Clinical approach to gastro-oesophageal reflux
in idiopathic pulmonary fibrosis

Rhys Thomas Jones

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Supervisors
Dr Chris Ward
Dr Ian Forrest
Professor Michael Griffin
Professor Jeffrey Pearson
Professor John Simpson
Abstract

Background

Idiopathic pulmonary fibrosis is a progressive condition with limited treatment options and median survival of 3-5 years. Gastro-oesophageal reflux (GOR) has been described in up to 90% of patients. Pulmonary aspiration has been suggested to contribute to IPF, with calls for aggressive antireflux therapy. Whilst medical therapy can usually control acid reflux, surgery may be required to control non-acid refluxate, which may also be harmful if aspirated into the lung. The risks of surgery in the IPF population are significant. There is no validated technique with which to measure aspiration in this group and furthermore, patient attitudes towards the treatment of reflux and aspiration in IPF are unknown. As a result, the population that might benefit from antireflux therapy has yet to be defined. The current study comprised two main aims. The first was to characterise reflux and aspiration in an IPF cohort. The second was to evaluate patient attitudes towards the burden of IPF disease as compared to the burden of antireflux therapy.

Methods

Symptoms of reflux and lung health were assessed using a panel of structured questionnaires. Oesophageal function and gastro-oesophageal reflux were objectively assessed using manometry and pH-impedance monitoring. A standardised bronchoscopy and bronchoalveolar lavage, with biochemical and cytological analysis, was used to assess pulmonary aspiration.

A separate group of individuals with IPF participated in an interview study. Respondents’ own health was evaluated using a visual analogue scale, the EuroQOL-5D-3L survey and a standard gamble utility analysis. Vignettes were constructed to describe mild- and moderate-severity IPF health states and adverse outcomes from medical and surgical antireflux therapy. Patient attitudes towards these four health states were assessed with a ranking exercise and a series of standard gambles.

Results

pH-impedance monitoring demonstrated supranormal levels of gastro-oesophageal reflux in 22 of 36 study subjects (61%). Eleven subjects had pre-existing evidence of gastro-
oesophageal reflux and questionnaire assessment suggested GORD in 29% of subjects. Oesophageal manometry identified abnormal oesophageal function in 56%.

Supranormal levels of pepsin were detected in bronchoalveolar lavage fluid in 16 subjects. The combination of pepsin quantification and oesophageal monitoring identified a subgroup of subjects with evidence of reflux and aspiration, but there was no correlation between levels of reflux and pepsin concentrations. Cytological staining results correlated poorly with gastro-oesophageal reflux. After formal multidisciplinary review, two patients who participated in the current study have undergone fundoplication. Both have enjoyed a stable disease course since surgery.

In the interview study, respondents recorded mean utilities of 0.611 to 0.798 for their own health. Amongst 59 respondents, 38 regarded both IPF health states as preferable to the outcomes of either antireflux therapy outcome; the remainder disagreed. An adverse outcome from antireflux surgery was generally regarded as the worst of the health states.

**Discussion**

Oesophageal physiology and BAL fluid analysis may be combined to investigate reflux and aspiration in IPF. The current data suggest that reflux is common and frequently asymptomatic. Aspiration may only be significant in the minority of patients. Oesophageal dysmotility, a relative contra-indication to fundoplication, was evident in the majority of subjects.

This is the first report of health state utilities for IPF and demonstrates a disease burden comparable to advanced lung cancer. Opinion was divided as to the relative burden associated with IPF disease and the potential outcomes of antireflux therapy.

In conclusion, it remains difficult to identify the IPF patients for whom antireflux surgery might be most beneficial. For a proportion, the risks of such treatment will be prohibitive. The complexity of surgical decisions in this group suggests a requirement for a standard of care that includes a multidisciplinary team, informed by objective aerodigestive physiology and imaging.
This work is dedicated to my wife, who has been tireless in her patience and support and without whom it would not have been possible.

For Suba
Sponsors

This work has been possible thanks to funding through a Knowledge Transfer Partnership in collaboration with Innovate UK. Funding originated largely from the Northern Oesophagogastric Unit, with additional support from the Medical Research Council. I am grateful to these organisations for this opportunity.

Acknowledgments

I am indebted to my supervisors for their guidance and expertise. Dr Ward and Dr Forrest have provided unfailing support and considered responses to innumerable questions, on both the academic challenges of my doctorate, and the broader experiences of the Knowledge Transfer Partnership. Professor Griffin has provided crucial assistance with this work and has made a huge contribution to my career since I started working at the Northern Oesophago-Gastric Unit. Professor Simpson and Professor Pearson have been invaluable co-supervisors and brought a wealth of research experience to my study.

I am grateful to Dr Laura Ternent and Dr Peter McMeekin, based at the Institute of Health and Society (Newcastle University) and the School of Health, Community and Education Studies (Northumbria University) respectively. They have provided crucial support with all stages of the standard gamble work.

I am grateful to my friend and colleague Mr Aman Krishnan, who initiated the characterisation study. His efforts and training made for a smooth introduction to the work.

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I am grateful to Mr Julian McGlashan for scoring the pharyngeal images for the study.

My thanks go to Ms Joy Candler who supported my training in oesophageal manometry and ambulatory monitoring. The medical and nursing staff in the Chest clinic at the Royal Victoria Infirmary have been hugely co-operative as I went about recruitment. I am also grateful to all the staff in the Endoscopy department for their assistance and co-operation with bronchoscopy and physiology assessments.
I am grateful to all the clinicians who have supported the aerodigestive MDT.

Finally, I would like to thank all the patients who participated in this research.
Declaration

This thesis is a presentation of my original research work. Wherever others have contributed, every effort has been made to indicate their involvement.

Dr Chris Ward and Dr Ian Forrest conceived the characterisation study. Mr Amaran Krishnan contributed to study design and secured ethical approval. Mr Krishnan studied the first 20 subjects. All bronchoscopy and broncho-alveolar lavage was performed by Dr Forrest.

I recruited the latter group of 16 patients to the characterisation study. I performed all oesophageal physiology monitoring this group. I completed cytological and bronchoalveolar lavage analysis on the latter group of 16 subjects. I performed all data analysis for the characterisation study.

I designed the patient attitudes study and secured all study approvals. I performed all patient interviews and data analysis for this section of the study.

I set up the Aerodigestive MDT within Newcastle Hospitals NHS Foundation Trust.
Publications


Al-momani H, Perry A, Stewart C, Jones R et al. Microbiological profiles of sputum and gastric juice aspirates in Cystic Fibrosis patients. Scientific Reports. (In press)

Presentations

Characterisation of reflux and aspiration in idiopathic pulmonary fibrosis; an integrated approach. British Thoracic Society, December 2014

Using questionnaires to measure the impact of gastro-esophageal reflux in chronic lung disease. American Thoracic Society, May 2014

Initial experience of an aerodigestive multidisciplinary team. European Society of Diseases of the Esophagus, November 2013

Initial experience of an aerodigestive multidisciplinary team. Association of Upper Gastrointestinal Surgeons of Great Britain and Ireland, September 2013

Grants and Awards

In January 2015, the Knowledge Transfer Partnership within which I developed the Northern Aerodigestive Group was awarded a grading of Outstanding

In 2012 we submitted a grant application to the British Lung Foundation based on data from the current study. A grant of £139 000 was secured for a randomised placebo-controlled trial of omeprazole in idiopathic pulmonary fibrosis.

In 2014, our research group secured a doctoral research grant of £322 000 from the Irish Health Board. The application included data from the current study and also benefitted from the training and collaboration I was able to provide on oesophageal physiology.

Also in 2014, I was awarded £5000 from the Joint Research Executive Scientific Committee at the Newcastle Hospitals, for an observational study of swallow competence in idiopathic pulmonary fibrosis.
Contents

Abstract .................................................................................................................. i
Sponsors ................................................................................................................... v
Acknowledgments .................................................................................................... v
Declaration ............................................................................................................... vii
Publications ........................................................................................................... vii
Presentations ........................................................................................................... ix
Grants and Awards ................................................................................................ xi
Contents .................................................................................................................. xi
Abbreviations ......................................................................................................... xxi

Chapter 1. Introduction ............................................................................................. 1
  1.1 Idiopathic pulmonary fibrosis ........................................................................ 1
     1.1.1 Introduction .......................................................................................... 1
     1.1.2 Epidemiology ....................................................................................... 3
     1.1.3 Assessment and diagnosis .................................................................... 3
     1.1.4 Treatment options in IPF ..................................................................... 4
     1.1.5 Aetiopathogenesis .............................................................................. 8
  1.2 Gastro-oesophageal reflux and aspiration .................................................... 14
     1.2.1 Gastro-oesophageal reflux disease ..................................................... 14
     1.2.2 Treatment of GORD .......................................................................... 20
  1.3 The association of reflux and IPF ................................................................. 23
     1.3.1 Pharmacological treatment of GORD in IPF ...................................... 29
     1.3.2 The role of antireflux surgery in IPF ................................................... 30
  1.4 Detection of proximal reflux and pulmonary aspiration .............................. 31
     1.4.1 Oropharyngeal pH measurement ....................................................... 31
     1.4.2 Scintigraphy ...................................................................................... 33
     1.4.3 Pepsin measurement in sputum and saliva ......................................... 34
     1.4.4 Bronchoalveolar lavage ..................................................................... 36
  1.5 Evaluating patient attitudes towards antireflux therapy in IPF .................... 41
     1.5.1 Measuring health .............................................................................. 42
     1.5.2 Scaling, value and utility ................................................................... 43
     1.5.3 Measurement of utilities .................................................................. 44
     1.5.4 Empirical support for measurement tools ......................................... 46
     1.5.5 Choice of respondents in health state valuation .............................. 49
  1.6 Unanswered questions about reflux, aspiration and IPF ............................. 51

Chapter 2. Hypothesis, Aims and Objectives ......................................................... 52
  2.1 Hypothesis .................................................................................................... 52
  2.2 Aims .............................................................................................................. 52
     2.2.1 Primary aim ...................................................................................... 52
     2.2.2 Secondary aims ............................................................................... 52
  2.3 Objectives .................................................................................................... 53

Chapter 3. Methods ................................................................................................ 54
  3.1 Characterisation of reflux and aspiration in IPF ........................................ 54
     3.1.1 Ethical approval ................................................................................ 54
3.1.2 Recruitment ................................................................. 54
3.1.3 Study overview ............................................................ 55
3.1.4 Selection of patient-reported outcome measures ...................... 56
3.1.5 Clinical data collection ..................................................... 62
3.1.6 Preparation for oesophageal physiology testing ....................... 63
3.1.7 Oesophageal manometry .................................................. 64
3.1.8 Ambulatory oesophageal monitoring .................................... 71
3.1.9 Bronchoscopy ............................................................... 74
3.1.10 Bronchoalveolar lavage fluid processing ............................ 75
3.1.11 Assessment of aspiration ................................................ 76
3.1.12 Formation of a novel aerodigestive multidisciplinary team ......... 79
3.1.13 Statistical analysis ......................................................... 79
3.2 Evaluation of patient attitudes towards the treatment of reflux in IPF 81
3.2.1 Ethical approval .............................................................. 81
3.2.2 Study overview .............................................................. 81
3.2.3 Study sample and recruitment .......................................... 81
3.2.4 Inclusion criteria ............................................................ 82
3.2.5 Exclusion criteria ........................................................... 82
3.2.6 Sample size .................................................................. 82
3.2.7 Standard gamble method ................................................ 82
3.2.8 Vignette design .............................................................. 84
3.2.9 The interview process ...................................................... 87
3.2.10 Statistical analysis ......................................................... 88
Chapter 4. Results of Characterisation Study .................................... 89
4.1 Study overview .................................................................. 89
4.2 Recruitment and demographics ............................................. 90
4.3 Assessment of gastro-oesophageal reflux .................................. 93
4.3.1 Subjective assessment ..................................................... 93
4.3.2 Oesophageal manometry .................................................. 94
4.3.3 Ambulatory monitoring ................................................... 98
4.4 pH-impedance analysis: inter-rater reliability ......................... 102
4.5 Assessment of laryngopharyngeal reflux and pulmonary aspiration 103
4.5.1 Subjective assessment ..................................................... 103
4.5.2 Bronchoscopy and bronchoalveolar lavage ......................... 108
4.6 Multidisciplinary review and subsequent management ............. 122
4.7 Summary ........................................................................ 123
Chapter 5. Results of Patient Attitudes Study .................................. 124
5.1 Interview summary and stratification by disease severity .......... 124
5.2 Summary statistics ............................................................ 126
5.3 Validation statistics ............................................................ 128
5.4 Health state ranking exercise .............................................. 129
5.5 Health state utility analysis ................................................ 130
5.6 Correlation between lung function and own health utility values 134
5.7 Summary ........................................................................ 136
Chapter 6. Discussion ................................................................ 137
6.1 Recruitment and feasibility ................................................... 138
6.2 Subjective assessment of reflux and aspiration ....................... 140
6.3 Oesophageal physiology monitoring ..................................... 142
6.4 Bronchoalveolar lavage ....................................................... 146
6.4.1 Pepsin ................................................................. 147
6.4.2 Bile salts .................................................................. 149

6.5 Bronchoalveolar cytology ............................................. 150
6.5.1 Differential cell counts ............................................... 150
6.5.2 Cell staining .............................................................. 150

6.6 Multidisciplinary review ................................................ 152

6.7 Evaluating patient attitudes: measuring the perceived burden of IPF and antireflux therapy ........................................... 154
6.7.1 Study design ............................................................. 154
6.7.2 Study subjects ........................................................... 156
6.7.3 Key findings .............................................................. 156
6.7.4 Validity, strengths and limitations ............................... 159

Chapter 7. Summary and Recommendations for Future Research .......................... 162

Appendix A Characterisation study appointment letter ....................... 166
Appendix B GerdQ questionnaire ........................................... 167
Appendix C Reflux Symptom Index questionnaire .......................... 168
Appendix D St George’s Respiratory Questionnaire .......................... 169
Appendix E Patient attitudes study - Demographic survey .................. 175
Appendix F EuroQOL-5D-3L .................................................. 179
Appendix G Standard operating procedure: Bronchoalveolar lavage processing .... 182
Appendix H Standard operating procedure: Geimsa staining .............. 187
Appendix I Standard operating procedure: Oil Red O staining ............ 190
Appendix J Standard operating procedure: Perls Prussian Blue staining .... 194

References .............................................................................. 198
List of tables

Table 1-1. Epidemiological studies of IPF from Europe and North America ........................................... 2
Table 1-2. Evolving techniques with which GORD has been investigated ............................................. 15
Table 1-3. Test-retest reliability of the standard gamble and time trade-off in studies reporting direct comparisons. Test statistic specified as intraclass correlation in studies indicated (*) and unspecified in the remainder ................................................................................................................................. 48
Table 3-1. Questionnaires rejected for assessment of symptoms of gastro-oesophageal reflux ........ 57
Table 3-2. Questionnaires considered for the symptomatic assessment of gastro-oesophageal reflux ......................................................................................................................................................... 58
Table 3-3. Questionnaires rejected for assessment of extra-oesophageal reflux symptoms .......... 61
Table 3-4. Classification of oesophageal peristalsis using conventional manometry assessment .... 67
Table 3-5. Comparison of conventional and high resolution oesophageal manometry assessment ... 67
Table 3-6. Defining features of an individual swallow ((Bredenoord et al., 2012)) ............................... 71
Table 3-7. Component scoring for the Reflux Finding Score, an instrument validated to assess the severity of laryngopharyngeal reflux (Belafsky et al., 2001) ......................................................................................................................... 75
Table 3-8. Iterative process through which health state scenarios were designed for the standard gamble. Superscript figures relate to notes detailed below the table ................................................................. 85
Table 4-1. Study subjects' breathlessness status at recruitment (Fletcher et al., 1959) ................. 92
Table 4-2. Respiratory health-related quality of life score recorded on and off PPI medication ...... 93
Table 4-3. Amplitudes of oesophageal peristalsis recorded at conventional 8-channel manometry. NA=not available. ........................................................................................................................................ 95
Table 4-4. Metrics used to evaluate oesophageal swallow integrity using the Chicago classification. 96
Table 4-5. The results of ambulatory pH monitoring. All figures are scaled so as to represent a 24 hour monitoring period. Long refluxes are defined as those in excess of five minutes. .............. 98
Table 4-6. Number of acid, weakly acid and non-acid refluxes recorded over a standardised 24 hour impedance monitoring period. ................................................................................................. 100
Table 4-7. Number of subjects identified to have high levels of reflux by pH and impedance monitoring. ........................................................................................................................................ 100
Table 4-8. Key pH-impedance results as reported by two independent raters. Rater A=Rhys Jones. Rater B=Amaran Krishnan, a surgical registrar and former research fellow who was blinded to the remainder of these individuals’ results. ................................................................. 102

Table 4-9. Bronchoscopic and symptomatic assessment of laryngopharyngeal reflux. Four of the eight components of the reflux findings scale (RFS) are included here for illustration. RSI=Reflux symptom index. Images for subject IPF25 could not be scored due to insufficient image quality. ..................... 107

Table 4-10. pH-impedance monitoring results and BAL pepsin concentrations in all study subjects. Normal values are included in brackets (Jamieson et al., 1992; Zerbib et al., 2013). .......................... 111

Table 4-11. Bronchoalveolar lavage fluid bile salt concentration measured in all 35 IPF patients using a spectrophotometric total bile salt assay. All measurements are in µmol/l. Three separate runs were initially performed and an average result determined. Positive readings were verified with a further set of readings. Zero values indicate readings lower than the limit of detection. ........................ 113

Table 4-12. Differential cell counts for bronchoalveolar lavage fluid retrieved from the 36 study participants. Values are expressed as mean (range). p-values refer to a one-sample T-test comparing the study data to the reference mean. .................................................................................. 114

Table 4-13. LLMI and haemosiderin scores enumerated from BAL cytospins ................................. 116

Table 4-14. Summary values for cell staining counts. Results are expressed as median (range). ...... 119

Table 5-1. Summary responses to the EQSD-3L survey .............................................................. 126

Table 5-2. Demographic summary statistics .................................................................................. 127

Table 5-3. Health state valuations. SD = standard deviation. OSG = own health evaluation by standard gamble. OE = own health evaluation by EQ5D). The terms “Top half” and “Bottom half” refer to a subdivision with respect to objective pulmonary function (section 5.1) ........................................................................... 130

Table 6-1. Reported prevalence of pathological gastro-oesophageal reflux detected by ambulatory monitoring. N/S=not specified ................................................................................. 146

Table 6-2. Published health state utilities for lung cancer ............................................................. 161
List of figures

Figure 1-1. The disease trajectory that has been postulated in IPF comprises a series of unpredictable exacerbations, separated by periods of stability ................................................................. 3

Figure 1-2. Schematic anatomy of the mouth, pharynx and upper aerodigestive tracts. Adapted from Cranial Nerves, 3rd edition. (Reproduced from (Pauwells et al., 1998)) ........................................ 14

Figure 1-3. The gastro-oesophageal junction. Left: 3D reconstruction, illustrating the intra-abdominal portion of the terminal oesophagus and its acute angle of entry into the stomach. An endoscope is seen in the retroflexed position. Right: Endoscopic view in retroflexion. Taken from Hill et al. (1996). ........................................................................................................................................ 16

Figure 1-4. The configuration of impedance and pH catheters within an intraluminal probe. The impedance drop illustrated is first detected distally before ascending to the most proximal channel. The pH trace, measured 5cm proximal to the LOS, remains above 4. This recording therefore demonstrates proximal, weakly acid reflux. [Oesophageal schematic taken from software licensed to MMS International (Enschede, The Netherlands)] ......................................................... 20

Figure 1-5. Schematic diagram to illustrate the anatomy of duodeno-gastric and gastro-oesophageal reflux (Modified from (UPMC, 2015)) ........................................................................................................ 24

Figure 1-6. Appearance of the hypopharynx at flexible endoscopy: 1) Normal  2) Diffuse erythema at the vocal folds and arytenoid walls  3) Severe oedema in the posterior larynx  4) Vocal fold granuloma. Images reproduced from (Park, 2013; Vivero, 2015) ............................................................................. 32

Figure 1-7. Pepsin lateral flow device showing the result of a gastric juice sample positive for pepsin. C=control band. T=test sample band. Reproduced from (Saritas Yuksel et al., 2012). ......................... 35

Figure 1-8. Decision tree for a standard gamble evaluating a chronic health state considered better than death. Reproduced from (Torrance, 1986) ............................................................................................................. 46

Figure 3-1. The orientation of side-holes in an 8-channel manometry catheter ...................................... 65
Figure 3-2. Oesophageal pressure topography illustrating a normal swallow. Colours corresponding to specified pressures are illustrated on the left of the figure. UOS = upper oesophageal sphincter; IBP rise = intra-bolus pressure rise; LOS = lower oesophageal sphincter. Taken from (Fox and Bredenoord, 2008).

Figure 3-3. Oesophageal pressure topography plot illustrating a normal water swallow as assessed by high-resolution manometry. Pressure scale indicated by the colour bar on the left (mmHg). UES = upper oesophageal sphincter; LES = lower oesophageal sphincter; CFV = contractile front velocity; DCI = distal contractile index (dashed pink box); DL = distal latency; IRP = integrated relaxation pressure; P=proximal pressure trough; CDP=contractile deceleration point. Adapted from (Zerbib and Omari, 2014).

Figure 3-4. Flow diagram to illustrate the Chicago classification system of high resolution oesophageal manometry (reproduced from (Bredenoord et al., 2012)).

Figure 3-5. The arrangement of pH and impedance sensors within the pHersaflex catheter.

Figure 3-6. Chance board used as a prop to illustrate decision-making within the standard gamble. Example vignette featured as “Choice B”.

Figure 4-1. Recruitment and completion figures for the characterisation study.

Figure 4-2. Histogram illustrating the percentage predicted vital capacity readings taken prior to participation in the study.

Figure 4-3. GORD symptom assessed by GerdQ scores. The individual value plot is overlaid with slope-graphs for those patients who completed GerdQ both on and off PPI therapy. The reference line indicates the score which has been reported as most discriminating as a cut-off in the diagnosis of GORD (Jones et al., 2009).

Figure 4-4. Chicago classification of HRM results.

Figure 4-5. Individual value plots for reflux index and DeMeester score. Horizontal reference lines indicate the upper limit of normal for a healthy control population.
Figure 4-6. 24-hour reflux events recorded at impedance monitoring. 95th centile reference lines indicate the published upper limits of normal for healthy controls (Zerbib et al., 2005). * indicates individuals with pathological levels of reflux on pH monitoring; †indicates individuals with pathological levels of proximal reflux. Subject number 21 was studied on PPI therapy. 101

Figure 4-7. Scatterplots to illustrate the level of agreement between key pH-impedance reflux episode counts. Rater details are included in Table 4-8. 102

Figure 4-8. Extra-oesophageal symptoms as assessed by Reflux Symptom Index in patients taking PPI therapy at the time of study recruitment. PPI was discontinued for two weeks prior to physiology testing. 104

Figure 4-9. Scatterplot to illustrate the relationship between levels of objectively measured proximal reflux and symptoms of laryngopharyngeal reflux. 104

Figure 4-10. Illustrative bronchoscopy images captured for an external assessor to evaluate evidence of laryngopharyngeal reflux using the Reflux Finding Score (Belafsky et al., 2001). 106

Figure 4-11. Standard curves for the ELISA used to quantify pepsin concentrations in BAL fluid. 109

Figure 4-12. Pepsin concentrations in IPF study subjects and healthy controls. In the IPF study subjects, the presence of gastro-oesophageal reflux was defined at pH-impedance monitoring by a high DeMeester score, a high total number of refluxes, or both. 110

Figure 4-13. Reference chart used to support LLMI grading. 115

Figure 4-14. Illustrative photomicrographs of cytospin cell staining with Giemsa, Perls Prussian Blue and Oil Red O stains. 117

Figure 4-15. Illustrative photomicrographs of cytospin cell staining with Giemsa, Perls Prussian Blue and Oil Red O stains. 118

Figure 4-16. The relationship between LLMI and DeMeester scores for the 36 study subjects. 120
Figure 4-17. LLMI calculated for study subjects grouped by DeMeester score; 14.7 is the upper limit of normal in healthy controls and values above this are consistent with a high level of gastro-oesophageal reflux ................................................................. 120

Figure 4-18. Haemosiderin scores for study subjects stratified by lung disease severity. Subjects were dichotomised with respect to the median figure for % predicted vital capacity. .......................... 121

Figure 4-19. Haemosiderin score calculated for study subjects grouped by DeMeester score........ 121

Figure 5-1. The frequency with which respondents ranked the health states in a specified order. Figures in brackets indicate respondents who ranked death as preferable to one of the descriptive health states ................................................................. 129

Figure 5-2. Mean health state evaluations recorded by standard gamble. P-values relate to 2-sample T-tests. Descriptive health states are as follows: W=mild IPF; X=moderate IPF; Y=surgical antireflux therapy; Z=medical antireflux therapy. Own health was also evaluated using the standard gamble.131

Figure 5-3. Mean health state evaluations recorded for respondent’s own current health. P-value refers to a 2-sample T-test ........................................................................................................ 132

Figure 5-4. Health state evaluations stratified by disease severity .............................................. 133

Figure 5-5. Scatterplot comparing respondents' own health valuations as measured by standard gamble and EQSD ........................................................................................................ 134

Figure 5-6. Scatterplot comparing the percentage predicted vital capacity with respondents' own health valuations as measured by standard gamble............................................................................. 135

Figure 5-7. Scatterplot comparing the percentage predicted vital capacity with respondents' own health valuations as measured by EQSD.................................................................................. 135
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>AEC</td>
<td>alveolar epithelial cell</td>
</tr>
<tr>
<td>AECII</td>
<td>type 2 alveolar epithelial cell</td>
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<tr>
<td>ASCEND</td>
<td>Assessment of Pirfenidone to Confirm Efficacy and Safety in Idiopathic Pulmonary Fibrosis</td>
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<tr>
<td>ATF-6</td>
<td>activating transcription factor 6</td>
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<td>ATS</td>
<td>American Thoracic Society</td>
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<tr>
<td>BAL</td>
<td>bronchoalveolar lavage</td>
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<tr>
<td>BALF</td>
<td>bronchoalveolar lavage fluid</td>
</tr>
<tr>
<td>BCT</td>
<td>bolus clearance time</td>
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<tr>
<td>BiP</td>
<td>binding immunoglobulin protein</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
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<tr>
<td>BOS</td>
<td>bronchiolitis obliterans syndrome</td>
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<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
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<td>CAPACITY</td>
<td>Clinical Studies Assessing Pirfenidone in idiopathic pulmonary fibrosis: Research of Efficacy and Safety Outcomes</td>
</tr>
<tr>
<td>CD4</td>
<td>cluster of differentiation 4</td>
</tr>
<tr>
<td>CD8</td>
<td>cluster of differentiation 8</td>
</tr>
<tr>
<td>CDP</td>
<td>contractile deceleration point</td>
</tr>
<tr>
<td>CFV</td>
<td>contractile front velocity</td>
</tr>
<tr>
<td>CHOP</td>
<td>CCAAT-enhancer-binding protein homologous protein</td>
</tr>
<tr>
<td>COX2</td>
<td>cyclooxygenase 2</td>
</tr>
<tr>
<td>CT</td>
<td>computed tomography</td>
</tr>
<tr>
<td>DCI</td>
<td>distal contractile index</td>
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<tr>
<td>DIAMOND</td>
<td>Dutch study on Initial Management Of Newly diagnosed Dyspepsia</td>
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<tr>
<td>DL</td>
<td>distal latency</td>
</tr>
<tr>
<td>DPX</td>
<td>distrene, plasticiser, xylene</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<td>EMT</td>
<td>epithelial-mesenchymal transition</td>
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<td>EQ5D-3L</td>
<td>European Quality Of Life-5 Dimension scale, 3 line version</td>
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<tr>
<td>ER</td>
<td>endoplasmic reticulum</td>
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<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
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</table>
FEV1  forced expiratory volume in 1 second
FH    functional heartburn
FVC   forced vital capacity
GERD-Q Gastro-Esophageal Reflux Disease Questionnaire
GERQ  Gastro-Esophageal Reflux Questionnaire
GI    gastro-intestinal
GOR (syn. GER) gastro-oesophageal reflux
GORD (syn.) gastro-oesophageal reflux disease
GERD)
GRACI Gastrooesophageal Activity Index
GSAS  Gastro-oesophageal reflux Symptoms Assessment Scale
GSFQ  GERD Symptom Frequency Questionnaire
H2RB  H2-receptor blocker
HO    hypersensitive oesophagus
HRCT  high resolution computed tomography
HRM   high resolution manometry
HRQL  health-related quality of life
IBP   intrabolus pressure
IL    interleukin
ILD   interstitial lung disease
INPULSIS Efficacy And Safety Of Nintedanib In Patients With Idiopathic Pulmonary Fibrosis
IPF   idiopathic pulmonary fibrosis
IRP   integrated relaxation pressure
LAP   latency-associated peptide
LLAM  lipid-laden alveolar macrophage
LLMI  lipid-laden macrophage index
LOS syn (LES) lower oesophageal sphincter
LOTUS Long-Term Usage of Esomeprazole vs Surgery for Treatment of Chronic GERD
LPR   laryngopharyngeal reflux
MDT   multidisciplinary team
mg/ml milligrams per millilitre
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>MII</td>
<td>multichannel intraluminal Impedance</td>
</tr>
<tr>
<td>MRC</td>
<td>Medical Research Council</td>
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<tr>
<td>MUC5B</td>
<td>mucin 5B</td>
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<tr>
<td>NAC</td>
<td>N-acetyl cysteine</td>
</tr>
<tr>
<td>NERD</td>
<td>non-erosive reflux disease</td>
</tr>
<tr>
<td>ng/ml</td>
<td>nanograms per millilitre</td>
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<tr>
<td>NICE</td>
<td>National Institute for Health and Care Excellence</td>
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<tr>
<td>NOGU</td>
<td>Northern Oesophagogastric Unit</td>
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<tr>
<td>NPV</td>
<td>negative predictive value</td>
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<tr>
<td>NRES</td>
<td>National Research Ethics Committee</td>
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<td>NSCLC</td>
<td>non-small cell lung cancer</td>
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<tr>
<td>NVQ</td>
<td>National Vocational Qualification</td>
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<tr>
<td>OE</td>
<td>own health state as assessed by EuroQOL-5D-3L</td>
</tr>
<tr>
<td>ONS</td>
<td>Office of National Statistics</td>
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<tr>
<td>OP</td>
<td>oropharyngeal</td>
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<tr>
<td>ORO</td>
<td>oil red O</td>
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<tr>
<td>OSG</td>
<td>own health state as assessed by standard gamble</td>
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<tr>
<td>PANTHER-IPF</td>
<td>Prednisone, Azathioprine, and N-Acetylcysteine: A Study That Evaluates Response in Idiopathic Pulmonary Fibrosis</td>
</tr>
<tr>
<td>PASS</td>
<td>Proton-Pump Inhibitor Acid Suppression Symptom test</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffer Solution</td>
</tr>
<tr>
<td>PDGF</td>
<td>platelet-derived growth factor</td>
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<tr>
<td>PFT</td>
<td>pulmonary function test</td>
</tr>
<tr>
<td>pH</td>
<td>potential for hydrogen ions; $-\log_{10}[H^+]$</td>
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<tr>
<td>PPI</td>
<td>proton pump inhibitor</td>
</tr>
<tr>
<td>PPV</td>
<td>positive predictive value</td>
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<tr>
<td>QALY</td>
<td>quality-adjusted life year</td>
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<tr>
<td>R&amp;D</td>
<td>Research and Development</td>
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<tr>
<td>RCT</td>
<td>randomised controlled trial</td>
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<tr>
<td>RDQ</td>
<td>reflux disease questionnaire</td>
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<tr>
<td>ReQuest</td>
<td>Reflux Questionnaire</td>
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<tr>
<td>ROC</td>
<td>receiver operating characteristic</td>
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rpm revolutions per minute
RSI Reflux Symptom Index
RVI Royal Victoria Infirmary
SAP symptom associated probability
SFPA2 surfacant protein A2
SFPC surfacant protein C
SG standard gamble
SGRQ St George's Respiratory Questionnaire
SI symptom index
SSI symptom sensitivity index
TBA Total Bile Acids
TERC telomerase ribonucleic acid component
TERT telomerase reverse transcriptase
TGF-β transforming growth factor beta
TH1 type 1 helper T-cells
TLOSR transient lower oesophageal sphincter relaxation
TMS tandem mass spectrometry
TNF tumour necrosis factor
TOMORROW To imprOve pulMOnaRy fibROsis With BIBF 1120
TTO time trade-off
UIP usual interstitial pneumonia
UOS syn (UES) upper oesophageal sphincter
VAS visual analogue scale
VC vital capacity
vNM utility von Neumann-Morgenstern utility
WHO World Health Organisation
WnT wingless-related integration site
WRAP-IPF Weighing Risks and benefits of IAParoscopic anti-reflux surgery in patients with Idiopathic Pulmonary Fibrosis
XBP-1 x-box binding protein 1
αSMA alpa smooth muscle actin
μg/ml micrograms per millilitre
μmol/l micromoles per litre
Chapter 1. Introduction

1.1 Idiopathic pulmonary fibrosis

1.1.1 Introduction

Idiopathic pulmonary fibrosis (IPF) is a progressive, scarring lung condition of unknown aetiology. It is the commonest of the interstitial lung diseases and, in contrast to several others, has well-defined clinical, radiological and histopathological features. The aetiology and pathogenesis of the condition are not well understood and treatment options are limited. Transplantation offers the only definitive treatment but few patients are eligible.

1.1.1 Epidemiology

The epidemiology of IPF has proved challenging for two main reasons. Clinically, the early features of IPF are often non-specific, which can lead to diagnostic uncertainty (Fell et al., 2010). From a research perspective, the nomenclature and disease classification of interstitial lung disease has evolved over the last 30 years, with significant inconsistencies between studies (Bradley et al., 2008).

Table 1-1 summarises the major studies of IPF incidence and prevalence from Europe and North America. The best estimates come from disease-specific registries and national datasets. Other studies have relied on clinic surveys which generate lower estimates and are more likely to underestimate disease occurrence (Karakatsani et al., 2009; Musellim et al., 2014).

