over the course of 24 hours. Both tests involve passing a narrow plastic tube through the nose which will sit in the gullet. Prior to the tests you will be required to complete a questionnaire so we can assess the nature of any reflux.

   The oesophageal manometry tube allows the passage of four channels of water into the gullet and over 20 minutes this will measure the pressure waves so that we can assess your swallowing. This tube will then be removed and the information used to place the impedance pH tube.

   The impedance pH tube is a much finer tube, and will also be passed into the gullet via your nostril. It is made up of small monitors along a fine tube that can detect the changes in electrical resistance present in liquids and gases. Thus it can detect the presence of gas and liquid in your gullet and whether you are swallowing this gas/liquid or whether it is travelling in the wrong direction. In summary this tube will allow us to accurately measure the reflux from your stomach. The impedance catheter will be placed in your gullet for 24 hours, after which it will be quickly removed and the information transferred to a computer.
3. **Collection of Sputum –** At the time you consent for the study, a sample pot will be provided. On the morning of your oesophageal study, a sample of sputum needs to be collected in the pot. If you have a physiotherapy regime for your chest, the sputum can be collected during these exercises.

What do I have to do?

An appointment will be made after you have consented to the study. You will be required to attend the oesophageal physiology laboratory. Before attending you will be required to be off your stomach medications for 2 weeks and 4 hours prior to the appointment have nothing to eat or drink. You will have the impedance tube secured in place for 24 hours and this will be recording the activity of the stomach through the box you shall carry on a belt. You may eat and drink normally with this tube in place. The following day the tube will be removed and the results transferred to a computer.

What is being tested?

- **Oesophageal Manometry and Impedance**

  The oesophageal manometry allows the assessment of how well the gullet is working, by observing over 20 minutes the changes in pressure as you swallow. It also provides the information required to place the impedance tube. The impedance device will measure the amount of stomach fluid travelling up the gullet toward the airways, providing us with an assessment of reflux.

- **Sputum sample**

  The sputum will be tested in laboratory for chemicals found normally in the stomach, called bile and pepsin. This will give an indication of the extent of reflux and aspiration that you have.

What are the side effects of the treatment?

The possible side effects of the manometry and impedance catheters are discomfort to the nose, throat or gullet. These are normally related to the manometry test which only lasts 20 minutes. For 2 weeks prior to commencing this test it is important that you withhold any antacid treatment (proton pump inhibitor –PPI). This will provide a more accurate assessment of any reflux.

What are the disadvantages and risks of taking part?

Involvement requires time to be spent at the hospital, presumably missing work or holiday. The procedures have some minor local side effects as outlined above.

What are the possible benefits of taking part?

The information gathered from these investigations may provide a direct benefit to you; the identification of significant reflux may warrant a specialist referral which may lead to treatment of the reflux. The information gathered may well provide benefit to patients in the future that are diagnosed with CF, by providing us with an understanding of the association of reflux with cystic fibrosis. Your participation in the study will have NO influence on your existing treatment.

What if something goes wrong?

In the unlikely event of a complication of this study occurring, you will be treated appropriately by the clinicians at the Hospital as an NHS patient. You have the right to claim against NHS Crown Indemnity for any injury that may arise and the normal NHS complaints mechanisms are open to you.

Will my taking part in this study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential. No identifiable data will be used in the study, and all data will be stored on a secure, encrypted database which is password locked. Any samples or information that leaves the hospital will have any information that identifies you removed. All samples will remain under the direct control of Professor Jeffrey Pearson and Dr Chris Ward at the University of Newcastle, in a secure facility. Outside the research team, your GP will be aware of your involvement in this study.

What will happen to the results of the study?

The results will be discussed at medical meetings and published in scientific journals as they emerge. You can request copies of any published results involving your information from your CF consultant. You will of course not be identified personally in any form of publication. At the end of this study, we may require the samples (unidentifiable) to be used for further related research.

Who is organising and funding the research?

The research project has been developed under the leadership of Professor Griffin at the Royal Victoria Infirmary. Funding has been provided through the Northern Oesophago-gastric Unit. There is no commercial involvement and no financial incentive to recruit any patient / volunteer exists.

Who has reviewed the study?

The Local Research Ethics Committee has reviewed the study.

Contact for further information

Study Co-Ordinator:	**Professor Griffin, Royal Victoria Infirmary** **Tel 0191 233 6161 ext 20240**
Principal Investigator:	Mr. Amaran Krishnan Oesophageal Laboratory Royal Victoria Infirmary Tel 0191 233 6161 ext 20240
CF Consultant Leads:	Dr A. Gascoigne and Dr S. Bourke Department of Respiratory Medicine Royal Victoria Infirmary Tel 0191 233 6161 ext 24776/20141
Independent Contact:	Amanda Tortice, Research and Development Office Royal Victoria Infirmary. Tel 0191 282 5959

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The Newcastle upon Tyne Hospitals NHS
NHS Foundation Trust

Patient Consent Form

STUDY TITLE: The use of impedance pH measurements to determine the effect of gastro-oesophageal reflux in patients with cystic fibrosis

Lead Investigator: Amaran Krishnan
Supervisors: Prof. S.M. Griffin, Prof. J. Pearson, Mr. Jon Shenfine, Dr. Chris Ward, Dr. Alistair Gascoigne, Dr. Stephen Bourke

1. I confirm that I have read and understand the information sheet
   I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that relevant sections of any of my medical notes and data collected during the study may be looked at by responsible individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
4. I understand that all data will be handled with the strictest of confidentiality. ☐
5. I understand that samples taken from me will be stored and maybe used for future related studies. ☐
6. I agree to my GP being informed of my participation in the study. ☐
7. I agree to take part in the above study ☐

Name of Patient	Signature	Date
Name of Person taking consent	Signature	Date

When completed, 1 for patient; 1 for researcher site file; 1(original) to be kept in medical notes.

Appendix 5

Successful Grant Application

- Trustees grant application letter of approval
- Trustees grant application

Newcastle University

Newcastle University
Fourth Floor, Cookson Building
Newcastle upon Tyne NE2 4HH

Chairman: Professor PF Chinnery
Administrator: Miriam Lowes

Tel: 0191 222 5101
Fax: 0191 222 5685

26 April 2010

Professor SM Griffin
NIHR
Paul O'Gorman Building
Newcastle University

Dear professor Griffin

Re: The use of impedance pH measurements to determine the effect of gastro-oesophageal reflux in patients with cystic fibrosis and idiopathic pulmonary fibrosis

Thank you for submitting your application to the JRESC. I am please to say that your grant application was favourably received and we would like to recommend funding your proposal. The reviewers did raise some important questions about where you plan to correlate your gastro intestinal measurements with the respiratory function measurements and it was strongly suggested that you work closely with a respiratory physician in developing the protocol along these lines.

Comments from the reviewers are shown below:

'This application addresses an important question and seeks to improve our understanding of the relationship between reflux and functional decline in patients with respiratory disease.
Two separate cohorts have been chosen which makes this an ambitious project. Would it not be better to concentrate on one group ?
More detail is needed on the phenotype of patient to be studied particularly in respect of disease stage and activity. I appreciate that patients will be recruited from NHS clinics but the CF group in particular offers opportunities to take patients of defined severity and genotype if necessary. I appreciate more difficult in the IPF group but there are wide variations in clinical phenotype under the umbrella term IPP and in a study like this some attempt to narrow is essential in my view.
I am still a little uncertain as to 1) whether the proposed study is a first shot at getting some data on impedance measurements and biomarkers of reflux in patients with CF and IPF , and trying to link with inflammatory cell profiles (not well described) . 2) An attempt to look at the inflammatory consequences of reflux and the effect on lung function (not well described) in two separate cohorts of patients'.

We will recommend that your application is funded, but please note that this is not an award letter. Our recommendation is subject to the approval of the charitable body, and in the current economic climate, these recommendations are not always acted upon. You will hopefully hear a final decision within six weeks.

I would be most grateful if you acknowledge support for the Newcastle Health Care Charity and the Newcastle upon Tyne Hospitals NHS Charity in all publications arising from this work.

Yours sincerely

Professor PF Chinnery
Chairman
Joint Research Committee

☎ (0191) 222 5101
✉ p.f.chinnery@ncl.ac.uk

JOINT RESEARCH EXECUTIVE SCIENTIFIC COMMITTEE

FORM OF APPLICATION FOR A RESEARCH GRANT TO THE NEWCASTLE HEALTHCARE CHARITY (RVI/NGH) AND NEWCASTLE UPON TYNE HOSPITALS NHS CHARITY (FH)

(v.1/1/09)

Q1	Name of Lead Applicant	Professor S Michael Griffin
	Appointment Held	Professor of Gastrointestinal Surgery
	Department	Northern Oesophago-Gastric Unit/ / Northern Institute for Cancer Research, Paul O'Gorman Building, Newcastle University
	Hospital/University	Royal Victoria Infirmary/ Newcastle University
	Address for Correspondence	Northern Oesophago-Gastric Unit Royal Victoria Infirmary
	Telephone Number	0191 282 0234

Q2	Name of Associated Research Worker(s) *(Enclose CV of any individuals for whom salary funding is requested)*	**(Co-investigators)**Mr. A. Krishnan, Dr. I. Forrest, Professor J. Pearson, Dr. C. Ward

Q3	Place of Research *(if different to Address for Correspondence))*	Northern Oesophago-Gastric Unit Royal Victoria Infirmary, Department of Respiratory Medicine Royal Victoria Infirmary, Institute for Cellular & Molecular Biosciences, University of Newcastle Upon Tyne

Q4	Title of Project (not more than 250 characters)
	The use of impedance pH measurements to determine the effect of gastro-oesophageal reflux in patients with cystic fibrosis and idiopathic pulmonary fibrosis

Q5	Period of Support (months)	12 Months
Q6	Proposed Start Date	April 2010
Q7	Total Support Requested	£24,283

Q8	Please list support given to ANY of the named applicants from the Trustees within the last 5-years. Please indicate the outcomes of this support (further grant applications, publications etc)

Professor Griffin:

The role of oesophageal impedance measurement and markers of aspiration in the detection of extra-oesophageal reflux disease and in the development of allograft dysfunction in human lung transplant recipients

Book Chapters

JP Pearson, S Parikh, AGN Robertson, R Stovold, IA Brownlee. Chapter 4 Pepsins. In: Effects, Diagnosis and Management of Extra-Esophageal Reflux Editors: Nikki Johnston and Robert J. Toohill ©2010 Nova Science Publishers, Inc. ISBN:978-1-61668-177-7

AGN Robertson, SM Griffin. Prophylactic antireflux surgery in lung transplantation. In "Difficult Decisions in Thoracic Surgery: An Evidence Based Approach." (Second Edition) Editor M Ferguson. Springer-Verlag 2010 ISBN.