Two consistent findings from these studies are that IPF is more common amongst men and that the incidence of the disease appears to be increasing. In the UK, Navaratnam et al analysed the Office of National Statistics mortality data in combination with a large primary care database over the period 1968-2008 (Navaratnam et al., 2011). Annual death certificate reporting rose six-fold (0.92-5.1 per 100 000) over the period and the incidence of IPF reported by General Practitioners rose from 5.8 to 8.0 per 100 000 between 2000 and 2008. The authors could find no firm evidence of improving survival rates, which makes ascertainment bias less likely. If milder cases were increasingly recognised over the study period then the survival rate be expected to increase over time.
Table 1.1. Epidemiological studies of IPF from Europe and North America.

<table>
<thead>
<tr>
<th>First author</th>
<th>Country and study period</th>
<th>Methodology</th>
<th>Incidence /100,000 person years</th>
<th>Prevalence /100,000</th>
</tr>
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<tbody>
<tr>
<td><strong>Europe</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Hodgson (Hodgson <em>et al.</em>, 2002)</td>
<td>Finland 1997-98</td>
<td>Nationwide survey of respiratory clinics and hospital databases (accuracy of coding validated within a sample and extrapolated to the study group)</td>
<td>-</td>
<td>16-18</td>
</tr>
<tr>
<td>Von Plessen (von Plessen <em>et al.</em>, 2003)</td>
<td>Norway 1984-98</td>
<td>Hospital registry</td>
<td>4.3</td>
<td>23.4</td>
</tr>
<tr>
<td>Gribbin (Gribbin <em>et al.</em>, 2006)</td>
<td>UK 1991-2003</td>
<td>Primary care database and ONS mortality records</td>
<td>4.6</td>
<td>-</td>
</tr>
<tr>
<td>Navaratnam (Navaratnam <em>et al.</em>, 2011)</td>
<td>UK 2000-09</td>
<td>Primary care database and ONS mortality records</td>
<td>7.44</td>
<td>-</td>
</tr>
<tr>
<td><strong>North America</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coultas (Coul tas <em>et al.</em>, 1994)</td>
<td>US 1988-90</td>
<td>Medical records, pathology reports, death certificates and autopsy reports</td>
<td>7.4 (F)</td>
<td>13.2 (F)</td>
</tr>
<tr>
<td>10.7 (M)</td>
<td></td>
<td></td>
<td></td>
<td>20.2 (M)</td>
</tr>
<tr>
<td>Raghu (Raghu <em>et al.</em>, 2006b)</td>
<td>US 1996-2000</td>
<td>Insurance claims database</td>
<td>6.8-16.3</td>
<td>14-42.7</td>
</tr>
<tr>
<td>Fernandez-Perez (Fernandez Perez <em>et al.</em>, 2010)</td>
<td>US 1997-2005</td>
<td>County-wide medical record linkage system</td>
<td>17.4</td>
<td>47</td>
</tr>
</tbody>
</table>

1.1.2 Clinical course

Patients generally present with worsening breathlessness between 50 and 70 years of age (Navaratnam *et al.*, 2011). Symptoms have often been present for many months or years by the time of specialist evaluation and diagnosis (du Bois, 2012). In time, the cardinal features of exertional dyspnoea and a non-productive cough develop. Eventually, patients will experience dyspnoea at rest as respiratory function declines. In the UK, the median survival from diagnosis is three to five years (NICE, 2013a).

The trajectory of disease progression in IPF remains a matter of debate. In a proportion of patients at least, periods of stability are punctuated by unpredictable exacerbations, with a
resultant loss of pulmonary function, Figure 1-1 (Collard et al., 2007). Acute exacerbations in IPF are defined as idiopathic, but risk factors have been identified. Functional and radiological markers of severity put patients at higher risk (Ryerson et al., 2015). Several triggers have also been suggested: exacerbations appear more common in the winter months, during periods of immunosuppression, and following infection (Wootton et al., 2011; Raghu et al., 2012; Collard et al., 2013b). Both thoracic and non-thoracic surgical procedures have also been cited (Yuksel et al., 2006; Ghatol et al., 2012).

Although the progression seen in IPF is often rapid, the chronic and variable nature of the disease course complicates research into pathogenesis. As a result, the significance of suspected aetiological factors and the potential benefits of therapeutic intervention are difficult to prove.

Figure 1-1. The disease trajectory that has been postulated in IPF comprises a series of unpredictable exacerbations, separated by periods of stability

1.1.3 Assessment and diagnosis

Plain chest radiograph most commonly reveals diffuse, reticular opacities over the lower lung zones. High resolution computed tomography (HRCT) scanning is increasingly used to evaluate parenchymal lung disease. HRCT can detect early changes in the context of a normal plain film and is better able to determine disease severity and differentiate between different lung pathology (Grenier et al., 1991). On HRCT, the changes of IPF appear as patchy, subpleural densities with a basal preponderance. Contiguous areas of reticulation
and normal lung give rise to a “geographical” pattern. In more advanced disease the typical pattern of honeycombing is seen as cystic air spaces develop between thickened interlobular septa. The accuracy of HRCT for diagnosing IPF is operator dependent but appears to be around 90% (Grenier et al., 1991).

In cases where the clinico-radiological diagnosis remains uncertain and confirmation will affect management, tissue may be sampled by means of a transbronchial or surgical lung biopsy. Histologically, IPF presents as usual interstitial pneumonia with a “patchwork pattern”, honeycombing and characteristic lesions known as fibroblastic foci (Katzenstein et al., 2008). As with HRCT, the patchy appearance arises from the temporal heterogeneity of fibrotic changes in adjacent sections of lung. Katzenstein and Myers coined the term “fibroblastic foci” to describe the hyperplastic reparative epithelium overlying aggregates of myofibroblasts (Katzenstein and Myers, 1998). Myofibroblasts are differentiated mesenchymal cells which contribute to the deposition of extracellular matrix and scar formation in both physiological and pathological states (Hinz et al., 2007). These are the cells mainly responsible for the excessive deposition of extracellular matrix seen in the IPF lung.

### 1.1.4 Treatment options in IPF

Until relatively recently, lung transplantation was considered to be the only effective treatment for IPF. Recent guidelines incorporate some new options, but the range of treatment options available remains extremely limited (NICE, 2013a).

#### Non-pharmacological options

**Lung transplantation**

Whilst the evidence base is weak, lung transplantation does appear to improve survival in IPF (Thabut et al., 2003). Long-term survival after transplantation for IPF is favourable compared to survival after transplantation for other indications (Keating et al., 2009). IPF is a disease of the elderly and age is a relative contraindication in lung transplantation. The proportion of patients who are eligible for transplantation and for whom a window of opportunity arises is small. The data from the latest survey of the International Society of Heart and Lung Transplantation suggest that 14.5% of adult sequential bilateral lung transplants are performed for IPF (Christie et al., 2009).
**Oxygen therapy**

Indirect evidence from studies of unselected patients with hypoxaemia suggests that long-term oxygen therapy improves exercise capacity. In obstructive lung disease, a survival benefit has been demonstrated (Nocturnal Oxygen Therapy Trial Group, 1980). There is no direct evidence of benefit in IPF but the use of long-term oxygen therapy has been recommended nonetheless (Raghu et al., 2011).

**Pulmonary rehabilitation**

Pulmonary rehabilitation combines physical conditioning, education and psychosocial support. In a controlled trial which randomised 57 ILD patients (34 IPF) to receive either supervised exercise training or weekly telephone support, those in the exercise group had a short-term benefit in terms of function and symptomatology (Holland et al., 2008). The benefit was not sustained at six months.

**Pharmacological options**

**Corticosteroids**

Prednisolone has immunosuppressive and anti-inflammatory effects. There is some evidence to support short-term use in a minority of IPF patients (Neuner et al., 2001; Richeldi et al., 2003). Overall, the response to corticosteroid therapy in IPF is almost universally poor. In clinical practice short-term trials are often used, but the side effect profile and limited evidence base led international guidelines to recommend against their use as monotherapy (Raghu et al., 2011).

**Combination therapy**

Azathioprine is an immunosuppressant that inhibits synthesis of the purine framework of the DNA bases adenine and guanine. N-acetyl cysteine (NAC), a precursor of the antioxidant glutathione, has also been used in the treatment of IPF. The combination was studied in a randomised controlled trial published in 2005 (Demedts et al.). The Idiopathic Pulmonary Fibrosis International Group Exploring N-Acetylcysteine I Annual (IFIGENIA) study randomised 182 patients to receive either high-dose NAC or placebo in addition to “standard therapy” with prednisolone and azathioprine. Those in the NAC group had a slower
deterioration of vital capacity (VC) and carbon monoxide diffusing capacity (DLCO). Limitations of the study include the lack of a placebo arm and a 30% dropout rate.

The same combination (NAC, azathioprine and prednisolone) was compared to placebo and NAC monotherapy in PANTHER-IPF (Prednisone, Azathioprine, and N-Acetylcysteine: A Study That Evaluates Response in Idiopathic Pulmonary Fibrosis) (The Idiopathic Pulmonary Fibrosis Clinical Research Network, 2012). This trial was terminated after interim analysis revealed an excess of hospitalisation and mortality in those receiving triple therapy. The placebo and NAC arms were continued but there was no difference in the primary endpoint of change in FVC over a 60-week period.

**Pirfenidone**

Pirfenidone is a small, orally available molecule that demonstrates anti-inflammatory and antifibrotic effects in vitro and in vivo (Schaefer *et al.*, 2011). Early mechanistic studies in hamsters demonstrated reduced expression of several profibrotic factors in lung tissue and or bronchoalveolar lavage (BAL) fluid. Pirfenidone has been shown to prevent the accumulation of hydroxyproline, procollagen I and III, inflammatory cells and transforming growth factor-beta (TGF-b) (Iyer *et al.*, 1995; Iyer *et al.*, 2000). Many of these findings have since been reproduced in different species and in human lung fibroblast cells in vitro (Kakugawa *et al.*, 2004; Nakayama *et al.*, 2008; Oku *et al.*, 2008; Conte *et al.*, 2014).

In a series of Japanese studies looking at the role of pirfenidone in IPF, patients in the treatment group exhibited better disease stability than those in the placebo arm (Taniguchi *et al.*, 2010). Two multinational RCTs were subsequently reported in concert (Noble *et al.*, 2011). Both CAPACITY (Clinical Studies Assessing Pirfenidone in idiopathic pulmonary fibrosis: Research of Efficacy and Safety Outcomes) trials used the same primary end-point of change in percentage predicted forced vital capacity (FVC). Initial analysis suggested that patients in the pirfenidone group had a significantly lower decline in FVC in only one of the two studies (CAPACITY 004). In the second study, patients in the placebo group had a lower than expected rate of decline, which may account for the negative findings (CAPACITY 006). A recent Cochrane meta-analysis evaluated the progression-free survival data reported as secondary endpoints in all three trials. In this analysis, pirfenidone reduced the risk of disease progression by 30% (Spagnolo *et al.*, 2010).
The US Food and Drug Administration ruled against pirfenidone approval in 2010. The ASCEND trial was then initiated (Assessment of Pirfenidone to Confirm Efficacy and Safety in Idiopathic Pulmonary Fibrosis) and randomised 555 IPF patients to receive either pirfenidone or placebo (King et al., 2014). In this study, the pirfenidone group had a better outcome with respect to the primary endpoint of change in FVC or death at week 52. This work adds to the body of evidence in favour of pirfenidone in selected cases of IPF, but the endpoints are of questionable clinical significance. The best study parameters have been described as those which measure how well patients feel, function or survive (Richeldi, 2013). To date, progression-free survival has not been properly validated as a marker of survival in IPF.

The National Institute for Health and Care Excellence approved the use of pirfenidone in a specified subset of IPF patients and the FDA followed suit in 2014 (NICE, 2013b; U.S. Food and Drug Administration, 2014).

**Nintedanib**

Several other novel agents are currently under investigation for the treatment of IPF (Woodcock and Maher, 2014). Amongst these, the drug most likely to feature in clinical practice in the near future is nintedanib, a tyrosine kinase inhibitor. Nintedanib has inhibitory effects on platelet-derived growth factor receptors, fibroblast proliferation and fibroblast-myofibroblast transformation (Wollin et al., 2014).

In the phase 2, double-blinded TOMORROW (To imprOve pulMOnaRy fibROsis With BIBF 1120) study, nintedanib was compared with placebo in a total of 432 patients with IPF (Richeldi et al., 2011). The patients in the treatment arm received one of four escalating doses. Compared with placebo, the difference in the primary endpoint of change in FVC failed to reach statistical significance for any of the treatment arms; p=0.06 for the highest dose (150mg twice daily) group. Treatment with nintedanib was associated with improved quality-of-life scores and fewer acute exacerbations.

Two replicate phase 3 trials of nintedanib have also been reported (Richeldi et al., 2014). The INPULSIS-I and -2 (Efficacy And Safety Of Nintedanib In Patients With Idiopathic Pulmonary Fibrosis) studies randomised a total of 1066 patients to nintedanib or placebo and recorded annual rate of decline in FVC as the primary endpoint. The patients in the treatment arms both had a significantly lower decline in FVC. In INPULSIS-2 alone, the patients in the treatment group had a longer time to first exacerbation and a lower decline in health-related
quality of life. There was no significant difference in survival but the treatment groups tended to better outcomes. The proportion of patients who died from any cause was 5.5% in the nintedanib group and 7.8% in the placebo group. Following publication of these data, nintedanib has been widely approved for clinical use in IPF (Raghu et al., 2015b).

Since its recognition as a distinct disease entity, drug development in IPF has been slow. These recent publications represent modest progress in the field, but meaningful advances remain elusive. Significant improvements in treatment for IPF rely on an improved understanding of the factors driving the fibrotic process.

### 1.1.5 Aetiopathogenesis

IPF results in a morphology of lung disease known as usual interstitial pneumonia (UIP) which is characteristic and well described. In contrast the causative agents resulting in IPF are unknown and the cellular pathogenesis is not well understood.

IPF was originally thought to be a disease of inflammation (Keogh and Crystal, 1982). Alveolitis was reported as a consistent feature of IPF lungs and was considered to provide a unifying pathogenic explanation for IPF and a range of other interstitial lung diseases (Carrington and Gaensler, 1978; Keogh and Crystal, 1982). After fibrotic foci became recognised in IPF, the evidence for alveolitis as the primary disorder came into question: inflammation is not a prominent finding in UIP and anti-inflammatory and immunosuppressive therapy fails to improve disease outcome (Selman et al., 2001).

#### Genetic studies

In 2001, Nogee et al reported the association of a mutation in SFPc, the gene encoding surfactant protein C, with interstitial lung disease in a mother and her child (Nogee et al., 2001). Other groups have since identified mutations in SFPa2 (encoding surfactant protein A2) and further mutations in SFPc. These surfactant proteins are expressed solely by type 2 alveolar epithelial cells (AECIIs).

In 2007, two independent groups studying familial pulmonary fibrosis identified mutations in TERT (encoding telomerase reverse transcriptase) and TERC (encoding the RNA component of the telomerase complex). Telomerase repairs damage that occurs as telomeres are shortened with each round of mitosis. Interestingly, short telomeres have been demonstrated in AECIIs in a high proportion of individuals with sporadic IPF, suggesting a
role for telomerase dysfunction in non-familial forms of the disease. The link between IPF and telomerase dysfunction may help to explain why the disease is predominantly a disease of older people (Tsakiri et al., 2007).

More recently, a whole genome linkage study of IPF identified a common polymorphism in the promoter of the mucin gene MUC5B (Seibold et al., 2011). This mutation was found in 34% of cases of familial pulmonary fibrosis, 38% of cases of sporadic IPF and 9% of healthy controls.

**Alveolar epithelial cells in the pathogenesis of IPF**

A common theme amongst all the mutations identified to date is that they affect genes that are expressed in type 2 epithelial cells (e.g. SFPC, SFPA, MUC5B) or lead to identifiable changes within them (TERT, TERC)(Wolters et al., 2014). AECIIs make up 60% of all alveolar epithelial cells (Crapo et al., 1982). In addition to their role in surfactant production, they contribute to immune defences and fluid balance in the lung (Pison et al., 1994; Fehrenbach, 2001). AECIIs have become a focus of research into IPF pathogenesis.

Within all cells, the endoplasmic reticulum (ER) is the site of newly synthesised secretory and membrane proteins. After translation, proteins must be appropriately folded and modified before they can be transported. These processes rely on chaperone proteins and a conducive environment within the endoplasmic reticulum. If the functional capacity of the ER is overwhelmed, an unfolded protein response (UPR) is initiated in an attempt to restore homeostasis (Zhang and Kaufman, 2004). Markers of the UPR include over-expression of BiP (binding immunoglobulin protein, an ER-associated chaperone protein) and the transcription factor X-box-protein 1 (XBP-1). A persistent state of stress will result in apoptotic cell death, which is mediated by the signalling proteins CHOP and ATF-6 (Zhang and Kaufman, 2004).

The SP-C mutation originally described by Nogee results in a failure of normal truncation of surfactant, prior to its release from the ER (Beers et al., 1998). In a human lung epithelial cell line, SFPC mutations led to increased expression of BiP and XBP-1. Cells expressing mutant SFPC also had increased activation of caspase-4, an ER-specific caspase that triggers apoptosis in the setting of overwhelming ER stress (Mulugeta et al., 2005). Expression of these markers of ER stress has subsequently been demonstrated in AECIIs in both familial and sporadic IPF (Lawson et al., 2008). Furthermore, in stained sections of IPF lungs, 70-80% of AECIIs demonstrated ongoing signs of apoptosis (Korfei et al., 2008).
TGF-β activation

Transforming growth factor β (TGF-β) is a highly conserved, pro-fibrotic cytokine. It is released in an inactive form, bound to the latency-associated peptide (LAP) and latent TGF-β-binding proteins. Levels of active TGF-β are increased in IPF (Krein and Winston, 2002).

As lung fibrosis develops, AECs express increased levels of the integrin αvβ6, which can bind to LAP and activate TGF-β (Munger et al., 1999; Jenkins et al., 2006; Horan et al., 2008). Active TGF-β does not appear to be released in a soluble form, so the resultant signalling is localised within the lung. Given the spatial heterogeneity of usual interstitial pneumonia, this property makes for an attractive pathogenic candidate in IPF. Possible pro-fibrotic processes associated with TGF-β activation include differentiation of fibroblasts to myofibroblasts and activation of programming that promotes mesenchymal transition of epithelial cells (Kim et al., 2006; Scotton and Chambers, 2007).

Epithelial to mesenchymal transition

Epithelial cells are derived from the embryological endoderm. They are anchored on a basement membrane and have a polarity, with basal and apical surfaces contributing to function. Mesenchymal cells originate in the embryological mesoderm. Mesenchymal tissue is characterised by loosely associated cells surrounded by an extracellular matrix. They lack polarity and can migrate easily (Kalluri and Neilson, 2003; Lee et al., 2006).

Epithelial-mesenchymal transition (EMT) is a process by which epithelial cells transdifferentiate into mesenchymal stem cells. EMT is not required for normal tissue haemostasis but it does play a role in normal development and wound healing. In disease, EMT can result in fibrosis and malignancy.

In IPF lungs, immunohistochemical and RNA quantitation techniques have been used to demonstrate co-localisation of proteins associated with epithelial and mesenchymal cells. Thyroid transcription factor 1 and pro-surfactant protein B (AECII markers) were seen in association with α-smooth muscle actin (a mesenchymal protein) in the majority of AECs in IPF lungs; there was no co-localisation in normal lungs (Willis et al., 2005; Marmai et al., 2011).

Despite these reports, the role of EMT in IPF remains unproven. It is not clear whether epithelial cells acquire sufficient mesenchymal characteristics that they can be classified as
fibroblasts (Kage and Borok, 2012). It is also possible that cells undergo mesenchymal-to-epithelial transition. This alternative process of transformation may also give rise to similar patterns of co-localisation (Li et al., 2010).

**From epithelial cell injury to fibrosis**

The existence of fibroblastic foci in the IPF lung indicates a potential, much needed and logical pathophysiological mechanism in the aetiology of IPF. It also emphasises the significance of the lung epithelium. The links between genetic predisposition, epithelial cell injury, ER stress and fibrosis remain unclear. In mice, lung fibrosis can be induced using bleomycin, an antibiotic and chemotherapeutic produced by *Streptomyces verticillus*. This model has been used extensively in IPF research but does not correlate perfectly with human pathogenesis. Pulmonary fibrosis develops in the bleomycin mouse model over weeks and months rather than years, as is the case in humans (Wolters et al., 2014). In addition, the pattern of disease that is induced is airway-centric, in contrast to the sub-pleural predominance seen in the IPF lung (Baron et al., 2012).

Mutated forms of surfactant proteins A and C have been associated with up-regulation of the unfolded protein response and EMT in murine models (Bridges et al., 2006; Lawson et al., 2011; Tanjore et al., 2011). Increased levels of active TGFβ have also been demonstrated (Maitra et al., 2012). Furthermore, there is some evidence that ER stress can result in EMT (Zhong et al., 2011).

Whilst these links between genetic predisposition and EMT are intriguing, the end result of fibrosis has yet to be demonstrated in the mouse model. This may relate to the short murine life span but alternatively, it may indicate a role for external factors in the pathogenesis of IPF (Wolters et al., 2014). Indeed, the fibrotic response to bleomycin was accentuated in mice in which ER stress had been induced (Lawson et al., 2011). Similarly, the effect of herpes virus infection was more pronounced in aged mice, in which ER stress would be induced more easily (Sueblinvong et al., 2012).

Additional mechanisms have been proposed to explain how the initiation and propagation of fibrosis in IPF relate to the interaction between the epithelial cells and the mesenchyme. TGFβ, released by epithelial cells, can signal to fibroblasts and convert them to αSMA-producing myofibroblasts (Xu et al., 2003). AECs also produce higher than normal levels of
the cytokine platelet-derived growth factor (PDGF) in IPF (Bergeron et al., 2003). PDGF has been shown to promote fibroblast proliferation (Hetzel et al., 2005).

The dysregulated IPF epithelium may also play a role in cellular recruitment from the circulation. Fibrocytes are a unique population of blood-borne cells that display a specific cell-surface phenotype. They are thought to migrate to tissues to support wound healing and fibrotic tissue repair (Quan et al., 2006). Alveolar epithelial cells express ligands with corresponding receptors on fibrocytes, the levels of which are increased in IPF lungs (Mehrad et al., 2007; Andersson-Sjoland et al., 2008; Strieter et al., 2009).

Wnt proteins are also over-expressed in IPF lungs. These soluble glycoproteins are produced by epithelial cells and deposited locally in an insoluble form after release (Nusse, 2003). A series of studies led to the identification of Wnt3a as a stimulator of type I collagen production (Wolters et al., 2014). The related downstream signalling molecule has been localised in nuclei of fibroblastic foci (Chilosi et al., 2003; Kim et al., 2009).

Several other lines of investigation are currently underway. The matrix in IPF is grossly modified and it has been shown that the increased stiffness may contribute to increased levels of pro-fibrotic cytokines, with the potential for a feed-forward auto-amplified loop mediated by αSMA and COX2 expression (Liu et al., 2010; Booth et al., 2012; Marinkovic et al., 2012). There are also numerous parallels between the pathogenicity of IPF and cancer, with CD44 and hyaluronan synthase proposed as mediators of the invasive activated myofibroblast phenotype seen in IPF (Li et al., 2011). Lastly, a number of recent epigenetic studies may help to explain the durable phenotypic changes seen in IPF lungs (Pandit et al., 2010; Rabinovich et al., 2012).

In summary, some important observations regarding the pathogenesis of IPF have been made. The role of the alveolar epithelial cells and the complex interactions between AECIIs and the mesenchymal cells are better understood but the relative importance of the various molecular mechanisms remains unclear. The current paradigm holds that in a susceptible individual, a series of subclinical insults damages the alveoli over time. An aberrant repair response, with excess matrix deposition and architectural remodelling, gives rise to the typical clinical, radiological and pathological features (Selman et al., 2001; Murray and Nadel, 2005).
Much of the current research in IPF is aimed at modifying this aberrant response within the lungs. Relatively little work has been done to identify the factors responsible for the initial damage.

**Aetiological factors**

The role of several environmental agents has been explored. Cigarette smoking is associated with the development of IPF. In one study the odds ratio for ever smoking was 1.6 (95% CI: 1.1 to 2.4) but no clear exposure-response pattern has been demonstrated (Baumgartner *et al.*, 1997). Epidemiological data also suggests an increased odds ratios for developing IPF after exposure to birds, livestock and several dusts (Kottmann *et al.*, 2009). Viruses have also been implicated in the pathogenesis of IPF, including Epstein-Barr virus, human herpes viruses 7 and 8 and cytomegalovirus (Yonemaru *et al.*, 1997; Tang *et al.*, 2003). To date, no causative role has been demonstrated for any of these agents.

Over the last forty years a body of evidence has emerged in support of an association between IPF and gastro-oesophageal reflux disease (GORD). Whilst the data to support a contributory role for GORD remains at an early stage, evidence for the link between the two conditions is increasingly persuasive (section 1.3). GORD is common and amenable to treatment and as a result, it has been identified as a research priority in IPF (NICE, 2013a). The following sections cover the assessment and treatment of gastro-oesophageal reflux and aspiration, and explore the link that has been suggested between these conditions and IPF.
1.2 Gastro-oesophageal reflux and aspiration

Aspiration occurs when oesophageal or oropharyngeal contents penetrate the larynx and then cross the glottis into the trachea, Figure 1-2. Aspiration from the oesophagus is the result of gastro-oesophageal reflux of material originating from the stomach and/or duodenum. The possibility that reflux might contribute to the pathogenesis of interstitial lung disease has been considered for over forty years (Pearson and Wilson, 1971).

Figure 1-2. Schematic anatomy of the mouth, pharynx and upper aerodigestive tracts. Adapted from Cranial Nerves, 3rd edition. (Reproduced from (Pauwells et al., 1998)).

1.2.1 Gastro-oesophageal reflux disease

Gastro-oesophageal reflux disease (GORD) is defined as reflux that causes troublesome symptoms, mucosal injury in the oesophagus, or both (Vakil et al., 2006). It is one of the commonest triggers for medical consultation in the developed world and its treatment accounts for the biggest single drug expenditure in the NHS. Prevalence estimates vary widely due to differing disease definitions, but the best estimates lie between 10 and 20% in the developed world (Dent et al., 2005; El-Serag et al., 2009).

The cardinal symptoms of GORD are heartburn and regurgitation. Heartburn is generally described as a burning retrosternal pain. It is the result of distal oesophageal acid exposure (Bredenoord et al., 2013). Regurgitation is defined as the backflow of gastric contents into the mouth, often with an acidic or bitter taste. Volume regurgitation, vomiting and dysphagia may also be experienced. Symptoms of extra-oesophageal reflux can arise from laryngopharyngeal reflux (LPR) or microaspiration. Voice change, pharyngitis, dental decay and chronic cough can all occur. In a large cohort study of reflux oesophagitis, elderly
patients had less typical and more-nonspecific symptoms than younger groups (Pilotto et al., 2006).

Accurate diagnosis can be challenging, as a significant proportion of symptomatic cases are associated with a normal endoscopy, a condition described as non-erosive reflux disease. Conversely, up to 20% of patients with endoscopic oesophagitis and/or Barrett’s oesophagus never experience heartburn (Sloan et al., 1992). Over the years, the concept of the disease has evolved in light of emerging diagnostic tools, each with their own strengths and limitations (Table 1-2).

Table 1-2. Evolving techniques with which GORD has been investigated

<table>
<thead>
<tr>
<th>Era</th>
<th>Modality of investigation</th>
<th>Abnormality identified</th>
<th>Limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1940s-</td>
<td>Plain radiography</td>
<td>Hiatus hernia</td>
<td>GORD can occur without hiatus hernia; hiatus hernia can exist without GORD</td>
</tr>
<tr>
<td>1960s-</td>
<td>Upper gastro-intestinal endoscopy</td>
<td>Mucosal inflammation and disruption</td>
<td>Non-erosive reflux disease not detected</td>
</tr>
<tr>
<td>1970s-</td>
<td>Ambulatory pH monitoring</td>
<td>Supranormal distal oesophageal acid exposure</td>
<td>Non-acid and weakly acid refluxate not detected</td>
</tr>
</tbody>
</table>

The antireflux barrier

Retrograde flow of gastric contents into the oesophagus is a physiological event that helps to vent the stomach. The symptoms and signs of GORD can occur when the frequency or volume of reflux is excessive, or the oesophageal mucosa is hypersensitive or frankly injured. The balance of factors that predispose to or protect against reflux are essentially mechanical. On the one hand the oesophagus is largely exposed to the negative pressure of the chest, whilst the stomach is normally exposed to the positive pressure of the abdomen. This is the only site within the gastrointestinal tract where a segment of the gut is in continuity with a distal region at higher pressure. Continuous gastro-oesophageal reflux is prevented by the structure and function of the gastro-oesophageal junction and the diaphragm. In addition, peristalsis is a barrier as it returns refluxate to the stomach.
Extra-oesophageal factors

The diaphragmatic crura are tendinous structures that attach inferiorly to the anterior aspect of the vertebral column. The right crus forms a sling that surrounds the distal oesophagus, creating a teardrop-shaped canal known as the oesophageal hiatus. This structure serves as an extrinsic sphincter.

In normal anatomy, the terminal oesophagus and the proximal stomach are orientated at an acute angle (Figure 1-3). This configuration is jointly maintained by the orientation of certain fibres within the wall of the stomach and also by the phreno-oesophageal ligament, running between the under-surface of the diaphragm and the oesophagus (Atkinson and Sumerling, 1959; Bombeck et al., 1966).

As a result, the terminal portion of the oesophagus is intra-abdominal. Pressure spikes are therefore transmitted to the oesophageal lumen, preventing a net gastro-oesophageal pressure gradient and protecting against reflux. In addition, the angle between the oesophagus and stomach creates a flap-valve mechanism that also prevents reflux (Figure 1-3).

Figure 1-3. The gastro-oesophageal junction. Left: 3D reconstruction, illustrating the intra-abdominal portion of the terminal oesophagus and its acute angle of entry into the stomach. An endoscope is seen in the retroflexed position. Right: Endoscopic view in retroflexion. Taken from Hill et al. (1996).
Lower-oesophageal sphincter

In addition to the external elements, there is a length of tonically contracted circular muscle within the terminal oesophagus that acts as an intrinsic barrier to reflux. Although the lower oesophageal sphincter cannot be defined anatomically, manometry reveals a high-pressure zone of around 1-4 cm that behaves as a physiological sphincter (Fox and Bredenoord, 2008). Relaxation is observed with swallowing and a hypertonic response is seen due to abdominal compression (bending, straining, coughing) or a drop in intrathoracic pressure (deep inspiration, sniffing) (Bredenoord et al., 2013). With the development of oesophageal manometry, the concept of a Transient Lower Oesophageal Sphincter Relaxation (TLOSRs) was introduced (Mittal et al., 1995). The resting tone of the crural diaphragm is inhibited due to a vagally mediated reflex triggered by gastric distension (Mittal and Fisher, 1990). TLOSRs serve to vent gas from the stomach.
Pathophysiology of GORD

The pathogenesis of GORD relates to the thoraco-abdominal pressure gradient and the constituents of the antireflux barrier.

With respect to the lower oesophageal sphincter, TLOSRs represent the most important cause of reflux. In healthy individuals, physiological reflux occurs almost exclusively as the result of TLOSRs, rather than from defective basal LOS pressure (Dent et al., 1980). In subjects with GORD, spontaneous reflux across a hypotonic LOS accounts for a greater proportion of reflux events, but TLOSRs remain the dominant mechanism (Dent et al., 1988).

Historically, the presence of a hiatus hernia was regarded as crucial to the pathogenesis of GORD. It is now understood that the majority of patients with a hiatus hernia are asymptomatic (Kahrilas et al., 1999). This may be explained by the action of a strong layer of endo-abdominal fascia known as the phreno-oesophageal ligament (Cohen and Harris, 1971). Whilst a portion of the stomach can be seen above the diaphragm on an x-ray, its encasement within this layer maintains the pressure gradient across the gastro-oesophageal junction.

The presence of a hiatus hernia is, nonetheless, a significant risk factor for GORD. As more of the stomach moves up into the chest, the flap-valve mechanism is lost. The length of the sphincter exposed to intra-abdominal pressure is also significant: DeMeester demonstrated that a low basal LOS pressure and/or a short intra-abdominal sphincter length resulted in a 90% incidence of abnormal GOR (Joelsson et al., 1982).

Post-prandially, an unbuffered “acid pocket” may be demonstrated within the proximal stomach, sitting above the meal (Beaumont et al., 2010). This results from the absence of peristaltic contractions in the proximal stomach. In the presence of a hiatus hernia, this acidic reservoir sits above the diaphragmatic crura, ready to reflux back into the oesophagus.

Above the gastro-oesophageal junction, dysfunction of the oesophageal body may also predispose to GORD. Prolonged acid clearance correlates with both the severity of oesophagitis and the presence of Barrett’s metaplasia (Gillen et al., 1987; Singh et al., 1992). Failed peristalsis and hypotensive contractions can both result in incomplete oesophageal emptying and prolonged mucosal exposure to refluxate (Kahrilas et al., 1988). Any condition that chronically increases intra-abdominal pressure will also amplify GOR. Pregnancy is a
physiological example. The association of obesity has also been proven (Derakhshan et al., 2012).

**Diagnosis of GORD**

Given that gastro-oesophageal reflux (GOR) is a physiological occurrence, it is not surprising that many people regard the milder symptoms of GORD as normal. Self-medication with over-the-counter treatments is common. For those patients who present to primary care with symptoms that respond to acid-suppressive medications, no further tests are required. Patients who present with “alarm” symptoms such as dysphagia, weight loss and haematemesis require investigation with flexible upper gastro-intestinal video-endoscopy. The test serves to rule out alternative pathology such as infection, eosinophilic oesophagitis and malignancy and also to identify oesophagitis and metaplastic change.

**Ambulatory monitoring**

GORD is routinely diagnosed using 24-hour oesophageal pH monitoring. Stationary oesophageal manometry is used to accurately locate the lower oesophageal sphincter and the pH probe is then inserted. The number and duration of pH drops are recorded using an electronic recording box and compared with values recorded from studies of healthy volunteers (Zerbib et al., 2005). The patient is asked to record symptom episodes by button-press so that symptom-event correlation may be calculated.

The major limitation of stand-alone pH monitoring is that weakly acid and non-acid reflux are missed. The solution to this problem came in the form of combined pH-impedance monitoring. The addition of 8 metal rings to the catheter allows measurement of the electrical resistance across 6 oesophageal segments. The resting impedance is that of the oesophageal mucosa and the passage of liquid and gas can be detected based on their impedance properties, Figure 1-4. In this way, the timing and proximal extent of any refluxate may be recorded be it acid, weakly acid or non-acid.
Figure 1-4. The configuration of impedance and pH catheters within an intraluminal probe. The impedance drop illustrated is first detected distally before ascending to the most proximal channel. The pH trace, measured 5cm proximal to the LOS, remains above 4. This recording therefore demonstrates proximal, weakly acid reflux. [Oesophageal schematic taken from software licensed to MMS International (Enschede, The Netherlands)].