Brief Communications/Research Letters

AGN Robertson, C Ward, JP Pearson, T Small, J Lordan, AJ Fisher, AJ Bredenoord, J Dark, SM Griffin, PA Corris. Longitudinal Changes in Gastro-Oesophageal Reflux from three months to six months post lung transplantation. Thorax 2009; Vol 64 (11): 1005-1007.

Review Articles

AGN Robertson, J Shenfine, C Ward, JP Pearson, JH Dark, PA Corris, SM Griffin. A call for standardisation of antireflux surgery in the lung transplantation population (Editorial). Transplantation 2009; Vol 87 (8): 1112-4.

AGN Robertson, SM Griffin, DM Murphy, JP Pearson, IA Forrest, JH Dark, PA Corris, C Ward. Targeting allograft injury and inflammation in the management of post-lung transplant Bronchiolitis Obliterans Syndrome (Invited review). American Journal of Transplantation 2009; 9(6): 1272-8.

AGN Robertson, C Ward, JP Pearson, PA Corris, JH Dark, SM Griffin. Lung Transplantation, Gastroesophageal Reflux, and Fundoplication (Review Article). Annals of Thoracic Surgery 2010; Vol 89 (2): 653-660.

International Meetings

"Aspiration in the immediate post lung transplantation period."
Poster Presentation. International Society for Heart & Lung Transplantation Annual Scientific Meeting 22-25th April 2009, Palais de Congres, Paris.*

"Aspiration secondary to gastro-oesophageal reflux but not duodenal reflux occurs in the immediate post lung transplantation period."
Poster Presentation. 14th Congress of the European Society for Organ Transplantation 30th August- 2nd September 2009, Palais de Congres, Paris.*

Poster Presentation. GASTRO 2009, Joint Meeting of the United European Gastroenterology Federation/World Gastroenterology Organisation /World Organisation of Digestive Endoscopy (OMED)/British Society of Gastroenterology, London

Q8 Please list support given to ANY of the named applicants from the Trustees within the last 5-years. Please indicate the outcomes of this support (further grant applications, publications etc)

GRANTS

2009: £5,600: British Lung Foundation: Trevor Clay Memorial Grant for:
The role of oesophageal impedance measurement and markers of aspiration in the detection of extra-oesophageal reflux disease in human lung transplant recipients.

2008: £35,000: Fellowship from the European Society for Organ Transplantation-Clinical Research Grant for:
The role of oesophageal impedance measurement and markers of aspiration in the detection of extra-oesophageal reflux disease and in the development of allograft dysfunction in human lung transplant recipients.

Q9 What is your Research question? (not more than 200 words)

We aim to identify the extent of gastro-oesophageal reflux in patients with idiopathic pulmonary fibrosis (IPF) and cystic fibrosis (CF) and determine whether there is a clear relationship between reflux, lung (micro) aspiration and deterioration of lung function. We shall use a combination of common medical investigations together with laboratory analysis of samples provided by these patients.

Q10 Summary of Proposed Research including key goals (not more than 200 words)

This research project will investigate the incidence and severity of gastro-oesophageal reflux disease in patients with idiopathic pulmonary fibrosis (IPF) and cystic fibrosis (CF) and understand its contribution to progressive lung damage in these patients. Using impedance pH catheters we will be able to measure reflux to a very accurate degree. These investigations will be compared to patients' lung function tests to determine the relationship between the two. Both groups of patients will have samples analysed in the lab for bile salts and pepsin; two chemicals, originating from the stomach and found in refluxed material. A questionnaire assessment of patients' symptoms will also be used.

KEY GOALS

1. To evaluate the relationship between impedance measurements, biomarkers of aspiration, and lung function in these patient groups
2. To evaluate the relationship between impedance measurements & symptoms of reflux
3. To set up a longitudinal study of reflux in IPF and CF patients
4. To use pilot data produced by this study to make an application to a substantative grant body

Q11 Why is it important for the health of patients in Newcastle upon Tyne? (not more than 200 words)
A recent review article published by Sweet in March 2009 [1], and evidence gathered from a study by Blondeau in March 2008 [7] identify that further information is required to determine the role of gastro-oesophageal reflux in patients with advanced lung disease and cystic fibrosis. In Newcastle we have specialist clinics for both IPF and CF and many patients have symptoms of reflux disease; but the true nature of this disease is undetermined. Gastro-oesophageal reflux is a disease that, once identified in these patients can potentially be treated; this therefore identifies the importance of this research and its potential contribution to improving the lung function in these patients. In addition, a large number of IPF patients are anecdotally placed on PPI treatment, with no evidence of the type of reflux, its extent and potential harm that may be caused. By formally testing these patients we can tailor treatment to individuals needs which may improve their lung function and quality of life. As life expectancy in people with CF increases it becomes much more important to develop our understanding so that we can improve the management of reflux in CF patients.

Q12 Details of Research Project (not more than three A4 pages)
(a) Aims (b) Work which has led up to the project (including pilot data) (c) Experimental design and methods (d) Timetable and milestones

AIMS OF STUDY

Purpose & Theory

We propose that both symptomatic and asymptomatic reflux is a common feature in patients with advanced lung disease. We hypothesise that, in patients with idiopathic pulmonary fibrosis (IPF) and Cystic fibrosis (CF), this reflux together with the subsequent (micro) aspiration of stomach /duodenal contents into the lungs can lead to long term deterioration of lung function. Detection of reflux using established techniques combined with laboratory measurements of biomarkers in refluxate will identify both the extent and severity of gastro-oesophageal reflux (GOR) in these patients. The translational significance of this is that there are both surgical and non surgical treatments available for reflux. The subsequent treatment of GOR identified patients could preserve long-term lung function and improve their quality of life.

Aims

- To measure impedance pH in patients with IPF and CF to objectively assess reflux disease
- To measure patient symptoms of reflux disease, using validated questionnaires
- To compare objective assessment of reflux disease (impedance pH) with patient experience of symptoms (questionnaire)
- To compare objective and clinical assessments of reflux and symptoms with markers of aspiration(pepsin, bile salts); using BAL samples (IPF group) and sputum samples (CF group)
- To correlate the above investigations of reflux with lung function
- To identify patients suitable for specialist referral and subsequent management of reflux disease; and assess the effect of the intervention with regular lung function assessment

WORK WHICH HAS LED TO THE PROJECT

Since the early 1960s several studies have demonstrated an association between interstitial lung disease (ILD) and gastro-oesophageal reflux (GOR) [1]. More recently it has been demonstrated through 24-h pH monitoring that GOR is highly prevalent compared to normal subjects but often clinically occult in

patients with ILD; and despite the use of standard dose proton pump inhibitors, reflux is not adequately suppressed [2]. Until recently the assessment and treatment of GOR focused on using conventional pH monitoring. Conventional pH measurement is limited to detecting only acid refluxing from the stomach. The addition of oesophageal impedance measurements allows the detection of non-acid and weakly acid reflux events (refluxate pH >4) [4]. A recent study [3] demonstrated using oesophageal impedance on subjects with systemic sclerosis associated ILD, that increased non-acid reflux episodes could be involved in the progression of pulmonary disease.

A relationship between IPF and GOR was first demonstrated by Mays et al [5] when they noted that hiatus hernia is more common in IPF patients. Tobin et al [6] demonstrated in 17 patients with biopsy-confirmed IPF, that 94% had reflux confirmed with 24-hour manometry, 75% of these patients showed no reflux associated symptoms. Recently this has been confirmed in a larger cohort of 65 patients by Raghu et al [2]. Their study demonstrated GOR was characterised on 24-hour pH monitoring in 87% of their subjects. Interestingly Raghu et al showed abnormal oesophageal acid exposure in 63% of their patients who remained on a proton pump inhibitor during the pH studies. The most recent guidelines *(BTS, 2008)* from the British Thoracic Society regarding ILD, recognises the complication of GOR in IPF, and encouraged further studies to determine the exact nature of the reflux and aspiration. GOR is thought to be highly prevalent in CF but has not been systematically studied with up to date methods such as impedance pH monitoring. About 1 in 5 newly diagnosed CF infants have pathological reflux, with a similar prevalence in adults [7]. Fathi et al [8] demonstrated that laparoscopic fundoplication was highly effective in controlling reflux in a small selection of CF patients, where medical treatment had failed.