1.2.2 Treatment of GORD

The treatment of GORD may be thought of as a ladder, ranging from lifestyle modifications through to surgery.

Lifestyle

Due to the mechanical nature of GORD, simple measures to address thoraco-abdominal pressure imbalance are often effective. Weight loss and the avoidance of tight clothing are advised as appropriate. Late meals should be avoided as oesophagogastric peristalsis is diminished overnight and the antireflux effect of gravity is also lost. For patients affected by nocturnal symptoms, elevating the head of the bed may help. Smoking and excessive alcohol consumption are associated with an increased risk of GORD but there is no evidence that cessation is helpful in controlling symptoms (Nilsson et al., 2004; Kaltenbach et al., 2006). Antireflux diets have been marketed but there is no strong evidence to support their use.
Pharmacological

Non-prescription treatments for GORD include simple antacids (e.g. Rennies) and alginate raft therapies (e.g. Gaviscon). Simple antacids work to buffer gastric acid, whilst raft therapies are designed to form a layer on top of gastric contents and provide a mechanical barrier to reflux. They may also protect the mucus and cells in the oesophagus (Mandel et al., 2000).

Acid-suppression therapies provide effective relief from GORD in the majority of patients. The introduction of histamine type 2 receptor blockers (H2RBs) in the 1970s revolutionised the treatment of duodenal ulcer disease, but are less effective for GORD. Proton-pump inhibitors (e.g. Omeprazole) were introduced into clinical practice in the late 1980s and are far more effective for both symptom control and for the healing of oesophagitis. A proportion of patients have symptoms refractory to maximum dose PPI, commonly those with worse oesophagitis (Hetzel et al., 1988). Despite initial treatment success, some patients will go on to develop breakthrough symptoms, often requiring higher doses to maintain symptom control. Inadequate acid suppression or the injurious effects of non-acid components may be responsible (Kauer et al., 1995).

Prokinetics are often used in the treatment of GORD, albeit in the absence of robust clinical evidence. Cisapride, which accelerates oesophagogastric emptying through increased acetylcholine release, was proven to be effective as a treatment for GORD (McCallum et al., 1988). Concerns regarding its cardiac safety profile have since led to its withdrawal in the UK and elsewhere (Committee on Safety of Medicines, 2000). Domperidone is an antidopaminergic prokinetic that is also used in GORD, but there is limited evidence to support its use (Halter et al., 1997; Veldhuyzen van Zanten et al., 2001).

Surgery

Patients with volume reflux and symptoms refractory to maximal medical therapy may be considered for antireflux surgery. Those reluctant to use PPI therapy lifelong will also be considered by some surgeons. The majority of procedures are now undertaken laparoscopically as faster recovery times and lower rates of incisional hernia have been demonstrated (Peters et al., 2009).

The original antireflux procedure was described in 1956 by Rudolf Nissen and the principles he set out are still respected today (Nissen, 1956). The gastric fundus is mobilised and
wrapped around the gastro-oesophageal junction so as to tighten it and anchor it within the abdomen. In a randomised controlled trial in which 372 patients were followed up for five years, the remission rates for laparoscopic antireflux surgery and esomeprazole PPI treatment were equivalent. By the end of the study period, acid regurgitation was more common in the PPI cohort and dysphagia, bloating and flatulence were more common in the fundoplication group (Galmiche et al., 2011).

The operation that Nissen originally described is a 360° posterior fundoplication but tailored wraps have also been used in an attempt to reduce the risk of dysphagia and gas-related symptoms. Over the last five years, several meta-analyses have compared the outcomes of anterior partial, posterior partial and total fundoplication. The results are conflicting. Partial fundoplication may carry a lesser risk of side-effects but the relative treatment efficacy remains unclear (Ramos et al., 2011; Ma et al., 2012; Broeders et al., 2013).

**Novel techniques**

A range of innovative techniques has been developed for the treatment of GORD. Endoscopic options include suturing devices, transmural staplers, and radiofrequency ablation. Surgical developments include the placement of a flexible band of magnetic beads and implantation of an electrical stimulator. Initial studies of the surgical techniques have demonstrated encouraging results but high quality trials with extended follow-up will be required before uptake is more widespread (Bonavina et al., 2010; Rodriguez et al., 2012).
1.3 The association of reflux and IPF

Reflux and aspiration have been recognised as precipitants of acute lung injury since early in the 20th century but the relevance to chronic lung disease has only been recognised more recently (Mendelson, 1946). In 1971, Pearson reported six cases of chronic pulmonary fibrosis within a series of 143 patients with hiatus hernia (Pearson and Wilson, 1971). Mays later reported that 26 of 48 patients with idiopathic fibrosis had fluoroscopic evidence of pathological gastro-oesophageal reflux, in comparison with 23 of 270 age-matched controls (Mays et al., 1976).

El-Serag referred to endoscopic records to provide more robust evidence of the association: in an analysis of population data, the odds ratio for pulmonary fibrosis in 100,000 patients with erosive oesophagitis was 1.36 (95% C.I. 1.25-1.48) (El-Serag and Sonnenberg, 1997).

In a retrospective study, the spatial and temporal patterns of fibrosis were analysed in a group of patients with IPF. A group of 32 patients was found to have asymmetrical disease across the right and left lungs. When compared to a control group with more symmetrical disease, these individuals had high levels of GOR and numerous acute exacerbations. The authors concluded that asymmetrical IPF may be related to locoregional factors including reflux (Tcherakian et al., 2011).

Tobin made the first use of ambulatory pH monitoring to compare the rate of GOR in IPF and other interstitial lung diseases (Tobin et al., 1998). This important study reported that 16 of 17 patients with IPF had abnormal distal and/or proximal acid exposure compared with four of eight controls. Significantly, only four of the IPF patients with GORD had typical reflux symptoms such as reflux and heartburn. This finding has been reproduced consistently in subsequent studies (Raghu et al., 2006a). In addition, Tobin’s report demonstrated proximal supine reflux to be commonplace. This may have clinical relevance as the resting upper oesophageal sphincter pressure is lower at night, so transit of refluxate into the oropharynx would occur more easily (Kahrilas et al., 1987). The significance of the findings was limited by the sample size and the technique used to site the pH probe; the lower oesophageal sphincter was identified according to the increase in pH from the stomach to the oesophagus. In cases of hiatus hernia, this technique can be unreliable (Mattox et al., 1992). More recent ambulatory pH studies have made use of oesophageal manometry, improving localisation of the lower oesophageal sphincter and providing data on oesophageal motility (Savarino et al., 2013).
Only two of these studies included comparison groups. Raghu et al studied 133 asthmatics with symptoms of reflux and Soares included 28 connective tissue disease-related ILD patients (Raghu et al., 2006a; Soares et al., 2011). There was objective evidence of GORD in 68% and 82% of these groups, respectively.

More recently, the concentration of gastroduodenal biomarkers have been analysed in BAL samples in an attempt to quantify microaspiration. The stomach contains a mixture of substances including food, acid and bile salts. The physiological origin of these potential aspirates is illustrated in Figure 1-5.

**Figure 1-5. Schematic diagram to illustrate the anatomy of duodeno-gastric and gastro-oesophageal reflux (Modified from (UPMC, 2015))**

In one study, 40 IPF patients had more gastro-oesophageal reflux and higher levels of BAL pepsin and bile salts than non-IPF ILD patients and healthy volunteers. Specific methodological issues limit the significance of these findings. Bile salts were measured using a commercially available spectrophotometric assay, for which the quoted lower detection limit was significantly higher than the bile salt concentrations reported in the paper (Savarino et al., 2013).
In another recent publication, BAL pepsin concentrations were measured with a commercially available ELISA and correlated with clinical follow-up data in 54 IPF patients. The measured pepsin levels in the patients who suffered acute exacerbations during the study period were significantly higher than the levels seen in the stable group (Lee et al., 2012).

**Cause and effect in reflux and fibrosis**

The studies discussed above provide preliminary evidence of an association between GOR and IPF, but the nature of the relationship remains unclear. GOR may contribute to IPF, but the possibility that fibrosis causes GORD in people with IPF must also be considered. There are several mechanisms that could account for this latter relationship: More negative intra-thoracic pressures are required for inspiration in fibrotic lungs and this amplifies the normal gastro-oesophageal pressure gradient. Significant intra-thoracic fibrosis may contribute to the oesophageal dysmotility seen in IPF. This would result in slower oesophageal bolus clearance and prolonged oesophageal volume exposure, in turn worsening reflux symptoms and ambulatory pH results. Finally, lung fibrosis may disrupt the relationship of the crural diaphragm and the lower oesophageal sphincter, weakening the antireflux barrier.

Given the mechanical nature of these effects it may be assumed that more advanced fibrosis would be associated with more severe reflux. Such a link has never been demonstrated.

Noth studied the link between hiatus hernia and IPF using multidetector CT scanning (Noth et al.). The study population included 100 patients with IPF, in whom hiatus hernia was identified more commonly than in control patients. There was no correlation between hiatus hernia and disease severity, as assessed by lung function tests.

In an important study describing the link between anti-reflux therapy and IPF survival outcomes, Lee et al also considered radiological disease severity and physiological measures of thoracic restriction (Lee et al., 2011). GOR medication use was associated with less radiologic fibrosis and there was no correlation between GOR medication use and thoracic restriction.

Lastly, studies of ambulatory pH monitoring have failed to correlate IPF disease severity with objective evidence of reflux (Tobin et al., 1998; Patti et al., 2005; Raghu et al., 2006a).
The results of these studies are contrary to the hypothesis that pulmonary fibrosis and mediastinal distortion significantly exacerbate gastro-oesophageal reflux but the evidence is scarce and indirect. Further study is required to clarify this issue.

**Experimental models of microaspiration**

The link between gastro-oesophageal reflux, aspiration and lung disease has also been explored through animal models and in vitro. Early work originated in anaesthetics with the observation that single large-volume aspiration events were associated with significant mortality (Mendelson, 1946). Early studies outlined the acute phase pathophysiology observed in aspiration pneumonitis: in small and medium-size animal models, intra-tracheal instillation of large volumes of acidic gastric juice resulted in endothelial cell damage, increased capillary permeability, and scattered intra-alveolar haemorrhage (Teabeaut, 1952; Greenfield et al., 1969). Several hours later, an acute inflammatory response follows, comprised primarily of alveolar neutrophils and macrophages. These observations provide a useful blueprint as to the effects of gastric aspiration into the lungs, but the volumes instilled limit their relevance to chronic microaspiration.

In a pig model, Popper et al described the longer term effects of aspiration in addition to the administration of putative treatments (Popper et al., 1986). Twelve pigs had gastric juice instilled into the right main bronchus and a further nine were given either hydrochloric acid, pepsin (either dissolved or neutralised) or bicarbonate-buffered gastric juice. In the “experimental” arm of the study, a further 25 animals were treated with acid suppression therapy, antacids or kallikrein inhibitor before or after gastric juice aspiration.

The initial insult involved necrosis of pneumocytes and bronchial epithelial cells. Inflammation was propagated via the complement cascade and arachidonic acid metabolites. Macrophages predominated in the early cell response and later, fibroblasts and myofibroblasts were seen. The neutralisation of gastric juice did not prevent the development of lung fibrosis and aspiration of both hydrochloric acid and pepsin also resulted in lung fibrosis. Consistent with the findings of a previous experiment in dogs (Schwartz et al., 1980), the work by Popper was one of the earliest reports to suggest that acidity may not be critical to the lung damage observed in aspiration syndromes. The same conclusion has been reached in studies employing models of chronic aspiration and alloimmunity (Hartwig et al., 2006; Downing et al., 2008).
The link with aspiration has been supported by the findings of more recent studies. Amigoni et al developed a low mortality mouse model in which repeated small volume aspirates resulted in scar formation that was histologically evident at 2 weeks post injury (Amigoni et al., 2008). More recently, Samareh Fekri et al used another mouse model to demonstrate that repetitive acid aspiration resulted in an increase in TGF-β1 in the bronchoalveolar lavage fluid, with associated increases in fibronectin and type III and IV collagen (Samareh Fekri et al., 2014).

The mechanisms driving this fibrosis were partially elucidated by Appel et al (2007). 48 rats underwent weekly instillation of either gastric juice or normal saline for up to 16 weeks. In this study, gastric juice resulted in giant cells, fibrosis and lymphocytic bronchiolitis. There were higher macrophage and T cell concentrations, an increased CD4:CD8 T cell ratio and higher concentrations of IL-1α, IL-1β, IL-2, TNFα and TGFβ, suggesting a TH1 cytokine-dominated profile. The authors propose that the resultant lymphocyte trafficking and activation could make for an inflammatory milieu, with the individual’s response influencing the phenotypic response in the lung. Most patients would respond with a normal reparative response whereas others may be more prone to persistent fibrosis.

Interestingly, the inflammatory responses reported in these animal models appear to be largely localised to the affected lungs. Downing et al subjected rats to weekly aspiration of isolated components of gastric juice or normal saline (Downing et al., 2008). In addition to histological evaluation, serum cytokine analysis was reported, which suggested that the effects of aspiration in the model were localised.

A number of studies have been designed to clarify the effect of specific components of gastric juice. Particulate food matter has consistently been shown to be harmful and is generally associated with an intense granulomatous response. The earliest animal studies concluded that the acidity of gastric juice is a significant factor but more recent studies provide compelling evidence that the effects of gastric juice aspiration are acid-independent. Pepsin, even in isolation, appears harmful; a recent report of a rat model of chronic aspiration suggested that pepsin aspiration resulted in more marked inflammation and fibrosis than comparable quantities of gastric juice or hydrochloric acid but these findings have not been widely replicated. The available data on the role of bile acids from rodent models of aspiration is also conflicting. Some investigators have reported a toxicity profile that exceeds comparable quantities of gastric juice and provided early descriptions of the
cytokine pathways that may be responsible (Porembka et al., 1993; Su et al., 2013), whilst others report a pulmonary response equivalent to the aspiration of normal saline (Downing et al., 2008).

In a canine model, use of a balloon-catheter to induce upper airways obstruction demonstrated a significant positive correlation between negative intrathoracic pressures and acid reflux (Boesch et al., 2005). This small study (n=4) may provide evidence as to how long-term pressure changes affect gastric aspiration.

Perhaps the most likely explanation to account for these various strands of evidence is that reflux and fibrosis may both contribute to each other (Brownlee et al., 2010). Ultimately, clinical trials of the effectiveness of antireflux therapy in IPF are required.
1.3.1 Pharmacological treatment of GORD in IPF

Published data on the treatment of GORD in IPF come from retrospective studies reporting on heterogeneous patient groups. To date, two have focused on the medical management of reflux disease.

In 2006, Raghu reported a series of four patients with IPF who declined conventional medical therapy with steroids or immunomodulation (Raghu et al., 2006c). Beyond home oxygen, the only treatment was directed at acid suppression: all patients were treated with proton pump inhibitors and one with fundoplication in addition. In contrast to the intractable decline in lung function normally seen in the disease, these patients demonstrated either stabilisation or improvement of their pulmonary function tests. The patients were followed up for a mean of 55 months, during which time no acute exacerbations were suffered and no specific treatment for respiratory problems was required. The significance of these results is clearly limited by the sample size. In addition, oxygen therapy is now recognised as an effective treatment adjunct in IPF (Morrison and Stovall, 1992; Raghu et al., 2011).

Lee et al reported a retrospective review of 204 IPF patients that sought to assess the impact of antireflux therapy (Lee et al., 2011). Use of antireflux medication was independently associated with lower radiological fibrosis scores and longer survival. The retrospective, uncontrolled design of the study precludes any formal comparison between the two groups. In addition, the use of acid-suppressive medication does not exclude gastro-oesophageal reflux; a significant proportion of patients with IPF have abnormal GOR despite taking PPI (Raghu et al., 2006a).

Recently, the effect of high dose acid-suppression therapy on cough was studied in a small group of IPF patients (n=14) (Kilduff et al., 2014). After eight weeks, there was a significant decrease in the number of acid reflux events but no change in cough frequency, as measured with a 24-hour cough monitor. Interestingly, non-acid reflux events increased (p=0.01).

One of the most challenging aspects of this area of clinical management is that PPIs and H2 receptor blockers (H2RBs) can only increase the pH of refluxate (Vela et al., 2001; Hemmink et al., 2008). The frequency of reflux events is unaffected and it is unclear whether the volume of refluxate is reduced. It is possible that reducing the volume of gastro-oesophageal refluxate may serve to concentrate non-acid components, resulting in a more injurious microaspiration.
1.3.2 The role of antireflux surgery in IPF

Effective gastro-oesophageal reflux therapy requires a mechanical barrier and the only proven technique to date is antireflux surgery. Such intervention requires general anaesthesia and mechanical ventilation for the duration of the procedure. People with IPF have impaired respiratory function and, often, significant comorbidity. Relatively few are good surgical candidates. In a disease as rare as IPF, these factors make the design of research studies challenging.

Nadrous et al reported the first study of fundoplication in interstitial lung disease in abstract form in 2003. Of the 15 patients included, 13 had IPF and their outcomes were mixed. Five patients had follow-up data available. Pulmonary function tests suggested that two stabilised and three deteriorated.

Linden et al reported on 14 IPF patients awaiting lung transplantation, in whom fundoplication resulted in a significant stabilisation in oxygen requirement when compared with 31 non-fundoplicated IPF controls (Linden et al., 2006). No significant differences in pulmonary function or 6-minute-walk tests were observed.

More recently, Hoppo reported an experience of 43 end-stage lung disease patients (including 14 with IPF) undergoing antireflux surgery before or after lung transplantation (Hoppo et al., 2011). FEV1 improved in both the pre- and post-transplant groups at one year post antireflux surgery (p<0.01). Episodes of pneumonia and acute rejection were significantly reduced in post-transplant patients (p=0.03) or stabilised in pre-transplant patients (p=0.09). The results are not stratified by lung disease and the proportion of patients transplanted for IPF (3 of 24) reflects the rarity with which this treatment option is deployed in IPF. In addition, the vagotomy often associated with lung transplantation generally results in a pattern of dysphagia not otherwise seen. The applicability of these data to the IPF cohort as a whole is therefore limited.

These preliminary reports suggest that there may be a role for antireflux surgery in a well-selected subset of IPF patients. The limited nature of the evidence base reflects the difficulties encountered in this area of research: IPF is rare and patients often have significant comorbidity. Furthermore, the natural history of the disease means that the benefits of surgery can be difficult to show (section 1.1.1).
Two key points emerge from this preliminary literature. In controlled studies of IPF, antireflux surgery has been associated with an improvement in measurable physiology, but not functional status or survival. Secondly, the benefits that have been shown across the groups who have undergone antireflux surgery as a whole have been modest. It is possible that more effective patient selection, for example using biomolecular markers of microaspiration, might yield more favourable results.

A multicentre randomised controlled trial to investigate antireflux surgery in IPF is ongoing in the United States (Collard *et al.*, 2013a). WRAP-IPF (Weighing Risks and Benefits of Laparoscopic Anti-Reflux Surgery in Patients With Idiopathic Pulmonary Fibrosis) randomises patients to receive either antireflux surgery or standard antireflux therapy, as per clinician discretion. The recruitment target is 58 and to be eligible, subjects must be willing to undergo antireflux surgery and fit enough to do so. The primary endpoint is decline in FVC between enrolment and 48 weeks.

This is an important study but it is unlikely that the findings will directly inform clinical practice. The paucity of pilot data indicates that the recruitment target is a pragmatic one. The likelihood of a positive result is therefore unclear. The relevance of antireflux surgery to the IPF patient group as a whole will be difficult to assess. In addition, no quality of life analysis is planned.

Even as better evidence becomes available, decisions will remain challenging for clinicians and patients alike. Section 1.5 below considers some of the techniques that may be used to explore patient attitudes and facilitate shared decision-making in this challenging area of clinical practice.

### 1.4 Detection of proximal reflux and pulmonary aspiration

#### 1.4.1 Oropharyngeal pH measurement

The detection of gastro-oesophageal reflux is established in clinical practice and helps to identify a group of patients who may be at increased risk of pulmonary aspiration. For aspiration to occur, the material must ascend the length of the oesophagus and pass through the pharynx. In theory therefore, more proximal detection of gastro-oesophageal refluxate is an appealing technique in the IPF population.
Laryngopharyngeal reflux (LPR) is the condition in which the detection of proximal reflux has been most extensively appraised. Sufferers are thought to experience their symptoms of hoarseness, cough and sore throat because of exposure of the sensitive laryngopharyngeal mucosa to gastric contents.

At flexible laryngoscopy, characteristic visual changes have been described in association with LPR. These include erythema, oedema, hypertrophy, mucus and granulation within the hypopharynx, Figure 1-6. The Reflux Finding Score was designed to record the severity of these changes and is well validated (Belafsky et al., 2002). The significance of these findings is not universally accepted as they have also been shown to occur in 64-86% of healthy controls (Reulbach et al., 2001; Hicks et al., 2002; Joniau et al., 2007).

Figure 1-6. Appearance of the hypopharynx at flexible endoscopy: 1) Normal  2) Diffuse erythema at the vocal folds and arytenoid walls  3) Severe oedema in the posterior larynx  4) Vocal fold granuloma. Images reproduced from (Park, 2013; Vivero, 2015)

The biochemical detection of supra-oesophageal reflux is also challenging: oropharyngeal refluxate has been shown to exist predominantly as an aerosol and conventional pH probes have poor sensitivity for the droplets of refluxate (Kawamura et al., 2004; Kawamura et al.,
Supra-oesophageal pH probes are also susceptible to drying effects which results in widespread artefact (Harrell et al., 2007). Pharyngeal reflux events have been demonstrated in 30-35% of normal controls (Merati et al., 2005; Ulualp et al., 2005).

The Dx-pH probe is a novel “ion-flow sensor” that has been developed to measure oropharyngeal (OP) pH (Restech, San Diego, CA). It is an ambulatory transnasal device which, according to its manufacturers, can detect the pH change resulting from aerosolised refluxate within the pharyngeal environment. Normative OP pH ranges have been defined although the studies are relatively small and the exact thresholds remain controversial (Ayazi et al., 2009; Chheda et al., 2009; Sun et al., 2009).

The results of early studies were promising. An in vitro model of supra-oesophageal reflux was used to demonstrate the Dx-pH probe provided faster, more accurate detection of pH than conventional pH probes (Yuksel et al., 2013). In another small clinical study of 15 patients, all pharyngeal “reflux” events were preceded by distal oesophageal pH drops (Wiener et al., 2009).

Larger analyses have since correlated the findings of the Dx-pH probe with the results of multilumen oesophageal pH-impedance monitoring and the findings are less convincing. In these studies, the proportion of pharyngeal refluxes that correlated with conventionally defined oesophageal reflux events was reported as 0-17%, 10-15%, 18% and 37-55%, respectively (Becker et al., 2012; Ummarino et al., 2013; Hayat et al., 2014b; Mazzoleni et al., 2014). Proponents of the technology would argue that this supports a higher sensitivity for the oropharyngeal probe. Concerningly, 5-35% of the refluxes defined by the Dx-pH probe were found to correlate with swallows, as demonstrated by impedance analysis (Ummarino et al., 2013; Mazzoleni et al., 2014).

The significance of a measurable drop in OP pH therefore remains unclear. The pH variation of exhaled breath and acid produced by OP bacteria may both interfere with results (Mazzoleni et al., 2014). To date, the ENT community have yet to recognise the clinical utility of dual-probe pH monitoring or the Dx-pH probe.

**1.4.2 Scintigraphy**

In both adults and children, radionuclide imaging has been assessed as a technique for the detection and quantification of gastro-oesophageal reflux. Typically, a volume of dilute
Technetium-99 is ingested and subsequently a gamma camera is used to assess the distribution of the radiolabel within the body. In some centres, gastro-oesophageal reflux scintigraphy is used routinely in paediatric practice but it has not gained widespread acceptance in adults (Thomas et al., 2003). The use of radionuclide imaging to assess pulmonary aspiration secondary to gastro-oesophageal reflux remains experimental.

Gastro-oesophageal reflux and pulmonary aspiration were assessed in a group of 51 patients with refractory respiratory symptoms, using a combination of ambulatory pH monitoring and Tc-99 scintigraphy (Ravelli et al., 2006). Thirteen of 51 had evidence of gastro-oesophageal reflux on pH monitoring. 25 of 51 had evidence of pulmonary aspiration based on scintigraphy. Interestingly, only 6 of the 25 patients with aspiration had abnormal pH results. The authors suggest non-acid reflux as an explanation for these findings (Zerbib et al., 2005).

The ability of scintigraphy to detect pulmonary aspiration was also assessed in two smaller studies of patients (combined n=23) with confirmed GORD and co-existent pulmonary symptoms (Ghaed and Stein, 1979; Fawcett et al., 1988). Neither supported the sensitivity or specificity of the technique in this role.

1.4.3 Pepsin measurement in sputum and saliva

The invasive nature of ambulatory probe testing has prompted the development of non-invasive methods. Peptest is a commercially-available, near-patient test which has been designed to provide a diagnosis of GORD (Strugala et al., 2007)(RD Biomed, Hull, UK). It is a lateral flow device which makes use of two unique human monoclonal antibodies to pepsin 3. The premise of the technique is that the presence of pepsin in a sample of sputum/saliva represents evidence of retrograde flow of gastric contents. Minimal laboratory equipment is required and the result is returned in around 15 minutes and read with the naked eye or a meter, in a manner similar to a urinary pregnancy test (Figure 1-7).
To date, the technology has not been extensively validated. Saritas Yuksel et al used a modified version of the Peptest which incorporated semi-quantitative colorimetry to differentiate between sterile water and gastric juice using a receiver operating characteristics (ROC) curve analysis (Saritas Yuksel et al., 2012). The sensitivity and specificity were reported as 87%.

Three clinical validation studies have analysed the performance of the test in comparison with the combination of ambulatory probe testing and endoscopy as a gold standard. In the second phase of the study mentioned above, the sensitivity and specificity of the lateral flow device to identify those patients with objective evidence of GORD, defined as supranormal pH and or endoscopic oesophagitis, was reported as 50% and 87%, respectively (Saritas Yuksel et al., 2012). Against a population prevalence of 47%, the positive predictive value was 85%.

Peptest has also been evaluated as a means to differentiate between patients with symptoms due to reflux and patients with symptoms related to reflux (Hayat et al., 2014a). The significance of this distinction is that the first group, comprising individuals with supranormal levels of reflux or a hypersensitive response to normal reflux, should be treated with medical or surgical antireflux therapy. In contrast, individuals in whom there is no correlation between symptoms and reflux events can be described as having functional heartburn (FH). Aggressive treatment is not indicated in this group.

De Bortoli et al compared the results of the lateral flow device with MII-pH studies in a small group of subjects with non-erosive reflux disease (NERD, n=16), hypersensitive oesophagus (HO, n=12) or without evidence of reflux disease (n=7)(de Bortoli et al., 2012). Peptest was

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Figure 1-7. Pepsin lateral flow device showing the result of a gastric juice sample positive for pepsin. C=control band. T=test sample band. Reproduced from (Saritas Yuksel et al., 2012).
positive in 93.7% of NERD, in 58.3% of HO, and negative in 100% of normal subjects. Whilst the sensitivity and PPV were both reported as 100%, the specificity and negative predictive value were significantly lower at 79% and 54%, respectively.

Hayat et al published a larger validation study, again comparing the lateral flow device with MII-pH monitoring (Hayat et al., 2014a). Three saliva samples were taken from each of 87 healthy volunteers and 111 subjects with typical symptoms of GORD. Based on ambulatory monitoring, the symptomatic group were subdivided into individuals with GORD, HO or FH. If at least one sample was positive, the test showed a sensitivity of 77.6% and specificity of 63.2%, with a negative predictive value of 80.4%. Using a modified version of the lateral flow device, the positive results were quantified and the highest readings (>210ng/ml) offered a specificity and positive predictive value in excess of 95% (D. Sifrim, personal communication).

Proponents of the lateral flow device suggest a potential for it to guide the use of invasive testing for GORD. With further development, such a role may be possible in the future.

1.4.4 Bronchoalveolar lavage

The most compelling evidence for recurrent pulmonary aspiration would come from direct bronchoalveolar detection and quantification of substances which originate exclusively in the stomach. At bronchoalveolar lavage, a narrow bore flexible endoscopy is used to inject and then aspirate a standardised volume of isotonic solution into the bronchoalveolar space. Analysis of the aspirate permits direct measurement of the environment within the distal airways. Cytological and biochemical measurement techniques have been used.

Cytological markers

In early paediatric studies, microscopy and lipophilic staining of bronchoalveolar lavage fluid was regarded as the gold standard for the detection of pulmonary aspiration. Oil Red O and Sudan Red G are the most commonly used stains (Corwin and Irwin, 1985; Colombo and Hallberg, 1987; Nussbaum et al., 1987).

Several studies demonstrated that the absolute presence or absence of lipid-laden alveolar macrophages (LLAMs) was not a good predictor of aspiration. As a result quantification systems have been described based on the number of LLAMs and the density of intracellular staining (Colombo and Hallberg, 1987; Kitz et al., 2012).
In many of the validation studies, the results of single or dual-probe pH monitoring has been compared with a specified index of LLAMs. The majority of studies report on children and demonstrate statistically significant associations between the results of pH monitoring and LLAM counts (Colombo and Hallberg, 1987; Nussbaum et al., 1987; Ahrens et al., 1999; Bibi et al., 2001; Sacco et al., 2006; Borrelli et al., 2010; Hopkins et al., 2010).

Negative studies have also been reported (Sacco et al., 2000; De Baets et al., 2010; Kitz et al., 2012). In the largest of these, Kitz et al compared a LLAM index with dual probe pH in 446 children with bronchitis, bronchial hyper-reactivity or recurrent pneumonia. In their experience, there were no correlations between LLAM count and any reflux parameters, even when the analysis was restricted to those with reflux (Kitz et al., 2012).

To date, a single study has compared MII-pH monitoring with LLAM quantification (Borrelli et al., 2010). In a group of 21 children with suspected pulmonary aspiration, LLAM counts were positively correlated with the total number of reflux episodes, the number of non-acid reflux episodes and the number of proximal non-acid reflux episodes (coefficients r=0.73, 0.61 and 0.64, respectively.

**Pepsin**

Pepsin is an acid protease released in the stomach. Detection in the oesophagus provides evidence of gastro-oesophageal reflux and, with bronchoalveolar lavage, pepsin can be used as a biomarker for pulmonary aspiration.

Pepsin was the first human enzyme described and its nomenclature over the years has been somewhat confused (Florkin, 1957). High performance chromatography has led to the identification of six pepsin subtypes in humans (Roberts, 2006). Subtypes 1-4 are now collectively known as pepsin A and subtypes 5 and 6 are known as pepsin C (Roberts, 2006).

Pepsin is released from chief cells in the stomach as the precursor pepsinogen and then activated to pepsin by hydrochloric acid, released from gastric parietal cells. Pepsin exhibits maximal proteolytic activity from pH 2-4 (Johnston et al., 2003; Bulmer et al., 2010). It remains active up to pH 6, above which it becomes inactive. This change is still reversible up to pH 7.5 (Pearson et al., 2011). The stability and residual activity of pepsin at higher pH is clinically relevant as weakly acid reflux is common in IPF. Additionally, re-exposure to acid in the oesophagus, pharynx or lungs could lead to reactivation. This could occur with a second wave of reflux or an acidic drink.
Several groups have established pepsin quantification assays, which fall into two categories. Activity assays measure the rate of liberation of small soluble peptide fragments, which are easily detected by absorbance (Roberts, 2006). Activity can also be quantified by determination of liberated amino groups from cleavage of peptide bonds, the N-terminal assay (Hutton et al., 1990). Alternatively, pepsin concentration can be measured by immunoassay. Several techniques have been developed and commercial kits are also available (Metheny et al., 2002; Farrell et al., 2006; He et al., 2007; Stovold et al., 2007). In the experience of our research group, the lower limit of detection is as low as 1ng/ml (Stovold et al., 2007). The concentration of pepsin within gastric fluid has been measured at 100-600 µg/ml (Wallace, 1989; Gotley et al., 1991). Given that bronchoalveolar lavage results in a 100-200 fold dilution, concentrations of pepsin may be as low as 0.5-6 µg/ml. In studies of pepsin as a biomarker for aspiration, reported lower limits of detection range from 1 ng/ml to 1 µg/ml (Metheny et al., 2002; Stovold et al., 2007).

To date, pepsin has been utilised as a biomarker of aspiration in the study of lung transplantation, laryngopharyngeal reflux and a range of non-transplant lung conditions. Quantification in bronchoalveolar lavage fluid is nonetheless associated with a number of limitations. Standardisation of any biochemical quantification in BAL is challenging. The pulmonary airways are a complex anatomical space and the volume and distribution of fluid within them is variable. In addition, the methodology of bronchoalveolar lavage has not been standardised so the results of different experiments are difficult to compare (Wells, 2010). Secondly, the decay of pepsin within the pharynx and lungs is not well understood. The temporal significance of pepsin detection is therefore unclear (Bohman et al., 2013).

Production of pepsin within the lung has been reported. In vitro, pepsinogen C has been identified as a product of type 2 alveolar epithelial cells, thought to play a role in the processing of surfactant protein B (Gerson et al., 2008). Pepsin C has also been detected in the bronchoalveolar fluid of subjects with no clinical suggestion of reflux: Elabiad et al performed post-mortem immunohistochemical staining on neonatal pulmonary and gastric tissues (Elabiad and Zhang, 2011). Pepsinogen A was detected in 12 of the 13 stomach sections, mainly in the chief cells, but not in any lung sections. Pepsinogen C was detected in all stomach sections in chief and mucus cells and in 9 of the 16 lung sections, mainly in type II pneumocytes. In an adult study, 51 adult patients undergoing elective orthopaedic surgery
had pepsin A and pepsin C concentrations derived from a small volume bronchoalveolar lavage (Bohman et al., 2013). Eleven individuals (22%) had measurable pepsin concentrations, all of which had pepsin C but not pepsin A. Whilst endotracheal intubation introduces the possibility of iatrogenic aspiration, this is unlikely to be a major confounder in patients who underwent uncomplicated intubation for elective surgery (Bohman et al., 2013).

**Bile acids**

Bile acids are steroid acids synthesised in the liver and released into the duodenum. They act as detergents, converting dietary fats into particles (micelles) which can be absorbed in the intestine. Duodenogastric reflux is a physiological event within the normal population, so bile acids can also be used as a marker of gastro-oesophageal reflux and pulmonary aspiration (Klokkenburg et al., 2009).

Cholic acid and chenodeoxycholic acid are the two primary bile acids produced from cholesterol in the liver. They are dehydroxylated into the secondary bile acids deoxycholic acid and lithocholic acid by bacteria in the colon. Bile acids are then conjugated with the amino acids taurine and glycine in the liver to form bile salts. In humans taurocholic acid and glycocholic acid (from cholic acid) and taurochenodeoxycholic acid and glycochenodeoxycholic acid (from chenodeoxycholic acid) are the major bile salts in bile (Hofmann, 1999). The concentration of these four components is roughly equal.

As a biomarker for aspiration, bile acids have been studied extensively within the lung transplant literature. Most studies have used one of two commercially available spectrophotometric detection methods for quantification. These rely on the action of a steroid dehydrogenase generating reduced nicotinamide adenine dinucleotide (NADH). Both methods were developed for serological assessment of liver disease and their use in the measurement of bronchoalveolar lavage fluid samples is contentious. In serum, the normal range of total bile acids is quoted as ≤6 to ≤10 µmol/l (Korman et al., 1974; Wong et al., 2008). The kit manufacturers claim lower limits of detection of 1 µmol/l. Despite this, major reports in the transplant literature include measured levels as low as 0.2 µmol/l using these techniques (D'Ovidio et al., 2005; Blondeau et al., 2008).

The most accurate method with which to detect bile acids is tandem mass spectrometry (TMS), which is widely used in the setting of neonatal screening in high volume reference
laboratories. Parikh et al compared the results of TMS and commercially available spectrophotometric techniques in the quantification of bile acids in gastric juice and bronchoalveolar lavage fluid (Parikh et al., 2013). In the latter study, the lower limits of detection for TMS and spectrophotometric analysis were 0.1 and 5 µmol/l, respectively. This latter figure is consistent with the findings of Klokkenburg et al (2009).
1.5 Evaluating patient attitudes towards antireflux therapy in IPF

As discussed above, the investigation and management of gastro-oesophageal reflux in idiopathic pulmonary fibrosis is a challenging area of practice. There are no validated techniques with which to quantify chronic aspiration. Definitive therapy, in the form of antireflux surgery, is associated with greater risk in an elderly population with significant comorbidities. It is therefore essential that patients are properly informed about the limitations of the current evidence base before they agree to invasive tests and, potentially, to surgical treatment.

For clinicians, the role of reflux and aspiration in lung disease is emerging. The ability to effectively communicate the risks and benefits is hampered by a weak evidence base and limited clinical experience. Within the constraints of a time-limited medical consultation, it would be difficult to fully convey the uncertainties of therapy in such a way that patients could make an informed choice about their care. Similarly, it is crucial that patient attitudes inform the direction of future research.

The logical response to this challenge is to investigate patients’ attitudes towards the potential risks and benefits associated with possible therapies, by exploring how IPF patients value their own quality of life and to assess the level of risk they would accept for a chance to stabilise their future health.

There are two sides to this question. The first relates to the burden of IPF as a disease state and the second to the risks and benefits that may be attributed to antireflux therapy. For patients with IPF and minimal reflux symptoms, the benefit of antireflux therapy may be closely linked to their pulmonary symptoms or the prognosis attached to their lung disease.

At present, the natural history of IPF remains unclear, and the respiratory benefits of antireflux surgery are even less well understood. For these reasons, a balanced assessment of patient attitudes towards antireflux therapy in IPF is not feasible. Nonetheless, if clinicians could be afforded a better appreciation of how their patients perceive the respiratory burden of their disease as compared with the burden of antireflux surgery, a more informed consultation might result. The following section explores the measurement of health and healthcare and describes how such an analysis might be constructed.
1.5.1 Measuring health

With ageing populations, diminishing resources and increasingly complex therapies, healthcare budgets are under ever greater pressure. Quantifying the perceived value of a given health state, or the benefit derived from an intervention, is increasingly important. Governments rely on such analyses in order to maximise efficiency in healthcare spending. These pressures have contributed to significant developments in health economics in recent years. Before resources can be deployed, health must first be measured.

Certain indicators are unambiguous; infant mortality rate would be an example. As expectations of healthcare have risen over the years, perceptions of health have shifted away from mortality, through functional ability and on to quality of life. The measurement of these concepts is increasingly complex, and modern day health economics draws influences from psychometrics, consumer theory and decision analysis.

When two different treatments for the same disease are to be compared, a cost-effectiveness analysis may be undertaken. Two different antihypertensives may be compared in terms of pounds per mmHg blood pressure reduction. In contrast, evaluating the effectiveness of joint replacement as a treatment for arthritis would require a different unit of measurement. Deciding how much of a limited healthcare budget to spend on competing interventions requires a method of comparing the generic value they provide. For the purposes of the current study, the concept of generic valuation is crucial. The morbidity associated with IPF is very different from that associated with reflux disease and its treatment.

How to measure that generic value has been the subject of extensive debate. One available method is contingent valuation, a survey-based approach in which people are questioned on their willingness to pay for a specific resource (Diamond and Hausman, 1994). Valuation is contingent upon a specified hypothetical scenario. Contingent valuation has not been widely used to evaluate healthcare provision because, in general, healthcare is not directly paid for at the point of access. Secondly, willingness-to-pay reflects an individual’s wealth, which can skew results (Gold et al., 1996).

The more common approach is the quality-adjusted life year (Weinstein et al., 1996; National Institute for Health and Care Excellence, 2012). QALYs measure the quantity and quality of life that results from a healthcare intervention. A lung transplant might be deemed
to increase quality of life by 25% for ten years would be said to offer 2.5 QALYs (Torrance and Feeny, 1989). Further, by calculating the total cost of a lung transplant, the cost per QALY could be defined. QALYs have now become de facto currency with which to measure health effects in the NHS (National Institute for Health and Care Excellence, 2013).

The use of QALYs requires the assignment of weights to represent the quality of the health state in question. Such valuations should reflect the experience of those individuals affected by the condition in question, as well as the preferences of the population who provide the relevant funding.

### 1.5.2 Scaling, value and utility

The simplest way to quantify estimates of healthiness is to ask directly for a numerical answer. Subjects might be asked how bad their pain is on a scale of 0 to 100. The answers from such tasks will be highly subjective and do not provide easily comparable data. Measurement thus requires the assignment of numerical scores to some form of description. There are a wide variety of scaling techniques, with different forms generating data that might be nominal, ordinal or interval in nature.

Scaling procedures generally fall into two main categories, which can be considered psychometric and econometric (McDowell, 2011). Psychometric scaling procedures generally rate feelings, opinions or attitudes and concern the present. These techniques make use of values, which refers specifically to preferences expressed under conditions of certainty. Econometric scaling, by contrast, derives from studies in economics and decision analysis of consumer choices between products, often under conditions of risk or uncertainty. For health economists, the concept of uncertainty is inherent to the judgments and preferences that relate to the future as well as the present. Practically, utilities are simply numbers ranging on a scale from 0 (the worst) to 1 (the best).

In healthcare evaluation, utility is generally defined in accordance with von Neumann and Morganstern’s Theory of Games and Economic Behavior (von Neumann and Morgenstern, 1944). Often denoted as vNM utility, the model explains how a rational individual “ought” to make decisions when faced with uncertain outcomes so as to achieve the best outcome (Torrance and Feeny, 1989; Dolan et al., 1996b).
When QALYs are used to measure a health benefit, time provides the measure of quantity and utilities provide the measure of quality.

1.5.3 Measurement of utilities

Amongst the various methods used to measure health state utility, three predominate: the rating scale, the time trade-off and the standard gamble. The following sections outline the processes involved and the theoretical basis for these tools. The empirical evidence supporting them is discussed in section 1.5.4 below.

Rating scale

The rating scale is the most straightforward technique and consists simply of a line, with anchoring points defined at the two ends. This is sometimes represented as a thermometer. The two extremes might be identified as zero or death at one end and 100 or perfect health at the other. Respondents have a health state described to them and they simply draw a mark on the line to express their perception of the health-related quality of life between the two anchoring states provided. The physical location of the mark on the line is taken to represent the respondent’s utility for the specified health state.

The theoretical validity of this method has been challenged as there is no explicit choice or sacrifice involved (Brazier, 2008). The rater does not have to justify their response in terms of time, money or any other unit of value, so the response may be highly arbitrary. Utilities determined using a rating scale fail to satisfy the criteria of an interval scale: the difference between utilities of, for example, 0.2 and 0.3 cannot be taken as equivalent to the difference between utilities of 0.8 and 0.9 (Richardson, 1994). In addition, there is no transparent relationship between the units on the scale, on the one hand, and the measure of welfare or preference that is believed to be appropriate to resource allocation (Green et al., 2000).

Time trade-off

In a time trade-off (TTO) experiment, respondents are asked whether they would prefer to spend a given length of time in a defined health state of less-than-perfect health or an alternative duration in perfect health, after which the outcome is death. At the start of the experiment, the respondent is asked to choose between ten years in perfect health and ten years in a health state representing less-than-perfect health, and would be expected to opt
for the former. The duration of time available in perfect health is then incrementally decreased until the respondent is indifferent between the two options. The point of indifference indicates the utility score: if a patient was indifferent between 10 years in a defined health state and six years of perfect health, the utility for that health state would be recorded as 0.6.

TTO incorporates a choice based on sacrifice, as respondents must consider the opportunity cost of extra years of life. Respondents are asked directly about the specific number of years in full health which they value as equal to a (longer) period in the health state being measured. Thus it collapses the relationship between the duration and the value of a health state into a single measure. Critics of the technique argue that TTO relies on the unproven assumption that longevity is proportional to utility (Green et al., 2000).

A further criticism of TTO relates to the assumption that individuals are consistently prepared to trade a proportion of their remaining years of life in order to improve their health status. Empirical data suggest this may not be true (Dolan et al., 1996b).

**Standard gamble**

In the standard gamble respondents are asked to choose between two alternatives, where one is the certainty of a defined health state (\(i\)) and the other is a gamble with two possible outcomes (Torrance, 1986). The two outcomes in the gamble are defined such that one is more favourable and one is less favourable than the health state in question. Conventionally, the two outcomes are a return to full health (with probability \(p\)), and immediate death (probability \(1-p\)).

At the outset the probability of the favourable outcome is set at 100%. At this stage, respondents would be expected to opt for the gamble. As the interview proceeds, the relative probabilities of the two gamble outcomes is altered. The conclusion is the point at which the respondent is indifferent between the certain outcome (the defined health state) and the gamble. At this point, the probability \((p)\) of the favourable gamble outcome is recorded as the utility value for the health state in question.
The inclusion of a gamble, with two possible outcomes, means that respondents make their decisions under uncertainty. As a result, the standard gamble is generally considered as most appropriate to the measurement of vNM utility and thus commonly regarded as the gold standard for cost-utility analysis (Mehrez and Gafni, 1989; Torrance and Feeny, 1989).

This special status is not universally accepted. The strongest challenge relates to the “specific utility associated with risk, which varies between respondents” (Richardson, 1994). Richardson argues that many individuals would associate the process of gambling with anticipation and excitement and for others, it would confer feelings of anxiety and fear. The range of attitudes to risk can be said to introduce an additional factor, unrelated to the respondent’s utility for a given health state. Others argue that varying attitudes to risk are no different from varying attitudes towards time (Green et al., 2000).

One practical criticism is that respondents may struggle to understand risk as represented by probabilities. Standard gamble boards have therefore been developed to help visualise the effect of changing probabilities within the gamble outcomes. With the use of a board, good completion rates have been reported (Morss et al., 1993).

### 1.5.4 Empirical support for measurement tools

The choice-based methods are the focus of the remainder of this section, which considers the practical evidence to support the use of the standard gamble (SG) and the time trade-off (TTO).
Validity

Health state utility can be regarded as a theoretical construct, as preferences are never truly revealed. The absence of a gold-standard makes it difficult to validate the techniques with which utilities are derived. The fact that healthcare expenditure can be determined by the results these techniques generate makes this a significant challenge. The standard gamble, for example, tends to generate higher utility values than other methods (Torrance et al., 1996).

Studies designed to validate the different utility measurement techniques have largely relied on comparison with a predetermined rank ordering. Such a ranking might originate from an individual’s stated preferences, or from a logical comparison within a set of health state definitions. Another approach involves concurrent validation, which describes how well two methods agree with each other. If two techniques generate comparable results, they are likely to value similar aspects of health-related quality of life (Torrance, 1976; Read et al., 1984).

In a study of 350 respondents, Dolan et al made use of the EQ5D-3L multi-attribute scale to compare the standard gamble and the time trade-off (Dolan et al., 1996b). EQ5D-3L is a written health-related quality of life survey in which respondents select one of three statements for each of five domains best representing their health at the time of response. The domains relate to generic factors such as pain and mobility. Any combination of five responses can be taken to represent a hypothetical health state. The nature of the statements within EQ5D-3L is such that certain health states are inherently more attractive than others: respondents would be expected to prefer to have “no problems in walking about” than “some problems in walking about”. The extent to which the SG and the TTO evaluated the EQ5D-3L health states in accordance with their rational ordering was assessed. Whilst there was no significant difference in the results, TTO performed slightly better: reported logical consistency was 91.7% vs 83.8% for SG. The statistical significance of this result is not reported.

In a more recent study of 96 respondents with irritable bowel syndrome, the construct validity of the SG and TTO were compared through correlation of the derived utilities with the results of disease-specific and generic questionnaires (Puhan et al., 2007). In this analysis, the results of the SG were significantly better correlated with the results of the other tools than were the utilities generated by the TTO.
The convergent validity of the SG and TTO were compared with the results of the Short Form-36 HRQOL scale in another study of 878 subjects, made up of patients under treatment for heart disease and healthy volunteers (Lalonde et al., 1999). Overall, the correlation of the TTO was slightly higher than that of the SG with reference to SF-36 General Health Perception score (0.36 vs 0.30).

In a similar study of 25 patients with the condition systemic lupus erythematosus, Moore et al demonstrated that TTO had better correlation with SF36 subscales than SG (Moore et al., 1999).

When these results are assimilated, there is no clear signal from the published literature that either of the choice-based methods has superior validity.

**Reliability**

In cost-utility analysis, reliability measures the stability of a tool between two administrations assuming a constant health state. This can be assessed over time or between raters. The majority of studies have focused on test-retest reliability, as summarised in Table 1-3.

<table>
<thead>
<tr>
<th>Test-retest time period</th>
<th>Standard gamble</th>
<th>Time trade-off</th>
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<tbody>
<tr>
<td>≥1-week</td>
<td>0.80 (O’Connor et al., 1987)</td>
<td>0.87 (O’Connor et al., 1987)</td>
</tr>
<tr>
<td>6- to 16-week</td>
<td>0.63* (Dolan et al., 1996a)</td>
<td>0.83* (Dolan et al., 1996a)</td>
</tr>
<tr>
<td>1-year</td>
<td>0.53 (Torrance, 1976)</td>
<td>0.62 (Torrance, 1976)</td>
</tr>
<tr>
<td>Other (time-specified)</td>
<td>0.82 (Reed et al., 1993)</td>
<td>0.74 (Reed et al., 1993)</td>
</tr>
<tr>
<td></td>
<td>0.8* (Gage et al., 1996)</td>
<td>0.67-0.92* (Gage et al., 1996)</td>
</tr>
<tr>
<td></td>
<td>0.84* (Kim et al., 2012)</td>
<td>0.83* (Kim et al., 2012)</td>
</tr>
</tbody>
</table>

When the test-retest reliability was reported on the group level, as opposed to the individual level as in the studies summarised above, the results are significantly improved (Green et al., 2000).

Overall, comparative studies report slightly higher reliability for the time trade-off than the standard gamble, but it is not possible to choose one method over another based on this criterion.
To date, the majority of studies have employed the TTO but this may relate to the fact that it is quicker and therefore cheaper to administer. In the UK, the National Institute for Clinical Effectiveness published a statement that was mirrored by that of Panel on Cost-effectiveness in Health and Medicine in the U.S (Weinstein et al., 1996; National Institute for Health and Care Excellence, 2008). Both recommend the use of choice-based methods in the measurement of health state utilities, but neither specifies a single technique.

1.5.5 Choice of respondents in health state valuation

Health state utilities have been measured amongst patients, amongst clinicians and amongst sample populations drawn from the general public. Each method is associated with advantages and disadvantages.

Patients who have personal experience of a given condition will have the best understanding of what it means to live with that condition, and the effect that it has on their lifestyle and quality of life. This is reflected in a statement from NICE, suggesting that patients should ideally be used in the generation of utilities (National Institute for Health and Care Excellence, 2008).

One concern with patients as respondents is the so-called disability paradox: many people with serious and persistent disabilities report that they experience a good or excellent quality of life, when to most external observers their quality of life seems poor (Albrecht and Devlieger, 1999). Empirically, the health state utilities reported by patients with first-hand experience of a condition tend to be higher than the values assigned by members of the general public given detailed descriptions of that condition (Sackett and Torrance, 1978; Froberg and Kane, 1989).

Clinicians have been used in cost-utility analysis studies. After direct contact with patients affected by a given condition, clinicians provide a perspective on the range of health states that might be associated with a given disease. In addition, clinicians may be able to frame that perspective within the context of other health problems. Clinicians have been reported to overestimate the gravity of a condition, which may represent efforts to increase its status or resource allocation (Torrance and Feeny, 1989).

Non-patients have also been used to measure utility. In a publicly funded healthcare system, it is the general public whose resources are available for allocation, so their views should be
accounted for (Dolan and Olsen, 2002). As a study population, members of the public will provide aggregated utilities, unconstrained by vested interests (Gold et al., 1996).

In summary, the choice of study subjects boils down to the aims and objectives in hand. For resource allocation, the use of society’s preferences have been advocated (Gold et al., 1996). In guideline development and in individual decision-making, the use of utilities obtained from actual patients is generally preferred.
1.6 Unanswered questions about reflux, aspiration and IPF

In recent years, much has been written on the subject of reflux as a potential contributing factor in IPF. Earlier this year the National Institute for Health and Clinical Excellence identified the treatment of reflux as a research priority in IPF.

Most would agree that the quality and quantity of evidence available remains limited. To date, there has been no convincing demonstration of causality and no prospective data exist on the treatment of reflux in IPF. High quality, prospective studies of the prevalence of reflux in IPF are lacking and microaspiration remains difficult to quantify. When compared to conditions with a similar prognosis, the therapeutic advances in IPF have been modest. In this context, it is doubly important that a potentially treatable aetiology is properly appraised.

How the investigation and management of reflux in IPF might be translated to clinical practice is another topic that has received little attention to date. The widespread use of standard tests and surgery for reflux may be very different amongst the IPF population than it is in an adult surgical clinic. Patient-reported outcome measures may or may not play a role. Furthermore patient attitudes have yet to be explored. It is difficult to predict how patients with varying degrees of respiratory compromise will approach the risks and benefits of aggressive antireflux treatment.
Chapter 2. Hypothesis, Aims and Objectives

2.1 Hypothesis

**Hypothesis 1:** IPF patients are willing and able to undergo investigation and, in appropriate cases, surgical treatment of gastro-oesophageal reflux and microaspiration.

**Hypothesis 2:** Gastro-oesophageal reflux and microaspiration are commonly found when state-of-the-art investigative techniques are used.

**Hypothesis 3:** The investigation and management of reflux in idiopathic pulmonary fibrosis may be effectively delivered by a multidisciplinary team.

2.2 Aims

2.2.1 Primary aim

1. To characterise the frequency and nature of gastro-oesophageal reflux and aspiration in IPF

2.2.2 Secondary aims

1. To evaluate aerodigestive symptomatology and health-related quality of life in IPF

2. To evaluate the feasibility of selected clinical and experimental techniques for use in clinical investigation in this patient population

3. To explore attitudes towards antireflux surgery within the IPF population

4. To assess the feasibility of a novel multi-disciplinary team to investigate and manage reflux in IPF and related conditions
2.3 Objectives

A. Selection and use of a panel of validated questionnaires with which to assess symptoms and health-related quality of life in IPF

B. To perform high-resolution manometry and ambulatory impedance/pH monitoring in a consecutive series of IPF patients

C. Assessment of markers of aspiration and lung inflammation using per bronchoscopic bronchoalveolar lavage providing:
   a. total cell count
   b. differential cell count
   c. presence of lipid-laden macrophages
   d. presence of haemosiderin-laden macrophages
   e. in-house quantitative ELISA measurement of pepsin
   f. quantitation of bile acids by spectrophotometry

D. To use a standard gamble to measure health state utilities associated with the burden of IPF and the potential burden of antireflux surgery
Chapter 3. Methods

3.1 Characterisation of reflux and aspiration in IPF

3.1.1 Ethical approval

Ethical permission for the study was granted on 22nd February 2010 by the County Durham & Tees Valley 2 Research Ethics Committee, (reference number 10/H0908/9). The NHS sponsor for the study was the Newcastle-upon-Tyne NHS Hospitals Foundation Trust. Study approval was granted by the Trust Research and Development department on 18th May 2010 (ref 5183).

All participating individuals gave written informed consent after an opportunity to review approved study information sheets.

3.1.2 Recruitment

In an earlier phase of study conducted by a doctoral student within our research group, 20 subjects with idiopathic pulmonary fibrosis underwent oesophageal physiology assessment, bronchoscopy and bronchoalveolar lavage fluid (BALF) analysis. Study methodology was consistent with the current work. These data were analysed in conjunction with the results from the current group of study participants.

The previous group of 20 subjects also completed the Reflux Symptoms Index questionnaire, the results of which were pooled across the two phases of study.

The recruitment target for the current phase of the study was 15-20 patients. This was a pragmatic target based on the number of patients attending the clinic and the participation rate recorded from the previous phase of research.

IPF patient identification

Consecutive adult patients with a secure diagnosis of idiopathic pulmonary fibrosis were identified and recruited from the regional Interstitial Lung Disease (ILD) clinic with the support of clinicians with a specialist interest. Diagnosis was verified through discussion at
the regional ILD multi-disciplinary team, with input from respiratory physicians, thoracic radiologists and histopathologists with an interest in lung disease. The diagnostic criteria proposed jointly by the European Respiratory Society and American Thoracic Society were used, Box 1.

**Box 1. Consensus guidelines for the diagnosis of idiopathic pulmonary fibrosis. Reproduced from (Raghu et al., 2011).**

1. Exclusion of other known causes of interstitial lung disease (e.g. environmental exposures, connective tissue disease and drug toxicity).
2. The presence of a usual interstitial pneumonia pattern on high-resolution computed tomography (HRCT) in patients not subjected to surgical lung biopsy.
3. Specific combinations of HRCT and surgical lung biopsy pattern in patients subjected to surgical lung biopsy.

**Exclusion criteria**

- Lack of capacity to consent to the study protocol
- Use of long-term oxygen therapy
- Conditions precluding cessation of proton pump inhibitor medications, including Zollinger-Ellison syndrome and complicated Barrett’s oesophagus
- Co-existing respiratory disorder
- Overt congestive cardiac failure
- Patients regarded unfit for any other reason by their respiratory physician

**Control population**

A small population of healthy volunteers was recruited from the university staff in order to derive reference data for the bronchoalveolar lavage fluid analysis (one female; median age 39 years, range 32–46 years). All were non-smokers and none had any significant respiratory symptoms or existing respiratory diagnoses.

**3.1.3 Study overview**

Participants were asked to complete a panel of symptom questionnaires. Those taking proton-pump inhibitors discontinued therapy prior to study and then repeated the questionnaires after two weeks off treatment. On- and off-treatment responses were therefore recorded.
On the first study day written consent was recorded and a stationary oesophageal manometry assessment was performed in a designated oesophageal physiology lab. An oesophageal pH-impedance catheter was then inserted and ambulatory monitoring commenced.

The subject returned the following morning and the pH-impedance catheter was removed. Bronchoscopy and a standardised bronchoalveolar lavage were performed. Lavage samples were transferred to Newcastle University for processing, analysis and storage.

### 3.1.4 Selection of patient-reported outcome measures

The Ovid-Medline database was searched over the period January 1980 to May 2013 to identify questionnaires suitable for the symptomatic evaluation of gastro-oesophageal reflux disease and extra-oesophageal reflux disease in adults. Questionnaires were evaluated based on their development, psychometric evaluation and ease of completion.

During the study, questionnaires were either handed to patients in clinic or posted out. Questionnaires that were designed to be administered by an interviewer were therefore excluded. Given the two-week cessation period for proton-pump inhibitors, questionnaires that could only be used for longer recall periods were avoided.

### Symptoms of gastro-oesophageal reflux disease

The following search string was used within Medline: [gastro-oesophageal reflux OR GERD OR gastro-oesophageal reflux OR GORD] AND [questionnaire OR scale OR patient reported outcome OR instrument OR measure OR index] AND [symptoms OR diagnosis OR evaluation] AND [validity OR reliability or responsiveness OR psychometric properties]. The references of relevant review articles were also screened (Stanghellini et al., 2004; Mouli and Ahuja, 2011; Vakil et al., 2013). Instruments designed to assess health-related quality of life and instruments relating to reflux surgery were excluded.

A total of 20 tools were identified and evaluated using a priori selection criteria described in section 3.1.4. As a result, 17 were rejected (Table 3-1). The development and validation of the three remaining questionnaires were then reviewed in further detail (Table 3-2).
Table 3-1. Questionnaires rejected for assessment of symptoms of gastro-oesophageal reflux

<table>
<thead>
<tr>
<th>Name</th>
<th>Source</th>
<th>Basis for rejection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant gastroesophageal reflux</td>
<td>(Orenstein et al., 1993)</td>
<td>Specifically designed for use in infants</td>
</tr>
<tr>
<td>symptom questionnaire</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GERD symptom score</td>
<td>(DeMeester et al., 1980)</td>
<td>No validation available</td>
</tr>
<tr>
<td>GERD specific questionnaire</td>
<td>(Carlsson et al., 1998)</td>
<td>Validation incomplete: author-reported specificity of 19% at the chosen diagnostic cut-off</td>
</tr>
<tr>
<td>GERD score</td>
<td>(Allen et al., 2000)</td>
<td>Administered</td>
</tr>
<tr>
<td>Gastroesophageal Activity Index</td>
<td>(Williford et al., 1994)</td>
<td>Part-interview</td>
</tr>
<tr>
<td>(GRACI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leeds Dyspepsia Questionnaire</td>
<td>(Moayyedi et al., 1998)</td>
<td>Administered</td>
</tr>
<tr>
<td>Dyspepsia symptom score</td>
<td>(Buckley et al., 1997)</td>
<td>Unvalidated; administered</td>
</tr>
<tr>
<td>Gastrointestinal Symptom Rating</td>
<td>(Talley et al., 2001)</td>
<td>Designed to assess IBS and peptic ulcer disease. The reflux syndrome scale contains only two items and has not been evaluated in isolation.</td>
</tr>
<tr>
<td>Scale</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digestive Health Status Instrument</td>
<td>(Shaw et al., 2001)</td>
<td>Based on criteria for IBS and dyspepsia</td>
</tr>
<tr>
<td>Dyspepsia Symptom Severity Index</td>
<td>(Leidy et al., 2000)</td>
<td>Designed to assess dyspepsia</td>
</tr>
<tr>
<td>HK index of dyspepsia</td>
<td>(Hu et al., 2002)</td>
<td>Designed to assess dyspepsia</td>
</tr>
<tr>
<td>GERD specific esophageal</td>
<td>(Greatorex and Thorpe, 1983)</td>
<td>Reliability and responsiveness not assessed</td>
</tr>
<tr>
<td>symptom questionnaire</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GERD</td>
<td>(Ofman et al., 2002)</td>
<td>Administered</td>
</tr>
<tr>
<td>questionnaire</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSFQ</td>
<td>(Pare et al., 2003)</td>
<td>Regurgitation not assessed</td>
</tr>
<tr>
<td>Hearburn diary card</td>
<td>(Junghard and Wiklund, 2008)</td>
<td>4 week recall period</td>
</tr>
<tr>
<td>GERD Inventory</td>
<td>(Zimmerman, 2004)</td>
<td>4 week recall period</td>
</tr>
<tr>
<td>PASS</td>
<td>(Armstrong et al., 2005)</td>
<td>Exclusively assesses treatment-resistant symptoms</td>
</tr>
<tr>
<td>ReQuest</td>
<td>(Bardhan et al., 2004)</td>
<td>Lengthy – over 60 items</td>
</tr>
</tbody>
</table>
Table 3-2. Questionnaires considered for the symptomatic assessment of gastro-oesophageal reflux

<table>
<thead>
<tr>
<th>Name</th>
<th>Source</th>
<th>Strengths</th>
<th>Weaknesses</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>GERD Symptom Assessment Scale (GSAS)</td>
<td>(Rothman et al., 2001)</td>
<td>1 week recall</td>
<td>Validity demonstrated against HRQL tools</td>
<td>15 items</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Responsiveness tested with a 2 week window</td>
<td>No nocturnal symptoms</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Designed to be evaluative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reflux Disease Questionnaire (RDQ)</td>
<td>(Shaw et al., 2001)</td>
<td>Validity tested against physician diagnosis</td>
<td>Originally validated with a 4 week recall</td>
<td>12 items</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Previously used in a randomised controlled trial</td>
<td>Dyspepsia symptoms included</td>
<td></td>
</tr>
<tr>
<td>Gastro-esophageal reflux disease questionnaire (GerdQ)</td>
<td>(Jones et al., 2009)</td>
<td>Brief: 6 items</td>
<td>Reliability not assessed</td>
<td>Assesses symptom frequency alone</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Includes nocturnal symptoms and treatment response</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Validity demonstrated against a multimodal diagnostic workup</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Once the search had been narrowed to these three remaining tools, the strengths and limitations of each were reviewed in further detail:

**GERD Symptom Assessment Scale (GSAS)**

25 core symptoms of reflux disease were established through literature review, focus groups and expert consultation. A questionnaire was designed to assess the frequency, severity and distress of each of these symptoms. 185 patients with GORD were identified from a nationwide population based survey and, after an initial telephone assessment, were asked to complete the questionnaire at baseline and two weeks. They were also asked to complete four HRQL scales in addition.

Assessment of the psychometric properties of the GSAS was undertaken. Reliability and responsiveness were acceptable but validity was not assessed against any external standard:

“Validity was demonstrated by testing the hypotheses that first, symptom distress would be associated with lower general perceptions of health, and, second, symptom distress would be more strongly associated with patient perception of functioning and well-being than symptom frequency. Both were supported.” (Rothman et al., 2001)
Based on the results of the pilot survey, symptoms endorsed by less than 25% of the subjects were dropped from further analysis, yielding a 15-item symptom scale.

**Reflux Disease Questionnaire (RDQ)**

The RDQ aims to provide a validated patient-reported tool for the purposes of GORD. The domains assessed are regurgitation, heartburn and dyspepsia. The time recall in the original survey was four weeks but in a later validation study, for which the tool was translated into Swedish and Norwegian, a recall of one week was used.

Content validity was informed by literature review, expert opinion and cognitive interviews. Predictive validity analyses of all scales and items used gastroenterologist diagnosis as a gold standard. Good inter-rater agreement between physicians was demonstrated with the use of actors primed with scripts based on confirmed cases of GORD, irritable bowel syndrome and combinations of the two.

Responsiveness was assessed by “observed treatment effect” between two clinic attendances and by analysing those reporting at least moderate change. Stability was assessed by constructing intra-class correlation coefficients in patients with stable symptoms.

**Gastro-Esophageal Reflux Disease- Questionnaire (GERD-Q)**

The GERD-Q was designed as a tool with which to support accurate, symptoms-based diagnosis of GORD by general practitioners and also to monitor the effect of therapy over time. The development was based on data from the Dutch study on Initial Management Of Newly diagnosed Dyspepsia (DIAMOND), in which GORD was diagnosed on the basis of any one of the following criteria (Dent et al., 2010):

(i) Oesophageal pH<4 for >5.5% of a 24-h period on ambulatory pH monitoring;

(ii) Los Angeles grade A-D oesophagitis at endoscopy (Lundell et al., 1999);

(iii) Indeterminate 24-h oesophageal pH in combination with a positive response to 14 days’ esomeprazole (proton pump inhibitor) treatment;

(iv) Positive (>95%) Symptom Association Probability.
Based on Receiver Operating Characteristic (ROC) curve analysis, logistic regression and data from previous studies and qualitative interviews, six items from the different questionnaires were chosen for inclusion in the questionnaire. These included four positive and two negative predictive factors. Further ROC curve analysis indicated that a questionnaire score of 8 points or more provided the highest sensitivity and specificity for diagnosing GORD.

Focus group feedback suggested that patients preferred to score symptoms by frequency rather than severity, as it was easier to recall. In addition, ROC curve analysis suggested that symptom frequency scoring provided equivalent sensitivity and specificity for diagnosis when compared to the use of symptom frequency and severity in combination. As a result, all six items within GerdQ assess symptom frequency.

In order to assess the responsiveness of the GerdQ, the effect size and the standardized response mean of the pre- and post-treatment questionnaire results distributions were compared. Using these techniques, the GerdQ was found to be as responsive as the RDQ, a comparable but lengthier tool.

The evaluation methodology, brevity and reported performance of the GerdQ make it particularly attractive.

**Symptoms of extra-oesophageal reflux**

A further Medline literature review was undertaken, incorporating the terms “extra-oesophageal reflux” and “laryngo-pharyngeal reflux” into the string detailed above. Six tools were identified with which to evaluate the symptoms of extra-oesophageal reflux. Four were deemed unsuitable (Table 3-3).
The Reflux Symptom Index (RSI) is a brief questionnaire designed to assess laryngeal symptoms secondary to laryngopharyngeal reflux. It is well validated and the normal range of scores has been published (0-13) (Belafsky et al., 2002). The RSI has been used in our research group previously.

Assessing quality of life related to reflux and lung disease

The putative relationship between reflux and lung disease makes it more appropriate to assess health-related quality of life from a respiratory perspective. The St. George’s Respiratory Questionnaire is the best validated tool with which to assess respiratory-specific health-related quality of life (Jones et al., 1991). It was originally designed and validated for use in chronic obstructive airways disease. It has now been validated for use in idiopathic pulmonary fibrosis, bronchiectasis, asthma, and cystic fibrosis and used in numerous clinical trials (Wilson et al., 1997; Sanjuas et al., 2002; Schunemann et al., 2003; Jones, 2005; Padilla et al., 2007; Swigris et al., 2010; Swigris et al., 2014).