Patients with advanced lung disease are particularly vulnerable to aspiration events. A close relationship between reflux mediated microaspiration and chronic lung injury has been demonstrated in transplant patients. The concentration of bile salts and pepsin found in bronchoalveolar lavage samples had predisposed to Bronchiolitis obliterans syndrome (BOS) in these patients. Bile salts are thought to disrupt phospholipids and surfactant when present in the allograft in addition to disruption of the local and regional innate immunity, therefore predisposing the individual to infections. The presence of bile salts in the bronchoalveolar district decreases the time to the development of bronchiolitis obliterans significantly, in addition pepsin can cause damage to the lungs as it retains activity up to pH 6.5 [9].

Gastric aspiration may account for deterioration in lung function in adult patients with cystic fibrosis (CF) [7]. Few studies have been completed to elucidate the exact cause of reflux in CF patients, but an increased abdominal-thoracic pressure gradient during physiotherapy and periods of coughing may be a major contributing factor [8]. Although BAL samples can provide useful information about the gastric aspirate by analysing for specific markers such as pepsin; salivary samples have been shown to be a useful non-invasive surrogate for analysis in patients with advanced lung disease [10].

Over the last 6 years, the Institutes of Cell and Molecular Biology and Cellular Medicine at the University of Newcastle have been actively involved in research focusing on gastro-oesophageal reflux and its affect on lung tissue [11]. Over the last 3 years the Institutes have been working closely with the regional transplant centre and the Northern Oesophago-gastric Unit in order determine the incidence of reflux after lung transplantation.

Patients that had undergone a recent lung transplant were referred for oesophageal studies, using impedance pH monitoring; all had BAL as part of their routine lung transplant follow-up and analysis of bile and pepsin was performed. Patients demonstrating significant symptomatic reflux, or asymptomatic reflux correlating to a reduction in lung function were offered anti reflux surgery. Nine patients have been successfully treated this way with promising post-operative outcomes.

DESIGN AND METHODOLOGY

CF Patients will be recruited directly from designated specialist clinics. There are currently two specialists at the Royal Victoria infirmary, and patients will be approached directly by the primary investigator and provided with a patient information leaflet. Patients with IPF will be recruited with the aid of a national interstitial lung disease specialist, already closely involved in the study. Currently at the

Royal Victoria infirmary, ILD clinics are organised twice a month, recruitment of IPF patients will be by the primary investigator, directly from these clinics. Both groups of patients will have regular lung function assessment, and if they choose to participate in the study they will be requested to attend a routine lung function assessment at the start of the study to assess their lung function. In addition all patients will be provided with validated questionnaires to assess their reflux symptoms.

Over an 18 month period the study will be looking at reflux in patients using a combination of oesophageal impedance pH monitoring and manometry. Multichannel Intraluminal Impedance is a technology that measures changes in oesophageal intraluminal resistance and bolus transit. It consists of a catheter with several metal rings. Changes in resistance between these rings are detected. Gas causes an increase in resistance, liquids cause a decrease. The direction of these changes allows the direction of movement of the bolus to be determined (see fig. 1). This device also has a pH probe which allows reflux events to be classified as acidic, weakly acidic or non-acid. The MMS Omega system will be used, which is a state of the art facility for detecting oesophageal reflux with a high degree of sensitivity.

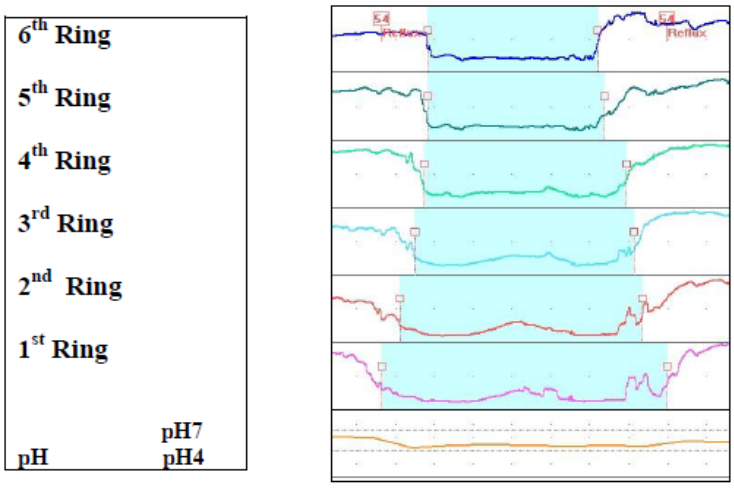

Figure. 1 A weakly acidic liquid reflux event showing a progressive decrease in resistance from ring 1 (lowest) to 6 (highest). The pH does not fall below 4. Time is on the x axis.

Impedance devices have been in use for over 10 years and the devices used in the study have been used in the UK for several years in both clinical and research settings. Impedance devices are used routinely throughout the UK and worldwide. We also use this device clinically at the Northern Oesophagogastric Unit in the Royal Victoria Infirmary for ongoing research studying reflux in lung transplant patients.

The degree of reflux detected (how often, how severe, and whether it is acid or not) will be compared with molecular measures of reflux. The detection of pepsin (a protein made in the stomach) and bile salts (from the liver via the small intestine) in the lung fluid and the presence of cells of inflammation in the lung fluid sample will be used to assess the relevance of the detected reflux episodes. These samples will be collected differently for the CF and IPF groups as follows:

CF patients will be encouraged to express sputum through their routine morning physiotherapy. A small aliquot will be used for analysis

IPF patients who consent to the study will have a flexible bronchoscopy and standardised 3 x 60 ml BALs will be performed. The samples will be sent for routine investigations to assist in the further management of the patient's lung disease, and a small aliquot will be retained for our lab based investigations characterising markers of aspiration and inflammatory cell count differentials.

The detection of pepsin and bile salts in bronchoalveolar lavage fluid and the inflammatory cell profile of the bronchoalveolar lavage fluid will be used to assess associations between GOR, markers of aspiration and lung inflammation. A locally devised ELISA, based on a mono-specific antibody to

pepsin will be used. The lower limit detection of pepsin is <1ng/ml. Bile acids will be measured using a commercial kit based on an enzymatic reaction using 3α-hydroxysteroid dehydrogenase and quantified using photospectrometry.

The oesophageal physiology studies, the lung function assessments and the laboratory studies will be compared to patients' own assessment of their reflux disease using three established questionnaires; The De Meester Reflux Related symptoms questionnaire, The Reflux Symptoms Index (RSI) and The Gastrointestinal Quality of Life Index (GIQOLI).

Outcomes

The study will provide us with a subjective score of symptoms, objective evidence of GOR physiology and laboratory based assessments of markers of aspiration in patients with IPF and CF. The information gathered from the studies above will be used to develop our understanding of the association between these lung diseases and gastro-oesophageal reflux.

Potential development from the study: Those patients with significant reflux that could warrant treatment may be offered referral to an upper GI specialist for the most appropriate management.

Time Table

March 2010	March - June 2010	June -Sept 2010	Sept-Dec 2010	December 2010 to March 2011
Recruit 20 patients both IPF and CF	Begin Oesophageal studies on first group	Complete 10 BAL IPF pt studies Complete 1st group oesophageal studies	Begin lab work on BAL and sputum samples	Lab work and follow-up those pts who need referral
	Recruit further 20 patients both IPF and CF	Begin Oesophageal studies on second group	Complete 10 BAL IPF pt studies Complete 2nd group oesophageal studies	Lab work and follow-up those pts who need referral
	Lab training	Lab training	Sample analysis	Sample analysis
			First Year Reports To deanery and Newcastle University	Submit work for presentation /publication June 2011
National grant applications	Finances allocated Use grant money to Initiate research	Further national and international grant Applications based on initial work	First year report to Grant committees	

Q13 Explain how this work will pump prime future grant proposals to continue this line of investigation (not more than 200 words)
If awarded the grant from the Trustees would be used to collect pilot data and show proof of principle. This would then be used to pump prime future grant proposals. We hope that the initial support from the Trustees will allow a future application for a MRC research fellowship. As noted from the trustees support last year, the related research project was successful in achieving further grant support and numerous publications. The research fellow's salary is fully funded and he is registered for a MD with the University. Being awarded this grant would therefore help him develop a career in academic surgery. This is an exciting new multidisciplinary proposal, with potential early benefits for patients. This project adopts the use of biomarkers and translational research. Therefore we hope the pilot data would enable us to be successful in our application for national and international grants.

Q14 Summary in simple language for the non-expert (including the research question, why it is important for the health of patients in Newcastle upon Tyne, an overview of the experimental approach, key goals and why this is likely to lead to external funding if appropriate, 200 words)

Patients with CF and IPF are known to suffer from reflux. This is when stomach contents travel up the gullet and then enter the airways causing significant lung damage. This reflux may or may not cause symptoms. However, the long term consequences of stomach content within lung tissue can result in severe deterioration of lung function, affecting patients' quality of life. There is very little understanding about how bad this reflux can be. We aim to use state of the art devices called impedance pH catheters to measure reflux to a very accurate degree. In addition we will use samples collected at bronchoscopy and from sputum coughed up after patients' physiotherapy to perform laboratory analysis for infection and evidence of stomach chemicals (pepsin and bile salts), to demonstrate the presence of reflux. The measurements made will be compared to patients' lung function and symptoms. We aim to see whether reflux exists in these patients and offer early treatment to suitable patients. We think that reflux can and should be reduced to improve quality of life and preserve lung function.

Q15 References (full citation)

1. Sweet M, Patti M, Hoopes C, Hayes S, Golden J. Gastro-oesophageal reflux and aspiration with advanced lung disease. Thorax 2009; Vol 64: 167-173.