A total score is calculated which summarises the impact of the disease on overall health status. Scores are expressed as a percentage of overall impairment where 100 represents worst possible health status and 0 indicates best possible health status. Normal ranges are described.
Summary and selection of questionnaires

For the current study, the Gastro-Esophageal Reflux Disease Questionnaire was used to assess typical symptoms of reflux, the Reflux Symptoms Index to assess extra-oesophageal reflux symptoms and the St George’s Respiratory Questionnaire to assess respiratory health-related quality of life.

This panel of questionnaires was combined and tested in a sample of five staff from the university. The median time for completion of all four questionnaires was five minutes and free-text feedback suggested that they were acceptable as a group. Questionnaires are reproduced in the appendices.

3.1.5 Clinical data collection

The results of key pulmonary function tests were extracted from clinical correspondence:

- Forced expiratory volume (1 second) = volume exhaled at the end of the first second of forced expiration
- Forced vital capacity = the determination of vital capacity from a maximally forced expiratory effort
3.1.6 Preparation for oesophageal physiology testing

I arranged for the current study subjects to attend the oesophageal physiology lab within the endoscopy department at the Royal Victoria Infirmary. I performed all tests and data analysis.

In preparation for these studies, I completed a programme of training. I began by shadowing the nurse practitioner who ran the physiology lab at the time of the study (Joy Candler). I observed her performing and analysing the tests, and studied the theory and practice of oesophageal manometry and ambulatory oesophageal monitoring. I familiarised myself with practice guidelines published by the British Society of Gastroenterology, the Association of Gastro-Intestinal Physiologists and the International High Resolution Manometry Working Group (Bodger and Trudgill, 2006; Bredenoord et al., 2012; Association of Gastrointestinal Physiologists, 2013).

Over the following six weeks I attended regular lists and performed tests and analysis with progressively greater independence. At the end of this process, I performed four combined manometry/pH-impedance tests independently and received feedback on my reports.

I subsequently travelled to Enschede in The Netherlands for a three-day residential training course on manometry and ambulatory monitoring. Teaching was delivered by Arjan Bredenoord, principal author of the Chicago Classification system of High Resolution Manometry (Bredenoord et al., 2012).

Before recruiting the first patient to this study, I was independently performing and interpreting clinical tests at the Royal Victoria Infirmary in Newcastle upon Tyne.

Once recruited to the study, subjects received instructions as to how to prepare for the tests. This was included within a standard letter (Appendix A).
In summary, participants were asked to:

- discontinue medications that affect the investigations in advance (2 weeks for proton pump inhibitors; 48 hours for H2-receptor blockers and antacid medication)
- fast for a minimum of 4 hours before the study appointments
- arrange to be accompanied home after the visit, in the event that sedation was used for bronchoscopy

### 3.1.7 Oesophageal manometry

A water-perfused system was used for all manometry recordings (Medical Measurement Systems, Enschede). For the first 11 subjects in the previous phase of study, manometry was assessed using a “conventional” 8-channel manometry system. Following an equipment upgrade in the physiology laboratory, the remaining tests were performed using a high-resolution 20-channel system. Investigations were undertaken in line with manufacturers’ instructions and in accordance with guidelines published by the Association of Gastro-Intestinal Physiologists (Association of Gastrointestinal Physiologists, 2013).

A single-use catheter was connected to the manometry system, comprising a low compliance pneumo-hydraulic perfusion pump and a manometer corresponding to each channel. Transducers relay the pressure signals to the analysis software (MMS, Enschede, The Netherlands).

The catheter was perfused with sterile water for ten minutes and air bubbles excluded from the system. The system was checked by elevating the catheter above the manometers and confirming a uniform pressure increase across all channels. The catheter tip was introduced through the nose after lubrication. The use of local anaesthetic was avoided, so as not to interfere with pharyngeal sensation. After insertion, the pressure readings are calibrated against the level of the midpoint of the subject’s oesophagus.
8-channel manometry - technique

In the 8-channel probe, the four channels closest to the tip are arranged radially at the same level, and the remaining four channels are arranged at intervals of 5cm. The measurement span of the probe is therefore 20cm in total (Figure 3-1).

Figure 3-1. The orientation of side-holes in an 8-channel manometry catheter

The catheter tip is inserted to a depth of 70cm. After insertion, the subject was asked to lie in a semi recumbent position, which has been used to derive the normative data for the procedure. Gastric placement is confirmed by observing a pressure rise with inhalation. The assessment is then conducted in two stages. The lower oesophageal sphincter (LOS) is assessed first and the oesophageal body subsequently.

As the tip is withdrawn from the stomach into the oesophagus, the high pressure zone of the lower LOS is demonstrated and may be assessed. With the four radially-orientated channels in the LOS, the subject is asked not to swallow and the mean resting pressure is recorded over a period of 10 seconds (baseline pressure). 5ml water is injected into the subject’s mouth and as they swallow, the pressure drop within the LOS is recorded. The mean residual pressure may be expressed in real terms (mmHg) and also as a percentage pressure drop relative to the resting pressure.

The catheter is then withdrawn so that the entire 20cm span is lying within the oesophageal body. The patient is then asked to swallow a 5ml water bolus a further ten times and the propagation of the peristaltic swallow wave is visualised. After ten swallows have been recorded the probe is removed.
8-channel manometry – analysis

The following parameters are calculated for each swallow.

**Lower oesophageal sphincter**

Location: from the point of insertion in the nose (cm)

Length: (cm)

Tone: Mean resting baseline pressure (mmHg)

Relaxation: Mean pressure on deglutitive relaxation (mmHg)

% relaxation: \[ \frac{\text{Deglutitive relaxation pressure} - \text{Mean relaxation pressure}}{\text{Mean relaxation pressure}} \]

**Oesophageal body**

Mean contractile amplitude relative to baseline pressure (mmHg)

Peak contractile amplitude relative to baseline pressure (mmHg)

Conduction velocity (cm/s)

Based on these metrics, the individual swallows are categorised according to a semi-automated analysis:

- Peristaltic contractions: the proximal oesophagus contracts before the distal oesophagus and contraction peaks are seen in all oesophageal body channels
- Dropped contractions: the proximal oesophagus contracts normally but there is no contraction in the distal oesophagus
- Interrupted contractions: contractions occur in the proximal and distal oesophagus, but are absent in the channels in between
- Non-transmitted contractions: absence of contractile activity in the oesophagus following a wet swallow

Analysis of the individual swallows was then combined to provide a global classification of oesophageal peristalsis, Table 3-4 (Spechler and Castell, 2001).
Table 3-4. Classification of oesophageal peristalsis using conventional manometry assessment

<table>
<thead>
<tr>
<th>Classification</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Peristals</td>
<td>Normal peristals &gt;70% of the time</td>
</tr>
<tr>
<td>Mild Ineffective Oesophageal Motility</td>
<td>Abnormal peristals 30-70% of the time</td>
</tr>
<tr>
<td>Severe Ineffective Oesophageal Motility</td>
<td>Normal peristals &lt;30% of the time</td>
</tr>
<tr>
<td>Aperistals</td>
<td>Abnormal peristals 100% of the time</td>
</tr>
<tr>
<td>Diffuse Oesophageal Spasm</td>
<td>&gt;10% of swallows simultaneous with mean amplitudes over 30mmHg</td>
</tr>
<tr>
<td>Nutcracker Oesophagus</td>
<td>Mean amplitude of peristals &gt;180mmHg</td>
</tr>
<tr>
<td>Hypertonic Lower Oesophageal Sphincter</td>
<td>&gt;45mmHg but relaxing</td>
</tr>
<tr>
<td>Hypotonic Lower Oesophageal Sphincter</td>
<td>&lt;10mmHg</td>
</tr>
<tr>
<td>Achalasia</td>
<td>Hypertonic LOS, absent or incomplete relaxations &gt;70-80% of the time. Simultaneous contractions or aperistals in the oesophageal body</td>
</tr>
</tbody>
</table>

High resolution manometry - technique

High-resolution manometry (HRM) was used for the latter patients in the study.

Conventional and high-resolution manometry are compared in Table 3-5.

Table 3-5. Comparison of conventional and high resolution oesophageal manometry assessment

<table>
<thead>
<tr>
<th>Technique</th>
<th>Conventional</th>
<th>High resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of channels</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>Calibre of probe</td>
<td>3.9mm</td>
<td>4.9mm</td>
</tr>
<tr>
<td>Span of measurement</td>
<td>20cm</td>
<td>35cm</td>
</tr>
<tr>
<td>Setup time</td>
<td>Shorter</td>
<td>Longer</td>
</tr>
<tr>
<td>Study time</td>
<td>Longer</td>
<td>Shorter</td>
</tr>
<tr>
<td>Assessment of LOS and oesophageal body</td>
<td>Sequential</td>
<td>Simultaneous</td>
</tr>
<tr>
<td>Oesophageal body assessment</td>
<td>10 x 5ml swallows</td>
<td>10 x 5ml swallows</td>
</tr>
<tr>
<td>Display</td>
<td>Line graphs</td>
<td>Colour density plot</td>
</tr>
</tbody>
</table>

The probe was inserted through the nose and positioned at around 55cm, such that the LOS, oesophageal body and upper oesophageal sphincter (UOS) could be visualised simultaneously.
The subject was asked not to swallow for a period of 10 seconds and subsequently, oesophageal function is assessed over the course of 10 x 5ml water swallows. The results of the study are expressed as a colour plot. Different colours represent different pressures. The components of the swallow, as visualised by HRM are illustrated in Figure 3-2. After ten swallows have been recorded the probe is removed.

Figure 3-2. Oesophageal pressure topography illustrating a normal swallow. Colours corresponding to specified pressures are illustrated on the left of the figure. UOS = upper oesophageal sphincter; IBP rise = intra-bolus pressure rise; LOS = lower oesophageal sphincter. Taken from (Fox and Bredenoord, 2008).
**High resolution manometry - interpretation**

Individual swallows are analysed according to the Chicago classification system (Bredenoord et al., 2012), a semi-automated analysis of the pressure pattern. In the same way that contours on a map link the points of a specified height, isobaric contours can be displayed as a spatiotemporal colour plot and used to demarcate individual components of a swallow. Figure 3-3 illustrates the way in which a swallow is analysed. Two key landmarks are used:

- *Proximal pressure trough (P)*: the physiological trough which results from the interdigitation of the striated muscle in the upper oesophagus and the smooth muscle in the lower oesophagus.

- *Contractile deceleration point (CDP)*: the inflection point along the 30 mmHg isobaric contour where propagation velocity slows. This represents the transition between the tubular oesophagus and the stomach.

The following metrics are calculated as per the Chicago classification system, as illustrated in Figure 3-3 (Bredenoord et al., 2012).

**Lower oesophageal sphincter (LOS)**

<table>
<thead>
<tr>
<th>Location</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tone</strong>:</td>
<td><strong>Resting pressure</strong> = mean resting pressure recorded within the LOS (mmHg)</td>
</tr>
<tr>
<td><strong>Relaxation</strong>:</td>
<td><strong>Integrated relaxation pressure</strong> = mean pressure within the oesophagogastric junction during the lowest pressure period of 4 seconds recorded at any time within the 10 seconds after swallow initiation (mmHg)</td>
</tr>
</tbody>
</table>

**Oesophageal body**

| Peristaltic amplitude: | *Distal contractile index* = Amplitude x duration x length of the distal oesophageal contraction >20 mmHg from proximal pressure trough and the contractile deceleration point (mmHg.s.cm) |
| Duration: | *Distal latency* = Interval between UOS relaxation and the contractile deceleration point (s) |
Propagation velocity: *Contractile front velocity* = Slope of the tangent approximating the 30 mmHg isobaric contour between P and the CDP (cm/s)

Classification of swallow: Timing of onset as well as velocity and amplitude of contraction are used to classify each swallow, as described in Table 3-6. The algorithm within the Chicago classification can then be used to summarise an individual’s swallow function based on the results of the ten recorded swallows (Figure 3-4).

Figure 3-3. Oesophageal pressure topography plot illustrating a normal water swallow as assessed by high-resolution manometry. Pressure scale indicated by the colour bar on the left (mmHg). UES = upper oesophageal sphincter; LES = lower oesophageal sphincter; CFV = contractile front velocity; DCI = distal contractile index (dashed pink box); DL = distal latency; IRP = integrated relaxation pressure; P=proximal pressure trough; CDP=contractile deceleration point. Adapted from (Zeribib and Omari, 2014).
Table 3-6. Defining features of an individual swallow ((Bredenoord et al., 2012))

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

Figure 3-4. Flow diagram to illustrate the Chicago classification system of high resolution oesophageal manometry (reproduced from (Bredenoord et al., 2012))

Hierarchical Analysis of Esophageal Motility

1. IRP ≥ upper limit of normal AND absent peristalsis
   - Yes
   - Achalasia
     - Type I: classic
     - Type II: with esophageal compression
     - Type III: peristaltic fragments or spastic

2. IRP ≥ upper limit of normal AND some instances of intact or weak peristalsis
   - Yes
   - EGJ Outflow Obstruction
     - Achalasia variant
     - Mechanical obstruction
     - May have 1" or 2" hypercontractility

3. IRP is normal AND absent peristalsis
   - OR reduced distal latency
   - OR DCI > 8000 mmHg-s-cm
   - Yes
   - Absent Peristalsis
     - Distal Esophageal Spasm (DES)
       - • ≥ 20% of swallows with reduced latency
       - Hypercontractile (Jackhammer) Esophagus
       - Any swallow with DCI > 8000 mmHg-s-cm

4. IRP is normal AND Peristaltic abnormalities
   - No
   - Weak Peristalsis
     - • Large or small breaks in the 20-mmHg isobaric contour
     - Frequent Failed Peristalsis
     - Hypertensive Peristalsis (Nutcracker Esophagus)
     - Rapid Contraction
       - ≥ 20% of swallows with rapid contraction

3.1.8 Ambulatory oesophageal monitoring

After the location of the LOS was determined, gastro-oesophageal reflux was assessed using a multilumen endo-oesophageal probe. It is a 1.9mm calibre single-use device consisting of six impedance channels and a pH probe, as illustrated in Figure 3-5 (pHersaflex catheter, Medical Measurement Systems, Enschede, The Netherlands). After a 10 minute pre-soak in deionised water, buffer solutions (pH 1 and 7) were used to calibrate the pH probe at room
temperature prior to nasal insertion. The probe is fixed such that the pH probe is located 5cm proximal to the upper border of the LOS.

Figure 3-5. The arrangement of pH and impedance sensors within the pHersaflex catheter

After insertion, the probe is connected to an Ohmega ambulatory data recorder (MMS, Enschede, The Netherlands). The recorder has buttons for 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. The recording box was then connected to the MMS software and the data 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.
**pH assessment**

pH measurements were recorded over a period of not less than 16 hours. The following parameters were recorded and used to calculate the Demeester score, a composite measure of GOR (Johnson and Demeester, 1974).

- Number of reflux events
- Length of longest reflux
- Number of reflux periods > 5 minutes
- Percentage of overall study time pH<4

**Impedance assessment**

Impedance analysis was compared to published normal ranges (Zerbib et al., 2005). The following parameters are recorded and analysed:

- Reflux events
  - Acid: pH<4
  - Weakly acid: pH 4-7
  - Non-acid: pH>7
- Number of proximal events i.e. recorded at the proximal channel, 17cm from the catheter tip
- % of the total reflux time during which proximal reflux was recorded
- Oesophageal volume exposure: % time for which liquid was detected at the most distal channel
- Bolus clearance time: mean delay between detection of liquid and detection of gas at the most distal channel
Symptom-event correlation analysis

Based on the subject’s button presses, the correlation of GORD-related symptoms and reflux events was analysed. Association is determined by the presence of a reflux event in the two minutes preceding symptom onset (Bredenoord et al., 2005). Three measures are used:

- Symptom index (SI): the proportion of symptom episodes associated with a reflux event (Ward et al., 1986).
- Symptom sensitivity index (SSI): the proportion of reflux events associated with a symptom (Breumelhof and Smout, 1991).
- Symptom-associated probability (SAP): a statistical analysis of the degree of association between the symptoms and events. SAP uses a Fisher’s exact test which analyses the total duration of the study as divided into in two-minute windows, according to a 2x2 matrix comparing symptoms (positive/negative) and reflux events (positive/negative)(Weusten et al., 1994).

3.1.9 Bronchoscopy

A standardised bronchoscopy and bronchoalveolar lavage (BAL) was performed by a single consultant respiratory physician within the Newcastle-upon-Tyne Hospitals NHS Foundation Trust (IAF). In addition to consent for study participation, consent is also recorded in line with routine clinical practice.

The BAL procedure was performed in line with previous reports from our group (Stovold et al., 2007). Participants are offered the option of premedication with intravenous midazolam. 4% lignocaine was applied topically to the nose, pharynx, larynx and subglottis. Supplemental oxygen was administered and pulse oximetry recorded. Transnasal bronchoscopy was then performed. One or more images of the hypopharynx and vocal cords were captured prior to tracheal intubation.
The tip of the bronchoscope was wedged into a right middle lobe subsegmental bronchus and a standardised 3 x 60ml lavage is performed. The quantity of fluid retrieved was recorded. 5ml of BAL fluid was sent to the hospital laboratory for routine culture.

**Visual assessment of laryngopharyngeal reflux**

Anonymised images were later reviewed by Mr Julian McGlashan, a Consultant Ear Nose and Throat surgeon with an interest in LPR. Images were scored using the Reflux Finding Score, Table 3-7. Scores greater than seven are said to indicate LPR (Belafsky et al., 2001).

<table>
<thead>
<tr>
<th>Component</th>
<th>Score</th>
</tr>
</thead>
</table>
| Subglottic oedema                  | 0 = absent  
|                                    | 2 = present |
| Ventricular                        | 2 = partial  
|                                    | 4 = complete |
| Erythema/hyperemia                 | 2 = arytenoids only  
|                                    | 4 = diffuse |
| Vocal fold edema                   | 1 = mild  
|                                    | 2 = moderate  
|                                    | 3 = severe  
|                                    | 4 = polypoid |
| Diffuse laryngeal edema            | 1 = mild  
|                                    | 2 = moderate  
|                                    | 3 = severe  
|                                    | 4 = obstructing |
| Posterior commissure hypertrophy   | 1 = mild  
|                                    | 2 = moderate  
|                                    | 3 = severe  
|                                    | 4 = obstructing |
| Granuloma/granulation tissue       | 0 = absent  
|                                    | 2 = present |
| Thick endolaryngeal mucus          | 0 = absent  
|                                    | 2 = present |

**3.1.10 Bronchoalveolar lavage fluid processing**

The BAL fluid sample was transferred on ice for processing at the Newcastle University research laboratories. BAL processing was performed in accordance with an established standard operating procedure (Appendix G). Samples were centrifuged and a total cell count
estimated from the reconstituted cell pellet using a haemocytometer. Cytospin preparations were prepared on glass slides, air-dried and stored at -20°C for later examination. A maximum of 6 x 1ml cell pellets were reconstituted in RNA lysis buffer (Qiagen) and 25 x 600μl aliquots of acellular fluid were stored at -80°C. A single cytospin was stained with Giemsa and a differential cell count determined.

As part of my lab training, I counted archived cytospins. Prior to recruitment, my neutrophil and macrophage counts were within 5% of those recorded by experienced lab personnel (GJ and KJ).

3.1.11 Assessment of aspiration

Cytology

Cells were stained with oil red O to visualise lipid-laden macrophages, according to the method of (Hopkins et al., 2010). Oil red O is a Polyazo dye with higher solubility in lipid than in solvent. The standard operating procedure is established in our lab. Oil red O stain was combined with 60% isopropanol to make up a working solution. Cytospins were fixed in formalin in preparation before oil red O staining and counterstaining with Cilest in counterstain. An aqueous mountant was used.

Using the method of Colombo and Hallberg, a lipid-laden macrophage index was calculated for each slide. 300 macrophages were enumerated as follows (Colombo and Hallberg, 1987):

0 = cytoplasm not opacified
1 = up to ¼ opacified
2 = up to ½ opacified
3 = up to ¾ opacified
4 = totally opacified cytoplasm

One in four slides was second-read by an independent observer who was blinded to subjects’ clinical histories and test results.

Additional cytospins were stained to demonstrate oxidative stress through the release of chemically active iron. The use of Perl’s Prussian blue, which stains ferric iron from protein-
bound tissue deposits, is also established within our group (Appendix J). Perl’s reagent was constituted with equal volumes of 2% hydrochloric acid and 2% potassium hexacyanoferrate. Cytospins were fixed in acetone before staining with Perl’s reagent and counterstaining with 1% Neutral Red. Slides were then dehydrated in alcohol, cleared in xylene and fixed in D.P.X. mountant. For each slide, a haemosiderin score was calculated as described by Kahn et al. (1987). 200 macrophages were enumerated as follows:

0 = no colour
1 = faint blue in one portion of cytoplasm
2 = deep blue in a minor portion of the cell
3 = deep blue in most areas of the cytoplasm
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. The percentage of cells staining positively was also recorded. Cell counting and scoring was repeated by a blinded observer.

**Pepsin measurement by enzyme-linked immunosorbent assay**

Pepsin concentrations were measured by a PhD student (GZ) within the research group using a protocol modified from an established, locally developed enzyme-linked immunosorbent assay (ELISA) (Tasker et al., 2002; Stovold et al., 2007).

At each step, wells were washed three times with 0.05% Tween 20 in PBS and incubated at room temperature with vigorous shaking (800rpm on a plate shaker unless specified). 100µl aliquots of unconcentrated sample and standard solution (0-100ng/ml porcine pepsin in PBS, Sigma, Dorset, UK) were incubated overnight in a 96-well plate.

**Blocking**

1% bovine serum albumin in PBS was added before incubation for 2 hours at 500rpm.

**Primary antibody binding**

Standards and samples were incubated at room temperature for 2 hours with primary antibody: positive wells were incubated with anti-pepsin antibody (1:100 dilution in 0.1%
BSA/PBS, Biodesign International, TN, USA) and negative wells with 100µl 0.1% BSA solution. (The primary antibody binds both pepsin and pepsinogen.)

**Secondary antibody binding**

All wells were incubated at room temperature for 2 hours with 1:1000 HRP-conjugated antisheep/goat in 0.01% BSA (Sigma).

**Colour development**

100µl of 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (Sigma) was added to each well and allowed to develop for at least 20 minutes at room temperature with light shaking (100rpm). The reaction was stopped with 50µl of 1% sodium dodecyl sulphate in deionised water.

**Plate-reading**

The plate was measured at 405nm with the Infinite 200 Pro plate reader, using i-control software (Tecan, Männedorf, Switzerland).

**Bile salt measurement by spectrophotometry**

Bile acid concentration was measured using the Total Bile Acids kit (Alere, Stockport, UK).

Three buffers are supplied in stable liquid formulation:

- **R1:** Thio-NAD >0.1mM, Buffer
- **R2:** 3-a-HSD >2kU/L, NADH >0.1mM, Buffer
- **Calibrator:** Conjugated cholic acids, Buffer

270µl R1 and 4µl of sample or calibrator was incubated at 37°C for 3 minutes in triplicate. Blank absorbance was measured at 405nm.

For each sample/calibrator, 90µl R2 was added to the 2 positive wells and 90µl heat-killed R2 was added to the single negative well. The absorbance was then measured at 405nm after 1 minute and 10 minutes. ΔA405nm was calculated as the difference between these two readings.

TBA concentrations were determined using the equation:

\[
\text{Sample } \Delta A_{405nm/10min} - \text{Blank } \Delta A_{405nm/10min} \times \frac{\text{Calibrator Standard } \Delta A_{405nm/10min} - \text{Blank } \Delta A_{405nm/10min}}{\Delta A_{405nm/10min}}
\]
3.1.12 Formation of a novel aerodigestive multidisciplinary team

The question of how best to investigate micro-aspiration in complex respiratory patients is crucial to the field of aerodigestive medicine. Greater experience of the clinical and experimental techniques used in the current study can help to define their role. At present, data generated in the course of this research must be handled sensitively so that all management decisions are appropriate.

In order to discuss this challenging patient group, we established a cross-site aerodigestive multidisciplinary team in the Newcastle-upon-Tyne Hospitals NHS Foundation Trust. There is regular input from upper GI surgeons, respiratory physicians, transplant physicians and anaesthetists. Team members’ attendance, the results of relevant investigations and decisions regarding further investigation and management were recorded prospectively. To our knowledge this is the first time an aerodigestive MDT has been formally convened.

3.1.13 Statistical analysis

Analysis was performed using Minitab 17 (State College, Pennsylvania, USA). Analysis was largely descriptive. Where necessary, normality of data was assessed using the Anderson-Darling statistic and statistical significance was accepted at $\alpha<0.05$.

Proportions were compared using the Chi-squared statistic.

For normally distributed continuous data, comparisons were made using paired T-tests and unpaired (Student’s) T-tests as appropriate. Correlation was assessed using Pearson’s test.

For non-parametric data, continuous datasets were compared using the Mann-Whitney U-test and the Kruskal-Wallis test as appropriate. Correlation was assessed using the Spearman Rank method.

Physiology data were analysed with reference to published data from healthy control populations (Zerbib et al., 2005). Unless otherwise specified, the upper limit of normal was taken as the upper limit of the 95% confidence interval.

Inter-rater agreement was assessed using appropriate correlation coefficients (Pearson r, Spearman and Intraclass) in addition to the Bland-Altman method (Bland and Altman, 1986). This latter method accounts for the mean difference between paired data points and the
standard deviation of the differences. Most of the differences will lie within 2 standard deviations of the mean difference. If the differences are Normally distributed then 95% of the values will lie within this range, termed the “limits of agreement”.

Statistical methodology was reviewed with Dr Kim Pearce, Senior Statistician in the Institute of Cellular Medicine, Newcastle University.
3.2 Evaluation of patient attitudes towards the treatment of reflux in IPF

3.2.1 Ethical approval

The study was considered by the Proportionate Review Sub-Committee of the London-Bromley NRES Committee on 18th March 2014 (ref 14/LO/0531). The NHS sponsor for the study was the Newcastle-upon-Tyne NHS Hospitals Foundation Trust. Study approval was granted by Newcastle-upon-Tyne Hospitals R&D on 6th May 2014 (ref 6956) and by Gateshead Health on 23rd April 2014 (ref 928/14).

3.2.2 Study overview

The aim of this analysis was to evaluate the burden of antireflux therapy in the context of idiopathic pulmonary fibrosis. Respondents’ valuations of their own quality of life and of a set of written scenarios were recorded. The scenarios described i) health states relating to differing severities of idiopathic pulmonary fibrosis, and ii) adverse outcomes related to medical and surgical antireflux therapy. (As the respiratory benefit of antireflux therapy has yet to be quantified, good outcomes from antireflux therapy were not evaluated.)

Study participants completed a standardised study interview which included a standard gamble in addition to a validated assessment of their performance status. Pulmonary function tests were extracted from patients’ medical records to help define disease severity.

The strengths and weaknesses of the choice-based methods for utility measurement are discussed in section 1.5.3. The standard gamble was selected because it entails a choice between an ongoing health state and an intervention, the outcome of which is uncertain. This framework is consistent with the decision that a patient with IPF would have to make in considering the pros and cons of a specific treatment, such as antireflux therapy.

3.2.3 Study sample and recruitment

Prospective participants were identified from the interstitial lung disease out-patient clinics at the Royal Victoria Infirmary in Newcastle and the Queen Elizabeth Hospital in Gateshead. A letter outlining the study was posted out. At the outset, patients who did not wish to take part were given the opportunity to opt out by email or telephone/voicemail.
After a minimum of 72 hours, prospective participants were contacted by phone to discuss the study in more detail. Individuals keen to participate were sent a patient information sheet and offered an appointment for the study interview. The interview was conducted in the outpatient department or as a home visit.

### 3.2.4 Inclusion criteria

- Diagnosis of idiopathic pulmonary fibrosis, as defined by international consensus guidelines (Raghu et al., 2011)
- Age 18 or over

### 3.2.5 Exclusion criteria

- Difficulty in understanding written or verbal information in English
- Deterioration in lung function (as defined by change in WHO performance status in the preceding 4 weeks)
- Cognitive impairment such that informed consent could not be given

### 3.2.6 Sample size

There is no previous work in this area to inform a power calculation. For analysis purposes, participants were divided into mild/moderate disease and moderate/severe disease. Stratification between these two groups was made with reference to the median adjusted total lung capacity for the overall study population. In accordance with published convention for pilot studies where no previous data is available, the recruitment target was 30 patients per group (Lancaster et al., 2004). The overall recruitment target was therefore 60 patients.

### 3.2.7 Standard gamble method

Standard gamble interviews were conducted with the use of a chance board (Figure 3-6). The chance board illustrates a choice with two alternative options: one option consists of a gamble between two uncertain outcomes; the other option is a single certain outcome. The board is divided into two sections. The top half of the board illustrates the uncertain Choice A (Alternative 1) with probability p of the most preferred health state and 1-p of the least
preferred health state. Two small windows in this part of the board indicate the probabilities corresponding to $p$ and $1-p$. The probabilities can be changed by turning the wheel on the left of the board. On the bottom part of the board is the certain outcome Choice B (Alternative 2). The chance board thus converts the probabilities into a percentage risk of a specified outcome.

Figure 3-6. Chance board used as a prop to illustrate decision-making within the standard gamble. Example vignette featured as “Choice B”.

To start, the chance board was set at a 90% chance of the preferred health state (eg returning to full health) and a 10% chance of the least preferred health state (eg immediate death). The individual was asked to make a choice between Choice A and Choice B. The probabilities were then altered using a ping-pong strategy, whereby probabilities are alternated back and forth between a low value and a high value until the individual is indifferent between Choice A and Choice B. The percentages on the chance board convert to the probabilities for $p$ and $1-p$. The probability at the point where the individual is indifferent between Choice A and Choice B is the utility value of the health state described in Choice B.

The vignettes were presented to the subjects in random order, to avoid order-of-study bias.
3.2.8 Vignette design

For the purposes of the standard gamble, it was necessary to construct a series of concise descriptions such that study respondents could evaluate the burden of disease associated with both idiopathic pulmonary fibrosis and with adverse outcomes of antireflux treatment.

There is no accepted methodological approach to the generation of vignettes for health state utility analysis. In order for a health state description to provide a useful and authentic representation of the disease state in question, the following factors would seem important:

- Concise description, but with sufficient detail to provide meaningful explanations
- Symptom domains identified by patients affected by the condition
- Symptom severity defined in line with accepted grading systems where appropriate
- Validated by clinicians with specialist experience
- Written in simple language, avoiding the use of jargon

The process through which vignettes were composed and ratified is outlined in Table 3-8.

The final version of the four vignettes is shown in Box 2.
Table 3-8. Iterative process through which health state scenarios were designed for the standard gamble. Superscript figures relate to notes detailed below the table.

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Antireflux therapy</th>
<th>Idiopathic pulmonary fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Factor generation</td>
<td>Fortnightly upper gastrointestinal surgery outpatient clinic interviews over 12 months (n=50) ¹</td>
<td>Following review of a qualitative research study undertaken on behalf of Boehringer-Ingelheim ²</td>
</tr>
<tr>
<td>2</td>
<td>Severity correlation</td>
<td>Summary of Product Characteristics, Omeprazole ³</td>
<td>Dyspnoea guidelines ⁴</td>
</tr>
<tr>
<td>3</td>
<td>Vignette composition</td>
<td>In collaboration with health economics supervisors at Institute of Health and Society ⁵</td>
<td>In collaboration with health economics supervisors at Institute of Health and Society</td>
</tr>
<tr>
<td>4</td>
<td>Clinician ratification</td>
<td>Interviews with consultant oesophago-gastric surgeons (n=4) ⁶ Round table discussion with three senior registrars ⁷</td>
<td>Interviews with consultant respiratory physicians with an interest in interstitial lung disease (n=3) ⁸ Interview with a senior registrar training in respiratory medicine ⁹</td>
</tr>
</tbody>
</table>

Notes

1. Northern Oesophago-Gastric Unit (NOGU), Royal Victoria Infirmary, Newcastle-upon-Tyne.

2. Boehringer-Ingelheim commissioned Brains and Cheek, a qualitative research agency, to undertake a piece of qualitative research exploring the impact of idiopathic pulmonary fibrosis on quality of life in people living with the condition. The project consisted of in-depth interviews with a total of 18 patients and carers. The results of the study were presented to Boehringer-Ingelheim in a series of slides, to which I was allowed access.

3. (Teva UK Limited, 2009)

4. (Fletcher et al., 1959)

5. Dr Laura Ternent, Senior Lecturer, Institute of Health and Society, Newcastle University

Dr Peter McMeekin, Reader in Health Economics, Northumbria University
6. Professor Michael Griffin, Mr Nick Hayes, Mr Daya Karat, Mr Arul Immanuel; all based at NOGU

7. Mr Sebastian Kwon, Mr Barry Dent, Mr Maziar Navidi; all based at NOGU

8. Professor John Simpson, Dr Ian Forrest; Department of Respiratory Medicine, Royal Victoria Infirmary, Newcastle-upon-Tyne
Dr Rob Allcock; Department of Respiratory Medicine, Queen Elizabeth Hospital, Gateshead

9. Dr Jit Dutta, Department of Respiratory Medicine, Newcastle-upon-Tyne


<table>
<thead>
<tr>
<th>Outcome W</th>
<th>Outcome X</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Good energy levels</td>
<td>• Reduced energy levels</td>
</tr>
<tr>
<td>• Manage your own washing and dressing</td>
<td>• Manage your own washing and dressing</td>
</tr>
<tr>
<td>• Short of breath when hurrying or walking up a slight hill</td>
<td>• Stopping for breath after about 100m or after a few minutes walking on the level</td>
</tr>
<tr>
<td>• Coughing, which is troublesome to you one or two days a week</td>
<td>• Coughing, which is troublesome for you on most days</td>
</tr>
<tr>
<td>• No need for oxygen support</td>
<td>• Using supplemental oxygen</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Outcome Y</th>
<th>Outcome Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Five scars on your stomach, each measuring 1cm in length</td>
<td>• Taking five extra tablets over the course of the day, plus Gaviscon liquid after meals. You may experience the following problems:</td>
</tr>
<tr>
<td>• Not able to belch, leading to bloating and abdominal discomfort</td>
<td>Common · Headache · Abdominal pain · Constipation/diarrhoea which require treatment with tablets · Nausea/vomiting</td>
</tr>
<tr>
<td>• Food sticking in your gullet at times</td>
<td>Rare · Reduced energy levels · Serious gut infections · Increased risk of broken bones</td>
</tr>
<tr>
<td>• Only managing small meals</td>
<td></td>
</tr>
<tr>
<td>• Risk of bleeding, infection and hernias from surgery</td>
<td></td>
</tr>
<tr>
<td>(A hernia is a bulge at the site of a scar, which may be uncomfortable or painful. Sometimes hernias need to be repaired with an operation.)</td>
<td></td>
</tr>
</tbody>
</table>

No definition of “Full Health” was volunteered, but if interview subjects asked specifically what Full Health entailed, they were told:

“You can think of Full Health as a scenario where you are entirely free of symptoms and do not require any ongoing medical treatment.”
3.2.9 The interview process

An interview manual and a response booklet were prepared so as to standardise instructions and data capture. The manual included an interview script which was read out verbatim.