2. Raghu G, Freudenberger T, Yang S, Curtis J, Spada C, Hayes J, Sillery J, Pope II C, Pellegrini C. High Prevalence of abnormal acid gastro-oesophageal reflux in idiopatjic pulmonary fibrosis. European Respiratory Journal 2006; Vol 27: 136 -142.

3. Savarino E, Bazzica M, Zentillin P, Pohl D, Parodi A, Cittadini G, Negrini S, Indiveri F, Tutuian R, Savarino V, Ghio M. Gastroesophageal Reflux and Pulmonary Fibrosis in Scleroderma: A Study using pH-Iimpedence Monitoring. Am. J Respir Crit Care Med 2009; Vol 179: 408-413.

4. Wise J, Murray J, Utilising multichannel intraluminal impedence for diagnosing GERD: a review. Diseases of the Esophagus 2007; Voil 20:83-88

5. Mays EE, Dubois JJ, Hamilton GB. Pulmonary fibrosis associated with tracheobronchial aspiration. A study of the frequency of hiatal hernia and gastroesophageal reflux in interstitial pulmonary fibrosis of obscure etiology. Chest 1976 Apr;69(4):512-5

6. Tobin R, Pope II C, Pellegrini C, Emond M, Sillery J, Raghu G. Increased Prevalence of Gastroesophageal Reflux in Patients with Idiopathic Pulmonary Fibrosis. Am. J Respir Crit Care Med 1998; Vol 158: 1804-1808

7. Blondeau K, Dupont L, Mertens V, Verleden G, Malfroot A, Vandenplas Y, Hauser B, Sifrim D. Gastro-oesophageal reflux and aspiration of gastric contents in adult patients with cystic fibrosis. Gut 2008; Vol 57: 1049 -1055

8. Fathi H, Moon T, Donaldson J, Jackson W, Sedman P, Morice AH. Cough in adult cystic fibrosis: diagnosis and response to fundoplication. Cough. 2009 Jan 18;5:1.

9. Robertson AG, Shenfine J, Ward C, Pearson JP, Dark JH, Corris PA, Griffin SM. A call for standardization of antireflux surgery in the lung transplantation population. Transplantation. 2009 Apr 27;87(8):1112-4.

10. Potluri S, Friedenberg F, Parkman HP, Chang A, MacNeal R, Manus C, Bromer MQ, Malik A, Fisher RS, Nugent T, Thangada VK, Kueppers F, Miller LS. Comparison of a salivary/sputum pepsin assay with 24-hour esophageal pH monitoring for detection of gastric reflux into the proximal esophagus, oropharynx, and lung. Dig Dis Sci. 2003 Sep;48(9):1813-7

11. Ward C, Forrest IA, Brownlee IA, Johnson GE, Murphy DM, Pearson JP, Dark JH, Corris PA. Pepsin like activity in bronchoalveolar lavage fluid is suggestive of gastric aspiration in lung allografts. Thorax. 2005 Oct; 60(10):872-4.

Q16 Financial Support Requested			
*** PLEASE CONTACT THE JOINT RESEARCH OFFICE (http://www.ncl.ac.uk/business-directorate/joint/) IF YOU REQUIRE ASSISTANCE WITH COSTINGS ***			
Salaries (inclusive of Superannuation and National Insurance)	First Year £	Second Year £	Total Over Period £
(a) Medical or Dental Staff Grade and Pay Scale % WTE			
(b) Scientific Assistance Grade and Pay Scale % WTE			
(c) Technical and Other Grade and Pay Scale % WTE			
(d) Clerical/Secretarial Assistance Grade and Pay Scale % WTE			
(e) Advertising			
(f) Materials and Consumables (please list) Impedances Catheters (40 @ £117.33) Bile Acid Assay Kits (4 @ £300.00) ELISA plates Pepsin Isolation & purification reagents Impedance devices (2@ £7000) BAL and Sputum Processing	£20,883 £4693.20 £1200.00 £140 £850 £14000 £3400		
(g) Bench fee (please explain in detail)	£0		
(h) Apparatus/Instruments			
TOTAL SUPPORT REQUESTED	£24, 283		

Q17 Other Financial Support	
Is this or a related application currently under consideration elsewhere?	YES
An application has been made to the Royal College of Surgeons of Edinburgh for a research grant and fellowship. A Decision is expected by May 2010	

Q18 Have you applied for matched funds?

No

Q19 Ethical Approval *(delete as appropriate)*

The Local Ethical Committee has given its approval to the project.

Q20 Has a University "Blue Form" been signed and approved?

YES

Q21 Do you have approval from the relevant NHS Trust?

R&D meeting outcome - Recommend to approve

Q22 Signatures

a) For projects to be carried out partly/entirely in NHS facilities.
This application includes appropriate costs (excess treatment and service support costs) likely to be incurred by the Trust during the study.
Signature (Clinical Director) .....

b) For projects carried out party/entirely in University facilities.
This application includes appropriate costs likely to be incurred by the University during the study.
Signature (Head of Institute) .....

Q23 If successful, who will be responsible for administering the grant?

Signed .....Grants & Contracts, University

Q24 Liaison with Administration

Has the project been submitted to the Trust's R&D Department for approval? YES

Has the project been approved by University administration (blue form) YES

SIGNED AND DATED BY LEAD APPLICANT AND ASSOCIATED WORKERS

Name	signature	date
1. **Prof. S.M. Griffin**		
2. **Mr. Amaran Krishnan**		
3. **Dr. Ian Forrest**		
4. **Dr. Chris Ward**		
5. **Prof. Jeffrey Pearson**		

NAME:	Selwyn Michael GRIFFIN	
ADDRESS:	Northern Oesophago-Gastric Cancer Unit Royal Victoria Infirmary, Newcastle Upon Tyne	
DATE OF BIRTH:	7th February 1955	
UNIVERSITY EDUCATION:	1973 - 1978	University of Newcastle upon Tyne Medical School
ACADEMIC QUALIFICATIONS:	1978	MB BS
	1983	FRCS
	1988	Certificate of Accreditation for Higher Surgical Training
	1989	MD University of Newcastle Upon Tyne
AWARDS:	1968	Open Scholarship to Fettes College
	1984	Wellcome Surgical Training Fellowship
	1988-89	Chinese University of Hong Kong Fellowship
	1995	Fellow of the College of Surgeons of Hong Kong (Honorary)
	1997 (Honorary)	Fellow of the Royal College of Surgeons of Edinburgh
	2000	**Professor of Gastro-intestinal Surgery**

Present Appointment: Consultant Oesophago-Gastric Surgeon, Royal Victoria Infirmary; Clinical Lead in Northern Oesophago-Gastric Cancer Unit; Professor of Gastro-intestinal Surgery, University of Newcastle upon Tyne

Selected National and International Appointments

President, Association of Upper GI Surgeons of Great Britain and Ireland, (AUGIS) 2004-2006
Chairman of JCHST Interface Group on the Future of Oesophageal Surgery (1998-2001.
Elected Member for Upper GI Surgery of the **Specialist Advisory Committee for General Surgery (SAC) (1997-2003)**
Full Council member of the Association of Surgeons of Great Britain and Ireland (1995-2001) and (2004-2006)
Founder Member of the International Gastric Cancer Association (1995)
Editorial Board of Gastric Cancer & British Journal of Surgery
Assessor for the Royal College of Surgeons of England to the Appointments Committee for Consultants in General Surgery with an interest in upper GI Surgery (1998 – present)
Elected Member European Surgical Association (2003 – present)
Elected member of James IV Associtation of Surgeons, Inc 2005

32 Research Grants Obtained: Total awarded over £3.5 million

Current Grant Funded Members of my Research Group: MD Students 2; Data manager 2

Books: I have edited 4 books for W B Saunders including

- A Companion to Surgical Practice, Upper Gastrointestinal Surgery, Volume 2.
  Editor Mr S M Griffin, Mr S A Raimes First & Second Edition **[ISBN Hb0 7020 2141 5]. [ISBN 0 7020 2587-9]**

Lead Articles - premier papers

1* SM Griffin, SA Raimes . Proton pump inhibitors may mask the diagnosis of early gastric cancer. British Medical Journal, 1998, 31,1606-1607

2* CS Robertson, SA Raimes, SM Griffin, SCS Chung, AKC Li. Prospective randomised trial comparing R1 Subtotal Gastrectomy with an R3 Total Gastrectomy for antral cancer.
Annals of Surgery, 1994, 220, 176-182,

Summary of all Publications and Presentations

Peer Reviewed Publications including those in press 124
Case Reports and Letters including those in press 30
Published Abstracts including those in press 235
Presentations to Learned Societies 252
Book Chapters 16

Selected Achievements indicating excellence

1997: Key note lecture at the BSG on Early Detection of Gastric Cancer. Guest lecturer and visting Professor to the National Cancer Centre, Tokyo. Visiting Professorships in Europe, Asia and Australia, the University of Southern California, USA. Invited to lecture at many International meetings including the IGCA International Workshop on Therapeutic Endoscopy 1991-1998, the RACS and the College of Surgeons of Hong Kong.
Chaired sessions at the IGCA, ASGBI, British Stomach Cancer Group, AUGIS, the European Surgical Association, the ISDE and the GSM of Royal Australasian College of Surgery.
The research team have won ten national and international awards for Best Paper Presentations from prestigious meetings including BASO, The Association of Surgeons, AUGIS and the European Society for Digestive Diseases.

Selected Teaching at National Level

Organiser of 1st annual Upper GI National Surgical Masterclass, Royal College of Surgeons of Edinburgh 1998-2005.

Selected Esteem Indicators

Invited to write leading articles in the BMJ and BJS. Referee grant applications for the RCS and the RHA, the Scottish Office and smaller organisations. Elected Member of Council of the SAC in General Surgery at RCSEd 1998-2002. Chairman of the Joint BSG & AUGIS National Clinical Guidelines Group for Management of Oesophagogastric Cancer. External examiner MD Theses: Bristol, London and Nottingham; Surgery & Medicine, Chinese University of Hong Kong

I have organised National Meetings in Newcastle Upon Tyne including: . British Stomach Cancer Group, June 1992; The Association of Upper Gastro-intestinal Surgeons of Great Britain and Ireland, September 2000

Name: Amaran Krishnan
Date of birth: 12th September 1979
Address: Flat 227 Baltic Quay, Mill Road, Gateshead NE8 3QZ

Qualifications:
2008 MRCSed Royal college of Surgeons of Edinburgh
2004 MBBS Faculty of Medicine, University of Newcastle upon Tyne
2003 BMedSci (HONS) First Class, University of Newcastle upon Tyne

Awards:
2002 Stage 4 poster prize for clinical audit, Newcastle University
1999 British Young Scientists Award, Royal Society for Science
1997 Gold Crest Award for Scientific Innovation, Crest Association

Appointments:
Current: Speciality Trainee Yorkshire Deanery (ST3). OOPE at Northern Oesophago-Gastric Cancer Unit, Newcastle upon TyneUnder the supervision of Professor S.M. Griffin.
Aug 2007 – Aug 2009
ST2 General Surgery (Upper GI and Vascular), Leeds Teaching Hospitals Trust
ST1 Generic Surgery (Orthopaedics, Urology and General Surgery), North East Lincolnshire NHS Trust
Aug 2006 – Aug 2007
ST1 (SHO) Surgery (General, Orthopaedics and A&E), North Tees and Hartlepool NHS Trust
Feb 2006 – Aug 2006
Senior House Officer (Accident and Emergency Medicine), Sunderland Hospitals Foundation Trust
Aug 2005 – Feb 2006
Anatomy Demonstrator, Faculty of Medicine, University of Newcastle upon Tyne
Aug 2004 – Aug 2005
Pre-Registration House Officer (Surgery and Medicine), Newcastle Teaching Hospitals NHS Trust

Recent Publications:
Krishnan A, Robertson AG, Dunn LJ, Robinson S, Hayes N, Griffin SM, *Treatment of oesophageal anastomotic leaks by temporary stenting with self-expanding plastic stents*, Br J Surg 2009; 96: 887-891
AGN Robertson, **A Krishnan**, C Ward, JP Pearson, PA Corris, JH Dark, J Shenfine[1], DK Karat[1], SM Griffin[1]. *Initial experience of Anti-reflux Surgery in Lung Transplant Recipients in a European Centre*
Krishnan A, Robinson S, Harris AM, Griffin SM. *A prospective Study of Taste and Smell Deficits after Oesophagectomy and Gastrectomy*.. The Surgeon Supplement, Journal of the Royal College Surgeons of Edinburgh and Ireland 2005 Jun; 3(3): S65
Krishnan A, Shanahan D *A Computer Assisted Learning Programme for Anatomy*. http://anatome.ncl.ac.uk/tutorials..
Chambers S, Evans L, **Krishnan A**. *Colorectal Cancer among users of Aspirin and Non-Steroidal Anti-inflammatory Drugs*. Epidemiology. 2001 Jul; 12 (4): 471-2.
Newton LJ, **Krishnan A,** Parapia LA. *Born To Clot*: The European Burden. British Journal of Haematology. 1999 Oct; 107 (1): 213.

International Presentations:
Dec 2006 - Routine use of a rinse after breast fine needle aspiration cytology improves the cancer rate. 13th Congress of the European Society of Surgical Oncology, Venice, Italy.
Sept 2006 - How the preclinical years influence motivation and interest in medical students? Association for Medical Education in Europe, Genoa, Italy.
June 2005 - A Prospective Study of Taste and Smell Deficits after Oesophagectomy and Gastrectomy. Quincentenary Congress Royal College of Surgeons Edinburgh.
Sept 1999 - A Study of Inherited Thrombophilia in Bradford. 11th European Union Contest for Young Scientists, Thessalonica, Greece

Other Achievements:
2004 - Judge for the British Association for the Advancement of Science, The National Young Scientists of the Year Competition, The Royal Society, London.
2004 - Ambassador for the Crest Association of Scientific Representatives

Appendix 6

Reflux Questionnaires

- Gastrointestinal Quality of Life Index (GIQLI)
- DeMeester Questionnaire
- Reflux Symptom Index Questionnaire (RSI)

The Gastrointestinal Quality of Life Index (GIQLI)

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

all of the time	most of the time	some of the time	a little of the time	never

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

all of the time	most of the time	some of the time	a little of the time	never

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

all of the time	most of the time	some of the time	a little of the time	never

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

all of the time	most of the time	some of the time	a little of the time	never

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

all of the time	most of the time	some of the time	a little of the time	never

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

all of the time	most of the time	some of the time	a little of the time	never

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

all of the time	most of the time	some of the time	a little of the time	never

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

never	a little of the time	some of the time	most of the time	all of the time

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

very much	much	somewhat	a little	not at all

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

extremely poorly	poorly	moderately	well	extremely well

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

all of the time	most of the time	some of the time	a little of the time	never

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

all of the time	most of the time	some of the time	a little of the time	never

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

never	a little of the time	some of the time	most of the time	all of the time

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

all of the time	most of the time	some of the time	a little of the time	never

15. How often during the past 12 weeks have you been tired or fatigued?

all of the time	most of the time	some of the time	a little of the time	never

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

all of the time	most of the time	some of the time	a little of the time	never

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

every night	5-6 nights	3-4 nights	1-2 nights	never

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

a great deal	a moderate amount	somewhat	a little bit	not at all

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

a great deal	a moderate amount	some	a little bit	none

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

a great deal	a moderate amount	somewhat	a little bit	not at all

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

extremely unfit	moderately unfit	somewhat unfit	a little unfit	fit

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

never	a little of the time	some of the time	most of the time	all of the time

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

never	a little of the time	some of the time	most of the time	all of the time

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

very much	much	somewhat	a little	not at all

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

very much	much	somewhat	a little	not at all

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

very much	much	somewhat	a little	not at all

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

all of the time	most of the time	some of the time	a little of the time	never

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

all of the time	most of the time	some of the time	a little of the time	never

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

all of the time	most of the time	some of the time	a little of the time	never

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

all of the time	most of the time	some of the time	a little of the time	never

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

all of the time	most of the time	some of the time	a little of the time	never

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

all of the time	most of the time	some of the time	a little of the time	never

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

all of the time	most of the time	some of the time	a little of the time	never

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

all of the time	most of the time	some of the time	a little of the time	never

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

all of the time	most of the time	some of the time	a little of the time	never

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

all of the time	most of the time	some of the time	a little of the time	never

Calculation of the score:

most desirable option: 4 points

least desirable option: 0 points

GIQLI score: sum of the points

DeMeester Reflux Questionnaire

1) In the last 2 weeks have you suffered from heartburn (i.e. a burning sensation in the chest)?

grade 0, no symptoms	grade 1, occasional episodes	grade 2, reason for medical visit	grade 3, interference with daily activities

2) In the last 2 weeks have you suffered from regurgitation (acid or stomach contents coming up into your throat, mouth or lungs)?

grade 0, no regurgitation	grade 1, occasional episodes	grade 2, predictable on position of straining	grade 3, episodes of pulmonary aspiration, nocturnal cough or recurrent pneumonia

3) In the last 2 weeks have you suffered from dysphagia (difficulty swallowing or food getting stuck)?

grade 0, no dysphagia	grade 1, occasional episodes	grade 2, require liquid-to-clear diet	grade 3, episodes of esophageal obstruction

Extra-Oesophageal Reflux Study
Reflux Symptom Index Questionnaire
Response Form

Patient Initials: _____ Screening Number: _____

Date: _ _ / _ _ / _ _ _ _

Within the **last Month** how did the following problems affect you	0 = No Problem					5 = Severe Problem
Hoarseness or a problem with your voice	0	1	2	3	4	5
Clearing your throat	0	1	2	3	4	5
Excess throat or postnasal drip	0	1	2	3	4	5
Difficulty swallowing food, liquids or pills	0	1	2	3	4	5
Coughing after you eat or after lying down	0	1	2	3	4	5
Breathing difficulties or choking episodes	0	1	2	3	4	5
Troublesome or annoying cough	0	1	2	3	4	5
Sensation of something sticking in your throat or a lump in your throat	0	1	2	3	4	5
Heartburn, chest pain, indigestion or stomach acid coming up	0	1	2	3	4	5
	RSI					

Appendix 7

Publication Related to thesis

- Anti-reflux surgery in lung transplant recipients: Outcomes and effects on quality of life. Robertson AG, **Krishnan A**, Ward C, Pearson JP, Small T, Lordan J, Corris PA, Dark JH, Karat D, Shenfine J, Griffin SM. Eur Respir J. 2012 Mar;39(3):691-7

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Anti-reflux surgery in lung transplant recipients: outcomes and effects on quality of life

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ABSTRACT: Fundoplication may improve survival after lung transplantation. Little is known about the effects of fundoplication on quality of life in these patients. The aim of this study was to assess the safety of fundoplication in lung transplant recipients and its effects on quality of life.