The following demographic details were recorded:

- Gender
- Date of birth
- Marital status
- Co-habitation
- Employment status
- Educational qualifications
- Ethnicity

Utility values for the four health states detailed above were calculated using standard gamble. The respondent’s own current health state was also measured.

Finally, respondents were asked to complete a multidimensional health-related quality of life tool, EuroQOL-5D-3L (0). EuroQOL-5D-3L is brief, self-completed health-related quality of life survey in which respondents select one of three statements in answer to questions relating to five domains. The domains are pain, mobility, mood, level of function and self-care. After registering the study with the EuroQOL group, syntax was provided for use in the statistical program SPSS (IBM, New York, USA). Each respondent’s answers to the five questions were mapped to a predefined utility score. These figures were generated through a large time-trade off study (Dolan, 1997).

Euroqol-5D also includes respondents to assess their global quality of life on a visual analogue scale. The validated nature of this tool provides a reference point with which to compare respondents’ responses to the standard gamble.
3.2.10 Statistical analysis

Statistical analysis was performed as described in section 3.1.13. In line with the convention of the health economics literature, utilities were analysed using parametric methods.
Chapter 4. Results of Characterisation Study

4.1 Study overview

Thirty-seven study subjects were recruited from the regional interstitial lung disease clinic at the Royal Victoria Infirmary, Newcastle-upon-Tyne. The first twenty subjects were recruited and studied by a previous doctoral research student (Amaran Krishnan). I studied the remaining 16 subjects.

Patients had already been screened to confirm their respiratory diagnosis. All had undergone clinical assessment, pulmonary function testing and CT scans. Clinic review was supported by formal multidisciplinary discussion. None of the patients underwent lung biopsy.

After informed consent had been recorded, patients completed a panel of study questionnaires to assess symptoms of gastro-oesophageal reflux, laryngo-pharyngeal reflux and respiratory health. Those individuals who were taking proton pump inhibitors were asked to discontinue their medication. In this group, questionnaires were repeated after a two-week period of cessation. Objective study assessments were performed after this wash-out period.

There were two study visits, held on consecutive days at the RVI. On the first day, subjects underwent oesophageal manometry and naso-oesophageal intubation with an endo-oesophageal probe, connected to an electronic data recorder. They were instructed to electronically record their symptoms, meal-times and supine periods over the forthcoming 24 hours of pH-impedance monitoring. One subject was unable to tolerate naso-oesophageal intubation and was excluded from all study analyses. The remaining 36 subjects completed all tests.

On the second day the probe was removed and data were transferred to the departmental database for analysis. Subjects then underwent bronchoscopy and bronchoalveolar lavage. Study samples were transferred to the university laboratories for processing and analysis.

A group of four control subjects were recruited from within the university staff and underwent the same bronchoscopy and lavage.
4.2 Recruitment and demographics

The recruitment of participants in this study was managed at the regional interstitial lung disease clinic at the Royal Victoria Infirmary in Newcastle-upon-Tyne. This is a weekly clinic which was established in 2010. Suitable patients were identified from clinicians running the clinic, based on the inclusion and exclusion criteria detailed in section 3.1.2. Those willing to discuss the work further were referred to the lead researcher and further information regarding the study was outlined. This was consistently undertaken in a different clinic room from that in which the patient was seen for their clinic appointment. As the clinic became increasingly busy, it was no longer practicable to discuss the research with patients face to face. Latterly, the study was outlined to potential participants by telephone after they had been identified by the clinical team.

Seventy-nine eligible subjects were invited to participate. 36 completed the study protocol. Study recruitment is summarised in Figure 4-1.
There were 27 males. Median age was 72.5 and the interquartile range was 64.5-77.7. Two subjects were active smokers at the time of recruitment. Ten patients stated they had never smoked. The majority were ex-smokers who had stopped more than 6 months ago; two had stopped within six months of recruitment.

Ten subjects were taking corticosteroids and eight were taking N-acetylcysteine. 25 subjects were taking a proton-pump inhibitor (PPI) at the time of recruitment.

 Eleven patients had pre-existing evidence of GORD documented either in clinical letters or at recent endoscopy within the preceding two years.

As a routine, pulmonary function testing is repeated at each visit to the regional ILD clinic. The spread of vital capacity recordings is illustrated in Figure 4-2. Median percentage-predicted vital capacity was 77.6% (range 47.9-146.4).
Subjectively, patients are graded in clinic according to the MRC dyspnoea scale. Study subjects were categorised as detailed in Table 4-1.

Table 4-1. Study subjects’ breathlessness status at recruitment (Fletcher et al., 1959)

<table>
<thead>
<tr>
<th>Grade</th>
<th>Degree of breathlessness related to activities</th>
<th>Number of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Not troubled by breathlessness except on strenuous exercise</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>Short of breath when hurrying on the level or walking up a slight hill</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>Walks slower than most people on the level, stops after a mile or so, or stops after 15 minutes walking at own pace</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>Stops for breath after walking about 100 yds or after a few minutes on level ground</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>Too breathless to leave the house, or breathless when undressing</td>
<td>3</td>
</tr>
</tbody>
</table>

Within the second phase of recruitment, subjects were asked to complete the St George’s Respiratory Questionnaire as a measure of respiratory health-related quality of life. Median SGRQ score off PPI was 44.15.
For the small group of subjects who completed these questionnaires both on and off-PPI, there was a significant difference in the scores after PPI therapy was discontinued, Table 4-2. In statistical terms, SGRQ scores decreased significantly after PPI was discontinued (p=0.005). This change is consistent with an improvement in respiratory health-related quality of life following cessation of PPI.

Table 4-2. Respiratory health-related quality of life score recorded on and off PPI medication

<table>
<thead>
<tr>
<th>Study ID</th>
<th>On PPI</th>
<th>Off PPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>71.07</td>
<td>64.60</td>
</tr>
<tr>
<td>23</td>
<td>45.06</td>
<td>35.86</td>
</tr>
<tr>
<td>25</td>
<td>50.77</td>
<td>42.66</td>
</tr>
<tr>
<td>27</td>
<td>42.33</td>
<td>44.03</td>
</tr>
<tr>
<td>28</td>
<td>66.69</td>
<td>60.68</td>
</tr>
<tr>
<td>30</td>
<td>21.17</td>
<td>7.49</td>
</tr>
<tr>
<td>31</td>
<td>73.07</td>
<td>60.54</td>
</tr>
<tr>
<td>33</td>
<td>59.69</td>
<td>32.24</td>
</tr>
<tr>
<td>34</td>
<td>69.75</td>
<td>61.94</td>
</tr>
</tbody>
</table>

The Minimal Important Difference for SGRQ is most commonly quoted at 4, as determined by a range of distribution and anchor-based methods (Jones, 2002). With respect to this figure, there was a significant improvement in respiratory symptomatology in 89% of subjects.

4.3 Assessment of gastro-oesophageal reflux

4.3.1 Subjective assessment

In the latter group of 16 subjects, the Gastro-esophageal reflux disease questionnaire (GerdQ) was used to assess classical symptoms of reflux disease (Jones et al., 2009)(Figure 4-3). A score of 8 or more supports a diagnosis of gastro-oesophageal reflux disease.

On PPI, two patients (22%) recorded positive GerdQ scores. This proportion increased to 29% when subjects were off PPI. The median scores for these groups was 6 and 6.5, respectively. Overall, 25% of respondents recorded GerdQ scores suggestive of reflux.
disease. All but one had pre-existing evidence of GORD based on their clinical assessment and/or endoscopy.

Paired T-tests revealed there was no significant difference in the on-PPI and off-PPI scores in the subjects who discontinued therapy (Figure 4-3).

Figure 4-3. GORD symptom assessed by GerdQ scores. The individual value plot is overlaid with slope-graphs for those patients who completed GerdQ both on and off PPI therapy. The reference line indicates the score which has been reported as most discriminating as a cut-off in the diagnosis of GORD (Jones et al., 2009).

4.3.2 Oesophageal manometry

Thirty-seven subjects gave informed consent and attended for oesophageal physiology. Thirty-six completed all components of oesophageal physiology. One was unable to tolerate naso-oesophageal intubation so manometric assessment was abandoned. He was excluded from all analyses within the study.

All study participants underwent oesophageal manometry prior to ambulatory pH-impedance monitoring. The first 11 subjects underwent conventional 8-channel manometry and the remaining 25 underwent 20-channel high-resolution manometry. There were no complications arising from these tests.

The analysis of data differs for these two forms of oesophageal manometry. With reference to published normal ranges, it was possible to establish the proportion of normal tests for both (Spechler and Castell, 2001; Bredenoord et al., 2012).
8-channel manometry

Lower oesophageal sphincter (LOS) pressure was within normal limits for 8 of the 11 patients with a mean of 21.9mmHg (range 13-32mmHg). In the remaining three patients the LOS was hypertonic. Only one patient had complete relaxation of the LOS with swallowing. Median percentage relaxation was 32% (range 0-100%).

Seven subjects had normal peristaltic activity. One from this group had no LOS relaxation with swallowing, leaving six who can be described as having normal oesophageal swallows.

The remaining four subjects had evidence of dysmotility. Three had a high proportion of simultaneous contractions and one had a mixture of simultaneous and dropped contractions, denoting non-specific oesophageal dysmotility.

Median peristaltic amplitudes are shown in Table 4-3. Two patients had hypotonic segments and the remainder had proximal and distal amplitudes within the normal range.

![Table 4-3. Amplitudes of oesophageal peristalsis recorded at conventional 8-channel manometry. NA=not available.](image)

<table>
<thead>
<tr>
<th></th>
<th>Median (mmHg)</th>
<th>Range (mmHg)</th>
<th>Normal values (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum Oesophageal Amplitude</td>
<td>23</td>
<td>12-51</td>
<td>NA</td>
</tr>
<tr>
<td>Maximum Peristaltic Amplitude</td>
<td>157</td>
<td>104-282</td>
<td>NA</td>
</tr>
<tr>
<td>Average Peristaltic Amplitude</td>
<td>65</td>
<td>40-44</td>
<td>30-180</td>
</tr>
<tr>
<td>Distal Oesophageal Amplitude (5cm above LOS)</td>
<td>53</td>
<td>32-109</td>
<td>30-180</td>
</tr>
<tr>
<td>Proximal Oesophageal Amplitude (15cm above LOS)</td>
<td>68</td>
<td>22-282</td>
<td>30-180</td>
</tr>
</tbody>
</table>

High resolution manometry

Twenty-five patients underwent HRM. In contrast to 8-channel manometry, HRM simultaneously evaluates the LOS and the peristaltic waves within the oesophageal body. Results for the key metrics used to assess oesophageal swallows are detailed in Table 4-4.
Table 4-4. Metrics used to evaluate oesophageal swallow integrity using the Chicago classification

<table>
<thead>
<tr>
<th>Metric</th>
<th>Median</th>
<th>Range</th>
<th>Normal Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distal Latency (s)</td>
<td>6.4</td>
<td>4.5 – 8.6</td>
<td>&gt;4.5</td>
</tr>
<tr>
<td>Distal Contractile Integral (mmHg.s.cm)</td>
<td>835</td>
<td>14 – 4074</td>
<td>&lt;8000</td>
</tr>
<tr>
<td>Contractile front velocity (cm/s)</td>
<td>5.2</td>
<td>&lt;-100 - &gt;100</td>
<td>≤9</td>
</tr>
<tr>
<td>Peristaltic Breaks (cm)</td>
<td>1.9</td>
<td>0 – 13.3</td>
<td>&lt;2cm</td>
</tr>
<tr>
<td>Integrated Relaxation Pressure (mmHg)</td>
<td>6.5</td>
<td>-3.8 – 27.6</td>
<td>&lt;15</td>
</tr>
</tbody>
</table>

These metrics are automatically combined by an algorithm within the manometry software to generate an overall label, as defined by the Chicago classification, Figure 4-4.
Ten of the 25 subjects were categorised as having a normal swallow. A further 10 subjects had abnormalities consistent with hypomotility. The remaining five were categorised as having distal spasm, rapid contractions or OGJ outflow obstruction.

Figure 4-4. Chicago classification of HRM results

In summary, 8-channel manometry and HRM revealed abnormal swallows in 45% and 60% of these respective groups.
4.3.3 Ambulatory monitoring

pH monitoring

All thirty-six study subjects underwent multichannel intraluminal pH-impedance monitoring using an endo-oesophageal probe positioned manometrically. The pH probe is placed 5cm proximal to the lower oesophageal sphincter. The median duration of monitoring was 23 h 4 min (range: 15h 55 - 24h 45).

Twenty-five subjects were taking proton pump inhibitors at the time of recruitment to the study. All were asked to discontinue this medication 2 weeks prior to oesophageal physiology assessment. All but one subject (IPF21) discontinued therapy as requested. The patient who did not stated that he had forgotten to do so. (Data from this subject were included for analysis and are highlighted in relevant figures.) Key results are detailed in Table 4-5.

Table 4-5. The results of ambulatory pH monitoring. All figures are scaled so as to represent a 24 hour monitoring period. Long refluxes are defined as those in excess of five minutes.

<table>
<thead>
<tr>
<th></th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Normal range</th>
<th>Number supranormal</th>
<th>% supranormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of refluxes</td>
<td>42.7</td>
<td>0</td>
<td>326.7</td>
<td>&lt;50.2</td>
<td>17</td>
<td>47.2</td>
</tr>
<tr>
<td>Number of long refluxes</td>
<td>3.1</td>
<td>0</td>
<td>39.4</td>
<td>&lt;3.2</td>
<td>17</td>
<td>47.2</td>
</tr>
<tr>
<td>Duration of longest reflux (minutes)</td>
<td>12.1</td>
<td>0</td>
<td>164.3</td>
<td>&lt;9.3</td>
<td>21</td>
<td>58.3</td>
</tr>
<tr>
<td>Total reflux time (%)</td>
<td>6.3</td>
<td>0</td>
<td>60</td>
<td>&lt;4.3</td>
<td>20</td>
<td>55.6</td>
</tr>
<tr>
<td>DeMeester score</td>
<td>18.5</td>
<td>0.2</td>
<td>201.6</td>
<td>&lt;14.72</td>
<td>20</td>
<td>55.6</td>
</tr>
</tbody>
</table>

The range of values recorded for the reflux index and the DeMeester score are illustrated in Figure 4-5. With reference to either parameter, the same 20 subjects (56%) were identified to have supranormal levels of reflux.
Impedance monitoring

The duration of monitoring for the impedance results is identical to that for the pH studies as the same probe is used. As with the pH figures, results were scaled so as to be comparable with the 24-hour normal ranges.

Based on impedance monitoring, the median number of refluxes over 24 hours was 31.0 (range 9.3-119.2).

Impedance and pH monitoring data are combined to evaluate the acidity of refluxate. The breakdown of total, acid (pH<4), weakly acid (pH 4-7) and non-acid (pH 7+) reflux is detailed in Table 4-6. Values for the individual subjects are illustrated in Figure 4-6. Seven individuals recorded high levels of weakly acid reflux; none recorded high levels of non-acid reflux.

Objectively high levels of gastro-oesophageal reflux were defined as a high DeMeester score, a high level of total reflux on impedance monitoring, or both. According to this definition, 22 of 36 study subjects had high levels of reflux (61.1%), Table 4-7. Two subjects with high levels of reflux on impedance monitoring had normal DeMeester scores of 2.0 and 4.0. Both had high levels of weakly acid reflux.
Only 29% of the subjects with objectively high levels of reflux had evidence of GORD from GerdQ questionnaire results.

Table 4-6. Number of acid, weakly acid and non-acid refluxes recorded over a standardised 24 hour impedance monitoring period.

<table>
<thead>
<tr>
<th></th>
<th>Median number of refluxes (in 24h)</th>
<th>Minimum value</th>
<th>Maximum value</th>
<th>Normal range</th>
<th>Number supranormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>31.0</td>
<td>9.3</td>
<td>119.2</td>
<td>&lt;75</td>
<td>4</td>
</tr>
<tr>
<td>Acid</td>
<td>17.0</td>
<td>0</td>
<td>86.8</td>
<td>&lt;50</td>
<td>3</td>
</tr>
<tr>
<td>Weakly acid</td>
<td>10.5</td>
<td>0</td>
<td>89.8</td>
<td>&lt;33</td>
<td>7</td>
</tr>
<tr>
<td>Non-acid</td>
<td>0.0</td>
<td>0</td>
<td>3.8</td>
<td>&lt;15</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 4-7. Number of subjects identified to have high levels of reflux by pH and impedance monitoring

<table>
<thead>
<tr>
<th>Level of reflux as assessed by pH monitoring</th>
<th>High</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level of reflux as assessed by impedance monitoring</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Normal</td>
<td>18</td>
<td>14</td>
</tr>
</tbody>
</table>

The exposure of the oesophagus to gastric refluxate is measured by the average bolus clearance time and the oesophageal volume exposure. Bolus clearance time was prolonged in 11 of 36 study subjects and volume exposure was prolonged in four subjects.

The proximal channel of the oesophageal probe is 17cm up from the tip. When episodes of reflux are recorded at this uppermost level they are described as proximal. A median of 6.4 proximal reflux episodes were recorded in the current study. The upper limit of the 95% confidence interval for a health population was reported as 30. As a proportion of the total number of refluxes recorded, a median of 10.6% were registered at the proximal channel (normal range 12-39). Only three individuals had supranormal levels of reflux over a 24-hour period.
Figure 4-6. 24-hour reflux events recorded at impedance monitoring. 95th centile reference lines indicate the published upper limits of normal for healthy controls (Zerbib et al., 2005). * indicates individuals with pathological levels of reflux on pH monitoring; † indicates individuals with pathological levels of proximal reflux. Subject number 21 was studied on PPI therapy.
4.4 pH-impedance analysis: inter-rater reliability

To protect against reporting bias, one in three pH-impedance studies from the latter cohort were re-reported by a rater blinded to the results of the questionnaires and BAL analysis. The key results of this comparison are detailed in Table 4-8 and Figure 4-7. Pearson r and intraclass correlation coefficients both indicate strong inter-rater reliability.

Table 4-8. Key pH-impedance results as reported by two independent raters. Rater A=Rhys Jones. Rater B=Amaran Krishnan, a surgical registrar and former research fellow who was blinded to the remainder of these individuals’ results.

<table>
<thead>
<tr>
<th>Study number</th>
<th>24h pH reflux episodes (A)</th>
<th>24h pH reflux episodes (B)</th>
<th>24h impedance reflux episodes (A)</th>
<th>24h impedance reflux episodes (B)</th>
<th>24h proximal reflux episodes (A)</th>
<th>24h proximal reflux episodes (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>36.0</td>
<td>36.7</td>
<td>43.7</td>
<td>50</td>
<td>9.8</td>
<td>11.1</td>
</tr>
<tr>
<td>24</td>
<td>7.3</td>
<td>9.0</td>
<td>59.7</td>
<td>64.1</td>
<td>7.3</td>
<td>7.4</td>
</tr>
<tr>
<td>27</td>
<td>53.2</td>
<td>53.6</td>
<td>16.8</td>
<td>19.7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>31</td>
<td>77.7</td>
<td>74.0</td>
<td>38.7</td>
<td>39</td>
<td>8.5</td>
<td>8.5</td>
</tr>
<tr>
<td>36</td>
<td>59.9</td>
<td>59.9</td>
<td>27.5</td>
<td>31.9</td>
<td>12.1</td>
<td>12.1</td>
</tr>
<tr>
<td>Pearson r</td>
<td>0.999</td>
<td>0.992</td>
<td>0.993</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intraclass correlation</td>
<td>0.997</td>
<td>0.995</td>
<td>0.993</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 4-7. Scatterplots to illustrate the level of agreement between key pH-impedance reflux episode counts. Rater details are included in Table 4-8.
4.5 Assessment of laryngopharyngeal reflux and pulmonary aspiration

4.5.1 Subjective assessment

Symptoms of laryngopharyngeal reflux were assessed with the Reflux Symptom Index (RSI). Nine items are graded from 0 (no problem) to 5 (severe problem) to give a maximum score of 45. The normal range in a healthy population was reported as 0-13 (Belafsky et al., 2002).

Twenty-five study subjects were taking a PPI at the time of recruitment. Twenty-three completed the RSI questionnaire before discontinuing their medication. Two subjects failed to answer at least one question and their responses were excluded. The median score for the on-PPI response was 14. Twelve subjects (48%) had scores greater than 13, suggesting evidence of laryngopharyngeal reflux.

Off PPI, the median RSI score was 10. Thirteen patients (37%) had positive RSI scores. The median difference when subjects’ on-PPI and off-PPI scores were compared was -1. In summary, the questionnaires suggested slightly higher levels of LPR when subjects were on PPI therapy than when they were off PPI therapy but the difference was small.

On and off-PPI scores are illustrated in Figure 4-8. There was no consistent pattern associated with PPI cessation.

There was no good correlation between the objective measurement of proximal reflux and the symptoms of laryngopharyngeal reflux, as assessed by the RSI questionnaire (Pearson correlation coefficient 0.047),

Figure 4-9. The upper limit for the 95% confidence interval for 24-hour proximal refluxes is 30. Only three subjects had high levels of proximal reflux in the current study.
Figure 4-8. Extra-oesophageal symptoms as assessed by Reflux Symptom Index in patients taking PPI therapy at the time of study recruitment. PPI was discontinued for two weeks prior to physiology testing.

Figure 4-9. Scatterplot to illustrate the relationship between levels of objectively measured proximal reflux and symptoms of laryngopharyngeal reflux.
Pharyngeal imaging

Endoscopic appearances of the hypopharynx were recorded at the time of bronchoscopy and later evaluated for evidence of LPR by an external assessor. The Reflux Finding Score (RFS) has been validated for this purpose, with a normal range described as 0-7 (Belafsky et al., 2001).

Representative images are illustrated in Figure 4-10.

Scoring was possible in 35 of 36 subjects. In one, the images were of insufficient quality. RFS and RSI scores are detailed in Table 4-9.

There was no clear association between RFS and RSI scores (Pearson coefficient 0.184, p=0.298). Similarly, there was relationship between RFS and objective measures of reflux (overall or proximal) or aspiration (BAL pepsin concentration).
Figure 4-10. Illustrative bronchoscopy images captured for an external assessor to evaluate evidence of laryngopharyngeal reflux using the Reflux Finding Score (Belafsky et al., 2001)
Table 4-9. Bronchoscopic and symptomatic assessment of laryngopharyngeal reflux. Four of the eight components of the reflux findings scale (RFS) are included here for illustration. RSI=Reflux symptom index. Images for subject IPF25 could not be scored due to insufficient image quality.

<table>
<thead>
<tr>
<th>Patient No</th>
<th>Vocal fold oedema</th>
<th>Diffuse laryngeal oedema</th>
<th>Posterior Commissure hypertrophy</th>
<th>Granuloma/ granulation tissue</th>
<th>RFS Total</th>
<th>RSI Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPF1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>IPF2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>IPF3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>IPF4</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>IPF5</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>11</td>
<td>34</td>
</tr>
<tr>
<td>IPF6</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>18</td>
</tr>
<tr>
<td>IPF7</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>IPF8</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>IPF9</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>11</td>
<td>39</td>
</tr>
<tr>
<td>IPF10</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>11</td>
<td>23</td>
</tr>
<tr>
<td>IPF11</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>IPF12</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>IPF13</td>
<td>3</td>
<td>2</td>
<td>1</td>
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4.5.2 Bronchoscopy and bronchoalveolar lavage

All subjects underwent a standardised bronchoscopy and bronchoalveolar lavage, using 3 x 60ml instillations of normal saline. Thirty-four experienced no complications. One participant, who was taking the antiplatelet agent clopidogrel, suffered a nosebleed and the procedure was abandoned and repeated after clopidogrel had been suspended. Another participant was admitted to her local hospital the evening of the procedure with symptoms of lethargy and malaise. Her symptoms settled without specific treatment and she was discharged the following day.

Biochemical quantification

Pepsin

Pepsin concentration was measured in bronchoalveolar lavage fluid using a validated in-house ELISA. Assays were performed in two batches; standard curves are illustrated in Figure 4-11.
Figure 4-11. Standard curves for the ELISA used to quantify pepsin concentrations in BAL fluid

The limit of detection for both experiments was 3ng/ml. The median pepsin concentration for the study subjects was 0ng/ml, which was equivalent to the median value of 1.1ng/ml for the control subjects. The mean pepsin concentration for the IPF subjects and the controls was 12.0ng/ml and 1.1ng/ml, respectively. Seventeen of the thirty-six study subjects (44%) had pepsin concentrations above the levels recorded in the control population. The values for these positive readings ranged from 7-44ng/ml. Pepsin concentrations for each subject are listed in Table 4-10 below.
There was no significant correlation between pepsin concentration and summary reflux parameters, including DeMeester score, reflux index or total number of refluxes. Pepsin concentrations for the IPF patients was significantly higher than those recorded in the control group.

The IPF group was then divided into those with pH-impedance evidence of GOR, and those without. Objective evidence of reflux was defined as a high DeMeester score, a high total number of refluxes at impedance monitoring, or both. The pepsin concentrations for these two groups, in addition to the control group, are illustrated in Figure 4-12.

**Figure 4-12.** Pepsin concentrations in IPF study subjects and healthy controls. In the IPF study subjects, the presence of gastro-oesophageal reflux was defined at pH-impedance monitoring by a high DeMeester score, a high total number of refluxes, or both.
Table 4-10. pH-impedance monitoring results and BAL pepsin concentrations in all study subjects. Normal values are included in brackets (Jamieson et al., 1992; Zerbib et al., 2013).

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Bile salts: spectrophotometry

BALF bile salt concentrations are detailed in Table 4-11. Only three subjects had detectable levels of bile acids using the TBA assay. An additional subject had only one valid reading generated at spectrophotometry. Samples from these four subjects were verified with a further set of readings.

One of these subjects had high levels of reflux on pH monitoring, and the other two had normal pH-impedance results.
Table 4-11. Bronchoalveolar lavage fluid bile salt concentration measured in all 35 IPF patients using a spectrophotometric total bile salt assay. All measurements are in µmol/l. Three separate runs were initially performed and an average result determined. Positive readings were verified with a further set of readings. Zero values indicate readings lower than the limit of detection.

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<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>34</td>
<td>0</td>
<td>1</td>
<td>0</td>
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<td>0</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td></td>
<td></td>
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<td></td>
</tr>
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<td>36</td>
<td>0</td>
<td>0</td>
<td>xxx</td>
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<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Cytology

Bronchoalveolar lavage fluid was captured after the tip of the bronchoscope was wedged into a right middle lobe subsegmental bronchus. The median fluid volume retrieved was 82.8ml. After 5ml of fluid was sent for microbiology, the remaining sample was transferred to university laboratories on ice for processing. Processing was undertaken within 1 hour of retrieval in all cases.

The median total BAL cell count was $19.2 \times 10^4$ cells/ml. Summary figures for BAL cytology are detailed in Table 4-12 with reference to values recorded in a group of healthy controls (Zheng et al., 2000). The IPF study subjects had higher total cell counts and a higher proportion of neutrophils. There were lower proportions of lymphocytes and eosinophils in the study subjects.

<table>
<thead>
<tr>
<th></th>
<th>IPF study patients</th>
<th>Normal values</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total BAL cell count</td>
<td>19.2 (1.8-236.0)</td>
<td>14 (12-16)</td>
<td>0.006</td>
</tr>
<tr>
<td>Macrophages (%)</td>
<td>87.9 (23.4-99.4)</td>
<td>73 (66-80)</td>
<td>0.376</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>6.8 (0.2-93)</td>
<td>2.1 (1.6-2.6)</td>
<td>0.001</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>3.0 (0.4-58.0)</td>
<td>20 (14-26)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>0.7 (0-12.0)</td>
<td>1.1 (0-2.2)</td>
<td>0.044</td>
</tr>
</tbody>
</table>

Lipid ingestion

Cytospin slides from each subject were stained with Oil Red O for enumeration of a lipid-laden macrophage index (LLMI). The cytoplasmic opacification of 300 macrophages was enumerated using a scale from 0-4. In order to improve the consistency of enumeration, a reference chart was produced using images from the current study subjects, Figure 4-13. LLMI scores are listed in Table 4-13.

One in four samples were second read by an experienced biomedical scientist in the research laboratories at the Freeman Hospital (KJ). The Pearson correlation coefficient for the paired readings was 0.913, conventionally regarded as a very strong positive relationship.
In addition, the Bland-Altman method for evaluating inter-rater agreement was used to assess the difference between the reported ORO scores, as described in section 3.1.13 (Bland and Altman, 1986). The mean difference between the two readings was -1.5 and the standard deviation of the differences was 2.7. The limits of agreement were therefore -6.8 to 3.9.

Figure 4-13. Reference chart used to support LLMI grading

Lipid-laden macrophage grading
Kitz et al. Pulm Med 2012

- Cytoplasm not opacified
- up to ¼ opacified
- up to ½ opacified
- up to ¾ opacified
- totally opacified cytoplasm
Oxidative stress

A further 200 macrophages were counted on separate cytospin slides after staining with Perls Prussian Blue. Haemosiderin scores are listed in Table 4-13. Illustrative photomicrographs are shown in Figure 4-14 and Figure 4-15.

Table 4-13. LLMI and haemosiderin scores enumerated from BAL cytopsins

<table>
<thead>
<tr>
<th>Study number</th>
<th>LLMI</th>
<th>Haemosiderin score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>52</td>
<td>19.5</td>
</tr>
<tr>
<td>2</td>
<td>23</td>
<td>236.5</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>4.5</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>19.5</td>
</tr>
<tr>
<td>5</td>
<td>139</td>
<td>9.5</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>7</td>
<td>13</td>
<td>67</td>
</tr>
<tr>
<td>8</td>
<td>57</td>
<td>115.5</td>
</tr>
<tr>
<td>9</td>
<td>12</td>
<td>89</td>
</tr>
<tr>
<td>10</td>
<td>34</td>
<td>11.5</td>
</tr>
<tr>
<td>11</td>
<td>30</td>
<td>109.5</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>13</td>
<td>73</td>
<td>207.5</td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>6.5</td>
</tr>
<tr>
<td>15</td>
<td>7</td>
<td>44</td>
</tr>
<tr>
<td>16</td>
<td>18</td>
<td>3</td>
</tr>
<tr>
<td>17</td>
<td>11</td>
<td>46.5</td>
</tr>
<tr>
<td>18</td>
<td>5</td>
<td>71.5</td>
</tr>
<tr>
<td>19</td>
<td>310</td>
<td>4.5</td>
</tr>
<tr>
<td>20</td>
<td>24</td>
<td>74.5</td>
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<td>21</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>22</td>
<td>3</td>
<td>417</td>
</tr>
<tr>
<td>23</td>
<td>19</td>
<td>69</td>
</tr>
<tr>
<td>24</td>
<td>0</td>
<td>12</td>
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<tr>
<td>25</td>
<td>18</td>
<td>248</td>
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<tr>
<td>26</td>
<td>73</td>
<td>10</td>
</tr>
<tr>
<td>27</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>28</td>
<td>10</td>
<td>344</td>
</tr>
<tr>
<td>29</td>
<td>0</td>
<td>78</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
<td>60</td>
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<tr>
<td>31</td>
<td>2</td>
<td>67</td>
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<tr>
<td>32</td>
<td>5</td>
<td>7</td>
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<tr>
<td>33</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>34</td>
<td>0</td>
<td>79</td>
</tr>
<tr>
<td>35</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td>36</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>
Figure 4-14. Illustrative photomicrographs of cytospin cell staining with Giemsa, Perls Prussian Blue and Oil Red O stains.
Figure 4-15. Illustrative photomicrographs of cytospin cell staining with Giemsa, Perls Prussian Blue and Oil Red O stains.
Summary values for cell staining are illustrated in Table 4-14. Thirty-three of 36 subjects had haemosiderin scores in excess of the normal range for healthy control subjects (Reid et al., 2001). With respect to Oil Red O staining, a marker of lipid-laden macrophages, only six of the study subjects had values in excess of the normal range.

Table 4-14. Summary values for cell staining counts. Results are expressed as median (range).

<table>
<thead>
<tr>
<th></th>
<th>Study patients</th>
<th>Published healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Reid et al., 2001; Basset-Leobon et al., 2010)</td>
<td></td>
</tr>
<tr>
<td>Haemosiderin score</td>
<td>39 (0-417)</td>
<td>0 (0-2)</td>
</tr>
<tr>
<td>Lipid-laden macrophage index</td>
<td>9 (0-310)</td>
<td>5.47 (0-49)</td>
</tr>
</tbody>
</table>

There was no good correlation between objective markers of reflux and the lipid-laden macrophage index. Figure 4-16 and Figure 4-17 illustrate the relationship between the DeMeester score and the LLMI, for which the Spearman Rho correlation coefficient was 0.056. The correlation coefficients relating to impedance monitoring parameters (total refluxes and proximal refluxes) were lower still.

As a group, haemosiderin scores for the IPF subjects in the current study were significantly higher than those reported in healthy controls (Reid et al., 2001). Scores in excess of the normal range were calculated in 33 of the 36 study subjects.

The relationship between disease severity and haemosiderin score was assessed by splitting the group into two with respect to the vital capacity. Subjects with percentage predicted vital capacity higher than the median value (77.6%) had lower haemosiderin scores (9.5 vs 71.5, Kruskal-Wallis p=0.038, Figure 4-18). As with the LLMI, haemosiderin scores were not associated with reflux status (Figure 4-19). There was no association with smoking status, age or N-acetyl cysteine use or BALF neutrophilia.
Figure 4-16. The relationship between LLMI and DeMeester scores for the 36 study subjects

Figure 4-17. LLMI calculated for study subjects grouped by DeMeester score; 14.7 is the upper limit of normal in healthy controls and values above this are consistent with a high level of gastro-oesophageal reflux
Figure 4-18. Haemosiderin scores for study subjects stratified by lung disease severity. Subjects were dichotomised with respect to the median figure for % predicted vital capacity.