Between June 1, 2008 and December 31, 2010, a prospective study of lung transplant recipients undergoing fundoplication was undertaken. Quality of life was assessed before and after surgery. Body mass index (BMI) and pulmonary function were followed up.

16 patients, mean±SD age 38±11.9 yrs, underwent laparoscopic Nissen fundoplication. There was no peri-operative mortality or major complications. Mean±SD hospital stay was 2.6±0.9 days. 15 out of 16 patients were satisfied with the results of surgery post fundoplication. There was a significant improvement in reflux symptom index and DeMeester questionnaires and gastrointestinal quality of life index scores at 6 months. Mean BMI decreased significantly after fundoplication (p=0.01). Patients operated on for deteriorating lung function had a statistically significant decrease in the rate of lung function decline after fundoplication (p=0.008).

Laparoscopic fundoplication is safe in selected lung transplant recipients. Patient benefit is suggested by improved symptoms and satisfaction. This procedure is acceptable, improves quality of life and may reduce deterioration of lung function.

KEYWORDS: Fundoplication, gastro-oesophageal reflux disease, lung transplantation

Chronic microaspiration, secondary to extra-oesophageal reflux, may contribute to bronchiolitis obliterans syndrome (BOS) after lung transplantation. Up to 75% of lung transplant patients have demonstrable gastro-oesophageal reflux disease (GORD) [1–5]. Elevated biomarkers, pepsin and bile salts, have been documented in the bronchoalveolar lavage fluid after lung transplantation, suggesting microaspiration [6–8]. Early anti-reflux surgery may lead to protection of lung function and increased survival through preventing microaspiration. Most of the impetus has been from Duke University (Durham, NC, USA), where the majority of evidence originates [5]. There is a lack of basic information in this patient group, including safety and assessments of quality of life. Such information is important because physiological post-operative complications are common afetr fundoplication, and may lead to a reduction in quality of life, despite resolution of reflux symptoms. Specific complications include temporary dysphagia, nausea [9, 10], discomfort from gas bloating and increased flatulence [2]. Only one study has looked at the effects of fundoplication on quality of life in this population, despite a high prevalence of foregut dysfunction [11]. This puts these patients at risk of physiological dysfunction and reduced quality of life after surgery. To date, no transplant studies have been performed assessing the response of extra-oesophageal reflux symptoms to fundoplication.

The aim of this study was to assess the safety of fundoplication in lung transplant recipients and its effects on quality of life.

METHODS

A prospective study of all lung transplant recipients undergoing anti-reflux surgery between June 1, 2008 and December 31, 2010 at the Northern Oesophago-Gastric Unit (Royal Victoria Infirmary, Newcastle-upon-Tyne, UK) was carried out. All lung transplant recipients in this unit are routinely prescribed prophylactic proton pump inhibitor (PPI) therapy to prevent steroid-induced ulceration. There was no distinction in patient management made between underlying pathologies (*e.g.* cystic fibrosis). Surgery was considered for patients with symptomatic reflux alone, refractory to PPI

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therapy, or for reflux associated with deteriorating lung function. Patients with asymptomatic reflux were only considered for surgery if there were concerns about microaspiration. Maximal medical therapy was not considered for failed PPI therapy or suspected microaspiration, as it was felt that a mechanical barrier to reflux would better protect the allografts from microaspiration. Ethical approval for patient follow-up was obtained from a local ethics committee (County Durham and Tees Valley 2 Research Ethics Committee). Written consent was obtained for patients to be observed overall post lung transplant, but not specifically for this study.

Reflux status was assessed on PPI therapy by oesophageal manometry, pH impedance (Ohmega; MMS^{TM}, Utrecht, the Netherlands) and endoscopy. Patients underwent a thorough pre-operative assessment to ensure fitness for surgery. Reflux status was defined by the presence of oesophageal or extra-oesophageal symptoms combined with objective evidence of GORD on pH impedance and/or endoscopy. Patients did not undergo a post-fundoplication pH impedance or endoscopic measurement of reflux status. Pulmonary function tests and bronchoscopy were routinely performed in the pre-operative work-up.

Patients were followed up clinically with emphasis on lung function, satisfaction with treatment and quality of life. The following questionnaires were used: the DeMeester Reflux Questionnaire, a validated standard reflux questionnaire; the Reflux Symptom Index (RSI) questionnaire, a validated laryngo-pharyngeal reflux questionnaire; and the Gastro-intestinal Quality of Life Index (GIQLI), a validated gastrointestinal-specific quality of life questionnaire [12–14]. These questionnaires covered oesophageal reflux symptoms (heartburn and dysphagia), extra-oesophageal reflux symptoms (cough and wheeze) and functional gastrointestinal symptoms that could be affected by fundoplication (bloating and flatus). These were assessed pre- and post-operatively. Pre- and post-fundoplication body mass index (BMI) were recorded. Patient satisfaction was assessed by direct questioning of patients.

Lung function was assessed in accordance with European Respiratory Society guidelines [15]. BOS scores were calculated using forced expiratory volume in 1 s (FEV_1) in accordance with International Society for Heart and Lung Transplantation guidelines [16, 17]. The rate of decline in FEV_1 was calculated in accordance with previous studies, namely, the measures of FEV_1 before fundoplication were plotted and the gradient between points from the baseline FEV_1 level to the time fundoplication was calculated in millilitres per month. The same was done for the FEV_1 measurements after fundoplication, the last FEV_1 being either the current one in patients still alive or the final FEV_1 in the patients who died [18].

In our unit, bronchoscopy is routinely performed at 1 week, and at 1, 3 and 6 months, and 1 yr post-transplant. Further bronchoscopies are carried out when clinically indicated by an unexplained drop in FEV_1. Pulmonary function tests are carried out routinely at every outpatient visit, on average every 3 months.

The RSI, DeMeester reflux and GIQLI questionnaires were completed pre-operatively, and 6 weeks and 6 months post-operatively. The GIQLI score was subdivided into symptomatic questions (n=17) and functional questions (n=19) to assess whether changes in quality of life were due to changes in symptoms or social functioning. Patients were asked about overall satisfaction with the result of surgery at 6 weeks and 6 months post-operatively. Questionnaires were completed by patients, with expert advice on hand to explain any concerns about questions and to offer one-to-one advice.

Surgical technique

Laparoscopic Nissen fundoplication was performed. Access to the abdominal cavity was *via* four ports and an epigastric stab incision for the Nathanson retractor to retract the liver. Initially, the oesophageal hiatus was dissected to mobilise the oesophagus. The posterior vagus was preserved and a window was created behind the oesophago-gastric junction. The posterior crura were repaired to tighten the hiatus, and a loose 360° wrap was tailored with three Ethibond^{TM} sutures (Ethicon, Somerville, NJ, USA). One further suture was used to anchor the wrap to the oesophagus and right crus. Percutaneous endoscopic gastrostomy (PEG) fistulae were repaired when present. These were divided with an Endostapler^{TM} device (Ethicon). The PEG wound was excised and the deficit in the abdominal wall and skin were closed. Local anaesthesia was inserted into the peritoneal cavity and infiltrated in the wounds at the end of the procedure.

Statistical analysis was carried out with the help of a statistician. Initially, a Kolmogorov–Smirnov test was performed to assess normality. Subsequently, paired t-tests and two-way ANOVAs were performed with a post-test Bonferroni correction. Figures were created using GraphPad Prism^{TM} software (GraphPad, San Diego, CA, USA).

RESULTS

During the study period, 109 lung transplants were performed. 17 patients were considered for fundoplication. One patient was managed conservatively due to lack of objective evidence of GORD on pH impedance and endoscopy. Of 17 patients offered fundoplication, 16 (10 females and six males) with a mean$\pm$SD age of 38.2$\pm$11.9 yrs, consented to and underwent fundoplication. Indications for lung transplant were: cystic fibrosis in 10; chronic obstructive pulmonary disease (COPD)/asthma in one; COPD in one; pulmonary fibrosis in three; and pulmonary fibrosis/asthma in one patient. 13 patients underwent single sequential lung transplant, two had a right single lung transplant and one had a left single lung transplant. Indications for fundoplication were objective evidence of GORD on pH impedance and/or endoscopy with either typical reflux symptoms (heartburn) (n=8) or typical (heartburn) and atypical extra-oesophageal symptoms (cough and wheeze) with deteriorating lung function (n=8). Symptoms occurred despite PPI therapy. Mean pre-operative BMI$\pm$SD was 23.8$\pm$4.4 kg·m^{-2}. Patient demographics are summarised in table 1.

All patients had a diagnostic gastroscopy. 15 out of 16 patients had a hiatus hernia (2–6 cm). eight out of 16 has oesophagitis: grade A, n=4; grade B, n=3; and grade C, n=1. One patient had a small tongue of Barrett's oesophagus confirmed on histological assessment. Three patients had oesophageal candidiasis, which was treated pre-operatively. A summary of pre-operative oesophageal physiology is shown in table 2.

TABLE 1 Demographics of study patients

Age yrs	38.2±11.9
Sex	
Male	6
Female	10
Underlying pathology	
Cystic fibrosis	10
Pulmonary fibrosis	3
Pulmonary fibrosis/asthma	1
COPD	1
COPD/asthma	1
Transplant	
SSLT	13
LSLT	1
RSLT	2
BMI kg·m^{-2}	23.8±4.4
FEV$_1$ L	2.4±0.97
FEV$_1$ % pred	80±5
ASA	
2	5
3	11

Data are presented as mean±SD or n. COPD: chronic obstructive pulmonary disease; SSLT: single sequential lung transplant; LSLT: left single lung transplant; RSLT: right single lung transplant; BMI: body mass index; FEV$_1$: forced expiratory volume in 1 s; % pred: % predicted; ASA: American Society of Anesthesiologists Physical Status classification.

Operation

Pre-operative American Society of Anaesthesiology score was 2 (n=5) or 3 (n=11). Mean±SD FEV$_1$ was 80±5% predicted or FEV$_1$ was 2.4±0.97 L. Fundoplication was performed at a mean of 1,053±881 days post-transplant.

Mean intra-operative time was 93±20 min. All patients had blood loss of <100 mL. Four patients had a PEG fistula excised and no patients required an intensive treatment unit stay, although five out of 16 patients were admitted electively to our high-dependency unit for observation for 24 h. Mean hospital stay was 2.6±0.9 days; longer stays were due to post-operative pain (in two patients with PEG fistulae repair), peri-operative dysphagia (one patient), a return to theatre or difficulty arranging transport home.

TABLE 2 Summary of oesophageal physiology

Lower oesophageal sphincter	
Pressure mmHg	24.6±14.2
Length cm	2.8±0.7
Mean distal peristaltic amplitude mmHg	64.3±20.4
Peristalsis	
Normal	14
Abnormal	2#
Reflux indices	
Acid exposure %	12.6±7.3
DeMeester score	49.5±27.9
Oesophageal volume exposure %	1.3±0.4
Total reflux events	66±27
Proximal reflux events	23±15

Data are presented as mean±sd or n. #: nonspecific dysmotility and diffuse oesophageal spasm.

Morbidity and mortality

There were no deaths or serious post-operative complications. Two patients developed post-operative dysphagia. One of these patients returned to theatre the following day and underwent a laparoscopy and minor revision of fundoplication, and subsequently made an uneventful recovery. In the other patient, barium swallow revealed no significant blockage and symptoms subsequently resolved spontaneously.

Overall satisfaction with fundoplication

Overall, 15 out of 16 patients reported being satisfied at 6 weeks and 15 out of 16 patients reported satisfaction at 6 months. At 6 weeks one patient was unsatisfied due to dysphagia. At 6 months, one patient was unsatisfied due to pain at the site of their PEG fistula and abdominal bloating.

Quality of life

There was a statistically significant improvement in symptoms and quality of life scores over the first 6 months post-fundoplication. Kolmogorov–Smirnov analysis revealed the questionnaire data to be normally distributed. Questionnaires were completed by 15 out of 16 patients. One patient, despite reporting high levels of satisfaction with their result, did not wish to spend time completing these questionnaires. Patient symptom and quality of life questionnaire scores are summarised in table 3.

RSI questionnaire

Pre-fundoplication RSI was positive in eight out of 15 patients, and this decreased to three out of 15 being positive for RSI by 6 weeks and two out of 15 being positive at 6 months. The two-way ANOVA revealed a statistically significant improvement in RSI score over the three time-points ($p<0.001$). Post-test Bonferroni correction revealed a statistically significant improvement in the mean±SD RSI score from 14±7.1 pre-operatively to 6.7±7.9 at 6 weeks post-fundoplication ($p=0.021$) and 5.9±6.5 at 6 months ($p=0.003$) (fig. 1a). The Bonferroni correction did not show a statistically significant difference between RSI scores at 6 weeks and 6 months.

TABLE 3 Summary of symptom and quality of life questionnaire scores

	Pre-operative	6 weeks	6 months
DeMeester	3.7±1.7	1.5±1.6	1.2±0.8
RSI	14±7.1	6.7±7.9	5.9±6.5
GIQLI	96.5±34.4	105.1±27.6	112.4±22.4
GIQLI subsets			
Symptoms	49.7±10.5	56.9±9.1	58.7±7.6
Functional	51.9±19.2	54±19.2	59.1±13.1

Data are presented as mean±SD. RSI: Reflux Symptom Index; GIQLI: Gastrointestinal Quality of Life Index.

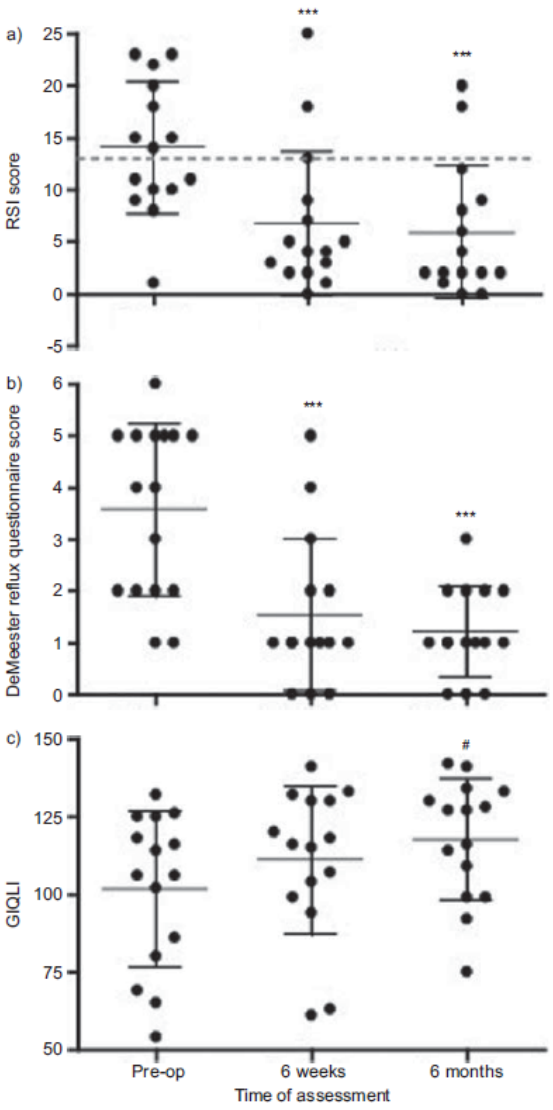

FIGURE 1. a) Reflux Symptom Index (RSI) score, b) DeMeester Reflux Questionnaire Score and c) Gastro-intestinal Quality of Life Index (GIQLI) score over the first 6 months post-fundoplication. The dotted line indicates a score of 13, the cut-off for a normal/abnormal score. Horizontal lines represent the mean and error bars represent the standard deviation. Pre-op: pre-operative. ***: $p<0.001$ compared to pre-op; #: $p=0.008$ compared to pre-op.

DeMeester reflux questionnaire score

The two-way ANOVA revealed a statistically significant improvement in DeMeester Reflux Questionnaire score over the three time-points ($p<0.001$). Post-test Bonferroni correction revealed a statistically significant improvement in the mean±SD DeMeester questionnaire score from 3.7±1.7 pre-operatively to 1.5±1.6 at 6 weeks post-fundoplication ($p=0.012$) and 1.2±0.8 at 6 months ($p=0.003$) (fig. 1b). The Bonferroni correction did not show a statistically significant difference between DeMeester questionnaire scores at 6 weeks and 6 months.

GIQLI

The two-way ANOVA revealed a statistically significant improvement in RSI score over the three time-points ($p=0.008$). Post-test Bonferroni correction revealed a statistically significant improvement in the mean±SD GIQLI score from 96.5±34.4 pre-operatively to 112.4±22.4 at 6 months ($p=0.036$) (fig. 1c). The Bonferroni correction did not show a statistically significant difference between GiQLI scores pre-operatively and at 6 weeks (mean±SD score 105.1±27.6), or at 6 weeks or 6 months ($p=0.1$).

GIQLI sub-analysis

Symptoms

The two-way ANOVA revealed a statistically significant improvement in symptom score from our GIQLI sub-analysis score over the three time-points ($p<0.001$). Post-test Bonferroni correction revealed a statistically significant improvement in mean±SD symptom score from our GIQLI sub-analysis from 49.7±10.5 pre-operatively to 56.9±9.1 at 6 weeks post-fundoplication ($p=0.03$) and 58.7±7.6 at 6 months ($p=0.006$). The Bonferroni correction did not show a statistically significant difference between symptom score from our GIQLI sub-analysis at 6 weeks and 6 months.

Functional

The two-way ANOVA revealed a statistically significant improvement in functional score from our GIQLI sub-analysis over the three time-points ($p=0.036$). Post-test Bonferroni correction did not reveal which pairs reached statistical significance in their improvement in mean±SD functional score from our GIQLI sub-analysis score from 51.9±19.2 pre-operatively to 54±19.2 at 6 weeks post-fundoplication and 59.1±13.1 at 6 months ($p=0.09$), although there was a mean improvement of 7.2 points from the pre-operative score to the score at 6 months. There was a trend to significance from the pre-operative to the 6-month score ($p=0.09$) and from the 6-week to the 6-month score ($p=0.11$).

Body mass index

Kolmogorov–Smirnov analysis revealed this data to be normally distributed. Mean±SD BMI significantly decreased from 23.8±4.4 $kg \cdot m^{-2}$ pre-fundoplication to 22.6±4.6 $kg \cdot m^{-2}$ at 6 months post-fundoplication ($p=0.01$) (fig. 2).

Lung function

Pre-fundoplication, nine patients had no evidence of BOS, whilst the remaining seven patients had BOS 0p (a new grade of BOS created in 2002 to denote "early BOS") (n=1), BOS score 1 (n=2), BOS 2 (n=1) and BOS 3 (n=3). Two patients had a worsening BOS score from BOS 0 to 1 and BOS 2 to 3. Despite a slowing rate of decline, the patient who deteriorated from BOS 2 to 3 died 482 days post-fundoplication from respiratory failure. The patient with BOS 0p had a reversal of this to BOS 0. All other patients remained stable.