Figure 4-19. Haemosiderin score calculated for study subjects grouped by DeMeester score
4.6 Multidisciplinary review and subsequent management

The participants in the current study volunteered to undergo invasive clinical testing. The oesophageal physiology data generated are equivalent to those which would guide the clinical management of patients in the upper gastrointestinal surgery clinic. The optimal management of gastro-oesophageal reflux in IPF remains unclear, so the appropriate response to these clinical data was not well defined.

As a result, an aerodigestive multidisciplinary team was convened to discuss the results generated in the current study. The team meets monthly and the core membership comprises oesophago-gastric surgeons, respiratory physicians, anaesthetists and research scientists. To the present day, the MDT continues to run. The majority of the cases discussed relate to:

- Reflux in IPF patients and lung transplant recipients
- Smaller numbers of patients with cystic fibrosis, systemic sclerosis, bronchiectasis
- Management of giant hiatal hernias with suspected pulmonary compromise
- Investigation and management of adult trachea-oesophageal fistula

The management of all 36 patients was formally discussed by the aerodigestive multidisciplinary team. Antireflux surgery was recommended for three patients; to date two have undergone surgery and the third declined surgery for the present time. Of the two that underwent fundoplication, one patient had received a lung transplant prior to fundoplication.
4.7 Summary

In the current study, 36 consecutive patients with a well-characterised diagnosis of IPF were recruited from the regional interstitial lung disease clinic at the Royal Victoria Infirmary in Newcastle upon Tyne.

Of those who were booked into the study, two individuals died before they were due to participate and two more did not attend due to disease progression. All but one subject were able to tolerate both oesophageal physiology monitoring and bronchoalveolar lavage.

From existing clinical assessments, 31% of subjects had prior evidence of GORD. Structured questionnaire (GerdQ) assessment suggested GORD in 29% of subjects off-PPI. In the small group of subjects who repeated structured assessments of respiratory HR-QOL questionnaires on-and off-PPI, significantly better scores were recorded after cessation of therapy. Extra-oesophageal reflux was evident from questionnaire assessment in 37% of subjects off-PPI.

The results of oesophageal manometry were normal in 44% and abnormal in 56%. The majority of subjects with abnormal swallows had weak peristalsis.

Ambulatory pH monitoring identified pathological levels of reflux in 56% of subjects and impedance monitoring demonstrated pathological reflux in 11%. In combination, pH-impedance monitoring suggested objectively high levels of reflux in 61% of subjects.

At bronchoalveolar lavage, bile acids were essentially undetectable. High levels of pepsin were detected in 44% of study subjects. The combination of pepsin quantification and oesophageal monitoring identified a subgroup with evidence of reflux and aspiration, but there was no correlation between levels of reflux and pepsin concentrations.

Cytological staining of BAL fluid samples suggested high levels of oxidative stress in 92% of subjects. Abnormal macrophage lipid uptake was only seen in 17%, half of whom had physiological levels of reflux on ambulatory monitoring.

After formal multidisciplinary review, two patients who participated in the current study have undergone fundoplication.
Chapter 5. Results of Patient Attitudes Study

5.1 Interview summary and stratification by disease severity

Subjects with a secure diagnosis of idiopathic pulmonary fibrosis were identified from the regional interstitial lung disease clinic and invited to complete a scripted interview. The interview comprised four stages:

1. Demographic survey

2. Respondents were asked to read six health states (Box 3. Health state descriptions used in the standard gamble. Outcome W: mild IPF. Outcome X: moderate IPF. Outcome Y: adverse outcome from antireflux surgery. Outcome Z: adverse outcome from medical antireflux therapy,) printed on cards and rank them. There were four descriptive health states in addition to the health states “Full health” and “Immediate death”

3. Standard gamble utility analysis for the four descriptive health states, in addition to the health state “Own health”

4. Euro-QOL 5D-3L

<table>
<thead>
<tr>
<th>Outcome W</th>
<th>Outcome X</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Good energy levels</td>
<td>• Reduced energy levels</td>
</tr>
<tr>
<td>• Manage your own washing and dressing</td>
<td>• Manage your own washing and dressing</td>
</tr>
<tr>
<td>• Short of breath when hurrying or walking up a slight hill</td>
<td>• Stopping for breath after about 100m or after a few minutes walking on the level</td>
</tr>
<tr>
<td>• Coughing, which is troublesome to you one or two days a week</td>
<td>• Coughing, which is troublesome for you on most days</td>
</tr>
<tr>
<td>• No need for oxygen support</td>
<td>• Using supplemental oxygen</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Outcome Y</th>
<th>Outcome Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Five scars on your stomach, each measuring 1cm in length</td>
<td>Taking five extra tablets over the course of the day, plus Gaviscon liquid after meals.</td>
</tr>
<tr>
<td>• Not able to belch, leading to bloating and abdominal discomfort</td>
<td>You may experience the following problems:</td>
</tr>
<tr>
<td>• Food sticking in your gullet at times</td>
<td><strong>Common</strong></td>
</tr>
<tr>
<td>• Only managing small meals</td>
<td>• Headache</td>
</tr>
<tr>
<td>• Risk of bleeding, infection and hernias from surgery</td>
<td>• Abdominal pain</td>
</tr>
<tr>
<td><em>(A hernia is a bulge at the site of a scar, which may be uncomfortable or painful. Sometimes hernias need to be repaired with an operation.)</em></td>
<td>• Constipation/diarrhoea which require treatment with tablets</td>
</tr>
<tr>
<td></td>
<td>• Nausea/vomiting</td>
</tr>
<tr>
<td></td>
<td><strong>Rare</strong></td>
</tr>
<tr>
<td></td>
<td>• Reduced energy levels</td>
</tr>
<tr>
<td></td>
<td>• Serious gut infections</td>
</tr>
<tr>
<td></td>
<td>• Increased risk of broken bones</td>
</tr>
</tbody>
</table>

Pulmonary function tests are routinely recorded at every visit to the interstitial lung disease clinic. At the time of the study interview, the most recent vital capacity (measured and predicted) was recorded from clinic letters. The median interval between interview and pulmonary function testing was 54 days (0-242). The percentage predicted vital capacity ranged from 41.7 to 133.3%. The median percentage predicted vital capacity was 78% (IQR 68.4-86.4). This midpoint was used to dichotomise the group into the “Top half” and “Bottom half”, with respect to objective pulmonary function.
5.2 Summary statistics

A total of 59 subjects were interviewed. Fifty-eight patients were able to complete all parts of the interview. One patient did not complete the standard gamble. He was awaiting an outpatient review and explained that he was frustrated about the delay in being seen.

The demographics of the respondents are summarised in Table 5-2. The mean age was 75.1 years, with a range of 51-92 and a standard deviation of 8.5.

The respondents were largely male, in a ratio of approximately 2:1. The overwhelming majority were retired. Half had no formal qualifications. All of the study subjects were White British.

The respondents’ assessment of their own functional status was recorded using EQ5D-3L, summarised in Table 5-1.

**Table 5-1. Summary responses to the EQ5D-3L survey**

<table>
<thead>
<tr>
<th>Domain</th>
<th>Frequency of response</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mobility</strong></td>
<td></td>
</tr>
<tr>
<td>No problems</td>
<td>12</td>
</tr>
<tr>
<td>Some problems</td>
<td>45</td>
</tr>
<tr>
<td>Confined to bed</td>
<td>1</td>
</tr>
<tr>
<td><strong>Self-Care</strong></td>
<td></td>
</tr>
<tr>
<td>No problems</td>
<td>45</td>
</tr>
<tr>
<td>Some problems</td>
<td>11</td>
</tr>
<tr>
<td>Unable to wash/dress</td>
<td>2</td>
</tr>
<tr>
<td><strong>Usual Activities</strong></td>
<td></td>
</tr>
<tr>
<td>No problems</td>
<td>17</td>
</tr>
<tr>
<td>Some problems</td>
<td>34</td>
</tr>
<tr>
<td>Unable</td>
<td>7</td>
</tr>
<tr>
<td><strong>Pain/Discomfort</strong></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>19</td>
</tr>
<tr>
<td>Moderate</td>
<td>32</td>
</tr>
<tr>
<td>Extreme</td>
<td>7</td>
</tr>
<tr>
<td><strong>Anxiety/Depression</strong></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>37</td>
</tr>
<tr>
<td>Moderate</td>
<td>21</td>
</tr>
<tr>
<td>Extremely</td>
<td>1</td>
</tr>
</tbody>
</table>
# Table 5-2. Demographic summary statistics

<table>
<thead>
<tr>
<th>Demographic</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>38</td>
</tr>
<tr>
<td>Female</td>
<td>21</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
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<tr>
<td>Married</td>
<td>40</td>
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<tr>
<td>Single</td>
<td>3</td>
</tr>
<tr>
<td>Divorced/separated</td>
<td>5</td>
</tr>
<tr>
<td>Widowed</td>
<td>11</td>
</tr>
<tr>
<td><strong>Employment status</strong></td>
<td></td>
</tr>
<tr>
<td>Employed</td>
<td>4</td>
</tr>
<tr>
<td>Retired</td>
<td>52</td>
</tr>
<tr>
<td>At home/not looking for work</td>
<td>1</td>
</tr>
<tr>
<td>Unable to work</td>
<td>2</td>
</tr>
<tr>
<td><strong>Qualifications</strong></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>28</td>
</tr>
<tr>
<td>GCSE (1-4)</td>
<td>2</td>
</tr>
<tr>
<td>GCSE (5+)</td>
<td>6</td>
</tr>
<tr>
<td>A-level (2+)</td>
<td>2</td>
</tr>
<tr>
<td>First Degree</td>
<td>6</td>
</tr>
<tr>
<td>Higher degree</td>
<td>3</td>
</tr>
<tr>
<td>NVQ level 3</td>
<td>1</td>
</tr>
<tr>
<td>NVQ level 4-5</td>
<td>3</td>
</tr>
<tr>
<td>Other</td>
<td>8</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
</tr>
<tr>
<td>White British</td>
<td>59</td>
</tr>
</tbody>
</table>
5.3 Validation statistics

Of the 59 respondents, 50 returned health state utilities which differentiated between the respective health states. The remaining 9 recorded the same utility values across each of the health states.

All respondents regarded Full health as preferable to all remaining scenarios in both the ranking exercise and the standard gamble utility measurement.

With respect to the specific scenarios, only the mild and moderate IPF disease states had an inherently ordinal relationship. For 53 of 59 respondents, utility measurements indicated that the mild IPF disease state was perceived as preferable to the moderate IPF disease state. Using a restrictive test, 31 of 59 respondents returned higher utility values for the mild IPF disease state than for the moderate IPF disease state. For the group as a whole, the mean utility value for the mild IPF state was higher than that for the moderate IPF disease state.

Test validity can also be assessed by comparing individual respondents’ rank preferences with the utility values they recorded through the standard gamble. Two formulae were used in Microsoft Excel:

Permissive test: =IF((HS#1>=HS#2)AND(HS#2>=HS#3)AND(HS#3>=HS#4),"True","False")

Restrictive test:
=IF((HS#1>HS#2)AND(HS#2>HS#3)AND(HS#3>HS#4),"True","False")

where HS#1...#4 represent the order of the health states as ranked by the individual respondent.

The permissive test therefore accepts greater than/equal to relationships between health states ranked adjacent to each other. According to this test, health state rankings were concordant with health state utility valuations for 41 respondents and discordant for 18.

According to the restrictive test, rankings and utility valuations were concordant for 2 of the 59 respondents.
5.4 Health state ranking exercise

Respondents were asked to put the four descriptive health states, in addition to the health states “Full health” and “Death”, into their personal order of preference. The majority of respondents (33 of 59) ranked the health states in the following order:

1. Full health
2. Mild IPF
3. Moderate IPF
4. Antireflux medication
5. Antireflux surgery
6. Death

Full results of the ranking exercise are illustrated in Figure 5-1.

**Figure 5-1.** The frequency with which respondents ranked the health states in a specified order. Figures in brackets indicate respondents who ranked death as preferable to one of the descriptive health states.

All respondents ranked the Full health state as preferable to all the remaining health states. Two respondents valued death as preferable to one of the descriptive health states. The majority of respondents (38 of 59) regarded both the mild and moderate IPF disease states as preferable to either of the adverse outcomes from antireflux therapy.

The vignette describing an adverse outcome from medical antireflux therapy was regarded as preferable to that for an adverse outcome from antireflux surgery by 51 of 59 respondents.

Of the descriptive scenarios, 46 respondents ranked antireflux surgery as the least preferable and eight ranked moderate IPF as the least preferable.
5.5 Health state utility analysis

The health state valuations recorded in the current study are detailed in Table 5-3 and Figure 5-2.

Table 5-3. Health state valuations. SD = standard deviation. OSG = own health evaluation by standard gamble. OE = own health evaluation by EQ5D. The terms “Top half” and “Bottom half” refer to a subdivision with respect to objective pulmonary function (section 5.1).

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Full sample (n)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild IPF (W)</td>
<td>0.862</td>
<td>0.108</td>
</tr>
<tr>
<td>Moderate IPF (X)</td>
<td>0.760</td>
<td>0.198</td>
</tr>
<tr>
<td>Antireflux surgery (Y)</td>
<td>0.679</td>
<td>0.230</td>
</tr>
<tr>
<td>Antireflux medication (Z)</td>
<td>0.759</td>
<td>0.204</td>
</tr>
<tr>
<td>Own health (OSG)</td>
<td>0.798</td>
<td>0.188</td>
</tr>
<tr>
<td>Own health (OE)</td>
<td>0.613</td>
<td>0.280</td>
</tr>
<tr>
<td><strong>Top half (n)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild IPF (W)</td>
<td>0.860</td>
<td>0.108</td>
</tr>
<tr>
<td>Moderate IPF (X)</td>
<td>0.752</td>
<td>0.201</td>
</tr>
<tr>
<td>Antireflux surgery (Y)</td>
<td>0.686</td>
<td>0.211</td>
</tr>
<tr>
<td>Antireflux medication (Z)</td>
<td>0.767</td>
<td>0.202</td>
</tr>
<tr>
<td>Own health (OSG)</td>
<td>0.821</td>
<td>0.174</td>
</tr>
<tr>
<td>Own health (OE)</td>
<td>0.656</td>
<td>0.236</td>
</tr>
<tr>
<td><strong>Bottom half (n)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild IPF (W)</td>
<td>0.864</td>
<td>0.111</td>
</tr>
<tr>
<td>Moderate IPF (X)</td>
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<td>0.201</td>
</tr>
<tr>
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<td>0.254</td>
</tr>
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<td>Antireflux medication (Z)</td>
<td>0.752</td>
<td>0.214</td>
</tr>
<tr>
<td>Own health (OSG)</td>
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<td>0.196</td>
</tr>
<tr>
<td>Own health (OE)</td>
<td>0.590</td>
<td>0.301</td>
</tr>
</tbody>
</table>
Consistent with the results of the ranking exercise, the mean health state utility recorded for mild IPF was significantly higher than that recorded for moderate IPF. The utility values for both of these states were significantly higher than those recorded for the adverse outcome from antireflux surgery. The scenario describing an adverse outcome from medical antireflux therapy was valued as similar to the moderate IPF health state but significantly worse than the mild IPF health state.

On average, respondents’ own health was rated worse than the mild IPF health state (p=0.03). The difference between the utility values for respondents’ own health and the moderate IPF health state was not significantly different (p=0.28).
Figure 5-3. Mean health state evaluations recorded for respondent’s own current health. P-value refers to a 2-sample T-test.

Own health utility values measured by standard gamble were higher than those measured by EQ5D, the utilities for which are generated with reference to the results of time trade-off interviews. The utility values recorded with the visual analogue scale within EQ5D were not significantly different from the values by the tool as a whole.
There were no statistically significant differences between the sub-groups of respondents after stratification by lung function but there were some trends: The mean utility values recorded for the IPF health states were slightly higher in those patients with worse pulmonary function, and the converse was true for the scenarios relating to antireflux therapy, Figure 5-4. The mean utility values that respondents recorded for their own health was slightly higher in those with better lung function. Again, this difference was not statistically significant (p=0.36 for EQ5D ratings).

As with the unstratified analysis, the health state utilities recorded by standard gamble were significantly higher than those recorded by EQ5D. These differences were statistically significant for the Top half and for the Bottom half (p=0.004 and p=0.006, respectively).
5.6 Correlation between lung function and own health utility values

Finally, reported utilities were assessed against physiological disease severity.

To begin, we compared the two subjective measures of own health. There was a weak correlation between the paired valuations for respondents’ own health, as measured by standard gamble and EQ5D (Figure 5-5); Pearson co-efficient of correlation 0.366, p=0.005. There were numerous examples of respondents who scored high scores with one assessment and low scores on the other assessment.

Figure 5-5. Scatterplot comparing respondents’ own health valuations as measured by standard gamble and EQ5D

![Scatterplot comparing respondents' own health valuations as measured by standard gamble and EQ5D](image)

Figure 5-6 and Figure 5-7 illustrate the correlation between respondents’ vital capacity and own-health valuations. The correlation between standard gamble-derived utilities and vital capacity was weak (Pearson correlation coefficient 0.129, p-value 0.342).

The relationship was marginally closer when the EQ5D scores were analysed (Pearson correlation co-efficient 0.191, p-value 0.16 (Figure 5-7).
Figure 5-6. Scatterplot comparing the percentage predicted vital capacity with respondents' own health valuations as measured by standard gamble.

Figure 5-7. Scatterplot comparing the percentage predicted vital capacity with respondents' own health valuations as measured by EQ5D.

5.7 Summary

The overwhelming majority of study subjects were able to complete the entirety of the interview, including multiple iterations of the standard gamble exercise.

The face validity of the defined health states was assessed with the use of logical checks. Similarly, the respondents’ own health states could be compared to their valuations of the defined IPF health states. On average, respondents’ own health was not significantly different to the health state defined as moderate IPF.

The use of Full health and Death as anchor states was largely supported by the respondents’ valuations, although this pattern was not universally applicable.

The majority of respondents regarded both IPF health states as preferable to either antireflux therapy outcome. An adverse outcome from antireflux surgery was generally perceived to be the worst of all the health states.

In this study, own health was valued higher by the standard gamble than it was when assessed by EQ5D, or the visual analogue scale that forms part of the EQ5D tool.

Health state valuations were not significantly different after stratification by physiological disease severity.

Vital capacity was poorly correlated with valuations for Own health.
Chapter 6. Discussion

Idiopathic pulmonary fibrosis is a progressive and disabling lung disease associated with high rates of mortality and morbidity, for which there are very limited treatment options. Evidence from the epidemiological literature suggests it is increasingly common (Navaratnam et al., 2011). The last decade has seen a significant increase in research efforts. Whilst the disease process is now better characterised, understanding the aetiology and the development of effective therapies has been more difficult.

IPF is largely a disease of the elderly. The association of significant morbidity is partly related to the advanced age of typical sufferers, but there is clear evidence of comorbidities occurring at higher levels than they do in age-matched controls. Coronary heart disease and diabetes are two well-documented examples (Raghu et al., 2015a).

Gastro-oesophageal reflux is common in IPF patients and pulmonary fibrosis is also common in those with reflux disease (El-Serag and Sonnenberg, 1997; Savarino et al., 2013). Whilst reflux may simply represent a comorbidity in IPF, several strands of evidence suggest that reflux may contribute to the parenchymal changes seen in IPF lungs:

- Bronchoalveolar lavage analysis suggests an association between microaspiration and high rates of acute exacerbations in IPF (Lee et al., 2012)
- Retrospective studies have reported a benefit of antireflux therapy in patients with IPF (Lee et al., 2011)
- Imaging studies also suggest a possible link between gastro-oesophageal reflux and disease severity, as evidenced by asymmetrical disease (Tcherakian et al., 2011)

This area of research has proved challenging for a number of reasons. The identification of gastro-oesophageal reflux identifies a risk of microaspiration, but the difficulty of sampling the intrapulmonary environment makes microaspiration more difficult to demonstrate directly. The damaging effect of weakly acid reflux in the lung means that definitive antireflux control can only be achieved mechanically. Given the age and comorbidities of the IPF population, high quality trial data is limited. Low mortality animal models have been developed, but their applicability to IPF in humans has been debated (Brownlee et al., 2010).
To date, a causative role for reflux and microaspiration in IPF has never been convincingly demonstrated.

Traditionally, respiratory and gastrointestinal clinicians work within distinct areas of clinical practice. Respective specialists train in distinct pathways and research efforts have been organised within distinct teams. Developing a better understanding of the interplay between gastro-oesophageal reflux, microaspiration and chronic lung disease demands a multimodality approach to both research and clinical practice. The traditional barriers between these different teams may provide another reason why progress in this area of practice has been modest.

Despite the limitations of the current IPF literature, the possibility that antireflux therapy might stabilise disease progression has been enthusiastically explored. One investigator concludes, “we do know that stopping reflux in IPF patients by a fundoplication can halt the progression of the disease” (Allaix et al., 2014). Unless better evidence can be found to support an aetiological link between reflux and the progression of fibrosis in IPF, this stance will remain contentious. In general, the age and frailty of people with IPF make them poor candidates for major surgery. The links between general anaesthesia, surgery and acute exacerbations indicate that an aggressive, surgical approach can cause more harm than good (Luppi et al., 2015).

In this early phase of research, the aim of the current study was to explore the feasibility of a systematic, multimodal assessment of reflux and aspiration in IPF. In the setting of a prospective research study, a consecutive series of patients with well-characterised IPF were invited to undergo a panel of investigations designed to analyse symptoms, oesophageal physiology and bronchoalveolar environment.

6.1 Recruitment and feasibility

In total, half of the 76 clinic attenders invited to participate in the study were recruited. During recruitment we explained to all patients that this was a research study, with invasive tests associated with a risk of morbidity and mortality. Whilst all participants had their investigations discussed by a dedicated MDT, it was made clear to all that in some cases, no change to clinical management would be made. Given the nature of the study, the 50% accrual rate attests to the enthusiasm that this group of patients have for research.
participation, which in turn relates to the paucity of effective treatment options that are currently available.

Of the 44 patients who indicated their preliminary agreement to take part in the study, 36 completed the study protocol. Four patients died from their disease before they were able to participate in the protocol, a figure which demonstrates the unpredictable and potentially rapid decline which may be seen. Clinical trials involving patients with IPF should be designed to reflect this finding; recruitment of these patients will generally be associated with an attrition rate, and the recruitment process must be sensitive to the fact that clinical deterioration can happen quickly.

Of those patients that commenced the study protocol, all but one were able to tolerate both the oesophageal physiology and the bronchoalveolar lavage. The protocol completion rate was 97%, which would be acceptable to any clinical bronchoscopy or oesophageal physiology lab. There were no complications associated with either oesophageal physiology or ambulatory oesophageal monitoring. Bronchoalveolar lavage resulted in one minor nosebleed and one subsequent hospital admission with symptoms of malaise and lethargy.

There were a number of logistical issues related to the study protocol in this group of patients, in whom the median age was 72.5 years and the oldest study participant was 85 years. Many were reliant on friends or family for transport to and from the hospital. One in ten patients remained as in-patients for the night in between the insertion of the oesophageal probe and the bronchoscopy. Those who stayed overnight all lived at least 20 miles from the hospital. Many patients were taking multiple medications at the time of their participation in the study. Cessation of proton pump inhibitors, H2-receptor blockers and over-the-counter antacids were all stipulated by the study protocol. Aside from these drugs, the only group of medications that required any specific consideration were anticoagulants. One patient (IPF22) was taking the antiplatelet agent clopidogrel as secondary prevention following a previous cerebrovascular accident. He suffered a nosebleed at the time of his bronchoalveolar lavage, which was abandoned. After consultation with his own stroke physician, it was deemed safe to discontinue the clopidogrel temporarily and the lavage was repeated without complication.

Overall, therefore, this IPF study population were able to tolerate the invasive protocol. The protocol could be employed amongst the wider IPF population in a larger study.
Subjective and objective assessments suggest that a broad spectrum of individuals participated in the study. Use of long-term oxygen therapy was listed within the study exclusion criteria. It is likely that clinicians involved in recruitment would also have passed over the frailest of individuals, given the invasive nature of the study protocol. Despite this, nearly half of individuals recruited had been classified as grade 3 or 4 on the MRC breathlessness scale (Fletcher et al., 1959).

Off-PPI, the mean score recorded for the St George’s Respiratory Questionnaire was 45. In a review study which considered the psychometric properties of SGRQ in IPF, the mean baseline score aggregated from the individual studies was also 45 (Swigris et al., 2014). The respiratory symptomatology of the current study population therefore appears comparable with the broader IPF population.

### 6.2 Subjective assessment of reflux and aspiration

In patients with IPF in whom gastro-oesophageal reflux can be identified, the typical reflux symptoms (heartburn and reflux) are known to be less common (Tobin et al., 1998). This group of patients will generally see a respiratory physician more commonly than a gastroenterologist or gastro-intestinal surgeon. As a result, the initial identification of gastro-oesophageal reflux can be challenging.

Amongst the 36 patients included in the current study, 11 had pre-existing evidence of GORD based on clinical correspondence or endoscopy findings. In the latter group of patients who completed the structured GerdQ questionnaire, only 4 of 17 patients (24%) returned scores consistent with a diagnosis of GORD disease. With respect to symptoms of extra-oesophageal reflux, 16 of 36 participants (44%) returned scores consistent with pathological laryngo-pharyngeal reflux. When compared with the 60% of participants identified to have pathological levels of reflux on objective testing, these proportions are all very low. If structured symptom questionnaires make any contribution to the diagnosis of gastro-oesophageal reflux disease, there remains a significant proportion of patients in whom high levels of reflux are clinically silent.

This is a significant finding: in an individual with high levels of reflux, microaspiration could still proceed in the absence of symptoms. In this patient population, it seems that objective oesophageal physiology testing should be used liberally, especially if there is any question that an individual might be a candidate for antireflux surgery.
Interestingly, the two-week cessation of proton-pump inhibitors had no consistent impact on reflux symptoms in the current study. The same was true for both gastro-oesophageal and extra-oesophageal symptoms. There are several possible explanations for this:

One possibility is that respondents were unable to accurately recall their symptoms over the preceding period and may simply have considered their normal pattern of symptoms. The RSI tool has not been widely validated for a two week recall period. It seems likely, however, that respondents would have been mindful of the fact that they discontinued their PPI medication two weeks previously and that this would provide an aide memoire in terms of their symptom chronology.

Another possibility is that a significant proportion of participants continued taking their PPI. This also seems unlikely as our study attrition rate, for an invasive protocol, suggests a motivated group of participants. In addition, the pH traces obtained at ambulatory monitoring suggest that the patients had indeed discontinued PPI therapy: the median reflux index indicates that, on average, oesophageal pH was acidic for over 6% of the monitoring period.

In reality, structured symptom assessment did not identify a high level of symptoms in this patient group, irrespective of PPI status. As a result, cessation of PPI would not be expected to have a big impact on symptoms.

Within the latter cohort of participants, respiratory health-related quality of life was assessed using St George’s Respiratory Questionnaire. Individual scores indicated an improvement in quality of life after PPI medication had been discontinued. This is contrary to the pattern of change that would be expected were acid reflux and aspiration contributing to respiratory symptoms.

In a study that made use of contrast-enhanced MRI, PPI use was shown to reduce the volume of gastric contents in the postprandial period (Babaei et al., 2009). It is possible that PPI use may be associated with a refluxate that has a lower volume but also a higher concentration of non-acid elements.

If non-acid elements, such as bile, were a more potent stimulus for respiratory symptoms than acid refluxate, PPI cessation could result in an improvement in symptoms. The association between non-acid reflux and respiratory symptoms has been demonstrated in a pH/impedance study in children with chronic respiratory disease (Rosen and Nurko, 2004).
In the current study, the number of respondents on which these observations are based is small, but further study into the association between PPI therapy and respiratory symptoms is warranted.

### 6.3 Oesophageal physiology monitoring

All study participants completed ambulatory oesophageal monitoring with a multichannel intraluminal impedance pH catheter, which was sited after formal manometric localisation of the lower oesophageal sphincter. At the time of study design, this technique was the “state of the art” for quantification of gastro-oesophageal reflux. To date, only three published studies have combined pH and impedance techniques to assess gastro-oesophageal reflux disease (Savarino et al., 2013; Kilduff et al., 2014; Gavini et al., 2015).

Prior to ambulatory monitoring, all patients underwent oesophageal physiology analysis. In the first 11 patients, this was achieved through conventional 8-channel manometry. In the latter 25, high-resolution manometry was used.

The key difference between these technologies is the number of manometers that are mounted within the catheter. As a result, HRM has a higher sensitivity for subtle dysmotility. The publication of normal ranges for the key study parameters means that subjects may still be dichotomised into normal and abnormal groups, with respect to the upper limits of the 95% confidence interval for respective control populations. To an extent, the manometry results from 8-channel and HRM may therefore be compared.

In those who were assessed using 8-channel manometry the combination of partial or complete LOS relaxation with swallowing and normal peristalsis was seen in 6 of 11 subjects (55%). In those assessed with HRM, ten of 25 subjects were defined as having a normal swallow by Chicago criteria (40%). Irrespective of which technique was used, a high proportion of subjects were demonstrated to have a degree of dysmotility.

Published reference ranges do not provide the ideal data to use for comparison. In the case of manometry, the main concern is that the reference data were measured in a younger group than that recruited to the current study (Zerbib et al., 2005). In routine clinical practice however, it is these generic reference ranges that are used to assess a patient’s suitability for antireflux surgery.

When individuals have oesophageal dysmotility, or failure of relaxation of the lower oesophageal sphincter with swallowing, there is a higher risk of complications following
antireflux surgery. Dysphagia and gas bloat are the major problems that result from an excessively tight LOS but malnutrition may also result. Dysmotility is therefore a relative contraindication to surgery. At worst, revisional surgery may be required and the likelihood of a good outcome is reduced with successive operations. In the frail IPF group, reoperation would be associated with significant risk of morbidity, and potentially, mortality.

Many surgeons would now offer a tailored fundoplication in this situation. There is an argument that a 180° or 270° fundoplication causes less of the symptoms that can result from a tight lower sphincter. To date, there is no good evidence that this practice actually results in better outcomes.

In previous IPF studies, the oesophageal manometry has been undertaken with conventional (5-9 channel) catheters and the results are mixed. Raghu reported the “majority” of subjects to be normal, whilst Kilduff reported only 17% to have ineffective oesophageal dysmotility (Raghu et al., 2006a; Kilduff et al., 2014). In contrast, Soares and Patti reported abnormal manometry in 64% and 75%, respectively (Patti et al., 2005; Soares et al., 2011). In a recent study of subjects with IPF, Savarino noted comparable patterns of dysmotility when compared with a group of healthy volunteers, who were only slightly younger (Savarino et al., 2013).

In summary, it seems likely that IPF patients have higher levels of oesophageal dysmotility. It is unclear whether this relates to age, comorbidities or the disease itself. In those considered for antireflux surgery, it would seem appropriate that modern manometry techniques are used and that the results are carefully considered.

All study subjects were asked to discontinue PPI medication prior to the study protocol. All but one complied with this request. In this isolated case (IPF21), the subject reported that he had continued to take his medication because he forgot to stop it, rather than because he was fearful of symptom recurrence. The pH trace from his ambulatory study confirmed an elevated baseline pH, which confirms that he had continued to take his medication. Interestingly, both the pH and impedance readings demonstrate high levels of gastro-oesophageal reflux. This case represents an example of GORD refractory to PPI therapy. In the IPF population, many of whom have asymptomatic GORD, it cannot be assumed that life-long PPI therapy will control reflux effectively.

Ambulatory monitoring was well-tolerated, as evidenced by the duration of monitoring that the study subjects managed. The shortest period captured was 16 hours, which is
conventionally regarded as the minimum required for representative results. The median monitoring period was 23 hours.

Taken in isolation, the pH results identified supranormal levels of gastro-oesophageal reflux in 20 of 36 study subjects (56%). This figure relates purely to acid reflux detected at the distal oesophagus. The single most important reading used to assess the degree of gastro-oesophageal reflux is the proportion of time for which the pH at this distal probe was lower than 4, sometimes referred to as the reflux index. In surgical practice this figure is often combined with the total number of refluxes, the number of long refluxes and the length of the longest reflux, to provide a composite marker of disease severity, the DeMeester score. In our study subjects, the reflux index and the DeMeester score identified the same 20 individuals as having high levels of pathological reflux. This finding adds weight to our definition of normal and abnormal results.

When the results of impedance monitoring were considered in addition, two further subjects were found to have objectively high levels of reflux. Both had high levels of weakly acid reflux. In total therefore, 22 of 36 subjects (61%) in the current study had objectively high levels of reflux after combined pH-impedance monitoring.

Overall, impedance monitoring only identified a pathological level of reflux in a small proportion of subjects, despite the high proportion for whom a high pH index was recorded. This relates, in part, to the way we have defined normality with respect to impedance analysis. Until recently, most reports of oesophageal impedance analysis have quoted the 75\textsuperscript{th} centile from a healthy control population as the upper limit of normal. With respect to the broader biomedical literature, this is unconventional. Ordinarily the upper limit of normal for a test result is taken to be 2 standard deviations above the mean, approximating to the upper limit of the 95\textsuperscript{th} centile. Recent publications, in addition to national association guidelines, support the use of the 95\textsuperscript{th} centile as the upper limit of normal (Association of Gastrointestinal Physiologists, 2015). This is the standard used in the current analysis.

With respect to the issue of oesophageal motility, impedance monitoring can be used to assess the efficiency with which the oesophagus clears a bolus of material. In the current study, mean bolus clearance time was prolonged in 11 of the 36 subjects. Eight of these individuals (73%) also had evidence of dysmotility at manometry, which provides some corroborative evidence that dysmotility is a significant issue amongst this patient population.
Proximal reflux was not commonly detected amongst the subjects in the current study. Only seven individuals had a high number of reflux events detected at the proximal channel on impedance. All but one had high levels of reflux overall, as assessed by the combination of pH and impedance.

In the current literature, there is no consensus as to how normality should be defined when pH-impedance data are analysed. Were parameters such as proximal reflux to be included within the definition of abnormality, the proportion of subjects with high levels of reflux would increase. As with many physiological tests, oesophageal monitoring can provide a vast array of data. Oesophageal volume exposure could be taken into account, as could sub-analyses based on supine/upright posture.

On balance it seems reasonable to combine the reflux index, from the pH trace, with the number of reflux events, from impedance monitoring, to provide an objective definition of pathological gastro-oesophageal reflux in the IPF population. Additional parameters may provide further information, without defining a disease state. Few of the papers in the IPF literature specify how pathological levels of reflux were defined at ambulatory monitoring.

Table 6-1 illustrates the prevalence of pathological reflux amongst IPF patients, as represented in the current literature. Whilst the higher prevalence figures are often quoted (Tobin et al., 1998; Raghu et al., 2006a), the figure of 61% in the current study is comparable to most published estimates.