Patients were followed up for a mean of 476±180 days post-fundoplication. FEV_1 was similar pre-fundoplication (2.4±0.97 L) and post-fundoplication (2.4±0.71 L) ($p=0.08$).

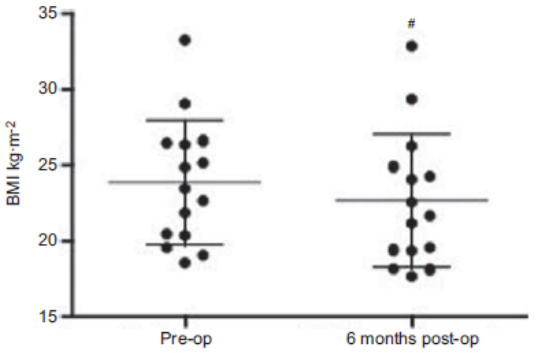

FIGURE 2. Body mass index (BMI) score pre-operatively (pre-op) and at 6 months post-fundoplication (post-op). Horizontal lines represent the mean and error bars represent the standard deviation. #: p=0.001 compared with pre-op.

Eight patients were operated on for deteriorating lung function. Of these eight, one patient had a reversal of BOS, two had a stabilisation of lung function and five had a decrease in the rate of deterioration. Kolmogorov–Smirnov analysis revealed this data to be normally distributed. In the eight patients operated on for deteriorating lung function, there was a statistically significant decrease in the rate of decline of FEV_1 per month post-fundoplication from a mean change±SD of -96.7±87.3 mL·month^{-1} pre-fundoplication to +9.5±26.5 mL·month^{-1} post-fundoplication (p=0.008) (fig. 3). Individual traces are shown in figure 4.

DISCUSSION

This study demonstrates that laparoscopic fundoplication in a transplant setting is safe. Patients reported a high level of satisfaction with the results of surgery at 6 weeks and 6 months. This study also demonstrated that, in this specialised patient population, laparoscopic anti-reflux surgery is effective in reducing symptoms of GORD and improves quality of life. Our study also supports the possibility that fundoplication may impact positively on the loss of lung function seen in BOS.

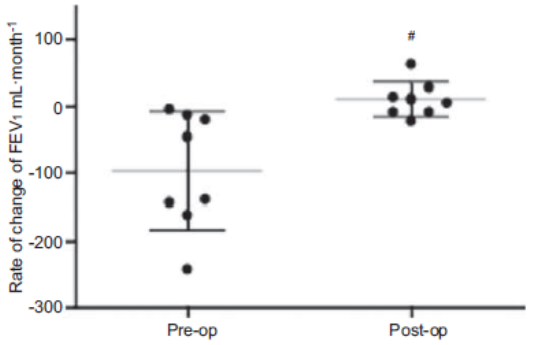

FIGURE 3. Rate of change of forced expiratory volume in 1 s (FEV_1) pre- and post-fundoplication (pre- and post-op) in patients with deteriorating lung function. Horizontal lines represent the mean and error bars the standard deviation. #: p=0.008 compared with pre-op.

These findings are important as there is little knowledge regarding laparoscopic fundoplication in these patients, and such surgery could potentially have negative effects. Our data demonstrating improvements in symptoms and quality of life are, therefore, reassuring. More speculatively, the reduction of decline in lung function observed in this open study supports the theory that fundoplication may protect the lung allograft from microaspiration injury, and suggests the need for further trials.

There is no consensus regarding fundoplication in lung transplant recipients [19]. Small series of fundoplication have been reported in patients with end-stage lung disease [20, 21]. Not all these patients will undergo transplant and there are significant risks associated with performing this procedure in patients with very poor lung function. We have adopted a pragmatic approach, operating in the post-transplant period on patients with symptomatic reflux and those with evidence of reflux and deteriorating lung function. Based on the available transplant evidence, laparoscopic Nissen fundoplication was favoured in our practice [22].

In the study of safety from Duke University, compared with the nontransplant population, there were no significant differences in operative time and blood loss [23]. Our study has comparable intra-operative data and no patients in our series have needed conversion to an open operation. No intra-operative or peri-operative deaths have been reported by the Duke University group [5, 23, 24], although, recently, one post-fundoplication death has been reported [25]. We have experienced no mortality to date and no major complications were encountered. The Duke group have reported increased length of stay in the transplant population and a higher readmission rate, due to transplant comorbidity [23]. Our results are comparable with this experience. The long post-operative stay may be partially explained by the fact that transplant patients have to travel greater distances than a local population. Overall, our results suggest that laparoscopic fundoplication is safe in selected lung transplant recipients.

Over the last 20 yrs, quality of life assessments have been established as end-point outcomes. The GIQLI questionnaire has been recommended by the European Association for Endoscopic Surgery for the assessment of quality of life after fundoplication [26]. The DeMeester reflux questionnaire is validated to assess reflux symptoms and the RSI has been validated in nontransplant patients as a marker of extra-oesophageal reflux [13], and has been used to assess the effects of fundoplication on extra-oesophageal reflux [27, 28].

In nontransplant patients, fundoplication has been shown to ameliorate reflux symptoms and improve quality of life [29]. This study showed that, in lung transplant recipients, there was an improvement in typical reflux symptoms. Although this may be expected, it has also shown an improvement in quality of life post-fundoplication, despite the high prevalence of foregut dysfunction in this population [11]. Our sub-analysis of the data showed that improvement in quality of life occurs *via* both amelioration of symptoms and improved social functioning.

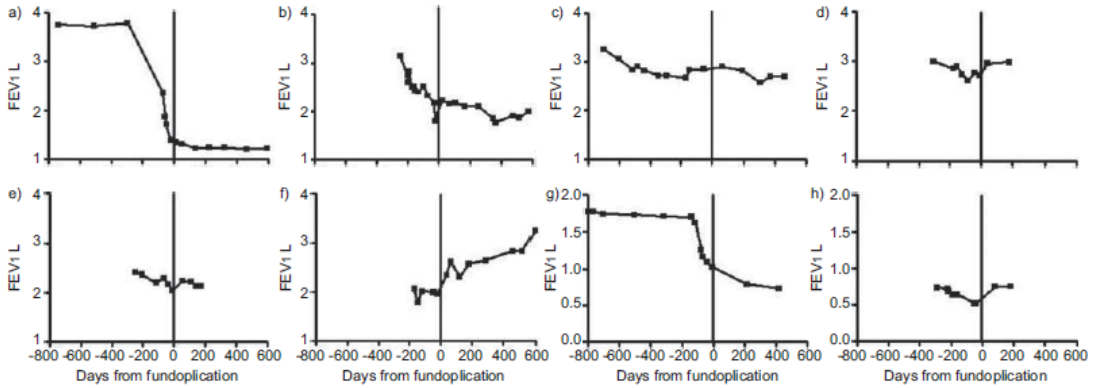

FIGURE 4. Changes in of forced expiratory volume in 1 s (FEV_1) over time pre- and post-fundoplication in patients with deteriorating lung function.

Questionnaires designed for the assessment of extra-oesophageal reflux have not previously been used in lung transplant recipients. Our finding of improvement in extra-oesophageal reflux symptoms in lung transplant recipients after fundoplication is, therefore, novel. These symptoms include cough and hoarseness, which can be caused by extra-oesophageal reflux, but may also represent primary respiratory symptoms. This finding further supports the theory that these patients experience laryngopharyngeal reflux [13, 27], which may precede micro-aspiration. It is unknown how the evolving changes in the lung transplant and BOS may affect extra-oesophageal reflux symptoms, but we believe improvements are possibly attributable to fundoplication.

The Melbourne group's study of fundoplication in lung transplantation described a decrease in mean BMI from 23 $kg \cdot m^{-2}$ 6 months pre-operatively to 21 $kg \cdot m^{-2}$ 6 months post-operatively. The current study's results are similar, with a decrease in mean BMI from 23.8 $kg \cdot m^{-2}$ to 22.6 $kg \cdot m^{-2}$ 6 months post-operatively. The significance of this is unknown, but, in selected patients, post-fundoplication dietary advice and intervention may have an important role.

The Duke University Transplant Group has published several papers [5, 23, 24, 30, 31], each an update of a continuing programme, with results suggesting that anti-reflux surgery may lead to increased survival and improved lung function post-transplantation [5]. Our study was not designed to assess the impact of fundoplication on lung function. In our series, mean FEV_1 did not deteriorate post-fundoplication. Those patients operated on for deteriorating lung function underwent a statistically significant reduction in the loss of lung function, and one patient had a reversal of a subtle defect in lung function.

Our current study has several limitations. The numbers involved were small and patients had a variety of indications for surgery. Fundoplication was performed at different times after transplant and no patients were operated on within 90 days of transplant, the suggested optimum time for intervention [5], although this study did not seek to define an optimum time for intervention. No control group was analysed and the study was not randomised. Further studies could include a focus on the effects of early fundoplication (within 90 days) on allograft function and long-term survival.

Almost all the evidence supporting fundoplication post-lung transplant originates from a single centre and only three other centres have published case series. Based on this evidence, we have tried to develop a series of pragmatic indications for those to be offered surgical interventions. The improvement in GORD symptoms and quality of life in these patients suggests that the developing indications for fundoplication post-lung transplant may include symptomatic GORD in fit patients. The reduction in deterioration of lung function post-fundoplication further supports a possible role of this therapy in the prevention of BOS, but further evidence is required, including formal trials. Our study suggests that, with careful design, such studies are possible and can be safe in an extended series of patients.

SUPPORT STATEMENT

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STATEMENT OF INTEREST

None declared.

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