There are other factors that may have influenced the prevalence estimate from this prospective study. The clinic patients who were approached were aware that the protocol involved a series of invasive tests, the results of which would be considered by a multidisciplinary team. The potential for additional therapy may have enticed those with more severe reflux symptoms to participate. This issue is inherent to many prospective clinical studies.

Albeit in a relatively small sample, the unselected, prospective nature of the current study adds validity to our prevalence estimate.
Table 6-1. Reported prevalence of pathological gastro-oesophageal reflux detected by ambulatory monitoring. N/S=not specified

<table>
<thead>
<tr>
<th>Reference</th>
<th>n</th>
<th>Recruitment source</th>
<th>Technique</th>
<th>Prevalence of pathological GOR (%)</th>
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<tbody>
<tr>
<td>(Tobin et al., 1998)</td>
<td>17</td>
<td>ILD clinic</td>
<td>pH</td>
<td>94</td>
</tr>
<tr>
<td>(Raghu et al., 2006a)</td>
<td>65</td>
<td>ILD clinic</td>
<td>pH</td>
<td>87</td>
</tr>
<tr>
<td>(Salvioli et al., 2006)</td>
<td>18</td>
<td>N/S</td>
<td>pH</td>
<td>67</td>
</tr>
<tr>
<td>(Sweet et al., 2007)</td>
<td>30</td>
<td>Pre-transplant swallow assessment</td>
<td>pH</td>
<td>67</td>
</tr>
<tr>
<td>(Bandeira et al., 2009)</td>
<td>28</td>
<td>ILD clinic</td>
<td>pH</td>
<td>36</td>
</tr>
<tr>
<td>(Soares et al., 2011)</td>
<td>16</td>
<td>Routine swallow assessment</td>
<td>pH</td>
<td>56</td>
</tr>
<tr>
<td>(Savarino et al., 2013)</td>
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<td>N/S</td>
<td>pH-impedance</td>
<td>83</td>
</tr>
<tr>
<td>(Kilduff et al., 2014)</td>
<td>18</td>
<td>Patients recruited for a study of PPI and cough</td>
<td>pH impedance</td>
<td>44</td>
</tr>
<tr>
<td>(Gavini et al., 2015)</td>
<td>54</td>
<td>Pre-transplant swallow assessment</td>
<td>pH-impedance</td>
<td>39</td>
</tr>
</tbody>
</table>

6.4 Bronchoalveolar lavage

In this study ambulatory oesophageal monitoring was combined with a standardised bronchoscopy and bronchoalveolar lavage (BAL) in all subjects. To date, this is the first report in which these techniques have been combined.

BAL was performed in accordance with a protocol that is now well established within our research group (Stovold et al., 2007). A volume of 180ml was used consistently. All studies were performed by the same respiratory physician, who has extensive experience in bronchoscopy and BAL (IAF).

For each subject, between 5 and 10ml was sent from the bronchoalveolar lavage fluid (BALF) and transported to the microbiology lab for processing. Subsequently, the median volume available for analysis was 78ml (range 35-113; IQR 64-87). Our median yield of 43% compares favourably with the recommended minimum yield of 30%, quoted in the American Thoracic Society’s guidelines (Meyer et al., 2012).

The control group for bronchoalveolar lavage is one of the key limitations of this study. The controls in the current study were healthy volunteers and younger than the study subjects. It
would have been more informative to compare the markers of aspiration from the study group with a control group of similar size and comparable age range. Ideally, the control group would have been selected from a population with a similar restrictive pattern of lung disease. At this early phase of research, it was difficult to justify a high volume BAL in such a group.

6.4.1 Pepsin

Clinically, the concentration of solutes in BAL fluid is most commonly measured after lung transplantation. The association of pathological reflux and accelerated onset of bronchiolitis obliterans syndrome (BOS) has been investigated using these solutes as a biomarker for microaspiration (Robertson et al., 2012). High levels of pepsin and bile salts have been correlated with adverse outcomes (Stovold et al., 2007). The proposed links between reflux, aspiration and the pathogenesis of IPF has prompted their use in interstitial lung disease, but this literature is less well established.

In the present study, pepsin concentrations were measured using a validated in-house assay with a limit of detection of 3ng/ml. In our small control group, the range of pepsin concentrations reported using the same assay was 0-2.3ng/ml, with a median of 1.1ng/ml. With reference to this upper figure, 17 subjects in the current study had high levels of pepsin measured in BALF. Once the group was dichotomised by reflux status, there was no difference in the proportion of subjects with high levels of reflux: 7 of 14 in the GOR-group vs 11 of 22 in the GOR positive group.

Two previous studies have investigated BALF pepsin concentrations in IPF. Lee et al reported median concentrations of 35ng/ml in 24 cases where BAL was performed around the time of an acute exacerbation and 47ng/ml in 30 “stable” patients (Lee et al., 2012). The authors conclude that aspiration may play a role in some cases of acute exacerbation. The design of this study is initially appealing, but there are some methodological issues. First, there is no objective assessment of gastro-oesophageal reflux. Second, the BAL was performed with “at least 100ml sterile saline” instilled. This variation makes the pepsin concentrations difficult to interpret. Third, there were seven subjects in which BAL was repeated, such that patients featured in both the “stable” and “acute exacerbation” groups. This duplication raises questions about comparisons between two groups. Six of the seven patients had similarly
low BAL pepsin levels at the two time-points, which challenges the validity of the authors' conclusions.

Savarino et al used the Peptest lateral flow device to assess the presence or absence of pepsin in saliva/sputum and BAL samples captured from 21 IPF patients (Savarino et al., 2013). Pepsin was detected in BALF in 67% of cases and in saliva/sputum in 61%. Bile acids were also measured. The authors report that all IPF patients with bile acids or pepsin detectable in BALF had abnormal impedance-pH test results. The detail of this statement is unclear from the manuscript, as the proportion of subjects with abnormal bile salts and pepsin within this group is not given.

In the current study the association between high pepsin levels and pathological GOR is less clear. The correlation between these data was low. There were individuals who had detectable pepsin but no pathological reflux, and vice versa. That said, 31% of subjects had pathological reflux and high levels of pepsin. It is possible that this microaspiration might contribute to lung disease in this subgroup. Using pepsin as a marker of aspiration remains attractive therefore, but a number of methodological barriers remain.

The sensitivity of pepsin as a marker of aspiration may be diminished as a result of the dilution inherent to BAL. In addition, a conventional BAL will only sample one subsegmental bronchus and the small airways with which it communicates. HRCT-guided lavage may improve sensitivity, but there is no good evidence base to support this modification.

The significance of ectopic pepsin production has yet to be fully resolved. Pepsin A is known to be specific to the stomach, but type II alveolar epithelial cells have been shown to produce pepsin C (Gerson et al., 2008; Elabiad and Zhang, 2011). These proteins are distinct gene products (Taggart et al., 1985; Hayano et al., 1988). The assay used in the current study is specific for type A pepsin so detectable BALF pepsin levels should accurately reflect gastro-oesophageal reflux and subsequent aspiration.

Once in the lung, the speed of pepsin clearance after an aspiration event is unknown. Internalisation of pepsin within hypopharyngeal epithelial cells has been demonstrated, but internalisation within the pulmonary epithelium has yet to be evaluated (Johnston et al., 2010). In the IPF lung, it is possible that fibrotic changes would influence epithelial permeability. Pepsin remains a promising biomarker for reflux and aspiration, but its behaviour with the lung must be clarified if its detection is to inform clinical practice.
The current data suggest that it is possible to combine oesophageal physiology monitoring and bronchoalveolar lavage analysis to identify a proportion of IPF patients with evidence of aspiration. With further validation of pepsin as a biomarker of aspiration, this combination of tests may provide a clinical insight into the IPF patients for whom aspiration is most significant.

6.4.2 Bile salts

In this study we used a commercially available spectrophotometric assay to quantify the concentration of bile salts in bronchoalveolar lavage fluid. This is the methodology that has been used most often in the studies that have established bile salts as a marker of aspiration, largely in the field of lung transplantation (D'Ovidio et al., 2005; Blondeau et al., 2008). Samples were run in duplicate to generate each measurement. This process was performed three times for each study subject. Three subjects were found to have detectable levels of bile salts. In each case, three further runs were performed so as to improve the reliability of the result. For one additional subject, only one of the readings from the first three runs was valid. Three further runs were performed in this case also. In total, four subjects had positive bile salt readings, of 0.7, 1, 2.3 and 8.5 µmol/l. The highest of these readings came from the one BALF sample that was turbid on visual inspection. The accuracy of the assay may be lower in these circumstances.

The results of a previous validation study indicate a lower limit of detection of 5 µmol/l for this specific assay. In this context, the current data suggest bile salts were not detectable in the BAL fluid.

The study by Savarino et al. is the first to evaluate BAL and saliva/sputum samples from IPF patients for total bile acids (2013). In keeping with previous attempts to evaluate bile acids as a marker of aspiration, a commercial assay (Bioquant, San Diego, USA) was used (D'Ovidio et al., 2005). Savarino et al. concluded that patients with IPF had more bile acids in BAL and saliva than non-IPF patients and controls. This assumes an accurate detection of levels below 1 µmol/L, which were not reproduced in the validation study (Parikh et al., 2013).

In a head-to-head comparison, Parikh et al reported a superior sensitivity for the TBA assay used in the current study, in comparison with the Bioquant assay used by Savarino et al (Parikh et al., 2013). This is due to the reversible nature of the reaction in the TBA method, which permits enzymatic recycling.
In general, spectrophotometric assays do not appear sensitive enough to detect bile salts in BAL fluid. This is unsurprising given that they were designed to detect far higher levels of bile salts in the serum of patients with liver disease. Lower concentrations of bile salts may be detectable using analytical chemistry techniques such as tandem mass spectrometry, but the physiological relevance of these lower concentrations requires clarification. In contrast to pepsin, aspiration of bile salts would also require duodenogastric reflux.

6.5 Bronchoalveolar cytology

6.5.1 Differential cell counts

In the current study total BAL cell counts were calculated with a haemocytometer. After standardised preparation of cytospins, differential cell counts were estimated by counting 500 cells and recording the number of neutrophils, lymphocytes, macrophages, eosinophils and basophils.

With reference to the values reported for healthy controls, the IPF subjects in this study had elevated levels of macrophages, and markedly elevated levels of neutrophils. These findings are consistent with the reported characterisation of IPF patients using BALF (Meyer et al., 2012).

6.5.2 Cell staining

In previous reports, pulmonary aspiration of gastric contents has been evaluated cytologically through selective staining of bronchoalveolar lavage fluid. Using an oil red stain, lipid uptake into macrophages can reportedly be measured (Colombo and Hallberg, 1987). The lipid-laden macrophage index (LLMI) gives a measure of the density of lipid uptake.

In the current study, only a minority of subjects had cytospins which demonstrated significant lipid uptake within pulmonary macrophages. The limits of agreement for the LLMI figures were -6.9 to +3.8. This inter-rater reliability suggests that the measurement error for the larger, positive LLMI values was acceptable. LLMI values less than 10 were less accurate.

With reference to the lipid-laden macrophage index (LLMI) for healthy controls, only six subjects had evidence of significant lipid uptake. The relationship between reflux and LLMI was assessed through correlation and by GOR status (normals vs refluxers). Neither analysis revealed any evidence of association.
Oil red staining has been used effectively within our research group in the past. I adjusted the standard operating protocol by using Cilestin Blue as a counterstain, in place of Harris Haematoxylin. This provided a more vivid result and enumeration of the requisite 300 macrophages was possible in all cases. To counter enumeration bias, a proportion of the slides were second read by an observer blinded to the physiology and pepsin results. Correlation analysis suggested a high level of inter-observer agreement.

Several authors have also reported negative results. Limited specificity of the test may be explained by the finding of lipid uptake into alveolar macrophages in non-aspiration lung disease, such as pneumonia (Kitz et al., 2012). Theoretically any process which leads to increased lysis of intrapulmonary cell walls, with subsequent release of lipids, could result in an increased LLMI. Conversely, the sensitivity of the LLMI for detection of aspiration relies on thorough sampling of the intrapulmonary environment, which is rarely possible with BAL. The half-life of alveolar macrophages is another area that is poorly understood, with data currently available only in animal models (Colombo et al., 1992). In the paediatric population, LLMI may have a role in identifying reflux and pulmonary aspiration, but the technique appears less useful in adults.

In addition, we assessed haemosiderin uptake in macrophages using a Perls Prussian Blue stain. Macrophage haemosiderin uptake has been used to explore oxidative stress in lung transplant recipients. Reid et al suggested that under inflammatory conditions, activated neutrophils release harmful free iron from ferritin (Reid et al., 2001). Alveolar macrophages sequester free iron as haemosiderin in order to prevent the generation of free radicals and reactive oxygen species.

In contrast to the results of LLMI evaluation, macrophage haemosiderin uptake was visible in the majority of the study subjects. With reference to haemosiderin scores reported for healthy controls, 33 of 36 subjects in the current study had high results (Reid et al., 2001). Haemosiderin scores were not associated with reflux status, but were found to be higher in subjects with more severe lung disease, as assessed by vital capacity.

The significance of iron deposition has been studied in IPF, in relation to putative associations with occult alveolar haemorrhage, angiogenesis and pulmonary hypertension (Kim et al., 2010; Puxeddu et al., 2014).

These issues seem increasingly pertinent in light of positive findings from recent clinical trials. Nintadenib, a tyrosine kinase receptor inhibitor, has potent angiostatic activity in both
in vitro and ex vivo studies (Hilberg et al., 2008). Pirfenidone has been shown to have antioxidant properties (Salazar-Montes et al., 2008). Techniques that characterise oxidative stress may be useful to personalise therapy in the future.

6.6 Multidisciplinary review

This research study was possible due to an ongoing collaboration between respiratory physicians, general surgeons and translational scientists with an interest in reflux and chronic lung disease. Whilst the design was largely observational, it was clear that the data generated could have implications for clinical management. Optimal management has yet to be defined in this area of practice, so we set up a multidisciplinary team to consider the study results and plan future management accordingly. In addition to surgical and respiratory input, the core clinical membership includes anaesthetic support. Only three patients from the current cohort were recommended for antireflux surgery; two have undergone fundoplication to date. One of these two patients was initially being considered for antireflux surgery but underwent urgent lung transplantation first, in light of declining pulmonary function.

In the context of the current literature, this approach to antireflux surgery may be regarded as overly conservative. However, the same MDT has demonstrated a positive approach to the management of reflux and aspiration in chronic lung disease, particularly in the setting of lung transplantation (Griffin et al., 2013; Jones et al., 2013).

Some of the barriers to antireflux surgery have included the risks of anaesthesia and major surgery in a frail elderly population, in addition to concerns about oesophageal dysmotility in many of the study subjects. These issues have been easier to confront in a multidisciplinary setting, where there is a growing awareness of the nuances of the IPF population. The proposed links between anaesthesia, surgery and acute exacerbation is one such example. Individual clinicians also find it easier to counsel patients on conservative therapy when this has been the joint perspective within the MDT.

For some patients, it was clear that the subclinical nature of their reflux meant that patients were reluctant to undergo treatment. Despite acknowledging the potential risks of persistent reflux and aspiration, many subjects were reluctant to consider treatment, medical or surgical, for something that was imperceptible to them.
When this range of attitudes came to light, the decision was taken to investigate this issue more closely. The data from the current study highlight the challenges of characterising reflux and aspiration in IPF. It will take time to clarify the possible role of aspiration in IPF. It is crucially important that we consider patients’ views as we continue to research this area and to better define the group of patients that might benefit from antireflux therapy.
6.7 Evaluating patient attitudes: measuring the perceived burden of IPF and antireflux therapy

In the work designed to characterise reflux and aspiration in IPF, around 50% of patients approached were willing to participate in a study which involved two different invasive tests. For an observational study, this represents an impressive proportion. Compliance with the study protocol was also high.

Conversely, around 50% of patients did not wish to take part. This might be consistent with a proportion of patients who were more conservative and risk averse. Such a dichotomy might also be expected for attitudes to treatments with a degree of risk.

Exploring the patient perspective in IPF involved a very different approach than the work to characterise reflux and aspiration. Evaluating attitudes towards health is a complex area but the theoretical and practical basis is increasingly well established within the health economics literature.

6.7.1 Study design

For the current study, the standard gamble was identified as the most appropriate tool to use. The impact of reflux on lung disease and the outcomes from antireflux therapy will always be associated with a degree of uncertainty. It was felt that the standard gamble was the best technique with which to frame that uncertainty.

Health state utility analysis has not been undertaken in idiopathic pulmonary fibrosis or any other interstitial lung disease previously. The unprecedented nature of this work made for some specific challenges. Whilst health state vignettes are available for many other chronic lung diseases, this is not the case in IPF. Therefore, the first step was to construct appropriate health states. These vignettes needed to be easily understood, provide a valid reflection of the disease and be concise enough to be incorporated into a standard gamble interview. Using longer vignettes may have provided a richer and more realistic account of living with the disease, but the concise nature of our health state cards made them accessible to the interview participants.

The most difficult aspect of designing the study came in integrating the diverse considerations of lung disease and antireflux therapy. The use of utilities made this comparison possible, but the standard techniques will only generate a utility for one health
state at a time. Colleagues from our own unit have described a chained standard gamble technique, in which health state vignettes are successively used within the gamble (McNamee et al., 2004). This technique is feasible because the utility recorded for each health state can be used to provide the anchor for the next standard gamble. In the current study, it was felt that a chained standard gamble would have introduced another, unwelcome layer of complexity and added burden for participants. Instead, four health states were measured consecutively using separate, conventional standard gambles.

Ideally, this study would have been designed to compare the burden of IPF with the range of possible outcomes associated with antireflux therapy. In time, this may provide an insight into patient perceptions of the risks and benefits of treating reflux in IPF. At present, the data on the pulmonary benefit of antireflux therapy in patients with IPF are essentially anecdotal. In the absence of high-quality study data with which to quantify potential benefit, the focus of the analysis shifted. The four rounds of the standard gamble were designed to compare the burden of disease in IPF with the potential burden of antireflux therapy. This latter concept can be readily derived from the experience of clinicians and patients in the upper GI surgery clinic. These groups informed the design and revision of the health state vignettes that were eventually used.

There is no accepted process for the design of health state vignettes in utility analysis. Some studies simply use the name of the condition or treatment outcome to characterise the scenario in question (Laccourreye et al., 2012). It is increasingly accepted that this is not the most robust technique as the details of the health state are left entirely to the imagination of the respondent, introducing a high degree of subjectivity. Alternatively it has been shown that study methodology becomes more reproducible if “word pictures” can be used (Hamilton et al., 2014).

This approach was utilised in the current study. Where possible, validated descriptors of disability were used. Qualitative study data were also incorporated where possible. Most importantly, the process was iterative, with successive rounds of input from patients and clinicians, until the vignettes became stable and acceptable.

The accuracy with which vignettes reflect a health state is difficult to prove but their validity is enhanced by the inclusion of explicit detail. As with more traditional biomedical studies, there are a number of techniques that can be used to assess validity, both of the vignette and the standard gamble process. In the current study, two key validation steps were the
inclusion of the ranking exercise and the comparison with EQ5D, which provides an indirect comparison with health state utilities derived from time trade-off.

6.7.2 Study subjects

The selection of interview respondents was another important decision in the design of the current study. At this early stage of research, the aim of this work was to capture the patient perspective, so IPF patients were chosen as the target audience. Almost all subjects preferred to participate in their interviews through home visits. Given the relative rarity of the disease, completing the interviews involved travelling across a large geographical area, including Greater Newcastle, North and South Tyneside, Northumberland and Gateshead. There are therefore resource implications for future studies of this type.

The study subjects appear to be representative of the broader IPF population in the North East of England. Median percentage predicted vital capacity was equivalent to that in the characterisation study population (78% in both studies). They were largely elderly, male, retired and with a relatively low level of formal qualifications; 11 of 59 participants had completed either A-levels or degrees and a further three had NVQ level 4-5. They were universally White British by ethnicity. Given the age and level of formal qualification within the group, the ease with which the interview could be completed would seem to be an important consideration.

In a previous postal study of diabetic subjects a standard gamble was administered both at interview and by post (Hammerschmidt et al., 2004). While the results showed good consistency, only about two-thirds of the completed postal forms were usable. The results of the current study demonstrate that a standard gamble is feasible in the IPF population. Telephone interviews or postal formats would have been more challenging as additional explanation was required at times.

6.7.3 Key findings

The majority of respondents rated both of the IPF health states as preferable to either of the antireflux therapy outcomes. A significant minority, however, regarded either one of the antireflux therapy outcomes as preferable (36% of respondents). This split indicates a genuine division of opinion across the group as a whole.
Almost all respondents (54 of 59) regarded either an adverse outcome from antireflux therapy or the moderate IPF health state as the least preferable health state.

Interestingly, five people in the study regarded the antireflux medication scenario as worse than moderately severe IPF and an adverse outcome from antireflux surgery. In the absence of any qualitative analysis, it is unclear from the present data whether respondents were more concerned about the prospect of having to take supplementary medication or about the risk of side effects.

The average utilities for the health states suggest that respondents rated mild IPF as the most preferable health state and an adverse outcome from antireflux surgery as the least preferable. The utilities recorded for the mild IPF state and for medical antireflux therapy were equivalent.

By the end of the interview, each respondent had evaluated his or her own health using three different techniques: standard gamble, EQ5D and the visual analogue scale, which is included within EQ5D. The mean utilities recorded by these latter techniques were equivalent (0.613 vs 0.611) but the utility recorded by the standard gamble was significantly higher (0.798).

The precise reason for these differences cannot be established from these data, but the subject of bias has been extensively discussed within the healthcare measurement literature. Bias occurs when responses result in a systematic departure from true values under consideration. When subjects are questioned about health, factors related to one’s own circumstances often have an influence. From my own observations, interview respondents would sometimes try to understand the vignettes by thinking about which elements they could relate to, or which were consistent with their own current health.

Bias can occur due to personality traits, attitudes, behaviours or might be more associated with the use of a specific measurement tool (McDowell, 2011). One respondent might exaggerate disability in an bid to delay their return to work, whilst another might play down their problems in a bid to return more quickly.

In the current study, there are several factors that could account for the observed pattern of results.

Stoicism is a personality trait that would be expected to inflate utility scores which were recorded for an individual’s own health. Defensiveness may have the same effect.
Hypochondriasm or a tendency towards attention-seeking behaviour would have the opposite effect.

Response shift is a phenomenon that has been described in individuals with chronic, progressive disease. Individuals have been shown to reframe their perceptions of disease severity as it progresses (Sprangers and Schwartz, 1999). Similarly, it has been shown that individuals living with a significant disability tend to report higher utilities for their situation than respondents considering the same health state objectively (Albrecht and Devlieger, 1999). This would explain the trend towards higher utilities recorded by the respondents with worse pulmonary function for the IPF health states.

Bias has also been reported for the use of devices where the middle section and end points are clearly visible, such as a visual analogue scale. End-scale aversion describes how some individuals will preferentially record responses at the extremes of the scale, whilst more conservative individuals will be drawn to respond in the mid-section of the scale (Parkin and Devlin, 2006).

Another explanation relates more specifically to the methodology of the standard gamble. At each stage, the respondent is asked to choose between the certainty of a defined health state, and the uncertainty of a gamble. Risk-averse individuals will be less happy to gamble, which has been shown to result in higher utility scores. The converse would apply to risk-takers. It is tempting to conclude that the issue of risk aversion might explain why the utilities from the standard gambler were higher. With respect to the figures generated by the visual analogue scale, this may be true.

The same argument is less applicable when the results of the standard gamble and EQ5D are compared. As discussed in section 3.2.9, EQ5D works by mapping a multi-dimensional assessment of health to a set of pre-calculated utility scores. The utility scores that have been calculated for the various permutations of EQ5D were derived from a time-trade off study. As discussed previously, the time trade-off can be thought of as simplified version of a standard gamble rather than a distinct technique. In the time-trade off, there is a group of people who are reluctant to trade, in a way that is analogous to the risk averse respondents in a standard gamble (Hamilton et al., 2014).

It seems more likely therefore, that the respondents in the current study displayed other behaviours which would give rise to inflated utilities for their own health state. Based on my experience of conducting the interviews, I was struck by the stoical nature of the group.
Various attempts have been made to measure and correct for bias in utility assessments, but no single approach has gained widespread support (McDowell, 2011). There is a counter-argument that the combined effects of different biases form an integral part of the health beliefs that are being studied.

Either way, utilities derived from a standard gamble have generally been shown to be higher than equivalent utilities derived from a time trade-off or visual analogue scale (Torrance, 1986). Conversion factors can be used to correct between the different tools.

6.7.4 Validity, strengths and limitations

In conventional biomedical research, the quality of data can often be assessed through direct comparison with pre-existing reference points. Physical data points might be compared to normal ranges, or assessed statistically to assess validity, reliability or responsiveness.

In health economics, many concepts are abstract so “true” values are never revealed. Scaling procedures and validated tools can provide more meaningful results, but the extent to which a measurement reflects the concept it is designed to reflect to will generally remain elusive.

In the absence of any previous utility analysis in interstitial lung disease, a number of internal checks were incorporated into the study design. These provide some logical reference points against which the validity of the data can be appraised.

With respect to the standard gamble, the anchor points “Full Health” and “Immediate Death” were defined as the extremes health states. The most important of these was Full Health, as this was set up as a “perfect” state. All 59 of the respondents ranked Full Health as the most preferable of all the states.

Interestingly, two of the respondents classed one of the descriptive health states as worse than Immediate Death. The concept of a state worse than death appears repeatedly in the literature and does not inherently challenge the fitness of the individual in question to complete the interview. In the current study, however, the standard gamble was predicated on extreme health states as the anchor points in the gamble. For this reason, the standard gamble-derived utilities for these two individuals were excluded from the analysis.

In the overwhelming majority of respondents, the utilities generated by the standard gamble differentiated between the respective health states. This demonstrates two points: Firstly,
the technique appears sufficiently sensitive to distinguish between the perceived quality of life associated with the different states. Secondly, it suggests that the respondents were able to express opinions as to their preferences between the different states.

This pattern is not universally reported in the literature. In a study of rhinoconjunctivitis, patients faced the choice of remaining in their current state for ten years or a gamble, where the possible outcomes were symptom relief and death. The utility values did not correlate well with other quality of life indices, as patients were unwilling to accept anything other than a minimal risk of death (Juniper et al., 2002).

There was also a reasonable degree of consistency between the opinions expressed in the ranking exercise and in the standard gamble. In 41 respondents (70%), health state rankings were concordant with utility estimates.

There were two further logical checks, both of which were satisfied. Firstly, the results of the ranking exercise and the standard gamble both indicated the mild IPF health state to be preferable to the moderate IPF health state. Secondly, the mean utility recorded for respondents’ own health was comparable to that for moderate IPF and worse than the mild IPF. This result is consistent with the inclusive nature of the study recruitment criteria.

Health state utilities are now available for an increasing number of diseases. The main use for these data is in health economics strategy, but they are increasingly used to provide an insight into individual patient preferences. The largest repository is the Cost-Effectiveness Analysis Registry, based at Tufts University in Massachusetts. With reference to this database, it appears that the current study reports IPF utility estimates for the first time. The disease burden associated with this condition can now be compared with that for some of the commoner pulmonary diseases, which will provide an opportunity to raise the profile of the condition.

In lung cancer, for example, health state utilities have been extensively reported, Table 6-2. Chouaid et al reports on EQ5D interviews with 263 patients with advanced non-small cell lung cancer (Chouaid et al., 2013). The study by Sturza et al is a meta-analysis of 23 original reports with varied measurement techniques and varied respondent groups. By comparison, the mean EQ5D utility of 0.613 in the current study demonstrates the significant burden of IPF disease.
Table 6-2. Published health state utilities for lung cancer.

<table>
<thead>
<tr>
<th>Author</th>
<th>Condition</th>
<th>Tool</th>
<th>Severity grading</th>
<th>Mean utility</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Chouaid et al., 2013)</td>
<td>NSCLC</td>
<td>EQ5D</td>
<td>Line of treatment</td>
<td>0.71, 0.74, 0.62</td>
</tr>
<tr>
<td>(Sturza, 2010)</td>
<td>Lung cancer</td>
<td>Mixed</td>
<td>Stage</td>
<td>0.57, 0.77, 0.87</td>
</tr>
</tbody>
</table>

Given utility estimates and prevalence data, pharmaceutical companies can start to calculate the cost per QALY and the potential returns which research and development can be weighed against in IPF.

There are significant limitations in the current study. Firstly, there was no clear reference point with which to say how bad the outcomes of the antireflux therapy should be. Patients who have an adverse outcome from a given treatment represent a broad spectrum. With respect to the medical vignette, it was possible to incorporate the conventional definitions of “rare” and “common” with reference to omeprazole’s product characteristics. Both clinicians and patients felt that the vignettes used gave a convincing account of a bad outcome, but this was not possible to quantify in any meaningful way.

Secondly, we made no assessment of reproducibility in the current study. This would seem particularly relevant to the standard gamble, with its relatively complex methodology. As discussed in section 1.5.4, several previous reports have included high levels of reproducibility for the standard gamble. This would seem to be an important area for future health state utility research in the IPF population.
Chapter 7. Summary and Recommendations for Future Research

In this study, I have completed a panel of assessments in order to characterise the nature of gastro-oesophageal reflux and pulmonary aspiration in 36 individuals with IPF. The findings suggest that a significant proportion of patients experience pathological levels of reflux, although reflux may not be as universal as previously reported in this group. In the majority, pathological levels of gastro-oesophageal reflux were clinically silent.

In addition to gastro-oesophageal reflux, there were high levels of oesophageal dysmotility. This finding is relevant because weak peristalsis is a risk factor for bloating, dysphagia and malnutrition after fundoplication. Dysmotility is regarded as a relative contraindication to antireflux surgery. Individuals with IPF must therefore undergo careful manometric assessment if they are to be considered for antireflux surgery.

In our study population, we used biochemical and cytological methods to look for direct evidence of aspiration in fluid retrieved at bronchoalveolar lavage. Of the techniques under review, pepsin provided the best evidence of aspiration in the study population, with high levels detected in the BAL fluid of 47% of subjects. There was no convincing correlation between markers of gastro-oesophageal reflux and any marker of aspiration. Whilst there was a reflux-positive, pepsin-positive subgroup, there were also groups who were reflux-positive, pepsin-negative and vice versa.

In a series of interviews with another 59 IPF patients, a standard gamble was used to generate health state utilities relating to IPF and to the treatment of gastro-oesophageal reflux disease. Data analysis indicated that respondents were comfortable with the methodology, engaged in the process and able to express clear opinions in the answers they gave. The own-health valuations confirmed, for the first time, the relative burden of disease that is associated with IPF. Comparable utility scores were also recorded for the descriptive IPF health states, indicating the validity of these vignettes.

Respondents were also asked to evaluate health states constructed to represent the adverse outcomes of antireflux surgery. The use of utility scores as a marker of preference enabled a direct comparison between the health states related to the burden of disease in IPF and to the outcomes of antireflux therapy. The majority of respondents regarded both the mild and
moderate IPF disease states as preferable to either of the antireflux therapy outcomes, whilst a significant minority (21 of 59 respondents) did not.

These findings do not allow for conclusions as to the level of risk and benefit that patients with IPF would accept before going ahead with antireflux surgery. There is a clear suggestion, however, that different individuals within a group have very different ideas on the subject. This finding is especially important in an area of medical practice where the balance of risk and benefit has yet to be defined.

At present, the literature on reflux and aspiration in IPF is difficult to interpret. Microaspiration may contribute to the aetiology of IPF but this remains unproven. With respect to clinical practice, some commentators assert that reflux is almost universal in IPF and antireflux surgery could help to stabilise lung disease.

The current findings do not support that statement: significant reflux and aspiration was only evident in the minority of patients, the group that would benefit from antireflux surgery are difficult to identify and a proportion of patients regard the potential burden of surgical complications as worse than that of living with IPF.

The aggregated results of this study demonstrate the importance of multidisciplinary team working and shared decision-making. In time we will develop a better understanding of the risks and benefits of treatment options in reflux and IPF. As clinicians we must communicate this information clearly to patients and listen carefully as they share their values and preferences.

From a research perspective further efforts must be made to identify the group of patients in whom reflux and aspiration may be contributing to the trajectory of lung disease. pH-impedance monitoring is now well established in clinical practice, but far less is known about the markers of aspiration that can be detected in bronchoalveolar lavage fluid.

In the absence of major developments with alternative biomarkers, the results of this study suggest that pepsin may be the most promising option available. An improved understanding of pepsin in the lungs would help to clarify discordant results from a given patient’s reflux and aspiration tests.

One approach to this would be to assess the pepsin subtypes found in gastric juice and BAL fluid. High-pressure liquid chromatography could be used to quantify the relative proportion
of pepsin subtypes in these two compartments. The same technique could be used to confirm the extent of cross-reactivity of pepsin subtypes when measured at BAL.

Secondly, it would be helpful to clarify the rate at which pepsin is cleared from the lungs. At present the relationships between pepsin, chronic micro-aspiration and acute exacerbations of IPF are poorly understood. Serial measurements in a low-mortality animal model would help to clarify the significance of detectable pepsin in BAL. This would be a more difficult study, but the current data suggest that oesophageal physiology alone is unlikely to identify those with IPF for whom aggressive antireflux therapy will be of most use.

Lastly, the results of BAL cytology suggested a negative correlation between objective lung function and oxidative stress: individuals with lower percentage-predicted vital capacity had higher levels of oxidative stress. Perls staining, as a simple marker of oxidative stress, could be used to inform studies of IPF pathophysiology and pharmacological therapy in the future.
Appendices
Appendix A  Characterisation study appointment letter
Appendix B  GerdQ questionnaire
Appendix C  Reflux Symptom Index questionnaire
Appendix D  St George’s Respiratory Questionnaire
Appendix E  Patient attitudes study - Demographic survey
Appendix F  EuroQOL-5D-3L
Appendix G  Standard operating procedure: Bronchoalveolar lavage processing
Appendix H  Standard operating procedure: Geimsa staining
Appendix I  Standard operating procedure: Oil Red O staining
Appendix J  Standard operating procedure: Perls Prussian Blue staining
References


226


227


Taggart, R.T., Mohandas, T.K., Shows, T.B. and Bell, G.I. (1985) 'Variable numbers of pepsinogen genes are located in the centromeric region of human chromosome 11 and determine the high-frequency electrophoretic polymorphism', *Proc Natl Acad Sci U S A*, 82(18), pp. 6240-4.


